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Carbohydrate Chemistry

Volume 5

A Review of the Literature Published during 1971

Senior Reporter

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Preface

In compiling this Report, the fifth of the series, our aim has been to cover the literature available to us between mid-January 1971 and mid-January 1972. However, it will be recalled that last year it was necessary to go to press without four of the chapters (General Methods, Plant Polysaccharides, Microbial Polysaccharides, and Physicochemical Properties) that are normally covered in Part II of these Reports. Our disappointment in the loss of continuity of the series has been mollified by the knowledge that the present Report contains complete literature coverage of these areas for both 1970 and 1971. This Herculean labour was unselfishly undertaken by Dr. R. J. Sturgeon, who has joined us as a Reporter.

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

We are indebted once again to Professor N. K. Kochetkov for providing the English abstracts of a large number of Russian papers and to Drs. L. W. Doner, C. W. Smith, and L. C. N. Tucker for reading and commenting on the whole of Part I.

J. S. B.

April 1972

Contents

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

Part II Macromolecules

Abbreviations

The following abbreviations have been used:

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

Part I

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

By

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1
Introduction

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

Although many notable syntheses were achieved during the year, the keynote in the area of synthetic carbohydrate chemistry has been one of consolidation rather than of innovation. There has been a marked increase in the number of papers dealing with nucleosides (Chapter 21) and one of the most significant achievements has been a synthesis of the fluorine-containing antibiotic nucleocidin by the Syntex group. Not unexpectedly, perhaps, there has been a fall in the number of reports on sugars containing heteroatoms other than oxygen in the ring.

The spate of papers dealing with the application of n.m.r. spectroscopy to the study of carbohydrates remains unabated, and all of the new developments in this area are covered in Chapter 23. Increasing use has been made of lanthanide shift reagents and pulse Fourier-transform ^{13}C n.m.r. spectroscopy, techniques that hold great promise for the future. Chapter 23 also contains a résumé of Durette and Horton's very detailed work on quantitative aspects of the conformational analysis of carbohydrates. Another interesting development has been an attempt by Perlin to correlate the rates of oxidation of aldopyranoses, by bromine water, with the electron density at the carbon atoms, as determined by ^{13}C n.m.r. spectroscopy. There are encouraging signs that ^{13}C n.m.r. spectroscopy will provide a valuable tool in structural and conformational work on di-, oligo-, and polysaccharides.

The International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB) have jointly published tentative and revised rules dealing with abbreviations and symbols for compounds (including carbohydrates) of biochemical interest.[1]

Several books of general interest have appeared.[2, 3] Professor L. L. Leloir's Nobel address on the biosynthesis of carbohydrates has been

[1] *Arch. Biochem. Biophys.*, 1971, **142**, 1.

[2] 'The Carbohydrates. Chemistry and Biochemistry', ed. W. Pigman and D. Horton, Academic Press, 1970, 2nd end., Volumes IIA and IIB.

[3] (*a*) J. F. Stoddart, 'Stereochemistry of Carbohydrates', Wiley–Interscience, 1971; (*b*) G. Dryhurst, 'Periodate Oxidation of Diols and Other Functional Groups', Pergamon Press, 1970.

published,[4] and the role of carbohydrates in pharmaceutical chemistry has been the subject of a review.[4a]

Volume 25 (1970) of *Advances in Carbohydrate Chemistry and Biochemistry* included an obituary of Professor S. Peat, F.R.S., and an obituary of Professor M. L. Wolfrom appeared in Volume 26 (1971).

The November issue[5] of *Carbohydrate Research* was dedicated to Dr. N. K. Richtmyer, in honour of his seventieth birthday.

[4] L. L. Leloir, *Science*, 1971, **172**, 1299.
[4a] J. M. J. Tronchet and B. Baehler, *Pharm. Acta Helv.*, 1971, **46**, 269.
[5] *Carbohydrate Res.*, Vol. 20, November, 1971.

2
Free Sugars

Reviews have been published on sucrose chemistry,[6] the uses of D-xylose in the food and pharmaceutical industries,[7] the sugars from honey,[8] and the connection between carbohydrates and dental caries.[9] The reactions of free sugars with aqueous ammonia have also been reviewed.[10] The use of a computerized system for retrieving carbohydrate references from the current chemical literature has been discussed.[11]

Tests for sweetness have shown that whereas α-D-mannose is sweet, the β-D-anomer is bitter.[12] On the evidence provided, reservations concerning the β-D-anomer must be expressed, since it was crystallized from acetic acid and no specification of purity was reported. However, since it took over six years to isolate β-D-mannose, there will be obvious delay if the sweetness comparison is to be repeated.

A carbon analyser has been used for monitoring the eluate from resins used to separate sugars and has been shown to be satisfactory for detecting p.p.m. of free sugars.[13]

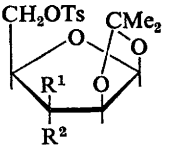
Isolation and Synthesis

Crystalline D-*glycero*-L-*gluco*-octulose and crystalline methyl D-*glycero*-α-L-*gluco*-octulopyranoside have been described.[14] The presence of sedoheptulose and D-*manno*-heptulose in the poppy capsule has been confirmed, and D-*glycero*-D-*manno*-octulose has also been found in this source.[15] The sugar components SF 666A and SF 666B, which were isolated from the fermentation broth of *Streptomyces setonensis nov.sp.*, have been identified as 7-deoxy-D-*glycero*-D-*gluco*-heptose and 7-deoxy-D-*altro*-heptulose, respectively. Both seem to behave as antimetabolites of D-glucose.[16] D-Allose has been isolated from a natural source; it is present

[6] D. W. Fewkes, K. J. Parker, and A. J. Vlitos, *Sci. Progr.*, 1971, **59**, 25.
[7] J. Saarnio, *Kem. Teollisuus*, 1971, **28**, 103 (*Chem. Abs.*, 1971, **75**, 6210f).
[8] I. R. Siddiqui, *Adv. Carbohydrate Chem.*, 1970, **25**, 285.
[9] T. H. Grenby, *Chem. in Britain*, 1971, **7**, 276.
[10] M. J. Kort, *Adv. Carbohydrate Chem.*, 1970, **25**, 311.
[11] G. G. S. Dutton and K. B. Gibney, *Carbohydrate Res.*, 1971, **19**, 393.
[12] R. A. Stewart, C. K. Carrico, R. L. Webster, and R. G. Steinhardt, *Nature*, 1971, **234**, 220.
[13] C.-M. Wu, J. S. Hudson, and R. M. McCready, *Carbohydrate Res.*, 1971, **19**, 259.
[14] N. K. Richtmyer, *Carbohydrate Res.*, 1971, **17**, 401.
[15] G. Haustvelt and J. K. Wold, *Acta Chem. Scand.*, 1970, **24**, 3059.
[16] T. Ito, N. Ezaki, T. Tsuruoka, and T. Niida, *Carbohydrate Res.*, 1971, **17**, 375.

in the leaves of *Protea rubropilosa* Beard in the form of 2′-hydroxy-4′-hydroxymethyl-phenyl β-D-allopyranoside as the 6-*O*-cinnamate (rubropilosin) or 6-*O*-benzoate (pilorubrosin) derivatives.[17]

D-Psicose (D-*ribo*-hexulose) and D-tagatose have been prepared from appropriate D-fructose derivatives by inversion of configuration using oxidation–reduction sequences. Details of the procedure for inverting the configuration of C-3 in 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose to give D-psicose have been described[18] and discrepancies in the results of this procedure observed by other workers have been explained. Similarly, D-tagatose was obtained by a procedure in which the key step involved inversion of configuration at C-4 in (1) to give (2).[19] A new synthetic



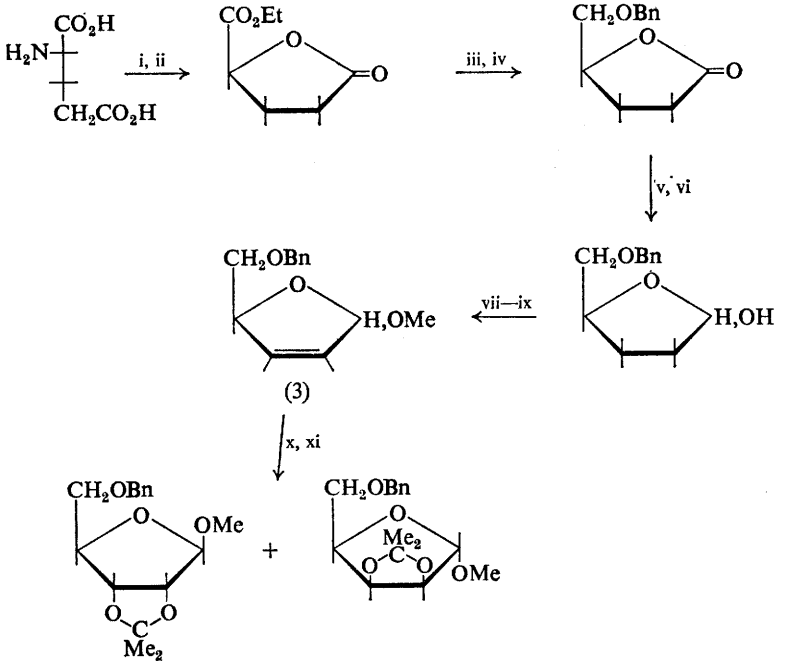
(1) R^1 = H, R^2 = OH
(2) R^1 = OH, R^2 = H

route to L-lyxose and some of its furanose derivatives has been described.[20] 1,2:5,6-Di-*O*-isopropylidene-α-D-gulofuranose (obtained in three stages from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) was benzylated and the 5,6-*O*-isopropylidene group was removed by mild acidic hydrolysis, whereafter successive periodate oxidation, borohydride reduction, catalytic hydrogenolysis, and acidic hydrolysis afforded crystalline L-lyxose.

D-Ribose and D-lyxose derivatives have been prepared from L-glutamic acid by the route illustrated in Scheme 1.[21] D-Ribose anilide was obtained when the acid hydrolysis product from the reaction of compound (3) with potassium permanganate was treated with aniline. A continuous procedure for the production of D-ribose from D-ribono-1,4-lactone has been devised.[22]

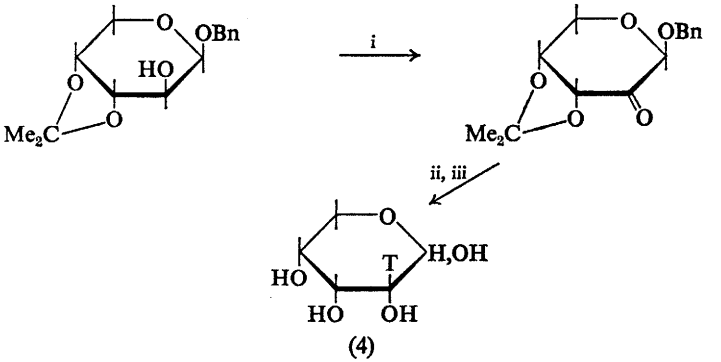
D-2-[^{3}H]Ribose (4) has been synthesized as illustrated in Scheme 2,[23] and both 2-*R*- and 2-*S*-deuterio-2-deoxy-D-*erythro*-pentoses [(6) and (7), respectively] have been synthesized stereospecifically.[24] The *R*-isomer (6) was prepared as illustrated in Scheme 3, while the *S*-isomer (7) was prepared in a similar manner starting from the β-anomer of (5). A total synthesis of DL-glucose has been achieved by the route shown in Scheme 4. Yields

[17] P. Beylis, A. S. Howard, and G. W. Perold, *Chem. Comm.*, 1971, 597.
[18] R. S. Tipson, R. F. Brady, jun., and B. F. West, *Carbohydrate Res.*, 1971, **16**, 383.
[19] A. A. H. Al-Jobore, R. D. Guthrie, and R. D. Wells, *Carbohydrate Res.*, 1971, **16**, 474.
[20] H. Kuzuhara, H. Terayama, H. Ohrui, and S. Emoto, *Carbohydrate Res.*, 1971, **20**, 165.
[21] K. Koga, M. Taniguchi and S. Yamada, *Tetrahedron Letters*, 1971, 263.
[22] E. I. Grigorashvili, N. S. Zolotarev, and G. I. Zaretsky, *Khim.-Farm. Zhur.*, 1971, **5**, 45.
[23] W. B. Gleason and R. Barker, *Canad. J. Chem.*, 1971, **49**, 1433.
[24] B. Radatus, M. Yunker, and B. Fraser-Reid, *J. Amer. Chem. Soc.*, 1971, **93**, 3086.



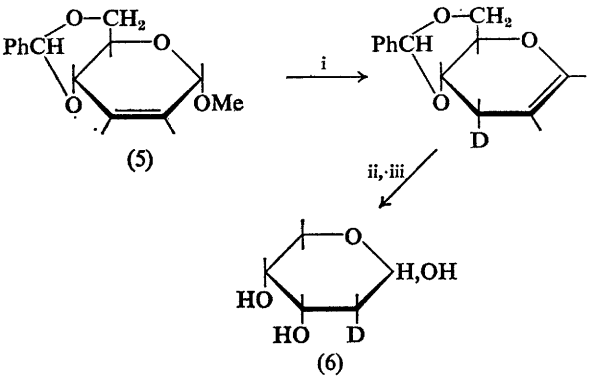
Reagents: i, HNO_2; ii, EtOH; iii, $NaBH_4$; iv, BnBr–Ag_2O; v, Na–HCO_2Et; vi, H_3O^+–dioxan; vii, H^+–MeOH; viii, Br_2–Et_2O; ix, MeONa–MeOH; x, $KMnO_4$; xi, Me_2CO–H^+

Scheme 1



Reagents: i, RuO_4; ii, $NaBT_4$; iii, H_3O^+

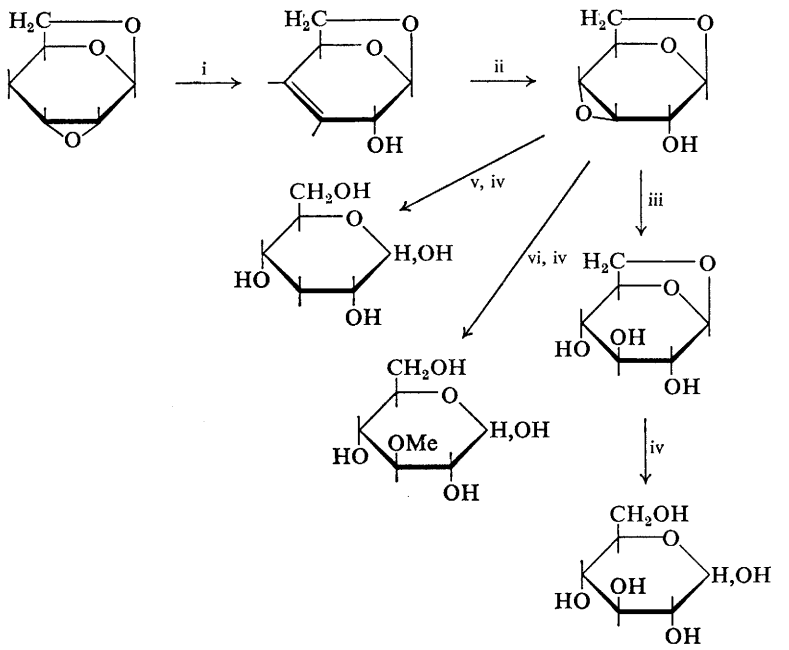
Scheme 2



(5) (6)

Reagents: i, $LiAlD_4$; ii, OsO_4–IO_4^-; iii, H_3O^+

Scheme 3

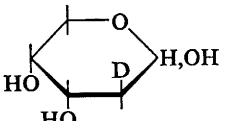


Reagents: i, BuLi–Et_2O; ii, *m*-Cl·$C_6H_4CO_3H$; iii, $Ba(OH)_2$; iv, HCl; v, $LiAlH_4$; vi, MeOH–H^+

Scheme 4

were high throughout and the common intermediate 1,6:3,4-dianhydro-DL-allopyranose was also converted into 3-*O*-methyl-DL-glucose and 3-deoxy-DL-*ribo*-hexopyranose.[25]

1-Deoxy-D-*glycero*-D-*galacto*-octulose has been prepared by hydrolysis of the product obtained when D-mannose is treated with nitroethane.[26] The octulose derivative has also been prepared from 2,3:5,6-di-*O*-isopropylidene-D-mannose (see Chapter 16). D-Idose and D-talose have been obtained by acyloxonium-ion rearrangements.[27]



(7)

The effects of rare-earth hydroxides as catalysts in condensations between formaldehyde and monosaccharides have been studied.[28] It was shown that $Eu(OH)_3$ at temperatures of 80—110 °C was an excellent catalyst, particularly for the formation of ketohexoses.[29] Many different chromatographic techniques have been used to separate the various sugars (trioses through to hexoses) formed from formaldehyde condensation mixtures.[30]

Physical Measurements

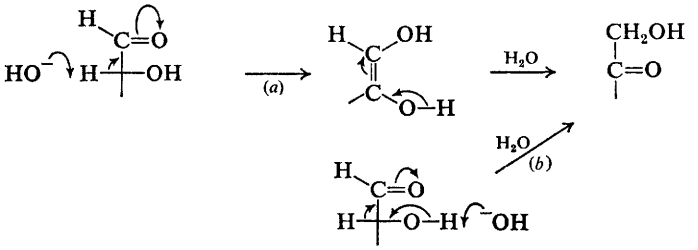
The solubility of several monosaccharides in a variety of alcohols has been investigated[31] and the compression properties of lactose[32] have been measured. ^{13}C Fourier-transform n.m.r. spectroscopy has been applied in determining the proportions of pyranose and furanose forms of D-fructose in solution (see Chapter 23).

The proportions of aldehydo- and keto-forms of sugars and sugar phosphates in solution have been estimated using i.r., u.v., and c.d. spectroscopy. It was found that the u.v. and c.d. procedures are inferior and generally less reliable than i.r. studies on D_2O solutions.[33] The results were compared with those obtained in previous studies (*e.g.* Vol. 4, p. 8).

A method has been developed for studying the enolization of sugars in D_2O by determining the DOH formed by i.r. spectroscopy. The method does not require isotopically labelled substrates and does not involve a

[25] U. P. Singh and R. K. Brown, *Canad. J. Chem.*, 1971, **49**, 3342.
[26] W. S. Chilton, W. C. Lontz, R. B. Roy, and C. Yoda, *J. Org. Chem.*, 1971, **36**, 3222.
[27] H. Paulsen, *Chimia (Switz.)*, 1970, **24**, 290.
[28] A. A. Berlin, O. V. Krylov, and Yu. E. Sinyak, *Kosm. Biol. Med.*, 1971, **5**, 33 (*Chem. Abs.*, 1971, **75**, 49 457s).
[29] A. A. Berlin, O. V. Krylov, and Yu. E. Sinyak, *Bull. Acad. Sci., U.S.S.R.*, 1970, 1592 (*Izvest Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 1679).
[30] T. Mizuzo, M. Asai, A. Misaki, and Y. Fujihara, *J. Agric. Chem. Soc. (Japan)*, 1971, **45**, 344.
[31] J. Saarnio and R. Kuusisto, *Paperi Puu*, 1971, **53**, 195 (*Chem. Abs.*, 1971, **75**, 6205r).
[32] O. Alpar, J. A. Hersey, and E. Shotton, *J. Pharm. Pharmacol.*, 1970, **22**, suppl. 15.
[33] C. A. Swenson and R. Barker, *Biochemistry*, 1971, **10**, 3151.

primary isotope effect. It can be applied to reducing sugars in general and to diverse reaction systems. By use of radioactivity measurements in conjunction with measurements of i.r. absorption, the isotope effect $K_{[^3H]}/K_H$ for the release of hydrogen atoms from deuteriated D-2-[^{3}H]glucose was found to be 0.13.[34] Enol–keto tautomerism of D-glucose, D-mannose, D-xylose, and D-arabinose in aqueous sodium hydroxide at room temperature has been studied by u.v. spectroscopy.[35] Studies of D-2-[^{3}H]ribose in aqueous alkali indicated that its isomerization occurs largely by a hydride-transfer mechanism rather than by reversible enolization,[23] *i.e.* pathway (*b*) in Scheme 5 was found to preponderate.



Scheme 5

The crystal structures of α-lactose,[36, 37] α-D-xylose,[38] α-L-rhamnose,[39] and of a 1 : 1 α-D-glucopyranose–urea complex [40] have been determined.

Ionization constants of D-glucose, D-ribose, 2-deoxy-D-*erythro*-pentose, D- and L-arabinose, and D- and L-xylose have been determined by potentiometric titration. It was shown that there is a distinct correlation between the pK values of these sugars and their tendency to undergo cyanogen-induced phosphorylation. The higher the pK value, the lower the yields of 1-phosphates that were obtained.[41]

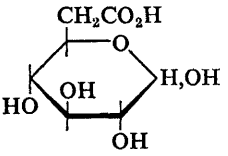
Laser-Raman spectra have been obtained for aqueous solutions of D-glucose, cellobiose, maltose, and dextran [(1 → 6)-α-D-glucan]. Assignments of lines related to the vibrational modes of OH, CH, and CH_2 have been made.[42]

Complex formation between D-galactose, D-glucose, D-mannose, and maltose with ethylenediamine has been studied by u.v. spectroscopy and by paper chromatography.[43] Paper electrophoresis in copper(II) acetate and basic copper(II) acetate solutions proved to be a useful procedure for

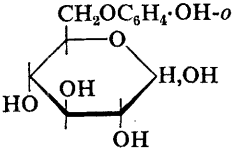
[34] H. S. Isbell, K. Linek, and K. E. Hepner, jun., *Carbohydrate Res.*, 1971, **19**, 319.
[35] T. V. Kleinert, *Holzforsch. Holzverwert.*, 1970, **22**, 76.
[36] D. C. Fries, S. T. Rao, and M. Sundaralingam, *Acta Cryst.*, 1971, **27B**, 994.
[37] C. A. Beevers and H. N. Hansen, *Acta Cryst.*, 1971, **27B**, 1323.
[38] A. Hordvik, *Acta Chem. Scand.*, 1971, **25**, 2175.
[39] R. C. G. Killean, J. L. Lawrence, and V. C. Sharma, *Acta Cryst.*, 1971, **27B**, 1707.
[40] R. L. Snyder and R. D. Rosenstein, *Acta Cryst.*, 1971, **27B**, 1969.
[41] C. Degani, *Carbohydrate Res.*, 1971, **18**, 329.
[42] P. D. Vasko, J. Blackwell, and J. L. Koenig, *Carbohydrate Res.*, 1971, **19**, 297.
[43] S. P. Moulik, A. K. Mitra, and K. K. Sen Gupta, *Carbohydrate Res.*, 1971, **19**, 416.

separating polyhydroxy-compounds. In general, tetritols, pentitols, hexitols, and reduced disaccharides of D-glucose formed cationic complexes, whereas free sugars (excepting ribose, xylose, and gulose) did not.[44] The principle was also applied to the separation of polyhydroxy-compounds over Amberlite IR 120 (Cu^{II}) resin. The kinetics of dissociation of a D-glucose bisulphite compound have been measured iodometrically and polarometrically.[45]

Micellar dodecylammonium benzoate and propionate have been shown to enhance considerably the rate of mutarotation of solutions of 2,3,4,6-tetra-*O*-methyl-α-D-glucose in benzene.[46] The rate of mutarotation of lactose is decreased by addition of sucrose,[46a] and the mutarotation of β-D-mannose is accelerated by a wide variety of metal ions.[47] It has been shown [48] that mutarotation of the anions of the D-glucose derivatives (8) and (9) is catalysed intramolecularly. It has been shown by detailed



(8)



(9)

thermal and chemical analysis that α-D-xylopyranose partially anomerizes on heating.[49] Continued heating beyond the melting point of the sugar, and of that of several β-D-xylopyranosides, caused rupture (heterolytic, it is suggested) of the glycosyl–oxygen bond and the formation of polymeric products, along with products of thermal decomposition. Differential thermal analysis, thermogravimetric analysis, and derivative thermal gravimetry results were reported in detail, but the reaction products were not described fully.

D-Glucose and cellobiose have similar specific thermal expansibility values, whereas the corresponding value for maltose is different.[50] It was suggested, therefore, that maltose folds in solution in such a manner that hydrophobic surfaces bond together.

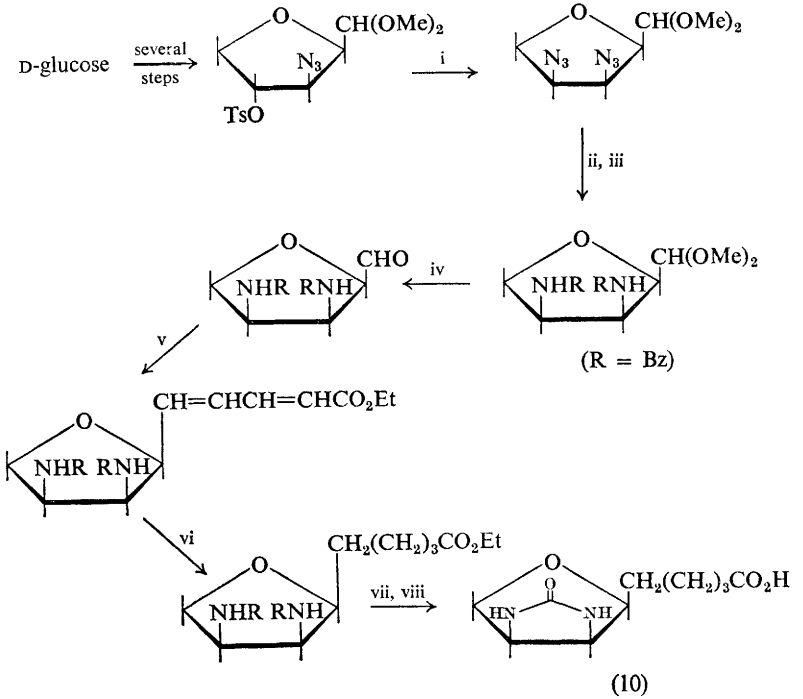
Reactions

Homogeneous oxidation of D-glucose and D-fructose with oxygen in aqueous alkaline solutions has been studied and a reaction scheme has been

[44] E. J. Bourne, F. Searle, and H. Weigel, *Carbohydrate Res.*, 1971, **16**, 185.
[45] M. A. Ivanov and G. V. Rachkov, *Nauchn. Trudy, Leningrad Lesotekhn. Akad.*, 1969, **121**, 102 (*Chem. Abs.*, 1971, **74**, 64 350z).
[46] E. J. Fendler, J. H. Fendler, R. T. Medary, and V. A. Woods, *Chem. Comm.*, 1971, 1497.
[46a] K. N. Patel and T. A. Nickerson, *J. Dairy Sci.*, 1970, **53**, 1654.
[47] R. Mitzner and E. Behrenwald, *Z. Chem.*, 1971, **11**, 64.
[48] B. Capon and R. B. Walker, *Chem. Comm.*, 1971, 1323.
[49] F. Shafizadeh, G. D. McGinnis, R. A. Susott, and H. W. Tatton, *J. Org. Chem.*, 1971, **36**, 2813.
[50] J. L. Neal and D. A. I. Goring, *Canad. J. Chem.*, 1970, **48**, 3745.

proposed which involves a number of well-known reaction pathways. Thus, it was suggested that formation of enolate anions is followed by (*i*) non-oxidative reactions involving double-bond migration and cleavage, and (*ii*) oxidative formation of peroxides leading to the formation of, amongst other products, D-arabonic acid, D-erythronic acid, D-glyceric acid, glycollic acid, and formic acid.[51] The reactions of pentoses with oxygen in dilute aqueous potassium hydroxide have been studied. The products were mainly formic acid, glyceric acid, glycollic acid, and tetronic acids, and the order of rates of decomposition was xylose > ribose > arabinose > lyxose. When potassium hydroxide was replaced by sodium carbonate, the order of rates was ribose > lyxose > xylose > arabinose.[52] Studies of the oxidation of D-glucose by a mixture of chromic and perchloric acids have been reported.[53]

Full details have been given[54] of the conversion of D-glucose into (+)-desthiobiotin (Vol. 4, p. 8), providing an example of the use of sugars



(10)

Reagents: i, NaN_3–DMSO; ii, Zn–H_2O–DMF; iii, BzCl; iv, H_3O^+; v, $Ph_3\overset{+}{P}-\overset{-}{C}HCH=CHCO_2Et$; vi, Pt–$H_2$; vii, $Ba(OH)_2$; viii, $COCl_2$

Scheme 6

[51] H. G. J. De Wilt and B. F. M. Kuster, *Carbohydrate Res.*, 1971, **19**, 5.
[52] W. B. Gleason and R. Barker, *Canad. J. Chem.*, 1971, **49**, 1425.
[53] S. Chandra and R. K. Mittal, *Carbohydrate Res.*, 1971, **19**, 123.
[54] H. Kuzuhara, H. Ohrui, and S. Emoto, *Agric. Biol. Chem. Japan*, 1971, **35**, 8.

as a source of asymmetric centres of known configuration [C-4 and C-5 from D-glucose were retained in (+)-desthiobiotin] in the synthesis of non-carbohydrate products. Similarly, D-glucose has been used as starting material for the synthesis of (+)-oxybiotin (10) (Scheme 6).[55] 6-Deoxy-L-mannose and D-mannitol have been used as sources of chirality in the synthesis of the enantiomeric acetyl α- and β-methylcholines [56] [(11) and (12), respectively].

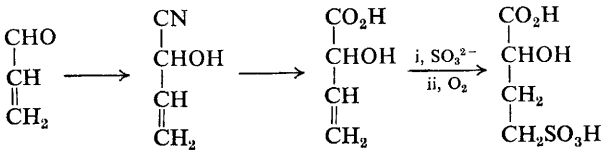
$MeCO_2CH_2CH(Me)\overset{+}{N}Me_3\ I^-$ (11) $MeCO_2CH(Me)CH_2\overset{+}{N}Me_3\ I^-$ (12)

Studies of the formation of carbocyclic compounds from reactions between carbohydrates and anisole in hydrogen fluoride have been continued (see Vol. 4, p. 11). The carbohydrates investigated have included D-mannose, D-galactose, and 2-deoxy-D-*arabino*-hexose.[57]

The products formed from D-glucose and sucrose in cigarette smoke have been studied.[58, 58a] Thirteen radioactive components were found in smoke from the burley portion of cigarette blend to which uniformly labelled D-[^{14}C]glucose and [^{14}C]sucrose had been added.

Certain monosaccharides and their simple derivatives have been investigated for their ability to act as specific precipitinogens of eel anti-human blood group H(O) antibody. Among the sugars examined, the best precipitinogens were 6-deoxy-3-*O*-methyl-D-galactose and 3-*O*-methyl-D-galactose. The nature of the interaction between the sugar and the antibody was discussed.[59]

It has been shown that D-erythrose afforded (13) and (14) as the main sulphur-containing products [60] when treated with aqueous sulphite. Compound (13) was also prepared as illustrated in Scheme 7. On treatment

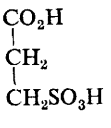


(13)

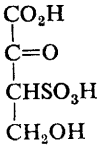
Scheme 7

with aqueous sulphite, D-xylose afforded (15) and (16); the mechanisms of these reactions were discussed.

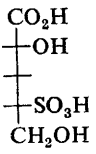
[55] H. Ohrui, H. Kuzuhara, and S. Emoto, *Agric. Biol. Chem. Japan*, 1971, **35**, 752.
[56] T. D. Inch and G. J. Lewis, *Carbohydrate Res.*, 1971, **16**, 455.
[57] F. Micheel and J. Staněk, jun., *Tetrahedron Letters*, 1971, 1605.
[58] F. L. Gager, jun., J. W. Nedlock, and W. J. Martin, *Carbohydrate Res.*, 1971, **17**, 327.
[58a] F. L. Gager, jun., J. W. Nedlock, and W. J. Martin, *Carbohydrate Res.*, 1971, **17**, 335.
[59] G. F. Springer and P. R. Desai, *Biochemistry*, 1971, **10**, 3749.
[60] H.-L. Hardell and O. Theander, *Acta Chem. Scand.*, 1971, **25**, 877.



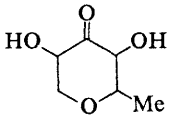
(14)



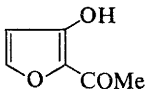
(15)



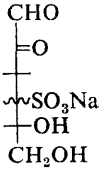
(16)



(17)



(18)



(19)

The identity of 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (17) as a product of hexose degradation has been confirmed and the ready rearrangement of (17) to isomaltol (18) has been described.[61]

3-Deoxy-D-glucosone and (19) were isolated from the products formed by heating together D-glucose, glycine, and sodium metabisulphite. Presumably, (19) is produced from 3-deoxy-D-glucosone by β-elimination of water followed by Michael addition of bisulphite.[62]

[61] P. E. Shaw, J. H. Tatum, and R. E. Berry, *Carbohydrate Res.*, 1971, **16**, 207.
[62] M. E. Knowles, *Chem. and Ind.*, 1971, 910.

3
Glycosides

O-Glycosides

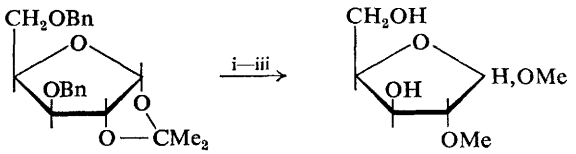
Synthesis.—A novel method for synthesizing α-glucosides has been applied in the preparation of methyl α-maltoside. Ethyl 1-thio-β-maltoside was converted into a mixture of anomeric methyl maltosides containing *ca.* 85% of the α-isomer by treatment with methanol, bromine, and silver carbonate, and, following acetylation, the α-maltoside fraction was enriched by preferential oxidation of the β-anomer with chromic acid. A 65% yield of methyl α-maltoside was produced in 97% anomeric purity.[63]

Direct methylation of 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methyl-α-L-mannopyranose (*cf.* Vol. 3, p. 15) with methyl iodide has been shown to give markedly different ratios of anomeric glycosides depending upon the conditions used. The β-anomer was formed predominantly with silver oxide, but addition of even small quantities of DMF or DMSO caused a reversal in the ratio of anomers. Methylation with sodium hydride–methyl iodide in benzene or THF gave *ca.* 90% of the β-glycoside, whereas addition of a 'crown' compound (a cyclic polyether) to complex sodium ions resulted in the formation of *ca.* 90% of the other isomer. These findings were rationalized. Similar studies were carried out on 6-deoxy-2,3-*O*-isopropylidene-5-*O*-*p*-tolylsulphonyl-α-L-mannofuranose, which was converted mainly into the α-glycoside by the Kuhn procedure and to the β-product with sodium hydride–methyl iodide.[64] α-Melibiose similarly has been converted into different products under different conditions of methylation. With ethereal diazomethane, the methyl β-glycopyranoside was formed specifically and was methylated completely with methyl iodide–barium oxide, whereas the last two reagents alone gave the permethylated methyl furanosides with the α-anomer predominating.[65]

The interesting observation has been made that the product ratios resulting from methanolysis of free sugars can be affected by the presence of metal ions. Normal methanolysis of D-allose, for example, afforded mainly the β-pyranoside, whereas the α-furanoside and α-pyranoside predominated when calcium chloride was added; their proportions depended largely upon the reaction times employed.[66] Methanolysis has also been

[63] W. E. Dick, jun., D. Weisleder, and J. E. Hodge, *Carbohydrate Res.*, 1971, **18**, 115.
[64] A. H. Haines and K. C. Symes, *J. Chem. Soc.* (*C*), 1971, 2331.
[65] M. E. Gelpi, J. O. Deferrari, and R. A. Cadenas, *J. Chem. Soc.* (*C*), 1971, 3354.
[66] S. J. Angyal and K. P. Davies, *Chem. Comm.*, 1971, 500.

used in the preparation of the anomeric methyl furanosides of 2-*O*-methyl-D-xylose (Scheme 8).[67]



Reagents: i, MeOH–H^+; ii, Me_2SO_4–NaOH–THF; iii, Na–NH_3

Scheme 8

A new synthesis of t-butyl glycosides is exemplified by the preparation of the anomeric t-butyl D-glucopyranosides by perchloric acid-catalysed t-butyl exchange between t-butyl acetate and 2,3,4,6-tetra-*O*-acetyl-β-D-glucose.[68] 2,3-Epoxypropyl β-D-glucopyranoside has been prepared from the acetylated allyl compound.[69]

Glycosyl halides continue to be the most frequently applied glycosylating agents and further examples of syntheses of 1,2-*cis*-related aldosides using halides having non-participating groups at C-2 have been reported. Thus, α- and β-D-xylopyranosyl chloride 2,3,4-tri(chlorosulphate), and the corresponding α-D-lyxose compound, on treatment with methanol in the presence of catalytic amounts of sodium iodide gave methyl pentosides, formed mainly by inversion at C-1. Methyl α-D-xylopyranoside was formed in *ca.* 90% yield by this procedure with 70% isolated as the triacetate.[70] The following α-D-glucopyranosides were prepared in related work from 3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl chloride in the presence of silver succinate: n-propyl (53%), benzyl (67%), isopropyl (41%), t-butyl (39%), phenyl (29%), and cyclohexyl (27%). These compounds were isolated as their tetra-acetyl derivatives.[71]

A more detailed examination has been reported of the reaction of acetobromoglucose with silver salts of hydroxycarboxylic acids. Glycosylation occurred at the hydroxy- and carboxy-groups and diglycosyl derivatives were also obtained. The stabilities of 1-*O*-acyl compounds bearing a hydroxy-group in the acyl functions were low when the hydroxy-group was in the γ- or δ-positions because of its participation in the hydrolyses.[71a]

Other β-glucosides have been synthesized by application of the Koenigs–Knorr procedure to 3-substituted *N*-(β-hydroxyethyl)pyridinium halides,[72] 2,3-dialkoxypropanol (where the alkyl groups were of the long-chain

[67] P. Kováč and M. Petríková, *Carbohydrate Res.*, 1971, **16**, 492.
[68] C. Wasielewski, E. Kasperowicz, and J. Rachon, *Roczniki Chem.*, 1971, **45**, 119.
[69] J. E. G. Barnett and A. Ralph, *Carbohydrate Res.*, 1971, **17**, 231.
[70] H. J. Jennings, *Canad. J. Chem.*, 1971, **49**, 1355.
[71] B. Helferich and W. M. Müller, *Chem. Ber.*, 1971, **104**, 671.
[71a] G. Wulff, W. Krüger, and G. Röhle, *Chem. Ber.*, 1971, **104**, 1387.
[72] F. Márquez and E. Jiménez-Caballero, *Anales de Quím.*, 1971, **67**, 623.

type),[73] *N*-tritylserotonin,[74] salicylanilide and its 4-chloro-derivative,[75] and various sterols, including androst-5-ene-3β,17β-diol.[76] Syntheses of analogous β-D-glucopyranosiduronic acids were described in the latter paper, and similar products have also been prepared from several aromatic sterols using cadmium carbonate as condensing agent. This development was claimed to represent a considerable improvement on earlier methods, but led to small proportions of α-products and *C*-glycosides as well as to the β-glycosiduronic acids.[77]

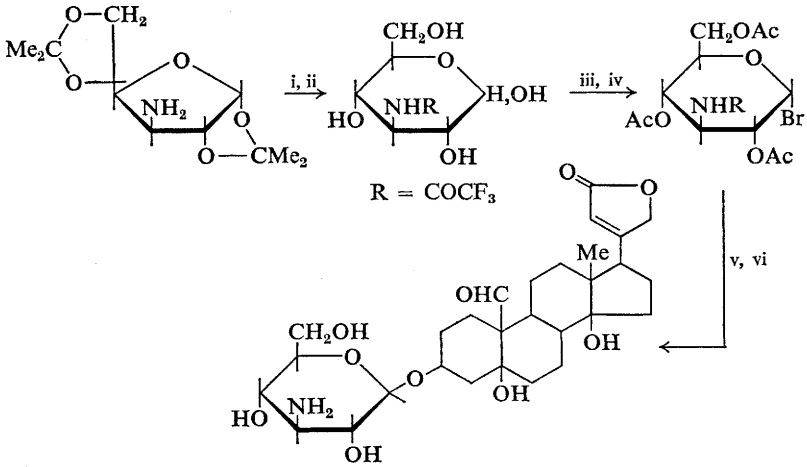
Several new disaccharide syntheses have been reported. Glycosyl halides with non-participating C-2 groups have again been shown to offer means of synthesizing 1,2-*cis*-linked products. Thus, 6-*O*-α-D-glucopyranosyl-D-galactose and the 3-α-linked isomer have been obtained by the reaction of 2-*O*-benzyl-3,4,6-tri-*O*-(*p*-nitrobenzoyl)-D-glucopyranosyl bromide with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactopyranose, respectively. α-Linked products were obtained irrespective of the configuration of the glucosyl bromide, suggesting that anomerization was faster than disaccharide synthesis and that the equatorial β-bromine atom was more susceptible to displacement.[78] Condensation between α-acetobromo-L-rhamnose and 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose was used as the key step in a synthesis of β-neohesperidose.[79]

Several non-reducing disaccharides have also been prepared. Sucrose and α-D-glucopyranosyl α-D-fructofuranoside have been isolated after debenzylation of the products of condensation of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose and 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride.[80] 6-Deoxy-$\alpha\alpha$-trehalose has also been prepared; the conformation of its peracetate has been determined and its substrate characteristics for a trehalase were examined.[81] The same deoxytrehalose and α-D-xylopyranosyl α-D-glucopyranoside were synthesized by enzymic methods.[82] A reduced disaccharide, 1-*O*-β-D-galactopyranosyl-D-mannitol, has also been obtained following Koenigs–Knorr glycosylation of either 1,2,3,4-tetra-*O*-acetyl-β-D-mannopyranose or its 6-trityl ether.[83] An improved procedure for preparing methyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranoside has been described; the glycoside was converted into α-cellotriose hendeca-acetate by treatment

[73] B. Czartoryska and J. Koscielak, *Roczniki Chem.*, 1971, **45**, 987.
[74] L. S. Krasavina, N. P. Kostjuchenko, L. I. Morozovskaya, and N. N. Suvorov, *Doklady Akad. Nauk S.S.S.R.*, 1971, **196**, 597.
[75] B. N. Stepanenko and L. W. Zhukova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **199**, 115.
[76] J. J. Schneider, *Carbohydrate Res.*, 1971, **17**, 199.
[77] R. B. Conrow and S. Bernstein, *J. Org. Chem.*, 1971, **36**, 863.
[78] H. M. Flowers, *Carbohydrate Res.*, 1971, **18**, 211.
[79] B. H. Koeppen, *S. African Med. J.*, 1968, **42**, 455.
[80] R. K. Ness and H. G. Fletcher, jun., *Carbohydrate Res.*, 1971, **17**, 465.
[81] E. R. Guilloux, J. Defaye, R. H. Bell, and D. Horton, *Carbohydrate Res.*, 1971, **20**, 421.
[82] E. Belocopitow, L. R. Marechal, and E. G. Gros, *Carbohydrate Res.*, 1971, **19**, 267.
[83] E. Solomon, K. Miyai, and R. W. Jeanloz, *Biochemistry*, 1971, **10**, 1803.

with hepta-*O*-acetyl-α-cellobiosyl bromide and subsequent acetolysis with acetic anhydride–sulphuric acid.[84]

For reasons of their significance in natural products, several glycosides (including disaccharides) of amino-sugars have been prepared. 2-Amino-2-deoxy-β-D-glucopyranosyl (−)menthol, (−)borneol, and thymol have been synthesized by the Koenigs–Knorr method, but the oxazoline route was generally preferable.[85] Similarly, analogous α- and β-derivatives of sterols have been reported,[86] and the 3-amino-3-deoxy-β-D-glucopyranoside of strophanthidin has been prepared as outlined in Scheme 9.[87] In the area



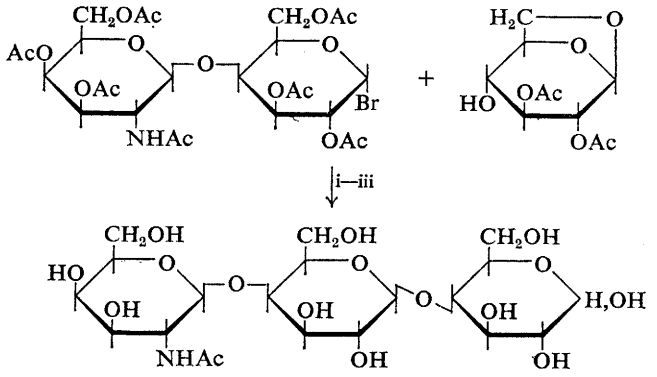
Reagents: i, $(CF_3CO)_2O$; ii, CF_3CO_2H; iii, Ac_2O–py; iv, HBr–HOAc; v, strophanthidin; vi, MeONa–MeOH

Scheme 9

of disaccharides, several compounds having amino-functions in the non-reducing moieties were described: 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose and -glucose have been synthesized by the oxazoline method,[88] and 2-acetamido-2-deoxy-3-*O*-(α-D-mannopyranosyl)-D-glucose was prepared (using 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide and benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside) to aid in the mass spectrometric analysis of glycoproteins.[89] Similarly, the 4-α-linked isomer of this disaccharide was obtained by glycosylation of the

[84] D. M. Hall and T. E. Lawler, *Carbohydrate Res.*, 1971, **16**, 1.
[85] S. A. Kozhin and E. G. Titkova, *J. Gen. Chem.* (*U.S.S.R.*), 1971, **41**, 698 (*Zhur. obshchei Khim.*, 1971, **41**, 700).
[86] J. P. Joseph, J. P. Dusza, and S. Bernstein, *Biochemistry*, 1971, **10**, 2941.
[87] W. Meyer zu Reckendorf, N. Wassiliadou-Micheli, and E. Bischof, *Chem. Ber.*, 1971, **104**, 1.
[88] T. S. Antonenko, S. E. Zurabyan, and A. Ya. Khorlin, *Bull. Acad. Sci. U.S.S.R.*, 1970, 2600 (*Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 2766).
[89] M. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1971, **17**, 193.

2,3-carbonate of 2-amino-2-deoxy-5,6-*O*-isopropylidene-D-glucose diethyl acetal.[90] In more complex work, glycosylation of appropriately protected monosaccharide derivatives with the acetylated glycosyl halide of methyl *N*-acetylneuraminate has afforded the following: α-*N*-acetylneuraminyl-(2 → 3)-D-glucose, α-*N*-acetylneuraminyl-(2 → 6)-D-galactose, and α-*N*-acetylneuraminyl-(2 → 6)-2-acetamido-2-deoxy-D-glucose and its 3-α-linked isomer.[91] Trisaccharide (20), the linear carbohydrate component of an abnormal ganglioside which accumulates in the brain of patients with Tay–Sachs disease, has been prepared as outlined in Scheme 10.[91a]



(20)

Reagents: i, $Hg(CN)_2$; ii, Ac_2O–AcOH–H_2SO_4; iii, MeONa

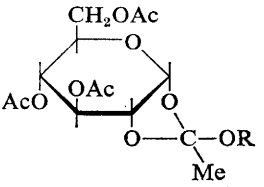
Scheme 10

Modified Koenigs–Knorr condensation of 2,3,4-tri-*O*-benzyl-6-deoxy-α-L-galactopyranosyl bromide with benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside in nitromethane–benzene (1 : 1) solution afforded, after removal of the protecting groups, 2-acetamido-2-deoxy-6-*O*-(6-deoxy-L-galactopyranosyl)-D-glucose (α : β, 7 : 3), which is a less usual disaccharide having the amino-function in the reducing moiety.[92] In the field of sugar–amino-acid compounds, Russian workers have prepared *O*-(di-*N*-acetylchitobiosyl)-*N*-benzyloxycarbonyl-L-serine by application of the oxazoline method. Here the disaccharide moiety has an amino-function in each sugar residue.[93]

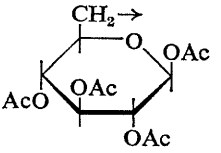
Important modifications of the orthoester glycosidation procedure have been reviewed. A two-step process involving transesterification of the initial orthoester with the alcohol to be glycosylated, followed by conversion

[90] M. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1971, **20**, 17.
[91] A. Ya. Khorlin, I. M. Privalova, and I. B. Bystrova, *Carbohydrate Res.*, 1971, **19**, 272.
[91a] D. Shapiro, A. J. Acher, Y. Rabinsohn, and A. Diver-Haber, *J. Org. Chem.*, 1971, **36**, 832.
[92] M. Dejter-Juszynski and H. M. Flowers, *Carbohydrate Res.*, 1971, **18**, 219.
[93] L. M. Likhosherstov, O. S. Novikova, and V. A. Derevitskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 652.

of this product into the required glycoside, was recommended. Thus, orthoester (21) was converted into (22) by reaction with 1,2,3,4-tetra-*O*-

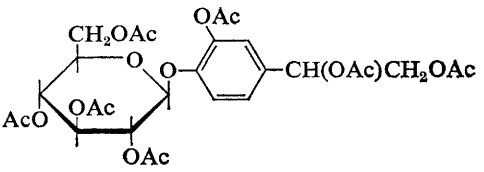


(21) R = Et

(22) R = 

acetyl-β-D-glucopyranose and, thereafter, into gentiobiose octa-acetate.[94] An interesting development of this work opens the way to solid-support syntheses of oligosaccharides. Copolymers of styrene with various 6-*O*-(*p*-vinylbenzoates) of D-glucopyranose derivatives were synthesized and the carbohydrate substituents on the polystyrene were converted into 1,2-orthoester derivatives in preparation for the formation of glycosidic linkages.[95]

Several new reports have appeared on the synthesis of aryl glycosides. Of general interest in this area is the development of conditions under which phenols and D-glucose penta-acetates can be condensed to give either α- or β-products with high stereospecificity.[96] Particular glycosides to have been synthesized by standard procedures were compound (23) and its



(23)

relatives,[97] *p*-nitrophenyl α-D-maltoside (which proved to be an effective substrate for α-amylase),[98] a wide range of substituted aryl β-D-galactopyranosides,[99] and 1-naphthyl α-L-arabinofuranoside.[100]

[94] N. K. Kochetkov, A. F. Bochkov, T. A. Sokolovskaya, and V. J. Snyatkova, *Carbohydrate Res.*, 1971, **16**, 17.
[95] R. D. Guthrie, A. D. Jenkins, and J. Stehlíček, *J. Chem. Soc.* (*C*), 1971, 2690.
[96] T. D. Audichya, T. R. Ingle, and J. L. Bose, *Indian J. Chem.*, 1971, **9**, 315.
[97] E. Lada and W. Wieniawski, *Acta polon. Pharm.*, 1971, **28**, 157.
[98] P. M. Barna, *Austral. J. Chem.*, 1971, **24**, 673.
[99] C. K. De Bruyne and J. Wouters-Leysen, *Carbohydrate Res.*, 1971, **18**, 124.
[100] A. H. Fielding and L. Hough, *Carbohydrate Res.*, 1971, **20**, 416.

Hydrolysis, Anomerization, and Related Reactions.—In a detailed study of the acid-catalysed hydrolysis of glycopyranosides, all evidence indicated that the reactions in aqueous hydrochloric acid proceeded by way of glycosyl carbonium ions generated unimolecularly from the conjugate acids. Measurements in other acids were not all in accord with this mechanism, but the discrepancies were interpreted as arising from deficiencies in the methods used rather than from a change of mechanism.[101]

A study has been carried out on the relative rates of hydrolysis of anomeric pairs of alkyl D-glucopyranosides and, in accord with previous findings, the α-compounds were found to be less reactive. Glycosides of primary alcohols were also found to hydrolyse more slowly than those of secondary alcohols. It was suggested that the rates of hydrolysis are primarily a function of the extent of protonation of the glycosidic oxygen atoms, and the lower rates of hydrolysis of alkyl α-D-glucopyranosides are due to the influence of a reverse anomeric effect, which destabilizes the conjugate acids derived from the α-species.[102] It has been demonstrated that esterification of methyl α- and β-D-glucopyranoside, maltose, methyl β-maltoside, cellobiose, methyl β-cellobioside, and amylose stabilizes the glycosidic linkage so that methanolysis (in acidified chloroform–methanol) did not occur until extensive deacylation had taken place. Methanolysis was also observed to occur with preponderant inversion, and it is notable that methanolysis of the glycosidic bonds of amylose was more facile than hydrolysis.[103]

In a more specific study, the detailed kinetics of the hydrolysis of 2-naphthyl β-D-glucuronide were investigated and comments were made on the care required in interpreting the results of uronoside hydrolyses already described in the literature.[104] Solvent effects on the acid-catalysed hydrolysis of sucrose have also been investigated.[105]

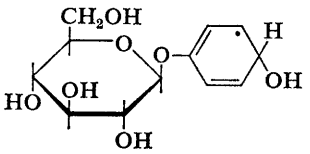
An interesting new route for the alkali-catalysed cleavage of certain aryl glycosides has been elucidated. Liberation of the *p*-nitrophenate ion from *p*-nitrophenyl α-D-glucopyranoside has been shown to occur by way of a O-1 → O-2 migration to give the 2-*O*-aryl ether (isolated), followed by a O-2 → O-3 migration (3-*O*-aryl ether isolated), and then β-elimination of phenate ion with concomitant production of saccharinic acids. The 2- and 3-*manno*-aryl ethers were found as by-products. Studies of the 3-*O*-methyl and the 2,3-di-*O*-methyl ethers of the glycoside confirmed the mode of reaction.[106]

Boron trifluoride-catalysed acetolysis of methyl α-D-xylopyranoside and its triacetate with acetic anhydride gave 1,2,3,4,5-penta-*O*-acetyl-1-*O*-methyl-*aldehydo*-D-xylose; both diastereoisomers were formed in equal

[101] C. K. De Bruyne and J. Wouters-Leysen, *Carbohydrate Res.*, 1971, **17**, 45.
[102] J. N. BeMiller and E. R. Doyle, *Carbohydrate Res.*, 1971, **20**, 23.
[103] G. Entlicher and J. N. BeMiller, *Carbohydrate Res.*, 1971, **16**, 363.
[104] B. Capon and B. C. Ghosh, *J. Chem. Soc.* (*B*), 1971, 739.
[105] J. W. Barnett and C. J. O'Connor, *J. Chem. Soc.* (*B*), 1971, 1163.
[106] D. Horton and A. E. Luetzow, *Chem. Comm.*, 1971, 79.

amounts and were separated. As with sulphuric acid-catalysed acetolyses, methyl pentosides were much more reactive than methyl hexosides. Methyl D-glucopyranoside tetra-acetates acetolysed fairly smoothly, but the corresponding reaction of methyl α-D-mannopyranoside tetra-acetate gave five products: *viz.* the penta-acetates of the pyranoses (60%), the epimeric, acyclic 1-*O*-methyl hexa-acetates (30%), and *aldehydo*-D-mannose hepta-acetate (10%). *C*-Acylations of the aromatic rings occurred with phenyl glycosides in addition to acetolysis.[107]

Other Reactions and Features of Glycosides.—Three papers have been devoted to the radiolysis of glycosides. The effects of γ-irradiation on crystalline aryl glycosides were examined and the major degradation process was cleavage of the glycosidic bond. Aryl glycosides were more stable under these conditions than alkyl glycosides, in contrast to their relative lability towards hydrolysis with acid or alkali. The effects of γ-irradiation of glycosides, thioglycosides, and glycosylamines were compared.[108] γ-Irradiation of aqueous solutions of phenyl β-D-glucopyranoside resulted in glycosidic-bond fission, with formation of equivalent amounts of D-glucose and phenol, and in hydroxylation of the aglycone. Pulse radiolysis indicated that the first intermediate was the radical (24), and two alternative



(24)

paths for its reaction were distinguished kinetically.[109] Studies of glycosidic bond cleavage of several substituted aryl glycosides in aqueous solution under the influence of hydroxyl radicals and hydrated electrons revealed that the latter could be regarded as nucleophiles, since they caused reactions with similarities to alkali-catalysed hydrolyses. On the other hand, reactions of the hydroxyl radical were comparable with acid-catalysed hydrolyses.[110] The products of radiolysis of liquid and frozen aqueous solutions of lactose have been shown to result from the action of hydroxyl radicals and only to a relatively slight extent from the action of hydrated electrons.[111]

The i.r. and n.m.r. spectroscopic and mass spectrometric properties of methyl 6-deoxy-α- and -β-L-mannopyranosides were measured for reference

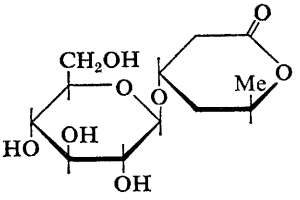
[107] F. W. Lichtenthaler, J. Breunig, and W. Fisher, *Tetrahedron Letters*, 1971, 2825.
[108] J. S. Moore and G. O. Phillips, *Carbohydrate Res.*, 1971, **16**, 79.
[109] G. O. Phillips, W. G. Filby, J. S. Moore, and J. V. Davies, *Carbohydrate Res.*, 1971, **16**, 89.
[110] G. O. Phillips, W. G. Filby, J. S. Moore, and J. V. Davies, *Carbohydrate Res.*, 1971, **16**, 105.
[111] L. I. Kudrjashov, S. M. Yarovaya, E. I. Bortsova, and Y. A. Sharpatyi, *Zhur. obshchei Khim.*, 1971, **41**, 2298.

during investigations of acetylated derivatives of naturally occurring carotenoid rhamnosides.[112] Many other references to applications of physical methods to glycosides are given in Chapters 23—25.

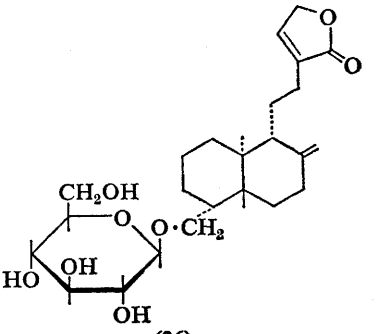
Methylamide *O*-galactosylserine dipeptides were prepared by DCC-condensations from the β-D-galactopyranosyl derivative of L-serine.[113]

Natural Products.—It should be recognized, as for previous Volumes, that coverage in this section is not comprehensive.

Two D-glucosyl lactones to have been discovered are parasorboside (25)[114] and neoandrographolide (26).[115] The former was isolated from



(25)



(26)

mountain ash; methanolysis caused lactone-ring opening but the glycosidic bond could be cleaved by enzymolysis. Both were fully characterized largely by spectroscopic methods. Closely related compounds having simple lactone rings have been reported by German workers.[116, 117] Rubescine is an alkaloid glucoside in which the D-glucosyl residue is esterified at C-3 with caffeic acid.[118] Reports of glycosides of carotenoids,[119] flavones,[120, 121] dilignols,[122] saponins,[123] and canarigenin[124] have also appeared.

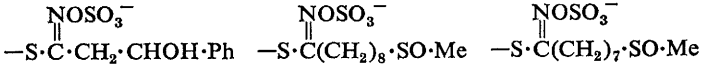
[112] E. Hemmer and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1970, **24**, 3019.
[113] V. A. Derevitskaya, I. M. Rotenberg, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 2092.
[114] R. Tschesche, H.-J. Hoppe, G. Snatzke, G. Wulff, and H.-W. Fehlhaber, *Chem. Ber.*, 1971, **104**, 1420.
[115] W. R. Chan, D. R. Taylor, C. R. Willis, R. L. Bodden, and H.-W. Fehlhaber, *Tetrahedron*, 1971, **27**, 5081.
[116] R. Tschesche, K. Struckmeyer, and G. Wulff, *Chem. Ber.*, 1971, **104**, 3567.
[117] R. Tschesche and H.-J. Hoppe, *Chem. Ber.*, 1971, **104**, 3573.
[118] W. P. Blackstock and R. T. Brown, *Tetrahedron Letters*, 1971, 3727.
[119] K. Schmidt, G. W. Francis, and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1971, **25**, 2476.
[120] M. Nógrádi, L. Farkas, and V. Olechnowicz-Stepien, *Chem. Ber.*, 1971, **104**, 3618.
[121] N. K. Sen, P. C. Ghosh, A. B. Kundu, and A. Chatterjee, *Chem. Ber.*, 1971, **104**, 3425.
[122] G. D. Manners and E. P. Swan, *Canad. J. Chem.*, 1971, **49**, 3607.
[123] R. Tschesche and P. Lauven, *Chem. Ber.*, 1971, **104**, 3549.
[124] E. V. Rao, D. V. Rao, S. K. Pavanaram, J. von Euw, and T. Reichstein, *Helv. Chim. Acta*, 1971, **54**, 1960.

A new tetrasaccharide, a fructosylraffinose, has been isolated from wheat bran,[124a] and ethyl α-D-glucopyranoside has been found in small concentration in saké.[125]

S-Glycosides

Treatment of pentopyranosyl chloride 2,3,4-tri(chlorosulphates) with sodium methanethiolate gave thioglycosides. Whereas the α-D-*xylo*-compound reacted with Walden inversion, the main products obtained from the β-D-*xylo*- and β-D-*lyxo*-isomers were formed with retention of configuration, presumably by way of 1,2-anhydrides.[70]

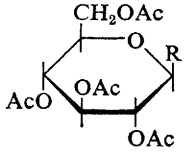
Three main glucosinolates have been isolated from seeds of the crucifer *Sibara virginica*. In these *S*-glycosides, D-glucose is attached to the groups (27)—(29).[126]



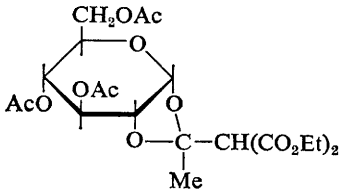
(27) (28) (29)

C-Glycosides

A new method of some general applicability to the synthesis of *C*-glycosides involved treatment of glycosyl halides with carbanions of malonic ester. The ester (30) was obtained from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and was converted into the acid (31), whereas the corresponding β-D-glucopyranosyl chloride gave the cyclic acetal (32) following participation of the C-2 group.[127]



(30) R = $CH(CO_2Et)_2$
(31) R = CH_2CO_2H

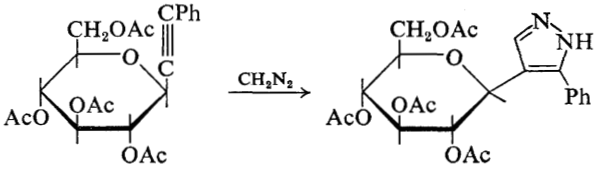


(32)

1,3-Dipolar addition of diazomethane to glycosyl acetylenes gave *C*-glycosyl heterocycles (Scheme 11).[128]

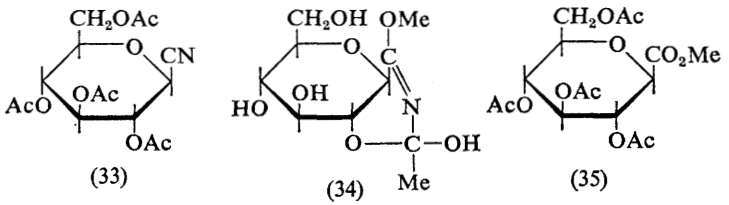
Treatment of the nitrile (33) with sodium methoxide in methanol gave a crystalline product assigned the structure (34). Acetylation with pyridine–

[124a] R. M. Saunders, *Phytochemistry*, 1971, **10**, 491.
[125] T. Imanari and Z. Tamura, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 321.
[126] R. Gmelin, A. Kjaer, and A. Schuster, *Acta Chem. Scand.*, 1970, **24**, 3031.
[127] S. Hanessian and A. G. Pernet, *Chem. Comm.*, 1971, 755.
[128] M. T. García-López, G. García-Muñoz, and R. Madroñero, *J. Heterocyclic Chem.*, 1971, **8**, 525.



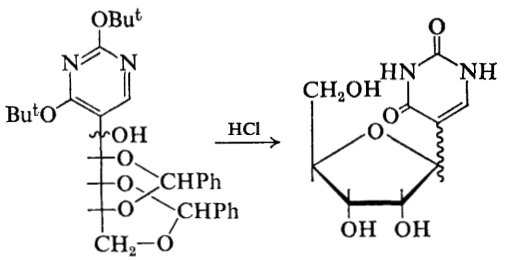
Scheme 11

acetic anhydride, followed by treatment with water, afforded the ester (35).[129] It should be noted that the products were not anomerically defined, so that some doubt may be associated with the allocated β-configurations



in view of the basic condition used for the first reaction. Analogous experiments were carried out in the D-galactose series.

A new approach to *C*-linked nucleosides is illustrated in Scheme 12.[130]



Scheme 12

In addition to *O*-glycosides, small proportions of *C*-glycosides were obtained on treatment of various aromatic sterols with glycosyl bromides.[77]

[129] B. Helferich and K. L. Bettin, *Chem. Ber.*, 1971, **104**, 1701.
[130] U. Lerch, M. G. Burdon, and J. G. Moffatt, *J. Org. Chem.*, 1971, **36**, 1507.

4
Ethers and Anhydro-sugars

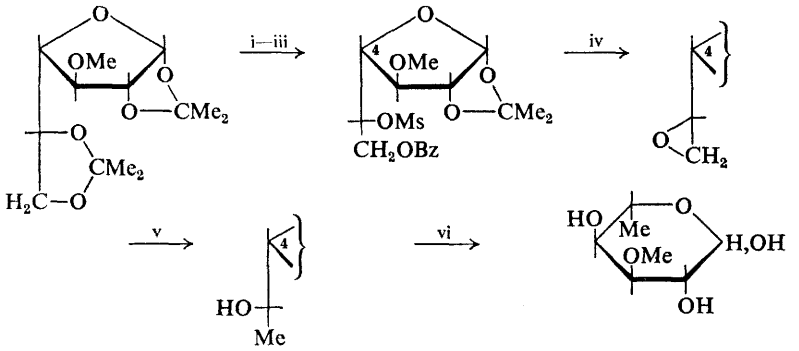
Ethers

Methyl Ethers.—New work on the synthesis of specific methyl ethers of sugars continues to be published. A new preparation of 3,4-di-*O*-methyl-D-glucose depended, in the key step, on methylation with diazomethane–boron trifluoride of the readily available methyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside.[131] An alternative synthesis of 3,4,6-tri-*O*-methyl-D-glucose used the 4,6-di-*O*-methyl ether, which was converted into the 1,2-*O*-isopropylidene derivative prior to methylation and removal of the acetal.[132] All seven possible mono-, di-, and tri-methyl ethers of 1,6-anhydro-β-D-glucopyranose have been prepared and their i.r. (with particular reference to intramolecular hydrogen bonding) and n.m.r. spectra were studied in detail.[133] The following methyl ethers of 2-deoxy-2-methylamino-D-glucose have been synthesized and their chromatographic properties recorded: 3-, 4-, and 6-mono, 3,4-, 3,6-, and 4,6-di, and 3,4,6-tri.[134]

^{1}H N.m.r. spectral data have been presented for the monomethyl ethers of D-galactopyranose, methyl α- and β-D-galactopyranoside, and galactitol.[135] In the D-mannose series, unambiguous syntheses of the 2,3,6- and 2,4,6-trimethyl ethers have been described,[135a] and 2,3,6-tri-*O*-methyl-L-idose has been prepared from the corresponding D-glucofuranoside triether.[136]

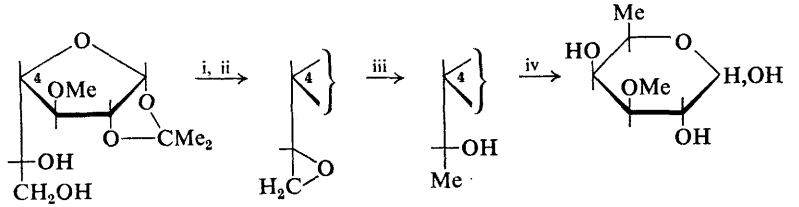
Several papers have reported on features of monodeoxy-monomethyl-hexoses, and references to other methylated deoxy-sugars are made in Chapters 13, 14, and 26. Full details have been given of syntheses of 6-deoxy-3-*O*-methyl-D-gulose and -L-mannose (L-acofriose) (see Vol. 4, p. 22),[137] and 6-deoxy-3-*O*-methyl-L-altrose (L-vallarose) and -D-galactose (digitalose) have been prepared as shown in Schemes 13 and 14, respectively.[138] 6-Deoxy-3-*O*-methyl-L-mannose has been found in hydrolysates of a lipopolysaccharide of a *Klebsiella*. Methylation analysis (using

[131] A. Lipták, *Acta Chim. Acad. Sci. Hung.*, 1970, **66**, 315.
[132] P. Kováč, *Chem. Zvesti*, 1970, **24**, 218.
[133] P. C. Wollwage and P. A. Seib, *J. Chem. Soc.* (*C*), 1971, 3143.
[134] P. A. J. Gorin and A. J. Finlayson, *Carbohydrate Res.*, 1971, **18**, 269.
[135] E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydrate Res.*, 1971, **20**, 357.
[135a] Y. M. Choy and A. M. Unrau, *Carbohydrate Res.*, 1971, **17**, 439.
[136] S. Morgenlie, *Acta Chem. Scand.*, 1971, **25**, 2353.
[137] J. S. Brimacombe, N. Robinson, and J. M. Webber, *J. Chem. Soc.* (*C*), 1971, 613.
[138] J. S. Brimacombe, I. Da'Aboul, and L. C. N. Tucker, *J. Chem. Soc.* (*C*), 1971, 3762.



Reagents: i, $AcOH–H_2O$; ii, BzCl–py; iii, MsCl–py; iv, MeONa; v, $LiAlH_4$; vi, H_3O^+

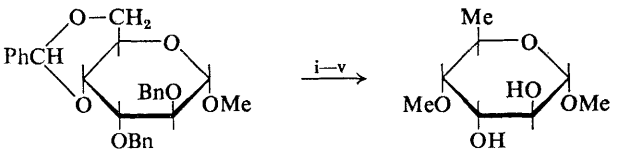
Scheme 13



Reagents: i, TsCl–py; ii, NaOMe; iii, $LiAlH_4$; iv, H_3O^+

Scheme 14

trideuteriomethyl iodide) and mass spectrometry showed that this sugar exists almost completely in terminal positions in the polymer.[139] An isomer of the foregoing sugars, 6-deoxy-4-*O*-methyl-D-altrose, was obtained from the antibiotic sordarin and its structure was proved by synthesis (Scheme 15).[140]



Reagents: i, H_3O^+; ii, TsCl–py; iii, $LiAlH_4$; iv, NaH–MeI; v, H_2, Pd–C

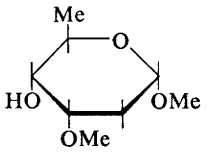
Scheme 15

In the pentose series, alternative syntheses of 4-*O*-methyl-β-L-arabinopyranose[141] and methyl 3-*O*-methyl(and 2,3-di-*O*-methyl)-D-xylofuranosides[142] have been published, and reports on the preparation of 2-*O*-

[139] H. Björndal, B. Lindberg, and W. Nimmich, *Acta Chem. Scand.*, 1970, **24**, 3414.
[140] A. M. Spichtig and A. Vasella, *Helv. Chim. Acta*, 1971, **54**, 1191.
[141] P. Kováč, *Carbohydrate Res.*, 1971, **20**, 418.
[142] P. Kováč and M. Pretíková, *Carbohydrate Res.*, 1971, **19**, 249.

methyl-L-lyxose, a component of everninomicins B and D, have appeared. The method employed used a descent of the series from 3-*O*-methyl-L-talose, which was prepared from 1,2-*O*-isopropylidene-3-*O*-methyl-5,6-di-*O*-methylsulphonyl-α-D-allofuranose.[143, 144] Limited oxidation of 3-*O*-methyl-D-galactose with periodate led to the preparation of the enantiomer of this antibiotic sugar.[145]

Lithium and ethylamine have been shown to lead to demethylation in the case of methyl cymaroside (36) and some steroidal monomethyl



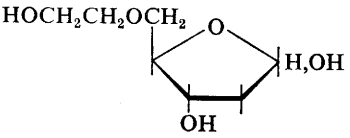
(36)

glycosides. The remarkable feature of this reaction is that glycosidic bonds are apparently unaffected.[146]

As part of an investigation into the probable origin of monomethylhexoses found in the polysaccharides of humus, leaves of various species were hydrolysed, and sugars with chromatographic mobilities greater than those of common, unsubstituted sugars were examined. 2-*O*-Methylxylose, 2-*O*-methylfucose, 3-*O*-methylgalactose, and probably 4-*O*-methylgalactose were identified, and apiose was also characterized. Leaf hemicellulose is apparently stabilized towards enzymic hydrolysis by the presence of these methylated compounds. These findings were taken to indicate that humus polysaccharides are of plant rather than bacterial origin.[147]

Substituted Alkyl Ethers.—The vinylation of methyl α-D-mannopyranoside has been investigated.[148] Methyl 4-*O*-alkyl-αβ-D-glucopyranosides (several simple alkyl groups were used) have been prepared from amylose by perbenzylation and methanolysis to give methyl 2,3,6-tri-*O*-benzyl-αβ-D-glucopyranoside, which was transformed into the products by alkylation, chromatographic separation of the anomers, and debenzylation.[149] 2-Deoxy-5-*O*-(2-hydroxyethyl)-D-*erythro*-pentose (37) has been synthesized from 6-*O*-(2-hydroxyethyl)-3-*O*-methyl-D-glucose by formation of the corresponding substituted saccharinic acid with alkali followed by Ruff degradation. Acetylation and fusion with 6-benzamidopurine gave,

[143] J. S. Brimacombe and A. M. Mofti, *Chem. Comm.*, 1971, 241.
[144] J. S. Brimacombe, A. M. Mofti, and L. C. N. Tucker, *J. Chem. Soc.* (*C*), 1971, 2911.
[145] J. S. Brimacombe, A. M. Mofti, and A. K. Al-Radhi, *J. Chem. Soc.* (*C*), 1971, 1363.
[146] Q. Khuong-Huu, C. Monneret, I. Kabore, and R. Goutarel, *Tetrahedron Letters*, 1971, 1935.
[147] J. S. D. Bacon and M. V. Cheshire, *Biochem. J.*, 1971, **124**, 555.
[148] V. L. Lapenko, N. L. Vasil'eva, and V. E. Sopina, *Trudy Voronezhsk. Gos. Univ.*, 1969, **73**, 75 (*Chem. Abs.*, 1971, **75**, 6206*s*).
[149] J. N. BeMiller, C. L. Collins, E. R. Doyle, and R. E. Wing, *Carbohydrate Res.*, 1971, **16**, 480.

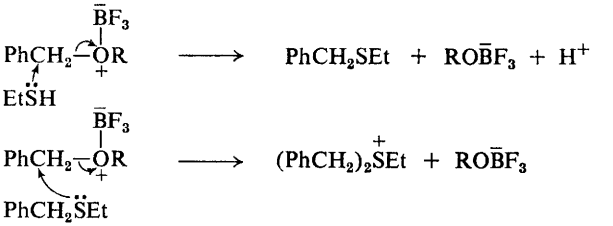


(37)

after de-esterification, 9-[2-deoxy-5-*O*-(2-hydroxyethyl)-α-D-*erythro*-pentofuranosyl]adenine, together with the β-anomer.[150]

Surface-active ethers of sucrose have been prepared by use of long-chain alkyl halides, epoxy-compounds, and chloromethylalkyl ethers.[151] Selective benzylation of sucrose with a molar proportion of benzyl bromide occurred at positions *O*-2 (86%), *O*-3′ (10%), *O*-1′ (3%), and *O*-3 (1%); it seems surprising that primary ethers were not more evident.[152]

An interesting report on demethylation has already been referred to, and other reports have appeared on debenzylation reactions. Treatment with boron trifluoride etherate and either ethanethiol or ethane-1,2-dithiol of, for example, 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose caused debenzylation to give a mixture of the anomeric ethyl 1-thio-D-glucopyranosides and the ethylene dithioacetals; dibenzylethylsulphonium tetrafluoroborate was the other product. The debenzylation mechanism outlined in Scheme 16 was proposed.[153] In a communication of related



Scheme 16

interest (but one which does not relate to carbohydrate derivatives), the use of trityl fluoroborate for cleaving benzyl ethers, carboxybenzyl esters, and tetrahydropyranyl acetals was described.[154]

The migration of a *p*-nitrophenyl group from position 1 to position 3 of D-glucose by a stepwise route has been referred to in Chapter 3.[106]

The effect of base concentration on the reaction of methyl D-glucopyranosides with *NN*-diethylaziridinium chloride has been investigated. It was shown that the rate of reaction at C-2, C-3, and C-4 decreased with

[150] S. David and J. C. Fischer, *Carbohydrate Res.*, 1971, **18**, 39.
[151] E. Reinefeld, A. Frehse, and K.-D. Heincke, *Zucker*, 1971, **24**, 95.
[152] E. Reinefeld and K.-D. Heincke, *Chem. Ber.*, 1971, **104**, 265.
[153] H. G. Fletcher, jun. and H. W. Diehl, *Carbohydrate Res.*, 1971, **17**, 383.
[154] D. H. R. Barton, P. D. Magnus, G. Streckert, and D. Zurr, *Chem. Comm.*, 1971, 1109.

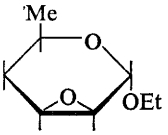
increasing concentration of base, whereas the reaction at C-6 remained largely unaffected by variation in base concentration. These observations were rationalized.[155] 1,2:3,4-Di-*O*-isopropylidene-6-*O*-[triethoxysilylpropyl]-D-galactose and the triethoxysilylethyl analogue, which are related to the diethylamino-compounds described in the last paper, have been prepared and were found to polymerize on treatment with acid.[156]

Silyl Ethers.—A review of the mass spectrometry of TMS ethers is mentioned in Chapter 24.

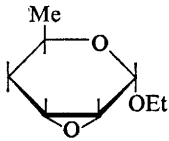
Intramolecular Ethers (Anhydro-sugars)

Epoxides.—A detailed review of the synthesis and ring-opening reactions of carbohydrate epoxides (oxirans) has appeared.[157] The acetolysis and benzolysis of several carbohydrate oxirans bearing vicinal acetoxy-groups have been studied. Cyclic acetoxonium ions were formed in acetic acid when the acyloxy-group was *trans*-related to the three-membered ring. In the presence of water, a *cis*-monoester was formed, whereas under anhydrous conditions three products were formed by attack at the different sites in the intermediate. The results were compared with those of Winstein's for non-carbohydrate systems.[158]

Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside was converted into methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside and the isomeric 2-*O*-methyl-α-D-altropyranoside, in the ratio 66 : 34, by iodine in methanol. The observed preponderance of the diequatorial product is in contradiction to the Fürst–Plattner Rule and possibly arises by unimolecular epoxide ring-opening after co-ordination to iodine.[159] Compounds DL-(38) and DL-(39) were opened in acidic media predominantly by



(38)



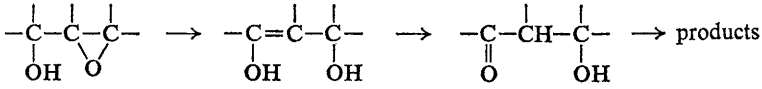
(39)

attack at C-3, whereas in alkaline media isomer (38) again showed mainly C-3 opening but the *ribo*-compound (39) also exhibited appreciable C-2 opening.[159a] All three possible 2,3:2′,3′-dianhydrides of 4,6:4′,6′-di-*O*-benzylidene-αα-trehalose (the D-*manno*,D-*manno*-, D-*allo*,D-*allo*-, and D-*allo*,D-*manno*-isomers) have been prepared by methods analogous to those used for the corresponding methyl D-glucoside anhydrides.[160]

[155] E. J. Roberts, C. P. Wade, and S. P. Rowland, *Carbohydrate Res.*, 1971, **17**, 393.
[156] J. Lehmann and H. Schäfer, *Carbohydrate Res.*, 1971, **16**, 225.
[157] N. R. Williams, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 109.
[158] J. G. Buchanan, J. Conn, A. R. Edgar, and R. Fletcher, *J. Chem. Soc.* (*C*), 1971, 1515.
[159] J. S. Jewell and W. A. Szarek, *Carbohydrate Res.*, 1971, **16**, 248.
[159a] A. Banaszek and A. Zamojski, *Roczniki Chem.*, 1971, **45**, 391.
[160] L. Hough, P. A. Munroe, and A. C. Richardson, *J. Chem. Soc.* (*C*), 1971, 1060.

The acid-induced ring-openings of methyl 3,4-anhydro-6-deoxy-α-D-galactopyranoside and its 2-acetate have been studied in detail and conditions for the preparation of methyl 6-deoxy-α-D-gulopyranoside have been optimized.[161]

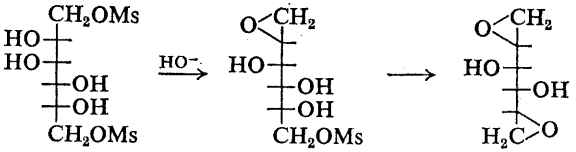
Investigations of hexose dianhydrides continue. 1,6:3,4-Dianhydro-β-DL-allohexopyranose has proved to be a convenient intermediate in total syntheses of DL-glucose and certain of its derivatives.[25] Treatment of 1,6:3,4-dianhydro-β-D-galactopyranose or its 2-acetate or 2-benzoate with hydrochloric acid in benzene gave only 1,6-anhydro-3-chloro-3-deoxy-D-galactose esters, so that ring opening must have involved initial formation of 2,3-acyloxonium-ion intermediates. Alternatively, with boron trifluoride etherate, the dianhydride 2-benzoate gave 1,6-anhydro-2-*O*-benzoyl-D-gulopyranose by hydrolysis of a benzoxonium intermediate.[162] Other Czech workers have studied the alkaline hydrolysis of 1,6:2,3- and 1,6:3,4-dianhydro-β-hexopyranoses. If the oxiran ring and the free hydroxy-group were *trans*-related, ring opening proceeded by migration of the epoxide ring, whereas if they were *cis*-related degradation occurred as proposed in Scheme 17. Little epoxide-migration occurred with dilute



Scheme 17

sulphuric acid and both anhydride rings opened to give free hexoses. Products of diaxial epoxide-ring opening were normally formed, but 1,6:3,4-dianhydro-D-allose gave D-glucose and D-gulose in equal amounts.[163] The use of a 1,6:3,4-dianhydride in the synthesis of 4-deoxy-4-fluoro-D-glucose is mentioned in Chapter 7 and the crystal structure of 1,6:2,3-dianhydro-D-gulose is noted in Chapter 24.

The rate constants of the anhydride formations outlined in Scheme 18 were determined and it was calculated that the maximum concentration of the monoepoxide that would accumulate was 43%. By careful control

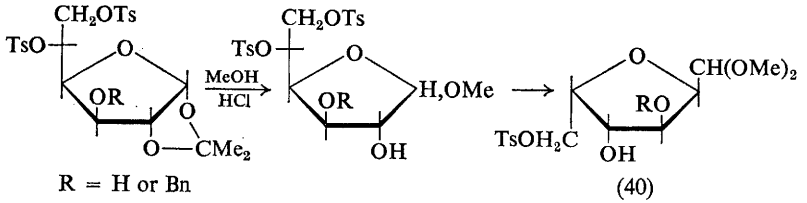


Scheme 18

[161] K. Čapek, I. Tíkal, J. Jarý, and M. Masojídková, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1973.
[162] M. Prystaš, H. Gustafsson, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1487.
[163] T. Trnka and M. Černý, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2216.

of pH, the monoepoxide, an effective antitumour agent, was obtained in 41% yield.[164] The related compounds 2,3:4,5-dianhydro-1,6-di-*O*-methylsulphonyl-L- and -D-iditol have been prepared and characterized; both compounds have significant cystostatic activity.[165] Closely related D-iditol and galactitol derivatives have also been reported.[166]

Other Anhydrides.—Reviews of alditol anhydrides[167] and 2,5-anhydrosugars have been published.[168] In the latter area, the 2,5-anhydro-L-iditol derivative (40) has been produced by solvolysis of 1,2-*O*-isopropylidene-5,6-di-*O*-*p*-tolylsulphonyl-α-D-glucofuranose (Scheme 19).[169] Similar re-



R = H or Bn (40)

Scheme 19

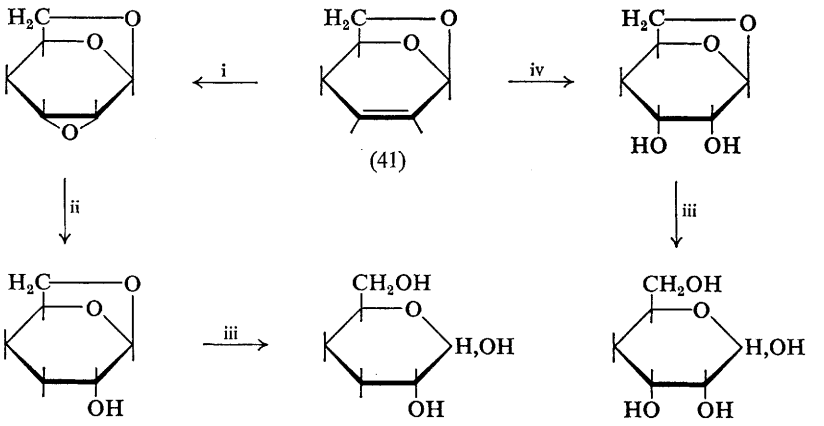
actions in the pentose series led to related 2,5-anhydropentose dimethylacetals. Different rates of anhydro-ring formation were interpreted in terms of steric factors operating during ring closure.[170] Treatment of 3,5-di-*O*-acetyl-1,6-dibromo-1,6-dideoxy-2,4-di-*O*-methylsulphonyl-D-mannitol with acid gave rise to the 2,5-anhydro-D-glucitol derivative.[166]

The crystal structure of 1,6-anhydro-β-D-glucopyranose is reported in Chapter 24. As referred to earlier, all the methyl ethers of 1,6-anhydro-β-D-glucopyranose have been prepared and selective esterifications were considered in the same work.[133] Other workers have examined the kinetics of acetylation of 1,6-anhydro-β-D-glucopyranose and also its reactions with phenyl isocyanate and with boron trifluoride etherate.[171] Differential thermal analysis and thermogravimetric analysis have been used to follow transformations such as solid-state transitions, melting, evaporation, polymerization, and degradation of this anhydride in the presence or absence of additives. Whereas zinc chloride promoted degradation and charring, sodium hydroxide enhanced molecular fragmentation.[172]

A series of DL-1,6-anhydro-deoxyhexoses and, thence, deoxyhexoses has been produced from the dihydropyran derivatives (41) and (42)

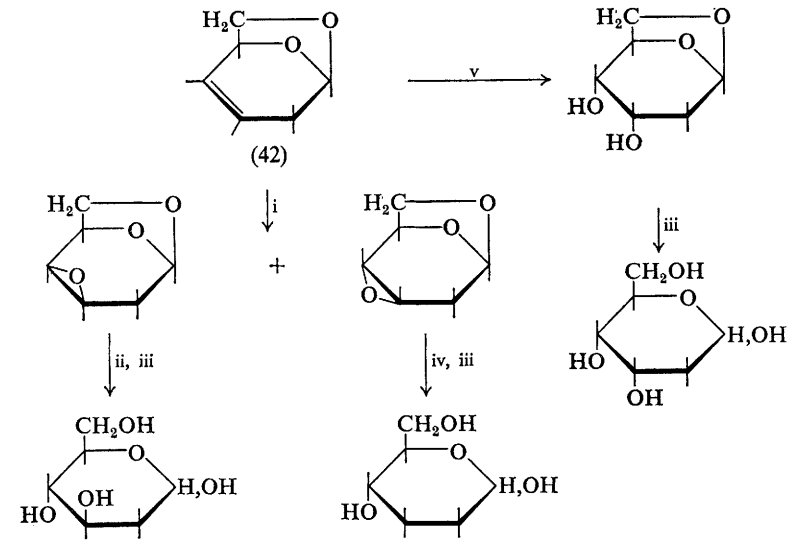
[164] M. J. Tisdale, *Carbohydrate Res.*, 1971, **19**, 117.
[165] T. Horváth and L. Vargha, *Carbohydrate Res.*, 1971, **16**, 253.
[166] J. Kuszmann and L. Vargha, *Carbohydrate Res.*, 1971, **16**, 261.
[167] S. Soltzberg, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 229.
[168] J. Defaye, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 181.
[169] J. Defaye and V. Ratovelomanana, *Carbohydrate Res.*, 1971, **17**, 57.
[170] J. Defaye, D. Horton, and M. Muesser, *Carbohydrate Res.*, 1971, **20**, 305.
[171] U. Stirna, R. Pernikis, and J. Surna, *Latvijas P.S.R. Zinatnu Akad. Vestis, Kim. Ser.*, 1970, 479 (*Chem. Abs.*, 1971, **74**, 23 074*u*).
[172] F. Shafizadeh, C. W. Philpot, and N. Ostojic, *Carbohydrate Res.*, 1971, **16**, 279.

(Schemes 20 and 21).[173] Somewhat surprisingly, treatment of the unsaturated glycoside (43) with hydrogen bromide in acetic acid gave the 1,6-anhydro-derivative (44) (Scheme 22).[174]



Reagents: i, *m*-$ClC_6H_4CO_3H$; ii, $LiAlH_4$; iii, H_3O^+; iv, OsO_4

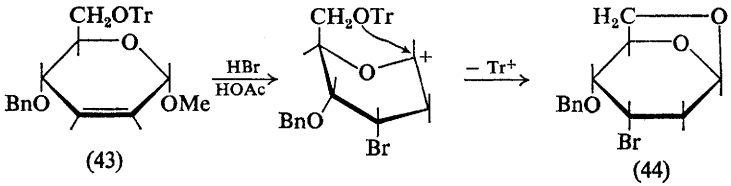
Scheme 20



Reagents: i, *m*-$ClC_6H_4CO_3H$; ii, KOH; iii, H_3O^+; iv, $LiAlH_4$; v, OsO_4

Scheme 21

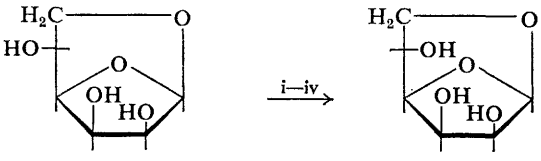
[173] T. P. Murray, U. P. Singh, and R. K. Brown, *Canad. J. Chem.*, 1971, **49**, 2132.
[174] S. Dimitrijevich and N. F. Taylor, *Carbohydrate Res.*, 1971, **20**, 427.



(43) (44)

Scheme 22

Vacuum pyrolysis of D-mannose is known to afford 1,6-anhydro-β-D-mannopyranose, but now the β-furanosyl isomer (45) (3.3% yield) is shown to be obtainable from the products by direct crystallization. Compound (45) was used to prepare the corresponding α-L-gulofuranose derivative (46) (Scheme 23).[175]



(45) (46)

Reagents: i, Me_2CO–$CuSO_4$; ii, RuO_4; iii, $LiAlH_4$; iv, H_3O^+

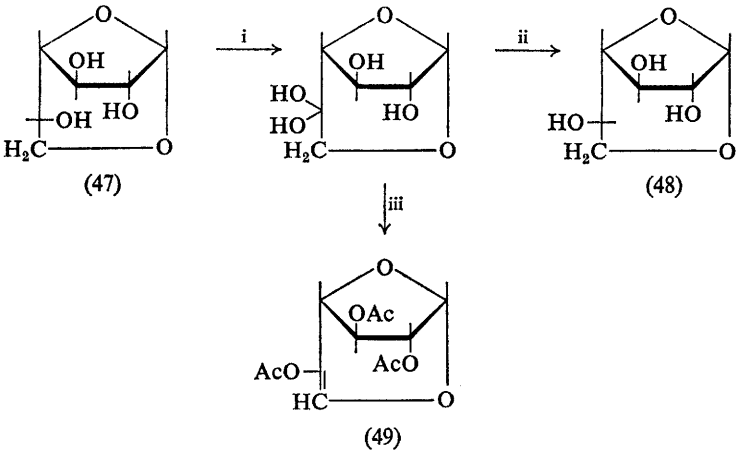
Scheme 23

1,6-Anhydro-β-D-glucofuranose was not selectively oxidized with platinum–oxygen, but the α-D-*galacto*-isomer (47) underwent oxidation at C-5. Reduction of the product led, with high selectivity, to the L-*altro*-anhydride (48) and acetylation gave the 5,6-unsaturated compound (49) (Scheme 24).[176] A detailed analysis of the n.m.r. spectra of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose and certain of its deuteriated derivatives has been reported (see also Chapter 23).[177]

1,6-Anhydrolactose hexa-acetate (50) has been prepared in good yield from lactose by a two-stage process and converted into the interesting thio-analogue (51).[178]

3,6-Anhydro-D-glucal has been described (Chapter 14) and mass spectrometric examination of a range of 3,6-anhydro-D-galactose derivatives has been reported; the nature and positions of substitution can be established readily.[179] 3,6:3′,6′-Dianhydro-αα-trehalose (52) underwent acid-catalysed rearrangement to the difuranoid isomer (53). The reaction presumably

[175] K. Heyns, P. Köll, and H. Paulsen, *Chem. Ber.*, 1971, **104**, 830.
[176] K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, 1971, **104**, 2063.
[177] D. Horton, J. S. Jewell, E. K. Just, and J. D. Wander, *Carbohydrate Res.*, 1971, **18**, 49.
[178] S. Tejima, *Carbohydrate Res.*, 1971, **20**, 123.
[179] O. S. Chishov, B. M. Zolotarev, A. I. Usov, M. A. Rechter, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **16**, 29.

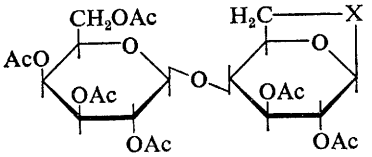


Reagents: i, $Pt-O_2$; ii, H_2–Pd–C; iii, Ac_2O–py

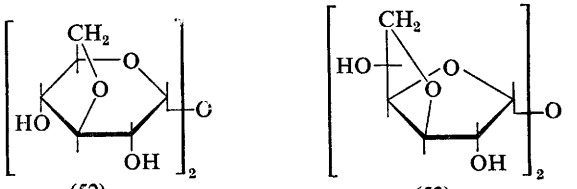
Scheme 24

parallels the known acid-catalysed isomerization of methyl 3,6-anhydro-α-D-glucopyranoside.[180]

The reaction of 2,5-di-*O*-acetyl-1,4:3,6-bis(thioanhydro)-D-iditol *RR*- (or *RS*-) disulphoxide (54) in acetic anhydride at 110 °C gave the heterocyclic compounds (55) and (56). These products were not formed similarly

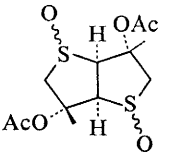


(50) X = O
(51) X = S

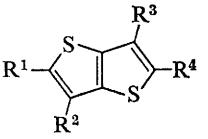


from (57), the product of Pummerer rearrangement of (54). Possible mechanisms for the elimination reactions were discussed and modifications of the reaction conditions, which led to the formation of other rearrangement products, were described.[181]

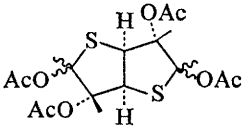
[180] G. Birch, C. K. Lee, and A. C. Richardson, *Carbohydrate Res.*, 1971, **19**, 119.
[181] J. Kuszmann, P. Sohár, and G. Horváth, *Tetrahedron*, 1971, **27**, 5055.



(54)

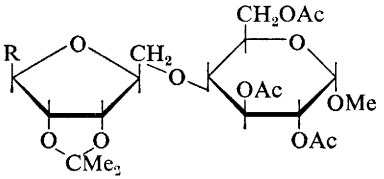


(55) R^1 = OAc, R^2 = R^3 = H, R^4 = Ac
(56) R^1 = R^2 = R^3 = H, R^4 = Ac



(57)

Two papers have appeared on unusual intermolecular anhydride disaccharide derivatives linked through non-glycosidic positions. In the first, a long discussion is given on various approaches to the synthesis of (58)[182] and, in the second, synthesis of the nucleosidic analogue (59) is described.[183]



(58) R = OMe
(59) R = adenyl

[182] M. Prystaš and F. Šorm, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1448.
[183] M. Prystaš and F. Šorm, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1472.

5
Acetals

Reactions and Properties

Acetone-sensitized photolysis of methyl 2,3-*O*-benzylidene-β-D-ribofuranoside gave 58% of the methyl 2(and 3)-*O*-benzoyl esters; similar treatment of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-D-xylofuranose gave a mixture of the 3- and 5-benzoates in good yield. The expected deoxy-sugar derivatives were not produced.[184]

The stereoselectivity of reactions between ketones and complexes of acetylenic Grignard reagents and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose was found to be less than when other Grignard reagents were used.[185]

Acetals Derived from Carbohydrate Carbonyl Groups

Several *aldehydo*-sugars, such as 3-*O*-benzyl-1,2-*O*-cyclohexylidene-α-D-*xylo*-pentodialdo-1,4-furanose, 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose, and *aldehydo*-D-glucose penta-acetate, have been converted into the diethylacetals by the Claisen procedure, involving treatment with triethyl orthoformate, absolute ethanol, and ammonium nitrate at room temperature.[186]

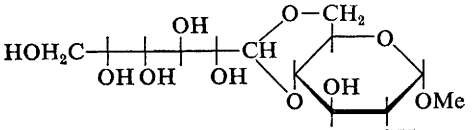
Acetals Derived from Carbohydrate Hydroxy-groups

A method of synthesis of acetals employing sulphonated polystyrene as catalyst and anhydrous calcium sulphate as desiccant has been described and the procedure should be applicable in the carbohydrate field.[187]

From Diol Groups on Cyclic Carbohydrates.—Modified conditions have been described for the preparation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside; although the conditions do not introduce any novel features, they represent improvements on frequently used procedures.[187a]

[184] K. Matsuura, S. Maeda, Y. Araki, and Y. Ishido, *Bull. Chem. Soc.* (*Japan*), 1971, **44**, 292.
[185] D. B. Cooper, T. D. Inch, and D. J. Sellers, *Tetrahedron Letters*, 1971, 2329.
[186] Yu. A. Zhdanov, G. N. Dorofeenko, G. V. Bogdanova, S. M. Lukhianov, V. G. Alexeeva, and Yu. E. Alexeev, *Carbohydrate Res.*, 1971, **19**, 287.
[187] V. I. Stenberg, G. F. Vesley, and D. Kubik, *J. Org. Chem.*, 1971, **36**, 2550.
[187a] J. W. Van Cleve, *Carbohydrate Res.*, 1971, **17**, 461.

Much more novel was the preparation of methyl 4,6-*O*-glucosylidene-α-D-glucopyranoside (60) and a number of its derivatives; the synthesis of the



(60)

parent compound was effected using methyl α-D-glucoside and acyclic *aldehydo*-D-glucose derivatives.[188] 4′,6′-*O*-Benzylidene derivatives of galactocerebrosides,[189] and corresponding acetals of epipodophyllotoxin β-D-glucopyranoside [190] and related glycosides have been reported.

1-(Dimethylamino)-ethylidene and -benzylidene acetals were obtained when methyl β-D-ribofuranoside was treated with *NN*-dimethylacetamide and *NN*-dimethylbenzamide, respectively.[191]

Details of the preparation of 1,2:5,6-di-*O*-cyclohexylidene-α-D-gulofuranose from the related D-*gluco*-compound have been given,[192] and the mechanism of acetonation of L-sorbose has been studied.[193] Mild acidic hydrolysis of the dibenzylidene tetra-*O*-benzoyl derivative of αα-trehalose has yielded the corresponding monobenzylidene tetrabenzoate, thereby offering a means of obtaining unsymmetrically substituted trehalose derivatives.[194]

The acetals obtained on acetonation of 3-deoxy-3-nitro-D-glucose are noted in Chapter 10.

From Diol Groups on Acyclic Carbohydrates.—Xylitol and acetylsalicylaldehyde combined in the presence of acidic catalysts to give mono- and bis-acetals having the 2,4- and 2,4:3,5-structures, respectively.[195]

Continued studies on the monoacetals formed from D-glucitol and n-butyraldehyde have involved an investigation of related deoxy-derivatives. 1-Deoxy-D-glucitol gave the 2,3-acetal as the kinetic product and the 2,4-acetal as the thermodynamic product. Similarly, '2-deoxy-D-glucitol' gave the 1,3-acetal followed by the 3,4-acetal, whereas the 3-deoxy-isomer gave the 2,4-product throughout.[196]

[188] F. Micheel, E. Velker, and E. A. Witte, *Tetrahedron Letters*, 1971, 451.
[189] A. Ya. Veinberg, G. I. Roslovtseva, S. Ya. Mel'nik, and G. I. Samokhvalov, *J. Gen. Chem.* (*U.S.S.R.*), 1970, **40**, 1891 (*Zhur. obshchei Khim.*, 1970, **40**, 1908).
[190] C. Keller-Juslén, M. Kuhn, A. von Wartburg, and H. Stähelin, *J. Medicin. Chem.*, 1971, **14**, 936.
[191] S. Hanessian and E. Moralioglu, *Tetrahedron Letters*, 1971, 813.
[192] S. L. Von Schuching and G. H. Frye, *Org. Prep. Proced.*, 1970, **2**, 83.
[193] L. O. Shnaidman and A. V. Fondarenko, *Khim.-Farm. Zhur.*, 1971, **5**, 55.
[194] A. C. Richardson and E. Tarelli, *J. Chem. Soc.* (*C*), 1971, 3733.
[195] V. V. Russanova, A. N. Anikeeva, and S. N. Danilov, *J. Gen. Chem.* (*U.S.S.R.*), 1970, **40**, 2754 (*Zhur. obshchei Khim.*, 1970, **40**, 2757).
[196] T. G. Bonner, E. J. Bourne, P. J. V. Cleare, R. F. J. Cole, and D. Lewis, *J. Chem. Soc.* (*B*), 1971, 957.

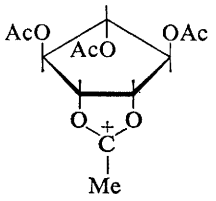
Acetalation of 6-*O*-benzoyl-2,4-*O*-benzylidene-D-glucose dialkyl dithioacetals gave the 2,4:3,5-diacetal with benzaldehyde and zinc chloride, whereas the debenzoylated precursor gave the 2,4:5,6-diacetal. Results of related acetalations were reported.[197]

[197] H. Zinner and R. Heinatz, *J. prakt. Chem.*, 1970, **312**, 561.

6
Esters

Acetates and Other Aliphatic Esters

Paulsen's group have continued their studies on acetoxonium-ion rearrangements and have studied model diol [198] and triol [199] systems; thermodynamic data for the isomerizations were reported. Extension to the cyclopentanepentol system (61) showed that the cyclic ion migrates continuously



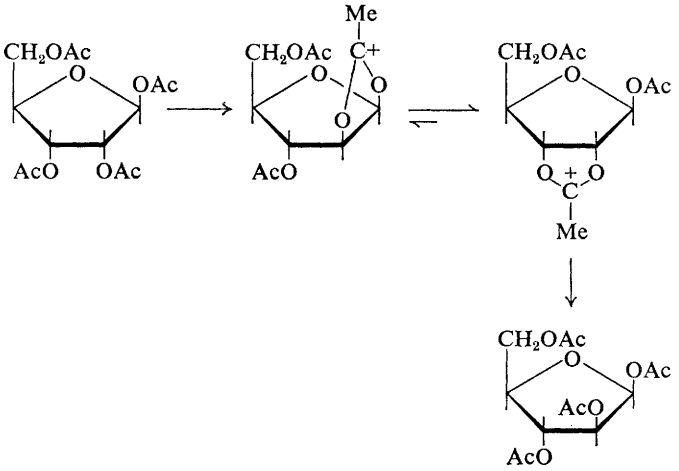
(61)

around the ring.[200] Acetoxonium-ion intermediates from the opening of epoxy-acetates have already been discussed [158] and related intermediates formed from amino-sugars are mentioned in Chapter 8.

Monosaccharides with a *cis*-configuration at C-2 and C-3 underwent inversion of configuration on acetolysis in acetic anhydride–acetic acid containing sulphuric acid. Thus, 2,3-*O*-isopropylidene-D-ribose gave D-arabinose after acetolysis and deacetylation [201] (Scheme 25). 2,3:5,6-Di-*O*-isopropylidene-D-gulofuranose, 2,3:6,7-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptose, D-ribose, and D-mannose were similarly epimerized on acetolysis.

The acetylation of 7-(3-deoxy-3-nitro-β-D-glycopyranosyl)theophyllines with the *gluco*, *manno*, and *galacto* configurations have been studied using acetic anhydride in the presence of boron trifluoride, perchloric acid, or phosphoric acid. The *gluco*-isomer gave the highest yield of triacetate with boron trifluoride. The *galacto*- and *manno*-compounds gave the triacetate with phosphoric acid or an excess of perchloric acid, but with only a trace

[198] H. Paulsen and H. Behre, *Chem. Ber.*, 1971, **104**, 1264.
[199] H. Paulsen and H. Behre, *Chem. Ber.*, 1971, **104**, 1281.
[200] H. Paulsen and H. Behre, *Chem. Ber.*, 1971, **104**, 1299.
[201] W. Sowa, *Canad. J. Chem.*, 1971, **49**, 3292.



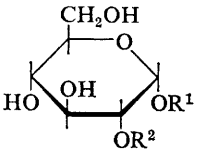
Scheme 25

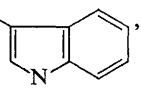
of perchloric acid gave the 4′,6′-diester.[202] A rationalization of the selectivity of 1,6-anhydro-β-D-glucopyranose towards acylation in pyridine was based on the observed hydrogen-bonding patterns of the hydroxy-groups.[133]

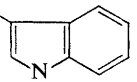
Acetyl (and benzoyl) derivatives of 1,2-*O*-isopropylidene-α-D-glucurone and methyl α- and β-D-glucurone were found to undergo acyl migration during ammonolysis.[203]

Detailed studies on the conformational equilibria of acetates of pentopyranoses are reported in Chapter 23.

Fatty-acid esters of xylitol, sorbitol, and sucrose have been reviewed.[204] Several 6-monoesters (*e.g.* laurate, palmitate, and stearate) of methyl α-D-glucopyranoside have been prepared by base-catalysed trans-



(62) $R^1 = -COCH_2$  , $R^2 = H$

(63) $R^1 = H$, $R^2 = -COCH_2$ 

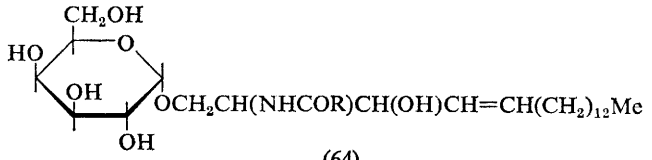
[202] T. Nakagawa and T. Takamoto, *Bull. Chem. Soc. Japan*, 1971, **44**, 192.
[203] H. Weidmann, K. Dax, and D. Wewerka, *Monatsh.*, 1970, **101**, 1831.
[204] J. Saarnio and L. Puupponen, *Kem. Teollisuus*, 1971, **28**, 107 (*Chem. Abs.*, 1971, **75**, 6211*q*).

esterification between the corresponding methyl ester and the parent glycoside.[205]

The preparation of (62) and its ready rearrangement to (63) have been described.[206]

Benzoates

Benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside has been selectively benzoylated at *O*-3, in high yield, by using *N*-benzoylimidazole.[207] Selective benzoylation of the D-galactosyl cerebroside (64) gave the 2′,3′,6′-triester,



(64)

and esterification of the aglycone also occurred.[208] 1,3,5-Tri-*O*-benzoyl-, 1,3,4,5-tetra-*O*-benzoyl-, and 1,3,5-tri-*O*-benzoyl-4-*O*-methyl-α-L-sorbopyranose and methyl 1,3,5-tri-*O*-benzoyl-4-*O*-methyl-α-L-sorbopyranoside have been prepared and characterized.[209] Preparations of the 2-, 3-, and 4-*O*-benzoyl derivatives of methyl α-D-glucopyranoside have been described.[205]

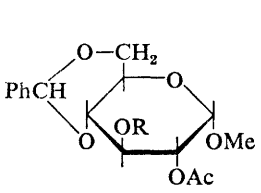
O → *N*-Transbenzoylation in 1-amino-2,3,4-tri-*O*-benzoyl-1-deoxy-D-erythritol led to 1-benzamido-3,4-di-*O*-benzoyl-1-deoxy-D-erythritol.[210] Hydrolysis of 1,2,3-tri-*O*-benzoyl-4-bromo-4-deoxy-L-erythritol in moist DMF gave 1,2,3-tri-*O*-benzoyl-L-erythritol and racemic 1,2,4-tri-*O*-benzoyl-erythritol.[211]

The value of benzoyl cyanide as a benzoylating agent in nucleoside chemistry has been emphasized.[212] Details of the conformational equilibria in pentopyranose benzoates are reported in Chapter 23.

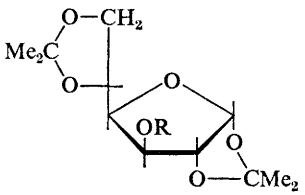
Carboxylic Orthoesters

N.m.r. evidence has been presented for a rigid conformation for tricyclic sugar orthoesters. The spectra of some α-D-xylopyranosyl compounds were compared with those previously reported for 3-*O*-benzoyl-α-D-ribopyranose 1,2,4-orthobenzoate.[213] Several bis-saccharide orthoesters, such as (65)

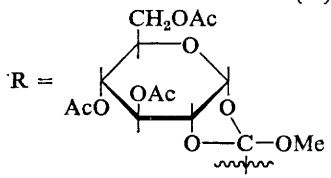
[205] G. N. Bollenback and F. W. Parrish, *Carbohydrate Res.*, 1971, **17**, 431.
[206] D. Keglević, *Carbohydrate Res.*, 1971, **20**, 293.
[207] G. J. F. Chittenden, *Carbohydrate Res.*, 1971, **16**, 495.
[208] A. Ya. Veinberg, S. Ya. Mel'nik, G. I. Roslovtseva, and G. I. Samakhvalov, *J. Gen. Chem.* (*U.S.S.R.*), 1970, **40**, 2738, 2742 (*Zhur. obshchei Khim.*, 1970, **40**, 2742, 2745).
[209] M. C. Teglia and R. A. Cadenas, *Carbohydrate Res.*, 1971, **19**, 223.
[210] I. Ziderman, *Carbohydrate Res.*, 1971, **17**, 224.
[211] I. Ziderman, *Carbohydrate Res.*, 1971, **17**, 220.
[212] A. Holý and M. Souček, *Tetrahedron Letters*, 1971, 185.
[213] A. F. Bochkov, V. M. Dashunin, A. V. Kessenikh, N. K. Kochetkov, A. D. Naumov, and I. V. Obruchnikov, *Carbohydrate Res.*, 1971, **16**, 497.



(65)



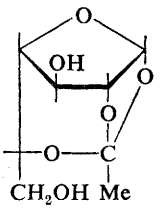
(66)



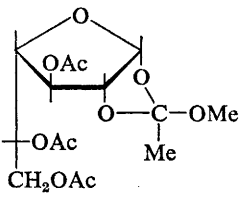
and (66), have been prepared by reaction of acetobromo-D-glucose with the appropriate hydroxy-compound in the presence of silver salicylate.[213a]

A variety of bicyclic orthoesters have been synthesized and their cyclization to tricyclic orthoesters has been studied.[214, 215]

Further studies on the use of orthoesters in the synthesis of glycosides have been reported.[94, 216] 1,2,5-*O*-Ethylidyne-α-D-galactofuranose (67) was one of the major products formed when 3,5,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactofuranose (68) was treated with methanolic sodium methoxide followed by neutralization with an acidic resin.[217]



(67)

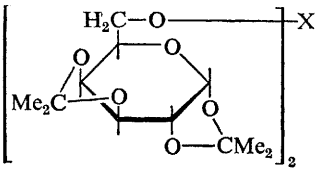


(68)

Sulphates

Sugar sulphates of type (69) have been prepared by reaction of the parent hydroxy-compound with thionyl chloride and oxidation of the intermediate sulphite (70) with permanganate.[218]

[213a] G. Wulff and W. Krüger, *Carbohydrate Res.*, 1971, **19**, 139.
[214] A. F. Bochkov, I. V. Obruchnikov, and N. K. Kochetkov, *Izvest. Akad. Nauk, S.S.S.R., Ser. khim.*, 1971, 1282, 1291.
[215] A. F. Bochkov, V. I. Snyatkova, Ya. V. Voznyi, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1971, **41**, 2776.
[216] A. F. Bochkov and T. A. Sokolovskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 2793.
[217] M. Bertolini and C. P. J. Glaudemans, *Carbohydrate Res.*, 1971, **18**, 131.
[218] K. Takiura and S. Honda, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 2125.

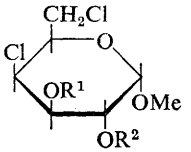


(69) X = SO_2
(70) X = SO

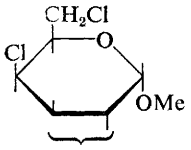
Preparations of D-glucose 6- and 3-sulphates have been described.[219] Direct sulphation of 6-deoxy-L-galactose with pyridine and sulphur trioxide yielded a mixture of mono-, di-, and tri-sulphates from which the 2-, 3-, and 4-monoesters were isolated. The 2-ester was also prepared from the 1,3,4-triacetate and from the methyl glycoside 2-sulphate.[220]

Several sulphated monosaccharides have been desulphated simply by heating in dioxan, pyridine, and either DMSO or DMF. Polysaccharides were also desulphated with DMSO–pyridine; the method gave higher yields and less decomposition than when methanolic hydrogen chloride was used for this purpose.[221]

Treatment of the bischlorosulphate (71) with sodium azide in DMF afforded a mixture of (72), (73), (74), and the azidosulphate (75).[222]



(71) $R^1 = R^2 = SO_2Cl$
(72) $R^1 = H$, $R^2 = SO_2Cl$
(73) $R^1 = SO_2Cl$, $R^2 = H$
(74) $R^1 = R^2 = H$



(75)

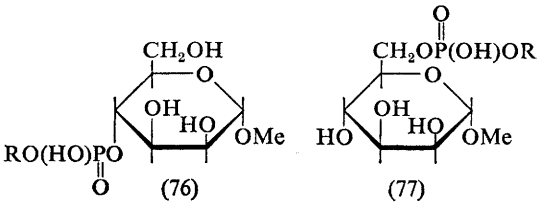
Glycoside 2-sulphates displayed a much higher susceptibility towards acid hydrolysis than did 3-, 4-, or 6-esters. There is also evidence that enhanced acid lability of acetal substituents in rings containing proximal sulphate groups is a general phenomenon.[223]

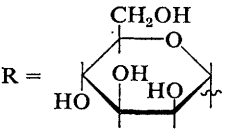
The reaction of sugars containing dialkylamidosulphochloride or chlorosulphate groups with amines gave sugar amidosulphates, representing new, stable derivatives of sugar sulphates.[224] L-Ascorbic acid sulphates are referred to in Chapter 17.

[219] M. Hranisavljevic-Jakovljevic, R. Dimitrijevic, and G. Markovic, *Glasnik Hem. Drustva Beograd.*, 1969, **34**, 245.
[220] P. F. Lloyd and P. F. Forrester, *Biochem. J.*, 1971, **24**, 21P.
[221] A. I. Usov, K. S. Adamyants, L. I. Miroshnikova, A. A. Shaposhnikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **18**, 336.
[222] H. Parolis, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1971, **19**, 97.
[223] P. F. Lloyd and P. F. Forrester, *Carbohydrate Res.*, 1971, **19**, 430.
[224] N. K. Kochetkov, A. I. Usov, and V. V. Derijabin, *Zhur. obshchei Khim.*, 1971, **41**, 1866.

Phosphorus Esters

Sugar Phosphates.—Syntheses of methyl α-D-mannopyranoside 4- and 6-phosphates, and also of (76) and (77), have been described.[225] Hydrolysis





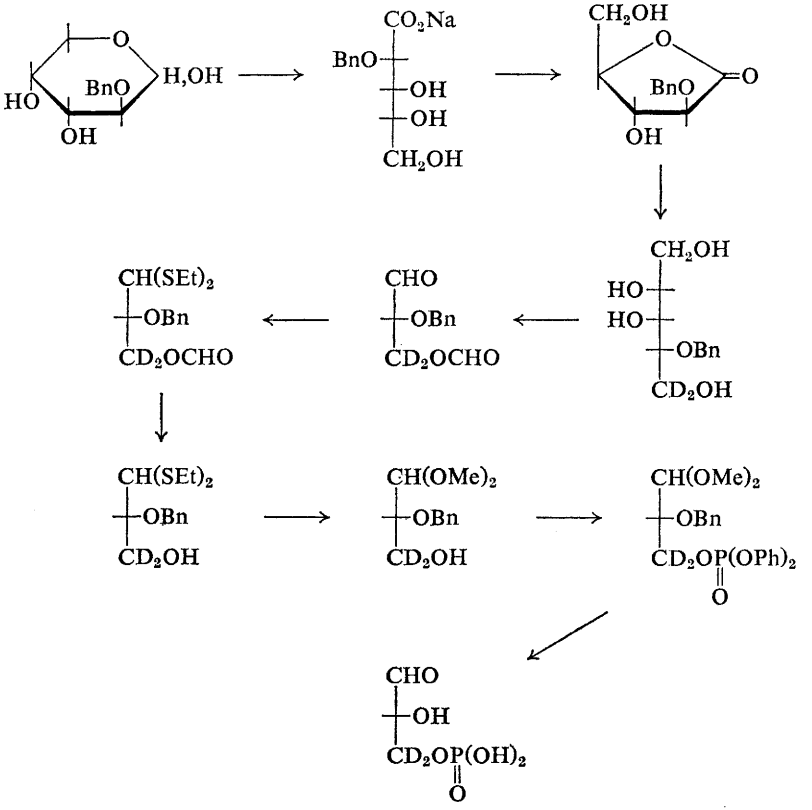
of these esters showed that phosphate migration from O-4 to O-6 did not occur and that the reverse migration occurred only to a limited extent. The synthesis of D-[3,3-$^2H_2$]glyceraldehyde 3-phosphate has been accomplished from 2-*O*-benzyl-D-arabinose, as illustrated in Scheme 26 (all transformations are standard).[226]

D-Glucose in aqueous solution (pH 6.7—8.8) is phosphorylated by orthophosphate and cyanogen to give the α- and β-1-esters and a phosphorylated disaccharide of unknown structure. This cyanogen-induced phosphorylation was shown to be general for a wide range of reducing mono- and di-saccharides, but not for non-reducing compounds.[227] The reaction is thought to proceed with formation of (78) as the phosphorylating agent by the mechanism shown in (79);[41] unless the hydroxy-group is sufficiently acidic, the key protonation of the nitrogen atom does not occur.

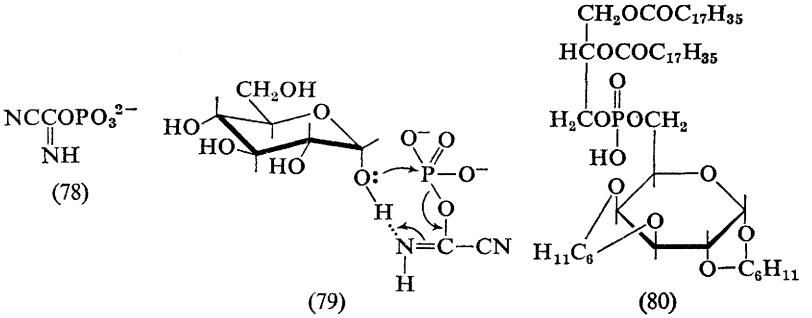
An enzymic synthesis of sedoheptulose 7-phosphate from D-ribose 5-phosphate has been described and the product was assayed by a newly developed enzymic procedure.[228] Syntheses of (80)[229] and phosphates of apiose (see p. 101) have been reported.

γ-Radiolysis of aqueous solutions of D-glucose 1- and 6-phosphates suggested that hydroxyl radicals play an important part in radiation-induced phosphate cleavage.[230] L-Ascorbic acid phosphates are discussed in Chapter 17.

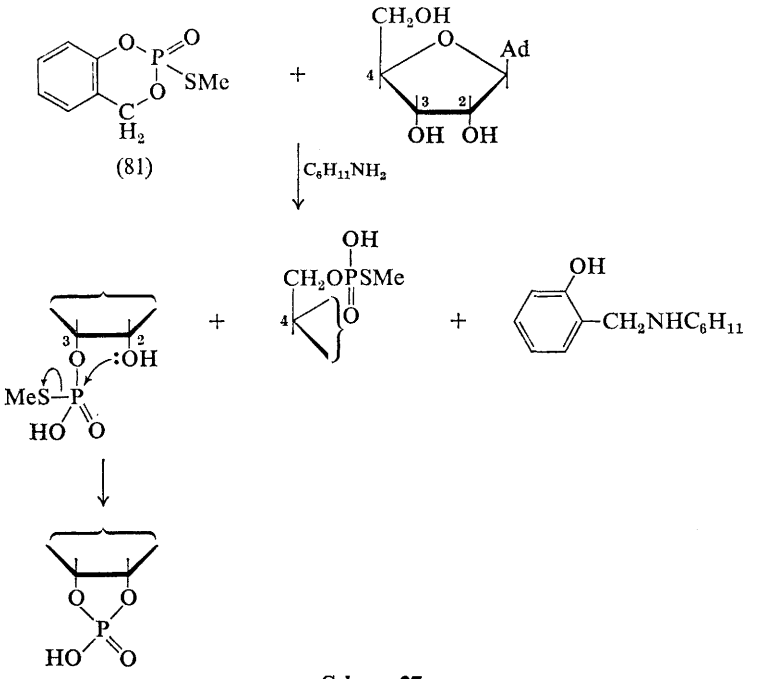
[225] T. N. Cawley and R. Letters, *Carbohydrate Res.*, 1971, **19**, 373.
[226] G. R. Gray and R. Barker, *Carbohydrate Res.*, 1971, **20**, 31.
[227] C. Degani and M. Halmann, *J. Chem. Soc.* (*C*), 1971, 1459.
[228] T. Wood and W. M. Poon, *Arch. Biochem. Biophys.*, 1970, **141**, 440.
[229] L. V. Volkova, M. G. Luchinskaya, N. A. Samoilova, and N. A. Preobrazhenskii, *J. Gen. Chem.* (*U.S.S.R.*), 1971, **41**, 437 (*Zhur. obshchei Khim.*, 1971, **41**, 446).
[230] N. K. Kochetkov, L. I. Kudrjashov, M. A. Chlenov, and L. P. Grineva, *Zhur. obshchei Khim.*, 1971, **41**, 2071.



Scheme 26



Nucleoside Phosphates.*—2′,3′-Cyclic phosphates of several pyrimidine nucleosides have been prepared *via* the 2′(3′)-phosphites.[231] 2-Methylthio-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (81) has been described as a potentially useful phosphorylating agent, as shown in Scheme 27.[232]



Scheme 27

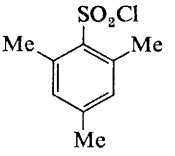
Phosphites.—Cyclic phosphites have been synthesized from various isopropylidene derivatives of D-mannitol.[233] Sorbitol and dulcitol cyclic phosphites have also been described.[233a]

Sulphonates

Selective tosylation of benzyl α-D-xylopyranoside gave a monoester claimed to be the 3-*O*-tosyl compound. Similar esterification of benzyl β-D-arabinopyranoside gave the 2-ester. Ditosylation of both compounds gave mainly 2,3-diesters.[234] Mesitylenesulphonyl ('mesisyl') chloride (82)

[231] A. Holý and R. Bald, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2809.
[232] M. Eto, M. Sasaki, M. Iio, M. Eto, and H. Ohkawa, *Tetrahedron Letters*, 1971, 4263.
[233] O. V. Voskresenskaya, P. A. Kirpichnikov, and E. T. Mukmenev, *Bull. Acad. Sci. U.S.S.R.*, 1970, 1580 (*Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 1668).
[233a] O. V. Voskresenskaya, P. A. Kirpichnikov, and E. T. Mukmenev, *Bull. Acad. Sci. U.S.S.R.*, 1970, 1578 (*Izvest. Akad. Nauk. S.S.S.R., Ser. khim.*, 1970, 1666).
[234] N. Friedmann, S. Cohen, and E. D. Bergmann, *Israel J. Chem.*, 1970, **8**, 663.

* See also Chapter 21.

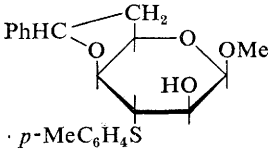


(82)

has been shown to be an excellent reagent for selective sulphonylation of vicinal glycol systems. Vicinal dimesisyl esters, in contrast to the corresponding ditosyl esters, were not affected by methanolic sodium methoxide.[235]

Selective tosylations of *myo*-inositol and its derivatives are discussed in Chapter 19.

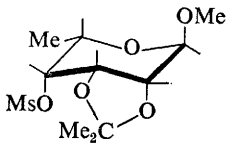
Further work (*cf.* Vol. 4, p. 45) on the reactions of lithium aluminium hydride with methyl 4,6-*O*-benzylidene-2,3-di-*O*-*p*-tolylsulphonyl-α-D-glycosides has been completed. The *altro*- and *ido*-compounds have now been investigated; the former gave the 3-deoxy-derivative, whereas the latter afforded the 2-deoxy-derivative.[236] The results were discussed in the light of previous work. In a further paper on the α-D-*galacto*-isomer, only 2—3% of a deoxy-compound was reported; the products were the 2-tosylate, the parent diol, and starting material.[237] The β-*galacto*-isomer gave the 3-deoxyidoside, presumably arising from the intermediacy of the *talo*-epoxide formed from the 2-tosylate. Both of the latter compounds were found among the products of reduction, which also included (83) formed by reaction of the *talo*-epoxide with thiocresylate ion derived by reduction of the tosyl group with lithium aluminium hydride. The structure of (83) was confirmed by independent synthesis.[237]



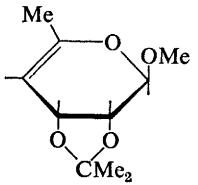
(83)

Displacement Reactions without Participation.—Methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methylsulphonyl-α-L-talopyranoside (84) gave the unsaturated sugar (85) on reaction with ammonia, and no basic product could be isolated. With hydrazine, (84) also gave (85) and a methoxymethylpyran of unknown structure. These results have to be compared with the reaction of (84) with azide ion (Vol. 2, p. 65) when the expected azido-sugar as well as (85) were formed. The results suggest that the more powerful azide nucleophile of low basicity favours substitution, whereas ammonia or

[235] S. E. Creasey and R. D. Guthrie, *Chem. Comm.*, 1971, 801.
[236] A. Zobáčová, V. Heřmánková, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1860.
[237] V. Heřmánková, A. Zobáčová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1971, **36**, 303.



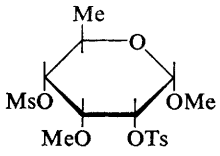
(84)



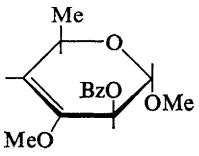
(85)

hydrazine of high basicity favours elimination from a system set up stereochemically for both reactions.[238]

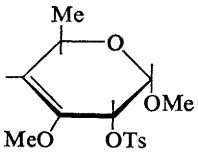
Reaction of (86) with sodium benzoate in HMPT gave the unsaturated product (87) formed, it was proposed, by way of the allylic tosylate (88).[239]



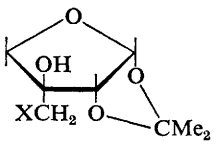
(86)



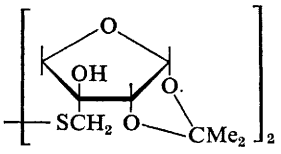
(87)



(88)



(89) X = OTs
(90) X = SAc

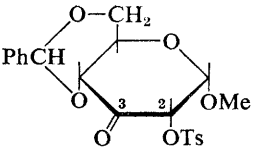


(91)

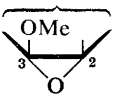
The apiose derivative (89), on treatment with potassium thioacetate in boiling ethanol, gave the disulphide (91), presumably formed by deacetylation and oxidation of the thiolacetate (90). A complex mixture of products was obtained when the displacement was conducted in aprotic solvents, owing to both *S* → *O*-acetyl migration and *S*-acetylation.[240] When treated with sodium iodide in acetone, 2,3,3′,4,4′-penta-*O*-acetyl-1′,6,6′-tri-*O*-*p*-tolylsulphonylsucrose gave the corresponding 6,6′-dideoxy-6,6′-di-iodo-1′-*O*-tosylate.[241]

Reaction of the α-ketotosylate (92) with methanolic trimethylamine gave (93) and (94); longer reaction times gave (95), formed at the expense of (93). Compound (94) was believed to be formed as shown in Scheme 28.[242]

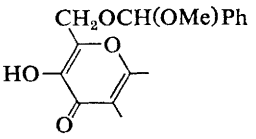
[238] J. Jarý and P. Novák, *Coll. Czech. Chem. Comm.*, 1971, **36**, 3046.
[239] A. K. Al-Radhi, J. S. Brimacombe, L. C. N. Tucker, and O. A. Ching, *J. Chem. Soc.* (*C*), 1971, 2305.
[240] M. H. Halford, D. H. Ball, and L. Long, jun., *J. Org. Chem.*, 1971, **36**, 3714.
[241] T. Suami, N. Kato, M. Kawamura, and T. Nishimura, *Carbohydrate Res.*, 1971, **19**, 407.
[242] A. Dmytraczenko, W. A. Szarek, and J. K. N. Jones, *Chem. Comm.*, 1971, 1220,



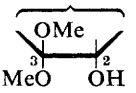
(92)



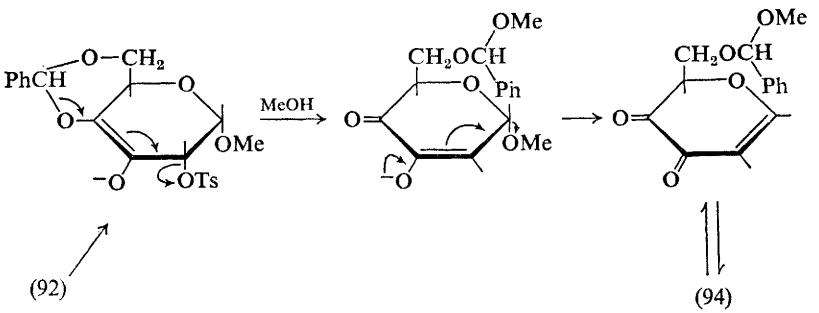
(93)



(94)



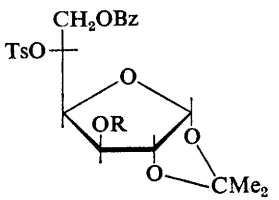
(95)



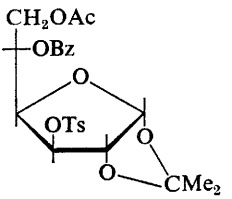
Scheme 28

Displacement Reactions with Participation.—Potassium acetate in DMF caused direct displacement of the 5-sulphonyloxy-group in (96). However, using acetic anhydride and Dowex 1 (AcO^-) or with potassium acetate in acetonitrile, neighbouring-group participation occurred to give (97) as the major product.[243] It was suggested that these reactions are kinetically controlled and that the greater solubility of the acetate ion in DMF favoured direct displacement, whereas its relative insolubility in acetonitrile favoured participation. Another study has been made of the displacement reactions of 6-*O*-benzoyl-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulphonyl-α-D-glucofuranose derivatives [244] with acetate or chloride ions in acetic anhydride, when participation by the benzoyloxy-group was again demonstrated: thus (98) gave (99). As noted previously, acetate displacements in DMF also occurred directly to give a mixture of 5-*O*-acetyl-6-*O*-benzoyl and 6-*O*-acetyl-5-*O*-benzoyl derivatives. Direct displacement occurred in (98) with sodium azide in HMPT.

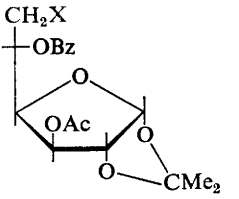
[243] M. Miljković, A. Jokić, and E. A. Davidson, *Carbohydrate Res.*, 1971, **17**, 155.
[244] R. C. Chalk, D. H. Ball, and L. Long, jun., *Carbohydrate Res.*, 1971, **20**, 151.



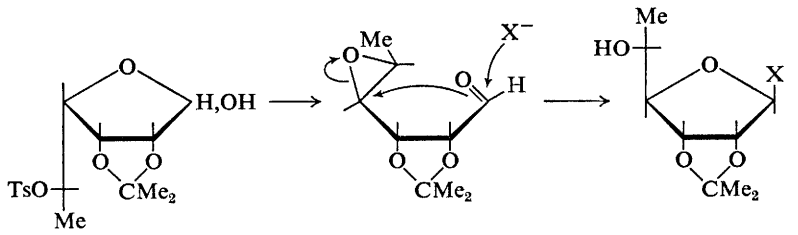
(96) R = Ts
(98) R = Ac



(97)



(99) X = OAc or Cl

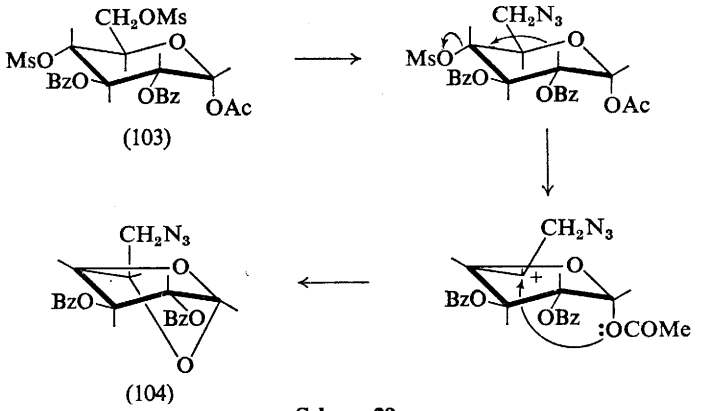


(100)

(101) X = OMe
(102) X = SEt

By analogy with the reaction of 6-deoxy-2,3-*O*-isopropylidene-5-*O*-*p*-tolylsulphonyl-L-mannose (100) and sodium methoxide to give the methyl allofuranoside (101), the reaction with sodium thioethoxide gave (102).[245]

Further details of some benzyloxy[246] and methoxy[247] participations have been given. Treatment of 1-*O*-acetyl-2,3-di-*O*-benzoyl-4,6-di-*O*-



(103)

(104)

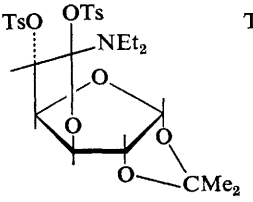
Scheme 29

[245] E. J. Reist and M. Tan, *Carbohydrate Res.*, 1971, **18**, 446.
[246] O. A. Ching Puente, *Bol. Soc. quím. Peru*, 1970, **36**, 60 (*Chem. Abs.*, 1971, **74**, 64 372*h*).
[247] O. A. Ching Puente, *Bol. Soc. quím. Peru*, 1970, **36**, 45 (*Chem. Abs.*, 1971, **74**, 64 368*m*).

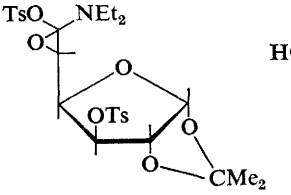
methylsulphonyl-α-D-glucopyranose (103) with sodium azide gave a monoazide shown to be (104); the mechanism shown in Scheme 29 accounts for its formation.[247a]

The cyclic orthoesters (105) and (106) have been postulated to account for the formation of monoesters on sulphonation of the amide (107).[248] Presumably, (108) is formed first and this is followed by intramolecular formation of the othoesters (105) and (106), which are hydrolysed by water to give the products.

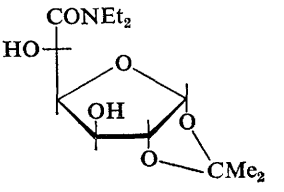
The 2-*O*-mesyloxy-group in (109) is easily displaced to yield the heterocyclic derivative (110).[249] Related participations are described in Chapter 12.



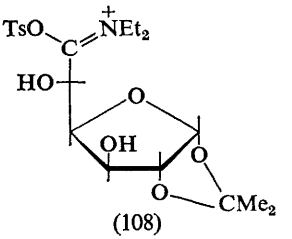
(105)



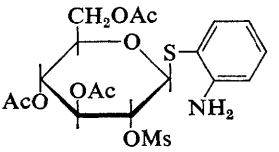
(106)



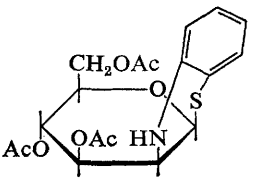
(107)



(108)



(109)



(110)

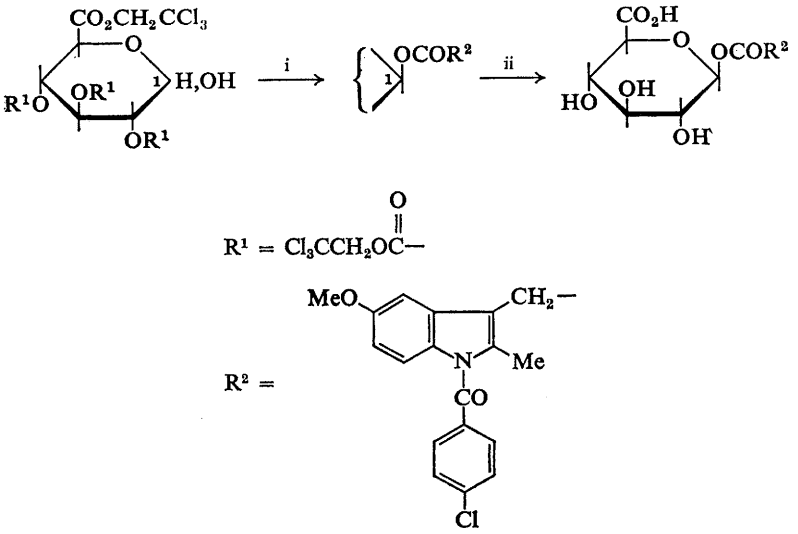
Miscellaneous Esters

G.l.c. of butane boronates of carbohydrates and their trimethylsilyl derivatives has been investigated.[250, 251] The following benzene boronates

[247a] C. Bullock, L. Hough, and A. C. Richardson, *Chem. Comm.*, 1971, 1276.
[248] M. Miljković, D. Miljković, A. Jokić, V. Andrejević, and E. A. Davidson, *J. Org. Chem.*, 1971, **36**, 3218.
[249] M. Sekiya and S. Ishiguro, *Tetrahedron Letters*, 1971, 431.
[250] F. Eisenberg, jun., *Carbohydrate Res.*, 1971, **19**, 135.
[251] P. J. Wood and I. R. Siddiqui, *Carbohydrate Res.*, 1971, **19**, 283.

of 1,6-anhydro-β-D-hexopyranoses have been characterized: 2,4-*gluco*, 3,4-*galacto* and -*altro*, and 2,3-*gulo* and -*manno*.[252] Uncharacterized borate esters have been prepared by the reactions, under anhydrous conditions, of methyl α-D-glucoside, methyl α-D-mannoside, cellobiose, cellulose, or *O*-methylcellulose with such boron alkoxides as boron ethoxide, boron propoxide, or boron isopropoxide. The reactivity of carbohydrates towards boron alkoxides was increased in pyridine or ethylenediamine.[253]

Use of the trichloroethoxycarbonyl (CCl_3CH_2OCO—, 'TROC') protecting group has been explored; such groups are resistant to acidic hydrolysis even when substituted on C-1. An example of its use is shown in Scheme 30.[254]



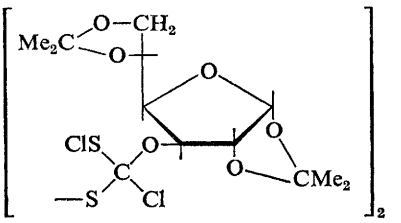
Reagents: i, R^2CO_2H–DCC; ii, Zn–AcOH

Scheme 30

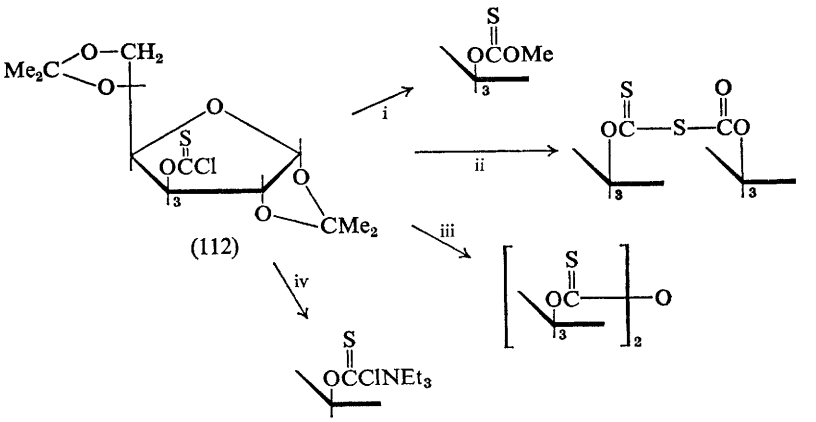
Chlorothioformates [*e.g.* (112)] have been formed by treatment of the bis(chloromethylsulphonyl chloride) disulphide (111) with sodium iodide.[255] The reactions of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose 3-chlorothioformate (112), shown in Scheme 31, have been developed.[255a]

The products from the reaction of methyl α-D-glucopyranoside and phenyl isocyanate, in varying proportions, have been separated and identified.[256]

[252] F. Shafizadeh, G. D. McGinnis, and P. S. Chin, *Carbohydrate Res.*, 1971, **18**, 357.
[253] M. S. Bains and J. C. Arthur, jun., *Carbohydrate Res.*, 1971, **19**, 365.
[254] R. Bugianesi and T. Y. Shen, *Carbohydrate Res.*, 1971, **19**, 179.
[255] B. S. Shasha and W. M. Doane, *Carbohydrate Res.*, 1971, **16**, 145.
[255a] B. S. Shasha, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1971, **17**, 444.
[256] Y. H. Yeh, K. P. Kringstad, and R. D. Gilbert, *Carbohydrate Res.*, 1971, **19**, 87.



(111)

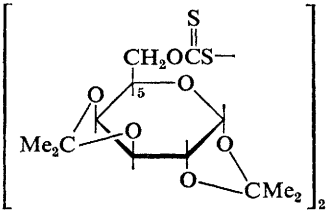


Reagents: i, MeOH; ii, py; iii, py (excess); iv, NEt_3

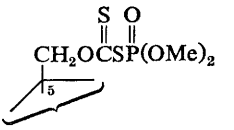
Scheme 31

Dinucleoside carbonates and trinucleoside dicarbonates have been prepared as nucleotide analogues.[257]

Treatment of bis-(1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose) 6,6′-[dithiobis(thioformate)] (113) with trimethyl phosphite afforded (114); the reactions of the latter compound were examined.[258]



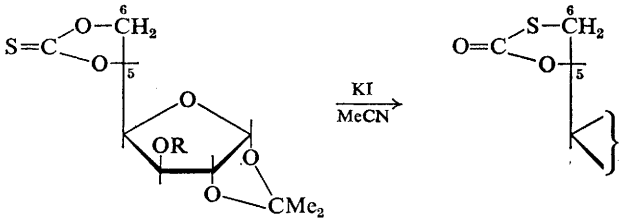
(113)



(114)

[257] J. R. Tittensor, *J. Chem. Soc.* (*C*), 1971, 2656.
[258] B. S. Shasha, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1971, **20**, 407.

It has been shown that thionocarbonate derivatives of sugars containing substituted hydroxy-groups can be isomerized to monothiocarbonates (Scheme 32); no isomerization occurred when the hydroxy-group was free.

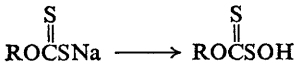


R = Ts or Ac

Scheme 32

Thionocarbonates involving only secondary positions underwent negligible isomerization.[259] The optical properties of a number of carbohydrate cyclic thionocarbonates have been examined [260] (see Chapter 25).

In model experiments designed to provide a better understanding of the reactions of cellulose dithiocarbonates with acrylonitrile–hydrogen peroxide to produce cellulose-acrylonitrile graft polymers, the *O*-(sodium thiocarbonyl) derivatives of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose have been converted into the corresponding hydroxythio(thiocarbonates) (Scheme 33). These are the first compounds reported with an



Scheme 33

—OC(=S)SOH group, which is acidic enough to be titrated with alkali, and, in the presence of hydrogen peroxide, is able to initiate graft polymerization of acrylamide.[261]

[259] D. Trimnell, W. M. Doane, C. R. Russell, and C. E. Rist, *Carbohydrate Res.*, 1971 **17**, 319.
[260] A. H. Haines and C. S. P. Jenkins, *J. Chem. Soc.* (*C*), 1971, 1438.
[261] B. S. Shasha, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1971, **18**, 251.

7
Halogenated Sugars

Glycosyl Halides

The hydrolysis of glycosyl fluorides by appropriate glycosidases has been studied; some enzymes operated by a mechanism which caused inversion of configuration at C-1, whereas retention of configuration was observed with others.[262] A detailed kinetic study of the exchange between acetobromosugars and lithium bromide in acetone suggested that the reactions were second order, even when there was evidence of neighbouring-group participation from the C-2 group.[263]

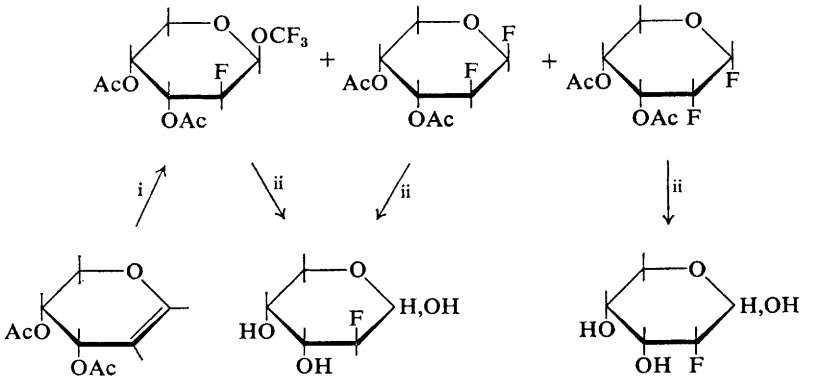
2,3,5-Tri-*O*-benzoyl-1-*O*-(*p*-nitrobenzoyl)-β-D-ribofuranose has been used as a convenient crystalline intermediate for the preparation of the corresponding chloride and bromide. On treatment of the starting material with either hydrogen bromide or hydrogen chloride in dichloromethane, *p*-nitrobenzoic acid was precipitated in virtually quantitative yield, thereby facilitating isolation of the amorphous furanosyl halides.[264]

The mechanism of formation of arabinofuranosyl halides has been studied for compounds with benzyl or *p*-nitrobenzyl groups on the ring. It is suggested that α-halides are formed under kinetic control and that they slowly anomerize to the β-forms. Participation by the C-5 group is invoked to account for initial formation of the α-anomer.[265]

Three papers dealing with fluoroglycosyl fluorides are treated in this section. Extended applications of the addition of trifluoro(fluoroxy)-methane to glycals (see Vol. 3, p. 59) are illustrated in Schemes 34,[266] 35,[267] and 36,[268] while similar addition to 2-hydroxy-D-arabinal triacetate gave the fluorinated compounds (115) and (116).[266] Other papers dealing with glycosyl fluorides encountered during work with unsaturated sugars are dealt with in Chapter 14.

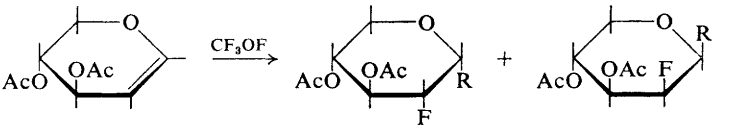
The reaction of acetobromoglucose with silver salts of hydroxycarboxylic acids and the reactions of pentopyranosyl chloride 2,3,4-tri(chlorosulphates) are mentioned in Chapter 3.

[262] J. E. G. Barnett, *Biochem. J.*, 1971, **123**, 607.
[263] M. J. Duffy, G. Pass, and G. O. Phillips, *J. Chem. Soc.* (*B*), 1971, 785.
[264] R. K. Ness, H. G. Fletcher, jun., and K. W. Freer, *Carbohydrate Res.*, 1971, **19**, 423.
[265] C. P. J. Glaudemans and H. G. Fletcher, jun., *J. Org. Chem.*, 1971, **36**, 3599.
[266] E. L. Albano, R. L. Tolman, and R. K. Robins, *Carbohydrate Res.*, 1971, **19**, 63.
[267] C. G. Butchard and P. W. Kent, *Tetrahedron*, 1971, **27**, 3457.
[268] J. Adamson, A. B. Foster, and J. H. Westwood, *Carbohydrate Res.*, 1971, **18**, 345.



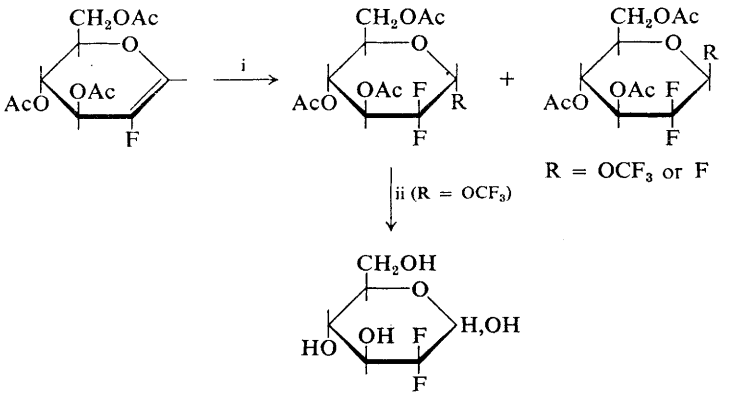
Reagents: i, CF_3OF; ii, H_3O^+

Scheme 34



R = OCF_3 or F

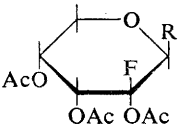
Scheme 35



R = OCF_3 or F

Reagents: i, CF_3OF; ii, H_3O^+

Scheme 36

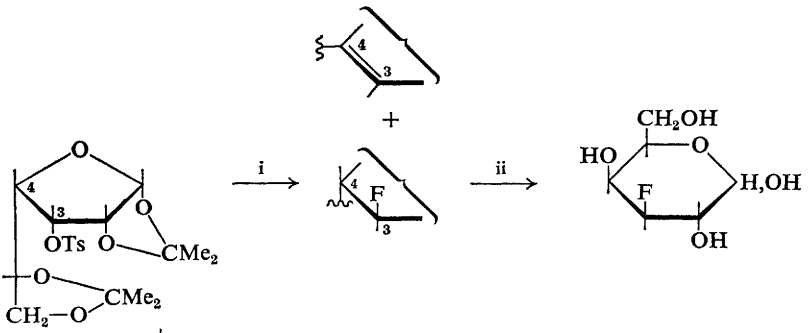


(115) R = OCF_3
(116) R = F

Other Halogenated Derivatives

An improved synthesis of 4-deoxy-4-fluoro-D-glucose utilized 1,6:3,4-dianhydro-β-D-galactopyranose (prepared by u.v.–base cleavage of the corresponding 2-tosylate) and potassium hydrogen fluoride. Detailed n.m.r. data for the sugar and its derivatives were discussed. It is exceptional that the ^{19}F resonance of the α-anomer occurs at lower field than that of the β-anomer, whereas the reverse situation obtains for the 2-, 3-, and 6-fluorinated analogues. A route to 2,4-dideoxy-2,4-difluoro-D-glucose was elucidated.[269] A much improved synthesis of 6-deoxy-6-fluoro-D-glucose utilized 1,2-*O*-isopropylidene-6-*O*-methylsulphonyl-D-glucofuranose as starting material. Various pyranose derivatives were prepared and studied in detail by n.m.r. spectroscopy.[270]

3-Deoxy-3-fluoro-D-galactopyranose was prepared as shown in Scheme 37; crystalline α- and β-tetra-acetates were obtained and their ^1H and ^{19}F n.m.r. spectra were interpreted using known stereochemical dependencies.[271] 4-Deoxy-4-fluoro-D-galactose was prepared similarly from



Reagents: i, $Bu_4\overset{+}{N}F^-$, MeCN; ii, H_3O^+

Scheme 37

[269] A. D. Barford, A. B. Foster, J. H. Westwood, L. D. Hall, and R. N. Johnson, *Carbohydrate Res.*, 1971, **19**, 49.
[270] E. M. Bessell, A. B. Foster, J. H. Westwood, L. D. Hall, and R. N. Johnson, *Carbohydrate Res.*, 1971, **19**, 39.
[271] J. S. Brimacombe, A. B. Foster, R. Hems, J. H. Westwood, and L. D. Hall, *Canad. J. Chem.*, 1970, **48**, 3946.

methyl 2,3-di-*O*-benzoyl-4-*O*-methylsulphonyl-6-*O*-trityl-α-D-glucopyranoside and the mass spectrum of its methyl α-glycopyranoside was discussed.[272] 5-Deoxy-5-fluoro-D-xylofuranose was likewise synthesized from 3-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulphonyl-α-D-xylofuranose.[272a] A synthesis of the fluorine-containing antibiotic nucleocidin has been reported (see Chapter 20).[273]

A stereospecific synthesis of 1-deoxy-1-fluoro-D-glycerol 3-phosphate has been effected from D-mannitol,[274] and 2-deoxy-2-fluoroglycerol has been prepared in four steps from glycerol by way of a displacement on 2-*O*-*p*-tolylsulphonyl-1,3-ditritylglycerol with fluoride ion. In this report, a further synthesis of 1-deoxy-1-fluoro-D-glycerol from D-mannitol was described.[275] 1-Deoxy-1-fluoro-L-glycerol could be obtained from its enantiomer by a sequence of reactions leading to inversion of configuration at C-2. Reports on the mass spectrometry of fluorinated compounds are noted in Chapter 24.

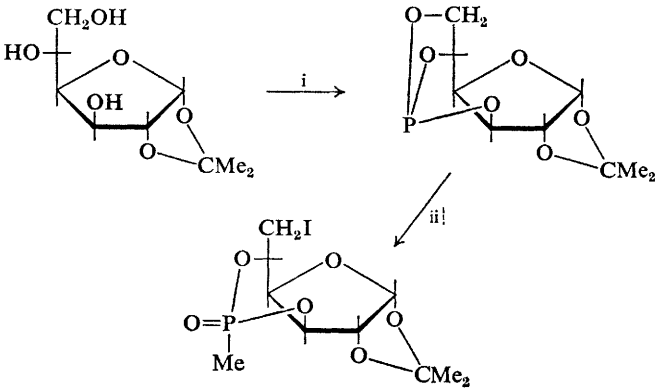
Chlorination of 2′,3′-*O*-isopropylideneinosine with triethyl phosphate and triphenylphosphine in carbon tetrachloride afforded the 5′-chloro-compound; bromination and iodination were similarly effected. When applied to 5′-*O*-acetylinosine, the 3′-chloro-3′-deoxy-D-*xylo*-nucleoside was obtained without formation of the 2-halogenated isomer.[276] Essentially the same chlorination procedure has been applied in the preparation of 1-chloro-1-deoxy-2,3:4,6-di-*O*-isopropylidene-L-sorbose, 1-chloro-1-deoxy-2,3:4,5-di-*O*-isopropylidene-D-fructose, 3-chloro-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and -β-D-idofuranose, and 6-chloro-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose. Replacement of secondary hydroxy-groups by chlorine in this reaction resulted in inversion of configuration. It was shown by use of suitably deuteriated materials that lithium aluminium hydride reduction of the 3-chloro-3-deoxy-D-glucofuranose derivative proceeded with retention of configuration at C-3.[277] Other chloro-sugars are mentioned in Chapter 13. A number of primary hydroxy-groups in carbohydrate derivatives have been selectively brominated by treatment with *N*-bromosuccinimide and triphenylphosphine in anhydrous DMF. For example, methyl 6-bromo-6-deoxy-α-D-glucopyranoside (as its triacetate) was obtained in 66% yield from methyl α-D-glucopyranoside.[278]

5,6-Anhydro-2,4-*O*-benzylidene-1-bromo-1-deoxy-D-mannitol has been used as an intermediate for syntheses of 1-bromo-6-chloro-1,6-dideoxy-D-

[272] D. M. Marcus and J. H. Westwood, *Carbohydrate Res.*, 1971, **17**, 269.
[272a] P. W. Kent and R. C. Young, *Tetrahedron*, 1971, **27**, 4057.
[273] I. D. Jenkins, J. P. H. Verheyden, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1971, **93**, 4323.
[274] G. S. Ghangas and T. P. Fondy, *Biochemistry*, 1971, **10**, 3204.
[275] W. J. Lloyd and R. Harrison, *Carbohydrate Res.*, 1971, **20**, 133.
[276] K. Haga, M. Yoshikawa, and T. Kato, *Bull. Chem. Soc. Japan*, 1970, **43**, 3922.
[277] C. R. Haylock, L. D. Melton, K. N. Slessor, and A. S. Tracey, *Carbohydrate Res.*, 1971, **16**, 375.
[278] M. M. Ponpipom and S. Hanessian, *Carbohydrate Res.*, 1971, **18**, 342.

mannitol, 1-bromo-1,6-dideoxy-6-iodo-D-mannitol, and 1-bromo-1-deoxy-6-*O*-methylsulphonyl-D-mannitol.[279]

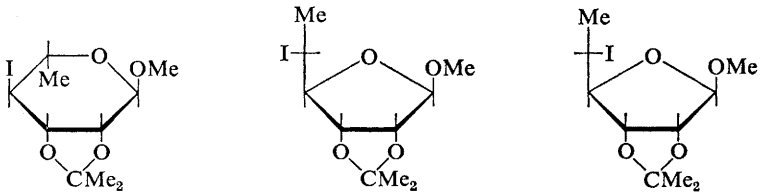
An ingenious method for preparing derivatives of 6-deoxy-6-iodo-D-glucose is illustrated in Scheme 38.[280] Further information on the reaction between triphenylphosphite methiodide and methyl 6-deoxy-2,3-*O*-



Reagents: i, $(PhO)_3P$–DMF; ii, MeI

Scheme 38

isopropylidene-α-L-mannopyranoside showed that compounds (117), (118), and (119) were produced in ratios of 13 : 14 : 1.[281] The products readily underwent replacement of the iodine atoms by thiobenzoyl groups with inversion of configuration.[282]



(117) (118) (119)

[279] J. Kuszmann and L. Vargha, *Carbohydrate Res.*, 1971, **17**, 309.
[280] S. Inokawa, K. Seo, H. Yoshida, and T. Ogata, *Bull. Chem. Soc. Japan*, 1971, **44**, 1931.
[281] N. K. Kochetkov, A. I. Usov, and K. S. Adamyants, *Tetrahedron*, 1971, **27**, 549.
[282] A. I. Usov, K. S. Adamyants, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 1740.

8
Amino-sugars*

Natural Products†

Full details of the isolation of 4-amino-4-deoxy-L-arabinose from *Salmonella* lipopolysaccharides have been given.[283] The polyene macrolide antibiotic amphotericin B has been shown to have a β-linked mycosaminopyranosyl unit.[284] Small amounts of 2-deoxy-3,6-di-*O*-methyl-2-methylamino-D-glucose were obtained after methylation and hydrolysis of baker's yeast mannan, and it was proposed that it arose from 4-substituted 2-acetamido-2-deoxy-D-glucose units linking the sugar moiety and peptide in a glycopeptide.[285]

Synthesis

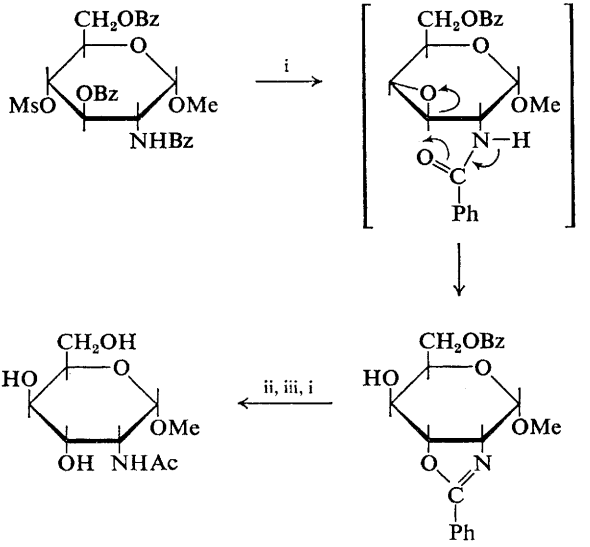
Synthetic and structural studies on amino-sugar glycosides have been reviewed (in Japanese).[286]

A route from 2-amino-2-deoxy-D-glucose to the D-*gulo*-analogue has been developed as shown in Scheme 39. N.m.r. studies showed that methyl 2-acylamido-2-deoxy-α-D-gulopyranoside assumes the C_1^4 conformation in solution.[287] Benzyl 2-acetamido-2-deoxy-α-D-gulopyranoside has been prepared by the route outlined in Scheme 40.[288]

3-Amino-3-deoxy-L-lyxose has been prepared by way of the key steps illustrated in Scheme 41.[289] 1-(4-Amino-4-deoxy-β-D-glucopyranosyl)-uracil has been synthesized; acetobromogalactose was condensed with 2,4-dimethylsiloxylpyridimidine to give a mixture of nucleosides yielding (120) after removal of the protecting groups, which was then used as shown in Scheme 42.[290] 2,5-Dideoxy-5-acetamido-D-*threo*-pentose has

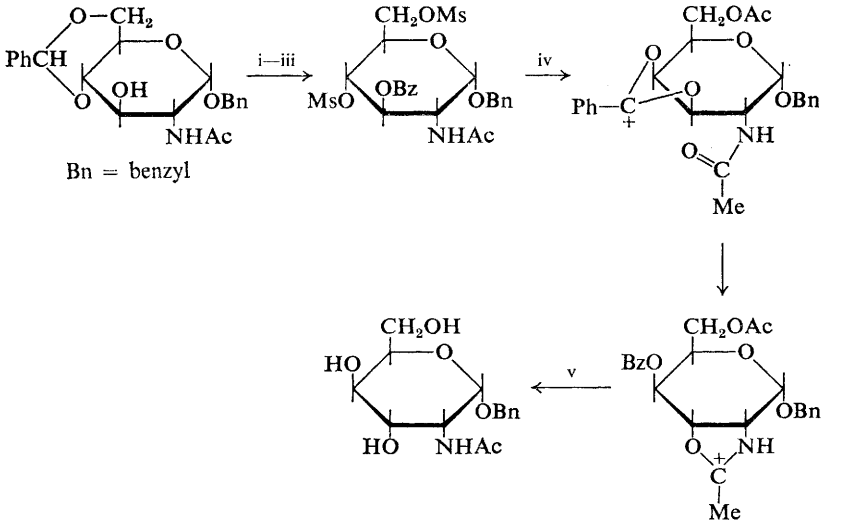
[283] W. A. Volk, C. Galanos, and O. Lüderitz, *European J. Biochem.*, 1970, **17**, 223.
[284] P. Ganis, G. Avitabile, W. Mechlinski, and C. P. Schaffner, *J. Amer. Chem. Soc.*, 1971, **93**, 4560.
[285] P. A. J. Gorin, *Canad. J. Chem.*, 1971, **49**, 527.
[286] S. Umezawa, *Yuki Gosei Kagaku Kyokai Shi*, 1970, **28**, 873 (*Chem. Abs.*, 1971, **74**, 23 070*q*).
[287] M. W. Horner, L. Hough, and A. C. Richardson, *J. Chem. Soc.* (*C*), 1971, 99.
[288] M. Parquet and P. Sinaÿ, *Carbohydrate Res.*, 1971, **18**, 195.
[289] J. S. Brimacombe, A. M. Mofti, and M. Stacey, *Carbohydrate Res.*, 1971, **16**, 303.
[290] T. Kondo and T. Goto, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 625.

* See also Chapter 12 of Part I.
† See also Chapter 20 of Part I.



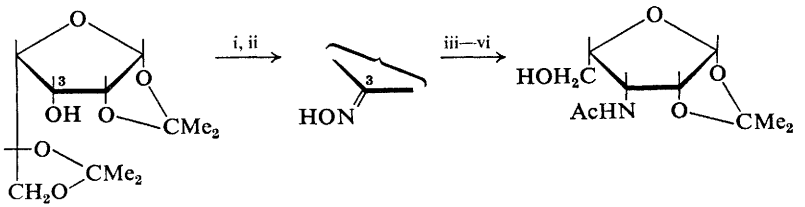
Reagents: i, MeONa–MeOH; ii, HCl–MeOH; iii, Ac_2O–MeOH

Scheme 39



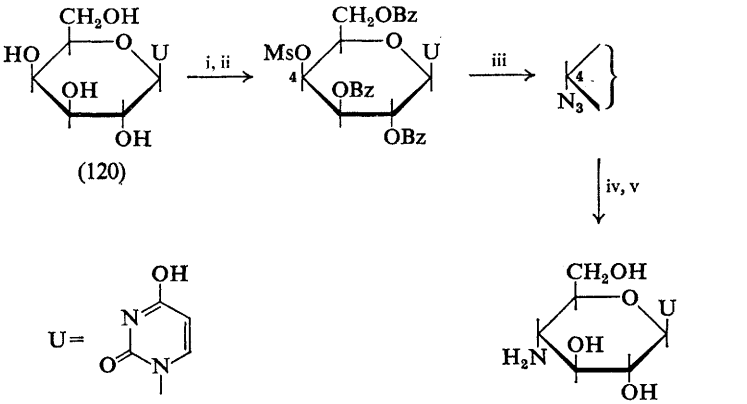
Reagents: i, BzCl–py; ii, aq. HCl; iii, MsCl–py; iv, KOAc–methylcellosolve; v, MeONa–MeOH

Scheme 40



Reagents: i, DMSO–Ac_2O; ii, NH_2OH,HCl; iii, $LiAlH_4$; iv, H_3O^+; v, IO_4^-; vi, $NaBH_4$

Scheme 41



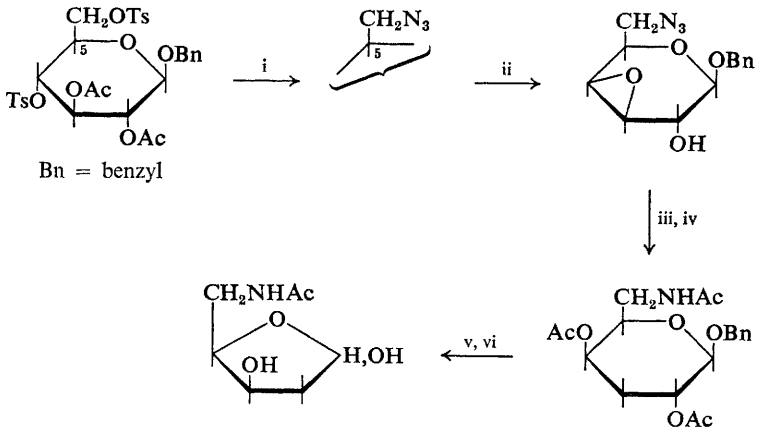
Reagents: i, 3BzCl–py (−12 °C); ii, MsCl–py; iii, NaN_3–HMPT; iv, MeONa–MeOH; v, H_2–Pt

Scheme 42

been synthesized from benzyl 2,3-di-*O*-acetyl-4,6-di-*O*-*p*-tolylsulphonyl-β-D-glucopyranoside by the route shown in Scheme 43.[291]

Various 5-*O*-methylsulphonyl-D-glucofuranosiduronic nitriles, used as possible precursors for 5,6-dideoxy-5,6-epimino-L-idose derivatives, unexpectedly yielded 6-amino-5,6-dideoxy products.[292] 5,6-Eneoses have been used in the synthesis of methyl 2-amino-2,6-dideoxy- and 3-amino-3,6-dideoxy-β-L-idopyranosides. All the enoses were prepared from D-glucose precursors and were converted into L-idose derivatives by catalytic hydrogenation.[293] Various αα-trehalose derivatives have been reported, including the 6,6′-diamino-3,3′:6,6′-tetradeoxy-[294] and the 6,6′-diamino-2,2′:6,6′-tetradeoxy-compounds.[295] Details of the synthesis of 6-[^{15}N]amino-6-deoxy-D-glucose derivatives have been given.[296]

[291] H. Weidmann and N. Wolf, *Monatsh.*, 1971, **102**, 747.
[292] H. Weidmann, E. Stieger, and H. Schwarz, *Monatsh.*, 1970, **101**, 871.
[293] D. Ikeda, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1971, **44**, 2529.
[294] L. Hough, A. C. Richardson, and E. Tarelli, *J. Chem. Soc.* (*C*), 1971, 2122.
[295] L. Hough, A. C. Richardson, and E. Tarelli, *J. Chem. Soc.* (*C*), 1971, 1732.
[296] B. Coxon, *Carbohydrate Res.*, 1971, **19**, 197.



Reagents: i, NaN_3–DMF; ii, MeONa–MeOH; iii, $LiAlH_4$; iv, Ac_2O–py; v, H_2, Pd–C; vi, IO_4^-

Scheme 43

Unsaturated amino-compounds are dealt with in Chapter 14 and amino-sugar nucleosides in Chapter 21.

Jeanloz's group have prepared several amino-sugar oligosaccharides, including 2-acetamido-2-deoxy-4-*O*-(6-deoxy-α-L-galactopyranosyl)-α-D-glucose,[297] 2-acetamido-2-deoxy-6-*O*-(α-D-mannopyranosyl)-D-glucose,[298] and *O*-α-D-mannopyranosyl-(1 → 6)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose.[299]

α- and β-Anomers of methyl 2-acetamido-2-deoxy-D-mannopyranoside have been described and their o.r.d. and c.d. spectra studied (see Chapter 25). Methyl ethers of 2-acetamido-2-deoxy-D-glucose and -galactose have been examined by g.l.c. (see Chapter 26).

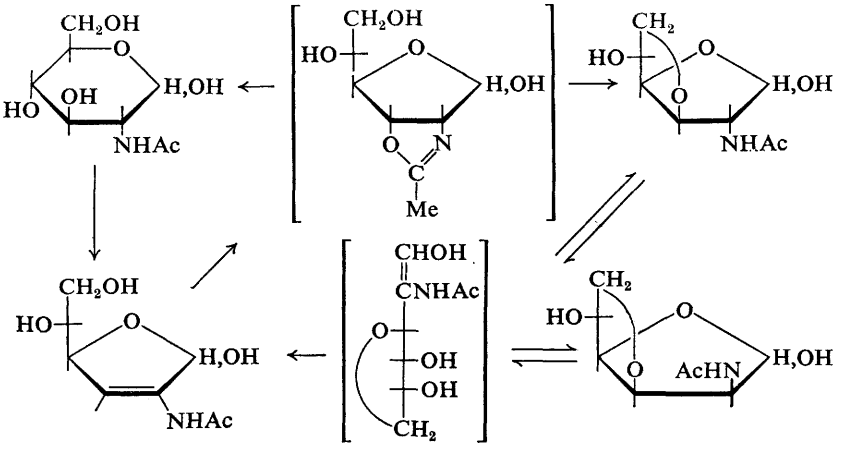
Reactions

The α : β ratio between the anomers of protonated 2-amino-2-deoxy-D-glucose and -galactose (equatorial amino-groups) was considerably higher than in the parent hexoses or the unprotonated forms. The increase in the anomeric effect in the protonated sugars was interpreted in terms of the electrostatic interaction with the C-1 dipoles. In protonated 2-amino-2-deoxy-α-D-mannose (axial amino-group), the glycosidic oxygen atom, which carries a partial negative charge, is 0.8 Å further away from the positively charged nitrogen atom than in the β-form, thus favouring the β-anomer. The α : β ratio was therefore lower in the charged species than in the parent sugar or the unprotonated species. The second ionization

[297] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1971, **20**, 399.
[298] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1971, **17**, 411.
[299] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1971, **19**, 311.

constant of both anomers of 2-amino-2-deoxy-D-glucose has also been measured. The anomeric effect on the glycosylate ions was not important.[300]

The sequence shown in Scheme 44 has been proposed to account for the formation of 2-acetamido-3,6-anhydro-2-deoxy-D-glucose and -mannose on



Scheme 44

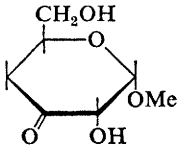
treatment of 2-acetamido-2-deoxy-D-glucose with alkali. It was suggested that these reactions have analytical value for the establishment of carbohydrate-chain structures, particularly since alkaline degradation of many glycoproteins is known to proceed with destruction of 2-acetamido-2-deoxyhexose components.[301]

Full details of the method for blocking vicinal hydroxyamino-groups have been given [301a] (see Vol. 4, pp. 64, 129), as have those for the deamination of methyl 4-amino-4-deoxy-α-D-glucoside with nitrous acid [302] (see Vol. 3, p. 72). Deamination of methyl 4-amino-4-deoxy-α-D-galactopyranoside gave principally (121) together with methyl α-D-glucopyranoside and -galactopyranoside and three unidentified products. Deamination of methyl 3-amino-3-deoxy-β-D-allopyranoside gave seven products. One of the two major products was methyl β-D-glucopyranoside and the other was (122), whose isolation was complicated by its epimerization to (123). A ring-contracted product (124) was also isolated.[303]

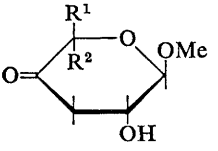
N-Nitrosoamide derivatives, prepared from acetamido-compounds and nitrosyl chloride, decomposed in aqueous acetone. Thus, methyl

[300] A. Neuberger and A. P. Fletcher, *Carbohydrate Res.*, 1971, **17**, 79.
[301] V. A. Dervitskaya, L. M. Likhosherstov, V. A. Schennikov, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **20**, 285.
[301a] S. Umezawa, Y. Takagi, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1971, **44**, 1411.
[302] N. M. K. Ng Ying Kin, J. M. Williams, and A. Horsington, *J. Chem. Soc.* (*C*), 1971, 1578.
[303] N. M. K. Ng Ying Kin and J. M. Williams, *Chem. Comm.*, 1971, 1123.

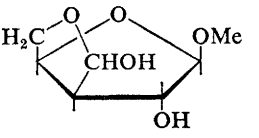
2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside gave 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose. The disaccharide (125) also gave the anhydromannose derivative and 1,2:3,4-di-*O*-isopropylidene-D-galacto-pyranose.[304]



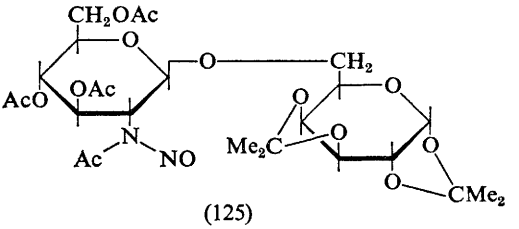
(121)



(122) $R^1 = CH_2OH$, $R^2 = H$
(123) $R^1 = H$, $R^2 = CH_2OH$



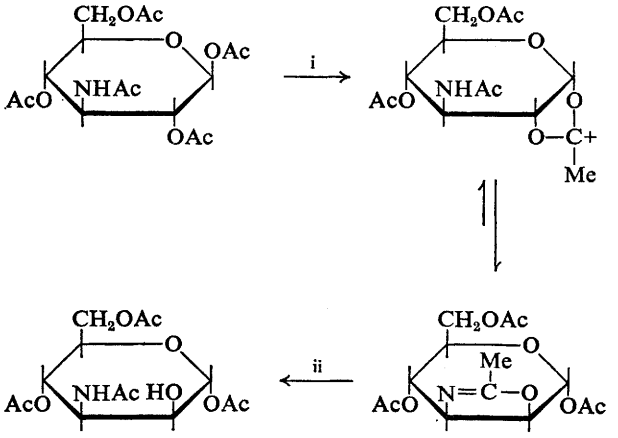
(124)



(125)

Acetoxonium-ion rearrangements have been reported for amino-sugar esters. The nitrogen-containing ions involved are much more stable than analogous acetoxonium ions, so that the equilibria are shifted to give the former, as in Scheme 45.[305]

The phthaloyl derivative (126) of 2-amino-2-deoxy-D-glucose, formed from the amino-sugar and phthalic anhydride in methanol, was converted



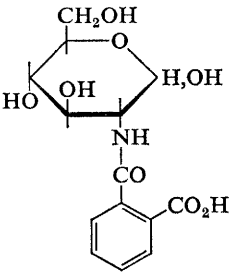
Reagents: i, $SbCl_5$; ii, HCl–THF

Scheme 45

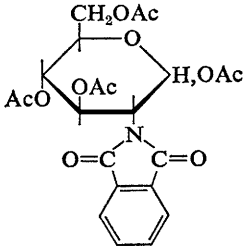
[304] J. W. Llewellyn and J. M. Williams, *Chem. Comm.*, 1971, 1386.
[305] H. Paulsen and C.-P. Herold, *Chem. Ber.*, 1971, **104**, 1311.

into the phthalimido-derivative (127) by reaction with acetic anhydride in pyridine.[306] The six possible mixed acetylated and benzoylated benzyl *N*-benzamido-β-D-glucopyranosides have been synthesized.[307]

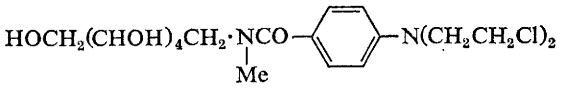
1-Amino-1-deoxy-D-erythritol has been converted into 2,3,4-tri-*O*-acetyl-1-amino-1-deoxy-D-erythritol *p*-tolylsulphonic acid by dissolving the salt in hot acetic anhydride; by contrast, benzoylation gave *O*- and *N*-substitution.[308] 1-Methylamino-1-deoxyhexitols (*gluco*, *galacto*, and *manno*) have been converted into compounds of type (128) of potential cytostatic activity.[309]



(126)



(127)



(128)

6-Deoxy-6-isocyanato-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose gave the expected carbamate when treated with ethanol. Reaction with 1,1-diphenyl-2-propyn-1-ol, however, gave a urea derivative.[309a]

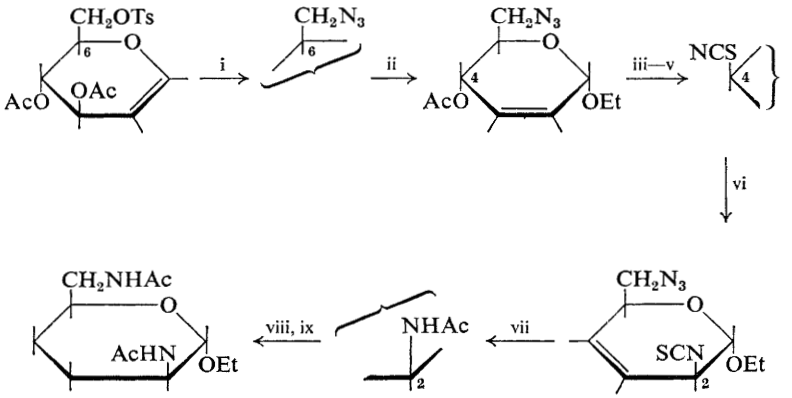
The synthesis of muramic acid 6-phosphate has been reported.[310] 7,9-*O*-Benzylidene and -ethylidene derivatives of *N*-acetylneuraminic acid have been described,[311] but attempts to prepare the 8,9-*O*-ethylidene compound were unsuccessful.[312] The eight-carbon analogue of *N*-acetylneuraminic acid, 5-acetamido-3,5-dideoxy-D-*galacto*-octulosonic acid, has been synthesized and the seven-carbon analogue, 5-acetamido-3,5-dideoxy-L-*arabino*-heptulosonic acid, was also prepared.[313]

The 3-amino-3-deoxy-β-D-glucoside of strophanthidin has been synthesized.[97]

[306] S. Hirano, *Carbohydrate Res.*, 1971, **16**, 229.
[307] H. Weidmann, H. Hönig, P. Stöckl, and D. Tartler, *Monatsh.*, 1971, **102**, 1028.
[308] I. Ziderman, *Carbohydrate Res.*, 1971, **18**, 323.
[309] H. Dorn and D. Arndt, *Z. Chem.*, 1971, **11**, 254.
[309a] E. M. Bessell and J. H. Westwood, *Carbohydrate Res.*, 1971, **19**, 389.
[310] Y. Konami, T. Osawa, and R. W. Jeanloz, *Biochemistry*, 1971, **10**, 193.
[311] L. Holmquist, *Acta Chem. Scand.*, 1971, **25**, 712.
[312] G. Ekborg and L. Holmquist, *Acta Chem. Scand.*, 1971, **25**, 1479.
[313] R. L. McLean, M. Suttajit, J. Beidler, and R. J. Winzler, *J. Biol. Chem.*, 1971, **246**, 803.

Di- and Poly-amino-sugars

Methyl 2,4-dibenzamido-2,4-dideoxy-α-D-glucopyranoside and -galactopyranoside have been prepared by routes involving azide displacements on appropriately substituted 4-sulphonates of 2-amino-2-deoxy-D-galactose and -glucose derivatives.[314] Ethyl 2,6-diacetamido-2,3,4,6-tetradeoxy-D-*threo*-hexoside has been synthesized as shown in Scheme 46.[315]



Reagents: i, NaN_3–DMF; ii, EtOH–BF_3; iii, MeONa–MeOH; iv, MsCl–py; v, KSCN–DMF; vi, heat–PhMe; vii, NaOAc–Ac_2O–AcOH; viii, H_2–Pt; ix, Ac_2O–MeOH

Scheme 46

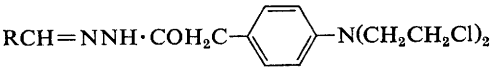
3,5-Diacetamido-3,5-dideoxypentoses with the D-*ribo*, D-*xylo*,[316] L-*arabino*, and L-*lyxo*[317] configurations have been described (see also Chapter 12). 4,4′:6,6′-Tetra-acetamido-3,3′:4,4′:6,6′-hexadeoxy-αα-trehalose has been prepared.[294]

Full details (*cf.* Vol. 3, p. 78) of the synthesis of 2,3,4-triamino-2,3,4-trideoxy-D-glucose derivatives have been recorded,[318] as well as those for the synthesis of 2,3,4,6-tetra-amino-2,3,4,6-tetradeoxy-D-galactose[319] (*cf.* Vol. 4, p. 66).

[314] M. W. Horner, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1971, **17**, 209.
[315] R. D. Guthrie and G. J. Williams, *Chem. Comm.*, 1971, 923.
[316] J. S. Brimacombe and A. M. Mofti, *Carbohydrate Res.*, 1971, **16**, 167.
[317] J. S. Brimacombe and A. M. Mofti, *J. Chem. Soc.* (*C*), 1971, 1634.
[318] T. Nakagawa, Y. Sato, T. Takamoto, F. W. Lichtenthaler, and N. Majer, *Bull. Chem. Soc. Japan*, 1970, **43**, 3866.
[319] W. Meyer zu Reckendorf, *Chem. Ber.*, 1971, **104**, 1976.

9
Hydrazones, Osazones, and Related Compounds

Several carbohydrate *p*-[di-(2-chloroethyl)amino]phenylacetyl hydrazones of general formula (129) have been prepared as potential antitumour agents from a number of peracetylated *aldehydo*-aldoses.[320]

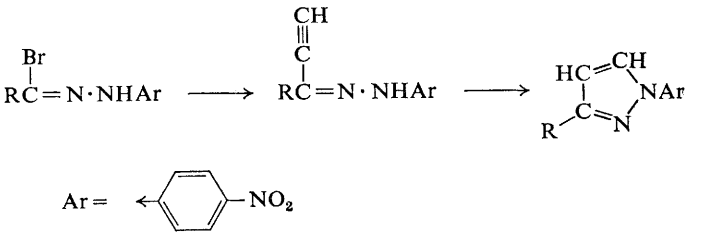


(129)

A new method for the synthesis of the 'β-phenylhydrazone' of D-glucose has been described in which, with pyridine as solvent, a hydrazone–pyridine complex is formed that is then decomposed to the hydrazone with water.[321] I.r spectroscopy suggested that this compound has a cyclic structure with the α-configuration. The use of benzoylhydrazone intermediates in the synthesis of 3-deoxyaldos-2-uloses has been extended (see page 111; also Vol. 4, p. 110).

N.m.r. spectroscopy has been used to study preferred rotamer states of various *aldehydo*-sugar hydrazones (see Chapter 23).

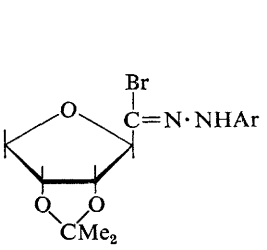
Aldehydo-sugar hydrazonyl bromides reacted with ethynylmagnesium bromide to give mainly α-ethynylhydrazones (Scheme 47), which underwent base-catalysed cyclization to pyrazoles.[322] Pyrazoles were also formed by direct addition of acetylenes to hydrazonyl bromides;[323] for example,

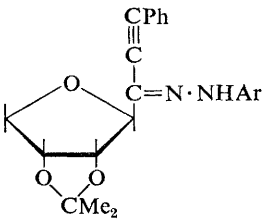


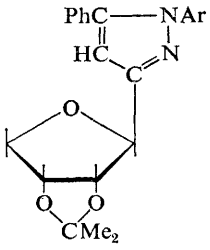
Scheme 47

[320] D. Dzhyuvene, Yu. Degutis, and B. Damaratskis, *J. Gen. Chem.* (*U.S.S.R.*), 1970, **40**, 1632 (*Zhur. obshchei Khim.*, 1970, **40**, 1645).
[321] A. Uliasz and J. Swiderski, *Roczniki Chem.*, 1971, **45**, 1463.
[322] J. M. J. Tronchet and A. Jotterand, *Helv. Chim. Acta*, 1971, **54**, 1131.
[323] J. M. J. Tronchet and F. Perret, *Helv. Chim. Acta*, 1971, **54**, 683.

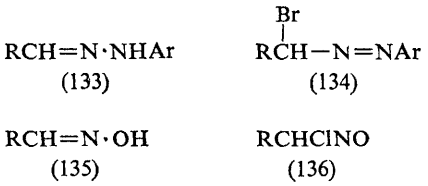
treatment of (130) with phenylacetylene afforded (131) and (132); isotope studies suggested that they were formed from (130) by independent routes.


(130)

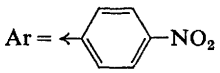

(131)

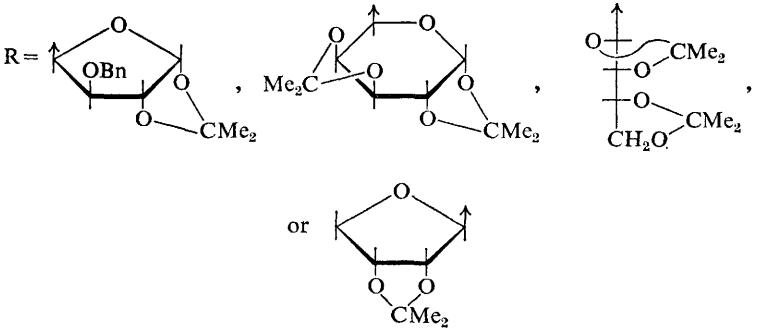

(132)

Hydrazonyl bromide derivatives were prepared[324] by bromination of *aldehydo-p*-nitrophenylhydrazones [*e.g.* (133) → (134)]. Similarly, carbohydrate aldoximes were chlorinated to give chloronitroso-derivatives [*e.g.* (135) → (136)]. Treatment of these products with phenylacetylene gave pyrazoles and isoxazoles, respectively.


(133) (134)

(135) (136)



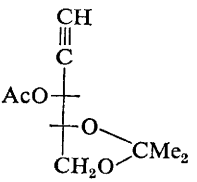


A number of reports on the formation of azole derivatives have been published. 1,3-Dipolar cycloadditions with acetylenic and azido precursors have been used in the synthesis of some triazole derivatives.[325] The

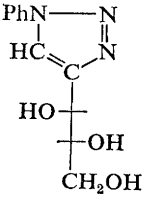
[324] J. M. J. Tronchet, B. Baehler, N. Le-Hong, and P. F. Livio, *Helv. Chim. Acta*, 1971, **54**, 921.

[325] H. El Khadem, D. Horton, and M. H. Meshreki, *Carbohydrate Res.*, 1971, **16**, 409.

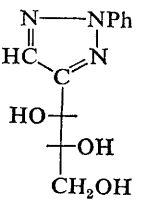
acetylenic derivative (137), for example, was converted into (138) by reaction with phenylazide and removal of the protecting groups; compound (138) is a positional isomer of D-*threo*-pentulose phenylosotriazole (139).



(137)

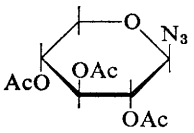


(138)

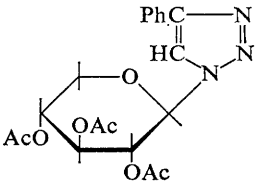


(139)

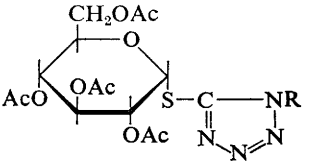
Phenylacetylene was also condensed with sugar azides [*e.g.* (140)] to give 1-substituted 4-phenyl-1,2,3-triazoles [*e.g.* (141)]. The o.r.d properties of the triazoles were described. Penta-*O*-acyl-D-galactononitriles have been converted into tetrazoles, in high yield, by treatment with ammonium azide in DMF at 25—35 °C,[326] and some 5-mercaptotetrazole tetra-*O*-acetyl-D-glucosides [*e.g.* (142)] and -galactosides have been prepared.[327]



(140)

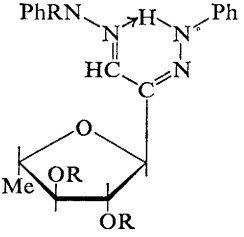


(141)

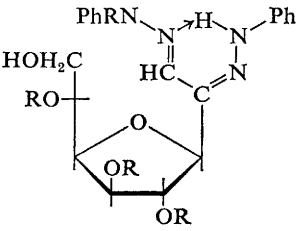


(142)

The 3,6-anhydro-osazones (143) and (144) were formed from 7-deoxy-L-*manno*-heptulose phenylosazone and D-*glycero*-D-*gluco*-octulose phenylosazone, respectively, on dehydration with methanolic sulphuric acid.[328]



(143) R = H
(145) R = Ac

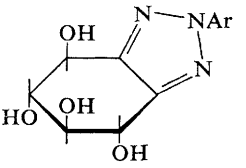


(144) R = H
(146) R = Ac

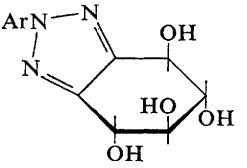
[326] J. O. Deferrari, A. M. Seldes, O. G. Marzoa, and I. M. E. Thiel, *Carbohydrate Res.* 1971, **17**, 237.
[327] U. Askani and R. Neidlein, *Deut. Apoth.-ztg.*, 1970, **110**, 1502.
[328] H. El Khadem, Z. M. El-Shafei, and M. A. E. Sallam, *Carbohydrate Res.*, 1971, **18**, 147.

The acetates (145) and (146) were prepared either by treating (143) and (144), respectively, with acetic anhydride or by acetylation of the parent phenylosazones.

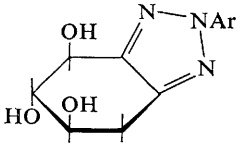
The (*p*-bromophenyl)osotriazoles (147), (148), and (149) have been prepared by treatment of the appropriate inositol phenylosazones with bromine.[329]



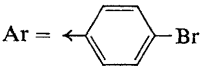
(147)



(148)



(149)



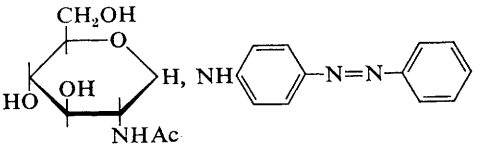
[329] A. J. Fatiadi, *Carbohydrate Res.*, 1971, **20**, 179.

10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines and Related Compounds

Further papers [330, 331] and a brief review (in Polish) [332] have appeared on the mutarotation of *N*-arylglycosylamines. The anomeric configurations of *N*-acetyl derivatives of 2,3,4-tri-*O*-acetyl-*N*-aryl-L-arabinosylamines [333] and of *N*-aryl-*N*-methyl-D-galactopyranosylamines [334] have been investigated.

The simple synthesis developed for 2,3-*O*-isopropylidene-β-D-ribofuranosylamine (*cf.* Vol. 4, p. 70) has been extended to D-xylose, D-mannose, and L-rhamnose analogues.[335] The reaction of 2-acetamido-2-deoxy-D-glucose with 4-aminoazobenzene gave (150), thought to have the β-pyranose configuration.[336]



(150)

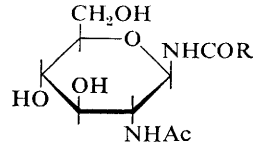
Ammonolysis of 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosylamine gave a mixture of D-glucose and D-glucosylamine, in high yield, as well as a small amount of 1,1-bis(benzamido)-1-deoxy-D-glucitol and traces of *N*-benzoyl-D-glucopyranosylamine. This result has a bearing on the mechanism of ammonolysis of acylated aldoses to give 1,1-bis(acylamido)-1-deoxyalditols in that it suggests that *O* → *N*-acyl migrations are not favoured.[337]

Changes in the o.r.d. spectra during mutarotation of *N*-aryl-β-D-galactopyranosylamines have been studied [338] and it has been shown that

[330] K. Smiataczowa, T. Jasinski, and J. Sokolowski, *Roczniki Chem.*, 1970, **44**, 2405.
[331] K. Smiataczowa, T. Jasinski, and J. Sokolowski, *Roczniki Chem.*, 1971, **45**, 329.
[332] K. Smiataczowa, *Wiad. Chem.*, 1971, **25**, 343 (*Chem. Abs.*, 1971, **75**, 88 848q).
[333] Z. Smiatacz and J. Sokolowski, *Roczniki Chem.*, 1971, **45**, 1431.
[334] S. Kolka and J. Sokolowski, *Roczniki Chem.*, 1970, **44**, 2311.
[335] N. J. Cusack, P. W. Rugg, and G. Shaw, *Chem. Comm.*, 1971, 190.
[336] M. Kalmanowa and J. Sokolowski, *Roczniki Chem.*, 1970, **44**, 2347.
[337] J. F. Sproviero, A. Salinas, and E. S. Bertiche, *Carbohydrate Res.*, 1971, **19**, 81.
[338] J. Szafranek and J. Sokolowski, *Roczniki Chem.*, 1971, **45**, 1213.

the sequences of oligosaccharides can be determined from the mass spectra of their *N*-arylglycosylamines.[339]

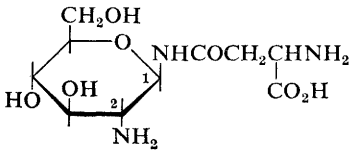
Reductive alkaline cleavage of glycoproteins containing 4-*N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine as the carbohydrate–peptide linkage has been shown to be a viable procedure for cleavage of this linkage. Model experiments on the latter compound and on 4-*N*-(β-D-glucopyranosyl)-L-asparagine were described.[340]



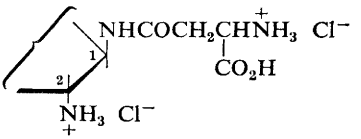
R = CH_2NH_2, $CH(Me)NH_2$, $CHPr^iNH_2$, $(CH_2)_2CH(NH_2)CO_2H$, or $CH_2CH(NH_2)CO_2H$

(151)

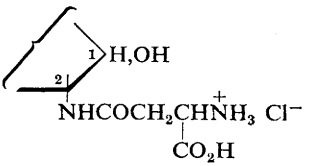
Derivatives [shown in (151)] of 2-acetamido-2-deoxy-β-D-glucopyranosylamine have been prepared. Utilization of the *N*-trifluoroacetyl blocking group has also enabled 2-amino-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (152) to be prepared.[341] Acid hydrolysis of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine to give 2-amino-2-deoxy-D-glucose hydrochloride has been shown to proceed mainly by way of 2-acetamido-2-deoxy-D-glucose and (153). However, the possibility that a small proportion of the hydrolysis proceeded *via* (154) could not be discounted.[342]



(152)



(153)



(154)

[339] N. K. Kochetkov, O. S. Chizov, N. N. Malysheva, and A. I. Shiyonok, *Org. Mass Spectrometry*, 1971, **5**, 481.
[340] B. M. Austin and R. D. Marshall, *Biochem. J.*, 1971, **124**, 14P.
[341] D. E. Cowley, L. Hough, and C. M. Peach, *Carbohydrate Res.*, 1971, **19**, 231.
[342] D. E. Cowley, L. Hough, and M. Y. Khan, *Carbohydrate Res.*, 1971, **19**, 242.

Azides

The role of azido-sugars in synthesis has been reviewed (in Japanese).[343] Routes for the synthesis of azides by additions to olefins have been surveyed, although no examples from carbohydrate chemistry were included.[344]

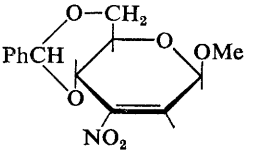
Glycosyl azides have been used as intermediates in the synthesis of heterocyclic derivatives (see p. 77).

The mass spectra of methyl 4-azido-4-deoxypentosides have been studied.[345] The 3,4,6,3′,4′,6′-hexa-azido-hexadeoxy-derivative of αα-trehalose has been described.[295] Rearrangements of allylic azides are referred to in Chapter 14.

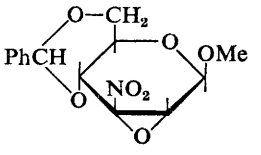
Nitro-compounds

Baer and his group remain very active in this area. The conformations of four methyl 3-deoxy-3-nitro-pentopyranosides and their sodium nitronates have been examined in aqueous solution, and the results have been interpreted in terms of the free energies of non-bonded interactions. In the case of the nitronates, the interaction between the nitrogen function and the adjacent groups ($A^{1,3}$ effect) was greater than 2 kcal mol^{-1}. Mechanisms of epimerization at the β-position to the nitro-group were considered in detail.[346]

Epoxidation of nitro-olefins with hydrogen peroxide in weakly alkaline media gave α-nitro-oxirans, in high yield, with the products having the oxiran ring *trans* to the aglycone predominating; for example, (155) gave (156). This type of product was also obtained on treatment of the 3-bromo-3-deoxy-3-nitro-analogues with base.[347]



(155)



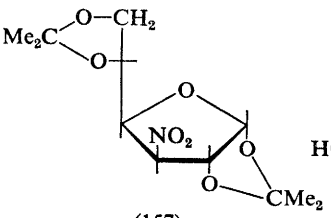
(156)

The acidities of various methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-hexopyranosides have been measured by spectroscopic methods and the results correlated with stereochemistry.[348]

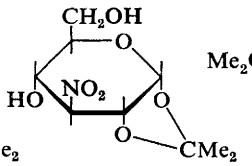
Treatment of 3-deoxy-3-nitro-D-glucose with acetone, triethyl orthoformate, and an acid catalyst gave (157), as the thermodynamically favoured product, as well as (158) and (159). Partial acid hydrolysis of (157) gave (160), whereas treatment with base caused epimerization at

[343] H. Kuzuhara, *Kagaku No Ryoiki*, 1971, **25**, 117 (*Chem. Abs.*, 1971, **74**, 125 934s).
[344] A. Hassner, *Accounts Chem. Res.*, 1971, **4**, 9.
[345] V. Kovácík, C. Peciar, Š. Bauer, and H. F. Grützmacher, *Carbohydrate Res.*, 1971, **19**, 169.
[346] H. H. Baer and J. Kovář, *Canad. J. Chem.*, 1971, **49**, 1940.
[347] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1971, **49**, 3192.
[348] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1971, **49**, 3197.

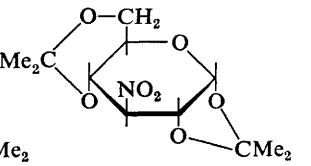
C-3 to yield the D-*allo*-compound (161).[349] Periodate oxidation, borohydride reduction, and acid hydrolysis of (160) afforded 3-deoxy-3-nitro-D-xylose.[350] Several other derivatives of 3-deoxy-3-nitro-D-glucose, namely (162), (163), and (164), have been described.[351]



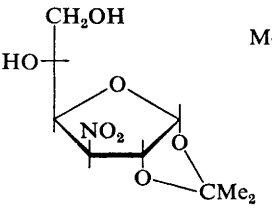
(157)



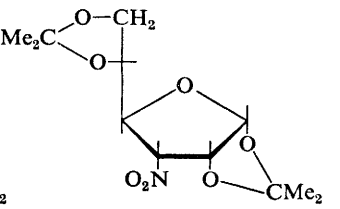
(158)



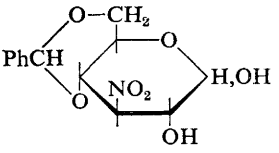
(159)



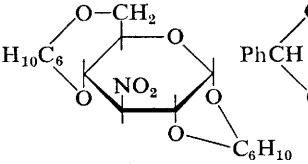
(160)



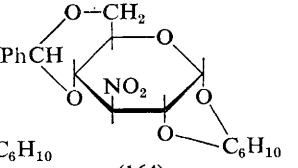
(161)



(162)



(163)

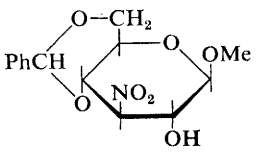


(164)

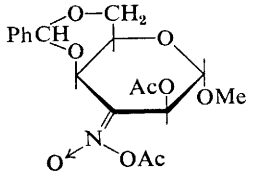
Acetylation of (165) or the *β-galacto*-isomer with acetyl chloride and triethylamine led to *O*-acetylation, as expected. With the *α-talo*-isomer, however, the product (166) was the 2-acetate of the mixed anhydride of the nitronic acid and acetic acid.[352]

A new route to nitro-sugars by oxidation of oximes with trifluoroperacetic acid has been described; for example, (167) gave (168) and (169).[353] 2,3:4,5-Di-*O*-benzylidene-L-*manno*-hexodialdose gave a mixture of diastereoisomers (170) with nitromethane in the presence of sodium methoxide.[354] Base-catalysed reaction of the unsaturated nitro-sugar (171) with sulphur nucleophiles yielded the thio-sugars (172).[355]

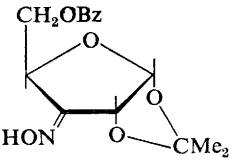
[349] J. Kovář and H. H. Baer, *Canad. J. Chem.*, 1971, **49**, 3203.
[350] J. Kovář and H. H. Baer, *Canad. J. Chem.*, 1971, **49**, 3238.
[351] T. Sakakibara, T. Takamoto, and T. Nakagawa, *Bull. Chem. Soc. Japan*, 1971, **44**, 865.
[352] W. Rank and H. H. Baer, *Canad. J. Chem.*, 1971, **49**, 3236.
[353] T. Takamoto, R. Sudoh, and T. Nakagawa, *Tetrahedron Letters*, 1971, 2053.
[354] I. Dijong and R. Bonn, *Tetrahedron Letters*, 1971, 1485.
[355] P. Wirz and E. Hardegger, *Helv. Chim. Acta*, 1971, **54**, 2017.



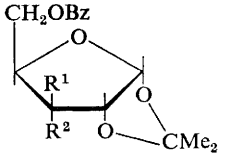
(165)



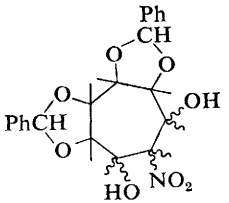
(166)



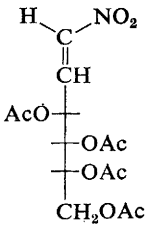
(167)

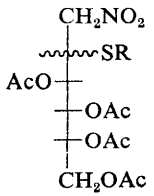


(168) R^1 = H, R^2 = NO_2
(169) R^1 = NO_2, R^2 = H



(170)





(171)

(172) R = Ac, Bn, or Bz

Heterocyclic Derivatives

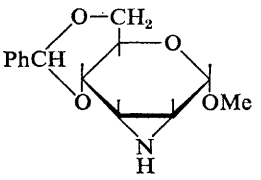
The synthesis of nitrogen heterocycles from sugar derivatives has been reviewed.[356]

N-Formylation and *N*-alkylation reactions of an epimino-sugar have been described. With chloral, (173) was converted into (174), which did not undergo elimination with sodium methoxide to give an *N*-formyl derivative, but gave instead the free epimine (173). Treatment of the epimine with methyl iodide and sodium carbonate afforded the *N*-methylepimine, which on further treatment with methyl iodide and silver picrylsulphonate gave (175). This compound was very reactive towards nucleophilic attack and gave (176) by conventional reactions.[357]

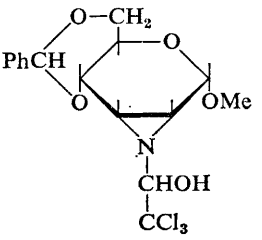
Glycosyl azides have been used as precursors of heterocyclic compounds. Reaction with methyl propiolate or propiolic acid gave *N*-glycosyl-*vic*-triazoles; both isomers were formed.[358] Reactions with

[356] H. El Khadem, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 351.
[357] C. F. Gibbs and L. Hough, *Carbohydrate Res.*, 1971, **18**, 363.
[358] G. Alonso, M. T. García-López, G. García-Muñoz, R. Madroñero, and M. Rico, *J. Heterocyclic Chem.*, 1970, **7**, 1269.

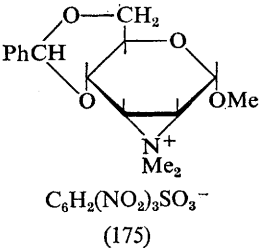
ynamines, ethoxyacetylene, and 1-ethylthio-2-phenylacetylene gave the appropriate *N*-glycosyltriazole. Compounds with the D-*gluco*, D-*galacto*, *malto*, and *cellobio* configurations were used; for example, (177) gave (178) (see also page 71).[359, 360]



(173)

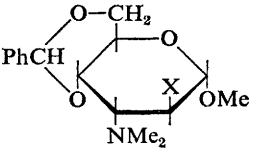


(174)



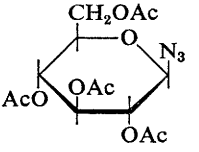
$C_6H_2(NO_2)_3SO_3^-$

(175)



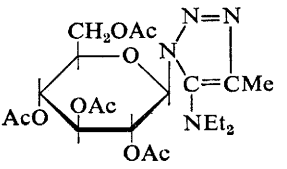
X = N_3, SCN, or SH

(176)



(177)

$Et_2NC{\equiv}CMe$



(178)

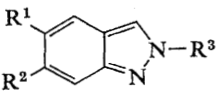
2-Glycosyl-imidazoles of the type (179) have been described.[361] N.m.r. and i.r. spectroscopic studies have been made on several aldosyl-pyrazoles and -imidazoles with a view to determining the anomeric configuration.[362] Oxazolidine derivatives, such as (180), have been prepared from benzaldehyde and 1-deoxy-1-methylaminoalditols.[363]

Acetylenic sugars have been used in the synthesis of triazoles. Thus, (137) yielded (138) on reaction with phenyl azide and subsequent removal of blocking groups. Phenylacetylene was also condensed with a wide range of sugar azides (primary, secondary, and glycosyl).[325]

[359] R. E. Harmon, R. A. Earl, and S. K. Gupta, *J. Org. Chem.*, 1971, **36**, 2553.
[360] R. E. Harmon, R. A. Earl, and S. K. Gupta, *Chem. Comm.*, 1971, 296.
[361] G. Alonso, G. García-Muñoz, and R. Madroñero, *J. Heterocyclic Chem.*, 1970, **7**, 1435.
[362] J. Jasinska, *Roczniki Chem.*, 1971, **45**, 1641.
[363] H. Dorn and D. Arndt, *Z. Chem.*, 1971, **11**, 306.

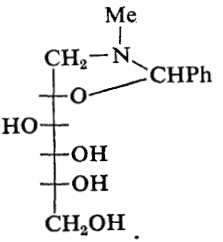
Compound (181) resulted from the reaction of 2-amino-2-deoxy-D-glucose with ethyl 4,4-diethoxy-3-oxobutanoate.[364] The same sugar with ethyl ethoxymethylenecyanoacetate gave (182) after acetylation.[365] The acetylated enamines were readily converted into pyrrole derivatives.[366]

The reactions of 1-amino-1-deoxy-D-fructose and its *N*-(n-butyl) and *N*-benzyl derivatives with a variety of β-dicarbonyl compounds have been shown to give polyhydroxyalkylpyrrole derivatives.[367]

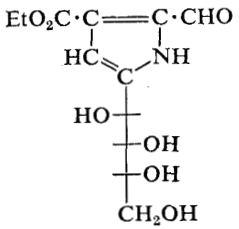


(179)

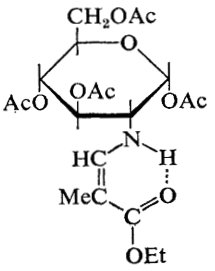
R^1 = H, R^2 = NO_2;
R^1 = NO_2, R^2 = H;
R^1 = R^2 = H;
R^3 = D-gluco- or galacto-pyranosyl



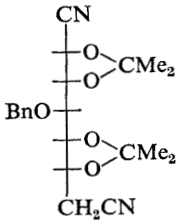
(180)



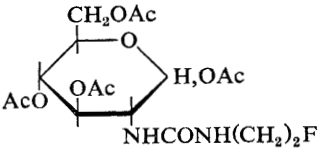
(181)



(182)



(183)



(184)

[364] F. García-González, J. Fernández-Bolaños, and F. Alcudia, *Anales de Quím.*, 1971, **67**, 383.
[365] A. Gómez-Sánchez, A. Cert Ventulá, M. Gómez-Guillén, and U. Scheidegger, *Anales de Quím.*, 1971, **67**, 545.
[366] A. Gómez-Sánchez, A. Cert Ventulá, and U. Scheidegger, *Carbohydrate Res.*, 1971, **17**, 275.
[367] F. García-González, A. Gómez-Sánchez, M. Gómez-Guillén, and M. Tena-Aldave, *Anales de Quím.*, 1971, **67**, 389.

When the α- or β-forms of *O*-acetylated enamines derived from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose and either 2,4-pentanedione or 1-phenyl-1,3-butanedione were treated with catalytic amounts of methanolic barium methoxide, de-*O*-acetylation occurred at C-1, accompanied by inversion at this centre with the β-anomer. Hydrolysis of the products with hydrochloric acid gave 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-D-glucopyranose hydrochloride.[368]

The sequence of monosaccharide units in oligosaccharides may be determined from the mass spectra of the phenylosotriazole derivatives.[339]

Oximes

Several aldulose oximes were shown to have negligible absorption due to C=N in their i.r. spectra, but this group gives rise to moderate to strong bands in the Raman spectra.[369] The *syn*- and *anti*-isomers of 3-*O*-benzyl-2,4-*O*-ethylidene-D-erythrose oxime have been separated and identified,[370] and the oxime derived from 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose was shown to be a mixture of *syn*- and *anti*-forms.[371] Oximes have been converted into nitro-sugars in good yield.[353]

Other Nitrogen-containing Compounds

Octa-*O*-acetylmaltobiononitrile has been obtained by treating maltose with hydroxylamine and subsequently acetylating the product.[372] Syntheses of (183)[373] and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[3-(2-fluoroethyl)ureido]-D-glucopyranose (184)[374] have been reported.

The effects of various acids and of reaction conditions upon 'fructosyl glycine' have been reported; whereas the proportions of *N*-(2-furacyl)-glycine to glycine formed were markedly different, no correlation was apparent between acid strength and product ratio.[375]

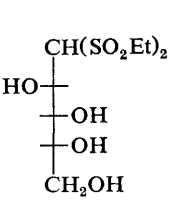
Allylic isothiocyanates are described in Chapter 14.

[368] A. Gómez-Sánchez, A. Cert Ventulá, and U. Scheidegger, *Carbohydrate Res.*, 1971, **18**, 173.
[369] D. Horton, E. K. Just, and B. Gross, *Carbohydrate Res.*, 1971, **16**, 239.
[370] A. Kampf and E. Dimant, *Carbohydrate Res.*, 1971, **16**, 212.
[371] M. Lamchen and R. L. Whistler, *Carbohydrate Res.*, 1971, **16**, 309.
[372] M. E. Gelpi, J. O. Deferrari, and R. A. Cadenas, *Carbohydrate Res.*, 1971, **17**, 478.
[373] I. Dijong and U. Wittkötter, *Chem. Ber.*, 1971, **104**, 2090.
[374] T. P. Johnston, G. S. McCaleb, P. S. Opliger, W. R. Laster, and J. A. Montgomery, *J. Medicin. Chem.*, 1971, **14**, 600.
[375] S. H. Lipton and R. C. Dutky, *Chem. and Ind.*, 1971, 1099.

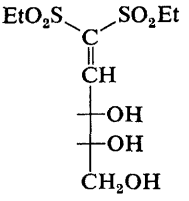
11
Thio-sugars

A crystallographic study[376] of ethyl 2-*S*-ethyl-1,2-dithio-α-D-manno-furanoside, obtained by nitrous acid deamination of 2-amino-2-deoxy-D-glucose diethyl dithioacetal, confirmed the structure previously deduced from chemical evidence.[377]

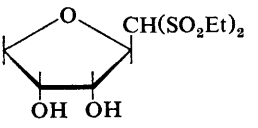
Oxidation of D-arabinose diethyl dithioacetal with perpropionic acid gave the acyclic bis-sulphone (185) which, with dilute acetic acid, was converted *via* (186) into (187); in solution, (187) is in equilibrium with a small proportion of (186).[378]



(185)



(186)



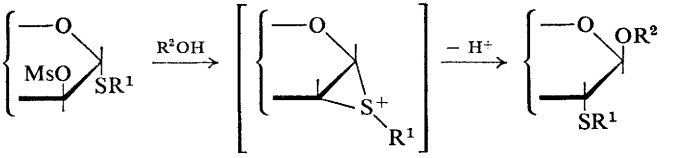
(187)

Conformational equilibria of 1-thio-D-aldopentopyranose peracetates are discussed in Chapter 23.

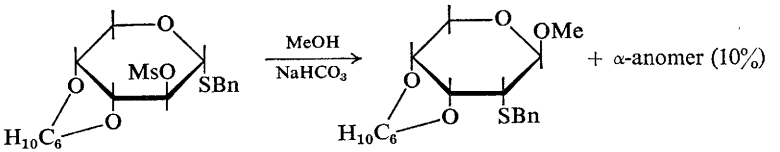
An ingenious method has been employed to prepare a series of 2-thio-D-ribose derivatives. Since 2,3-oxiran and 2,3-thi-iran rings are opened to give 2,3-*trans* products, this procedure could not be used, so that 1,2-episulphonium ion intermediates were used instead. The general approach is shown in Scheme 48, and was applied to both furanoid and pyranoid compounds as, for example, in Scheme 49. With the same system, acetolysis using acetic anhydride–acetic acid containing potassium acetate gave the β-glycosyl acetate as well as the α-anomer (10%) and small amounts of the glycal derivative (188).[379]

The novel 6-thiolactosan hexa-acetate (51) has been prepared from lactose.[178]

[376] J. Defaye, A. Ducruix, and C. Pascard-Billy, *Bull. Soc. chim. France*, 1970, 4514.
[377] J. Defaye, T. Nakamura, D. Horton, and K. D. Philips, *Carbohydrate Res.*, 1971, **16**, 133.
[378] A. Farrington and L. Hough, *Carbohydrate Res.*, 1971, **16**, 59.
[379] K. J. Ryan, E. M. Acton, and L. Goodman, *J. Org. Chem.*, 1971, **36**, 2646.



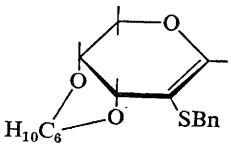
Scheme 48



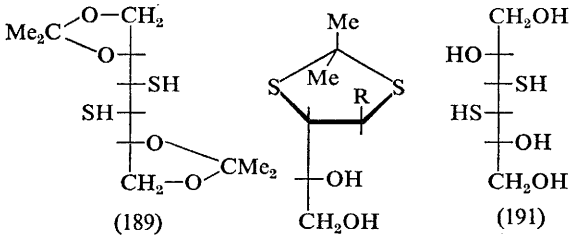
Scheme 49

Acidic hydrolysis of 1,2:5,6-di-*O*-isopropylidene-3,4-dithio-D-iditol (189) gave the 3,4-*S*-isopropylidene derivative (190) rather than the expected derivative (191). It was shown that the process was intermolecular rather than intramolecular.[380]

Acid-catalysed methanolysis of the thiol (192) gave (193), with migration of the acetal group.[240]

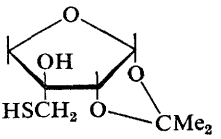


(188)

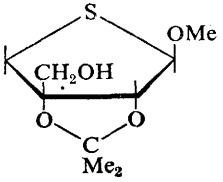


(189) (190) (191)

[380] G. E. McCasland and A. B. Zanlungo, *Carbohydrate Res.*, 1971, **17**, 475.



(192)



(193)

Mass spectra of 4-thioxylose derivatives have been determined and compared with those of the corresponding xylose compounds.[381] The u.v. spectra of forty-six carbohydrate derivatives bearing a thiocarbonyl group have been measured.[382]

Unsaturated thio-sugars, including thiocyanate derivatives, are covered in Chapter 14.

[381] V. Kováčik, P. Kováč, and R. L. Whistler, *Carbohydrate Res.*, 1971, **16**, 353.
[382] B. S. Shasha, D. Trimnell, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1971, **19**, 383.

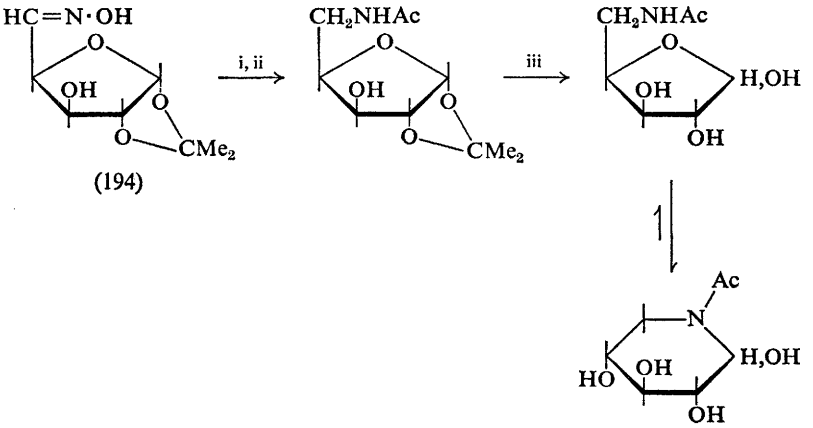
12

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

In 1971, there was a significant fall in the number of reports of sugars containing nitrogen, sulphur, or phosphorus in the ring.

Nitrogen Derivatives

5-Acetamido-5-deoxy-D-xylose has been synthesized by way of 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose oxime (194), as illustrated in Scheme 50. The oxime was shown to be a mixture of *syn*- and *anti*-forms.[371]



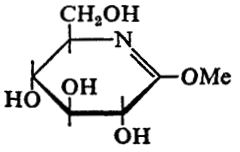
Reagents: i, $LiAlH_4$; ii, Ac_2O–MeOH; iii, H_3O^+

Scheme 50

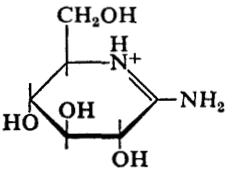
Nojirimycin and D-glucono-1,5-lactone have been shown to be powerful inhibitors of glucosidases, but poor inhibitors of *exo*-glucanases, *endo*-glucanases, and related enzymes. In connection with these studies, it was envisaged that compounds (195) and (196) might possess interesting inhibitory properties.[383]

[383] E. T. Reese, F. W. Parrish, and M. Ettlinger, *Carbohydrate Res.*, 1971, **18**, 381.

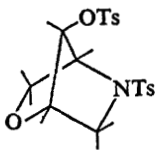
Carbohydrate derivatives have been used for the synthesis of several 2-oxa-5-aza-bicyclo[2,2,1]heptanes.[384] Thus, compounds such as (197), (198), and (199) were formed from (200), (201), and (202), respectively, by intramolecular displacements following reduction of the azido-groups. The structures of a variety of 2-oxa-5-aza-bicyclo[2,2,1]heptanes have been confirmed by n.m.r. spectroscopy.[384a]



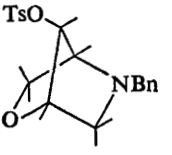
(195)



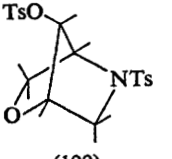
(196)



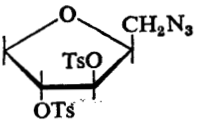
(197)



(198)



(199)



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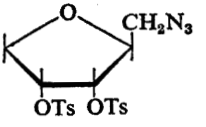
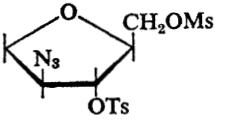


(201)



(202)

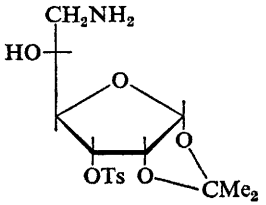
The ring-nitrogen compound (204) was prepared from (203) by treatment with sodium acetate in ethanol and subsequent *N*-acetylation.[385] The derived mesylate (205) was converted into (206) by treatment with sodium benzoate in DMF and on debenzoylation gave (207), which had been prepared previously by solvolysis of (208).

3,5-Diacetamido-3,5-dideoxy-D-ribose was found to exist in solution preponderantly in the furanoid form (209), whereas the pyranoid form (210) of the corresponding D-xylose derivative was preferred.[316] It was also shown that, whereas 3,5-diacetamido-3,5-dideoxy-D-arabinose favoured the furanoid form, the corresponding D-lyxose derivative favoured the pyranoid form.[317]

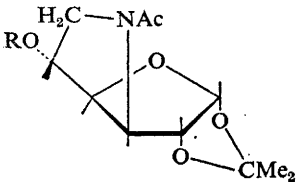
[384] J. Cléophax, J. Leboul, A.-M. Sepulchre, and S. D. Gero, *Bull. Soc. chim. France*, 1970, 4412.

[384a] J. Cléophax, A. Gaudemer, A.-M. Sepulchre, and S. D. Gero, *Bull. Soc. chim. France*, 1970, 4144.

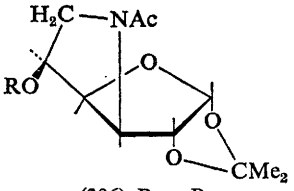
[385] J. S. Brimacombe and A. M. Mofti, *Carbohydrate Res.*, 1971, **18**, 157.



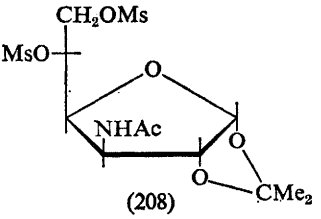
(203)



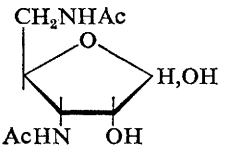
(204) R = H
(205) R = Ms



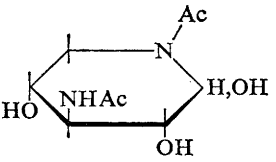
(206) R = Bz
(207) R = H



(208)



(209)

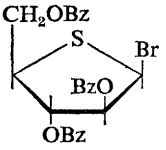


(210)

Sulphur Derivatives

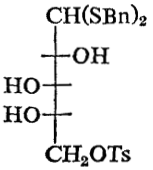
Anomeric mixtures of nucleoside derivatives of 4-thio-D-arabinose were formed when (211) was treated with trimethylsilylated pyrimidines.[386]

Benzyl 1,5-dithio-α- and -β-L-arabinopyranosides (213) were formed when 5-*O*-*p*-tolylsulphonyl-L-arabinose dibenzyl dithioacetal (212) was heated with sodium iodide in acetone.[387] Omission of the sodium iodide, or use of the corresponding diethyl dithioacetal, afforded the furanoside (214), suggesting that both the benzyl group and S_N2 conditions are necessary for dealkylation of the proposed cyclic sulphonium ion intermediate.



(211)

[386] R. L. Whistler, L. W. Doner, and U. G. Nayak, *J. Org. Chem.*, 1971, **36**, 108.
[387] J. Harness and N. A. Hughes, *Chem. Comm.*, 1971, 811.



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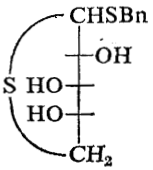
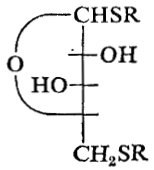


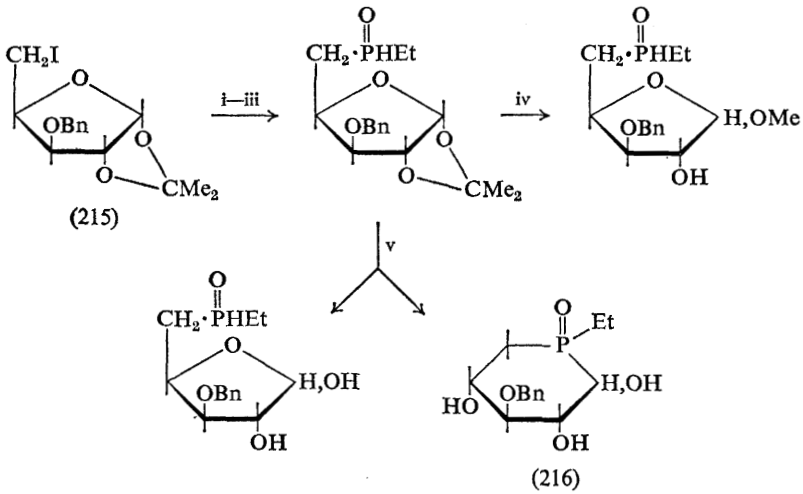
(213)



(214) R = Bn or Et

Phosphorus Derivatives

One paper has described a carbohydrate derivative with phosphorus in the ring. The synthetic sequences involved in the formation of 3-*O*-benzyl-5-deoxy-5-(ethylphosphonyl)-D-xylopyranose (216) from 3-*O*-benzyl-5-deoxy-5-iodo-1,2-*O*-isopropylidene-α-D-xylofuranose (215) are illustrated in Scheme 51.[388]



(215)

(216)

Reagents: i, $(EtO)_2PEt$; ii, $LiAlH_4$; iii, O_2; iv, MeOH–HCl; v, MeOH–H_3O^+

Scheme 51

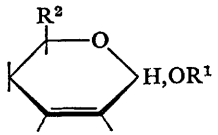
[388] S. Inokawa, Y. Tsuchiya, K. Seo, H. Yoshida, and T. Ogata, *Bull. Chem. Soc. Japan*, 1971, **44**, 2279.

13
Deoxy-sugars

Syntheses of several deoxy-sugars have been referred to already in Chapter 4.

Sodium cyanoborohydride ($NaBH_3CN$) in HMPT is a reagent which brings about rapid reductive removal of iodo-, bromo-, and *p*-tolylsulphonyloxy-groups and, as such, should be of use in carbohydrate chemistry.[389]

A sugar identified by chromatography and polarimetry as 6-deoxy-L-altrose has been isolated from the polysaccharide of *Yersinia enterocolitica*.[390] The 2,4-di-, 4,6-di-, and 2,4,6-tri-*O*-methyl derivatives of 3-deoxy-D-*xylo*-hexose have been described.[391] A series of racemic 2,3-unsaturated glycopyranoside derivatives (217) have been utilized in the preparation of 4-deoxyhexoses having the *lyxo*, *xylo*, and *arabino* configurations.[392] Related studies are reported in detail in Chapter 4 (see also Chapter 14).



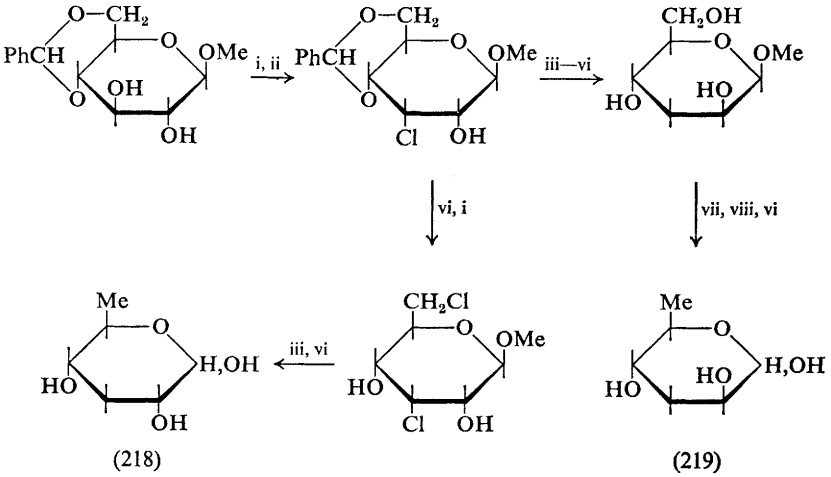
R^1 = Me, Et, Pr, or Bu
R^2 = CH_2OH or CO_2alkyl

(217)

Reductive ring-opening of 1,6:3,4-dianhydro-β-DL-allohexopyranose, followed by hydrolysis with acid, yielded 3-deoxy-DL-*ribo*-hexopyranose [25] (see Scheme 4).

In the area of dideoxyhexoses, paratose (218) and tyvelose (219), components of Gram-negative bacteria, have been synthesized as indicated in Scheme 52.[393] Oxidation of benzylidene acetals with *N*-bromosuccinimide by the Hanessian procedure was the key step in improved syntheses of methyl glycosides of 2,6-dideoxy-D-*ribo*-, 2,6-dideoxy-D-*arabino*-, and

[389] R. O. Hutchins, B. E. Maryanoff, and C. A. Milewski, *Chem. Comm.*, 1971, 1097.
[390] D. C. Ellwood and G. R. A. Kirk, *Biochem. J.*, 1971, **122**, 14P.
[391] H. Zinner and G. Wulf, *J. prakt. Chem.*, 1970, **312**, 635.
[392] A. Konowal and A. Zamojski, *Roczniki Chem.*, 1971, **45**, 859.
[393] E. H. Williams, W. A. Szarek, and J. K. N. Jones, *Canad. J. Chem.*, 1971, **49**, 796.



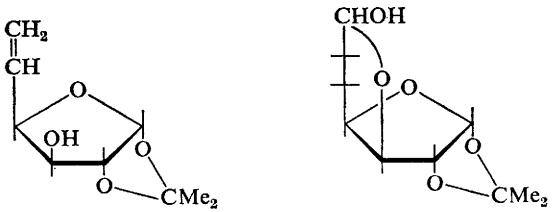
(218) (219)

Reagents: i, SO_2Cl_2; ii, NaI; iii, Ni–H_2; iv, RuO_4; v, $NaBH_4$; vi, H_3O^+; vii, TsCl–py; viii, $LiAlH_4$

Scheme 52

3,6-dideoxy-D-*arabino*-hexose.[394] 4,6-Dideoxy-L-*lyxo*-hexopyranose and the derived aldonolactone have been prepared from methyl 3,4-anhydro-6-deoxy-α-D-talopyranoside.[395] 3,3′-Dideoxy- and 3,3′,6,6′-tetradeoxy-αα-trehalose have been described.[294]

Hydroformylation of the olefin (220) was successfully accomplished to give the dideoxyheptose derivative (221) (51% yield), the 3-hydroxy-group effectively protecting the aldehyde initially formed from further reduction.[396]



(220) (221)

[394] M. Haga, M. Chonan, and S. Tejima, *Carbohydrate Res.*, 1971, **16**, 486.
[395] K. Kefurt, Z. Kefurtova, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1971, **36**, 1701.
[396] A. Rosenthal and G. Kan, *J. Org. Chem.*, 1971, **36**, 592.

14
Unsaturated Derivatives

This area still represents a rapidly expanding section of carbohydrate chemistry; the uses of unsaturated compounds in syntheses have already been illustrated in Chapters 7 and 13.

Glycals

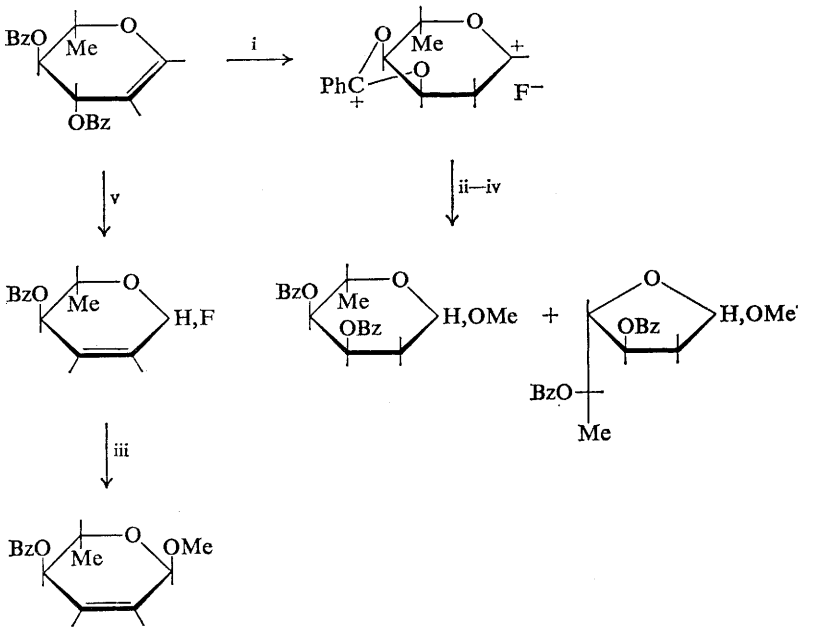
Glycal derivatives have been used extensively in the synthesis of fluorinated compounds (Chapter 7).

Pedersen and his colleagues have continued to explore the action of hydrogen fluoride on glycal esters and their results on additions, rearrangements, neighbouring-group participations, and solvolyses are summarized in Schemes 53,[397] 54,[398] and 55.[399] Following the work illustrated in Scheme 54, deuterium labelling was used to show that 1,3,4,6-tetra-*O*-benzoyl-2-deoxy-β-D-*arabino*-hexopyranose incorporated fluorine at C-1 and deuterium at C-2 on treatment with deuterium fluoride.[398] The same group has shown that boron trifluoride-catalysed reactions between glycal esters and methanol are sensitive to the proportions of alcohol used. With two molar equivalents of methanol and a short reaction time, glycal (222) gave (223) (19%) and (224) (73%), but when more methanol was used the methyl ethers (225) and (226) were formed by non-specific addition of methanol to the unsaturated intermediate (224).[400]

Further reports pertinent to the allylic rearrangements undergone by glycal esters have appeared. It has been confirmed that 3,4,6-tri-*O*-acetyl-D-glucal on treatment with purine derivatives (B) gives 2,3-unsaturated products (227), but now it has been observed that these are less favoured thermodynamically than the 3-linked unsaturated nucleosides (228). All the isomeric compounds obtained were studied in detail by n.m.r. spectroscopy.[401] Similar observations were reported by Spanish workers on related pentose nucleoside derivatives [401a] (see Vol. 4, p. 142), and Japanese workers have also reported finding 3-linked theophyllyl-glycals on fusing 3,4,6-tri-*O*-acetyl-D-galactal or a related uronic acid glycal with theophylline

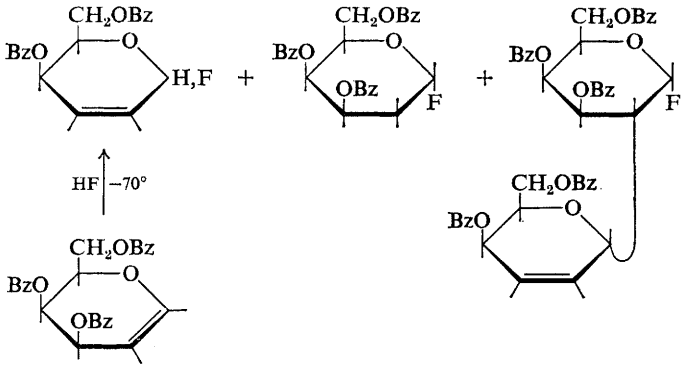
[397] I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 1971, **25**, 2320.
[398] I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 1971, **25**, 2749.
[399] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1971, **25**, 2757.
[400] K. Bock, J. K. Christiansen, and C. Pedersen, *Carbohydrate Res.*, 1971, **20**, 73.
[401] R. J. Ferrier and M. M. Ponpipom, *J. Chem. Soc.* (*C*), 1971, 553.
[401a] M. Fuertes, G. García-Muñoz, R. Madroñero, and M. Stud, *J. Heterocyclic Chem.*, 1971, **8**, 261.

in the presence of toluene-*p*-sulphonic acid.[402] Another 2,3-dideoxyglyc-2-enose–glycal rearrangement occurred on treating ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside with isocyanatosulphonyl chloride ($OCNSO_2Cl$), followed by potassium iodide and aqueous bicarbonate, to give the first reported 3-aminoglycal (229).[403]



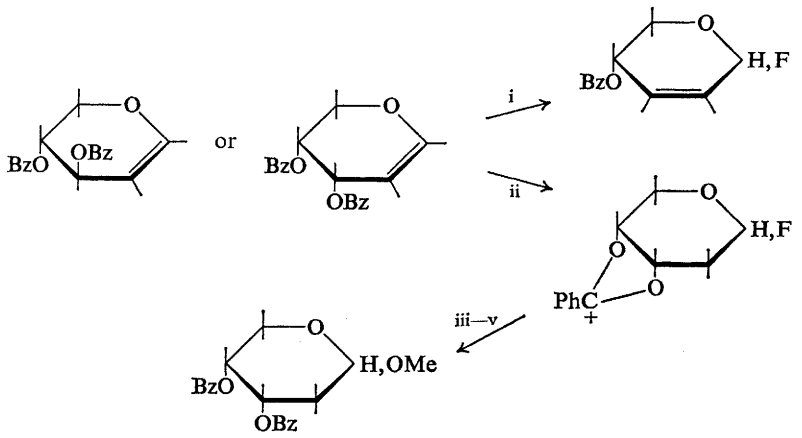
Reagents: i, HF; ii, H_2O; iii, MeOH; iv, BzCl–py; v, HF–C_6H_6

Scheme 53



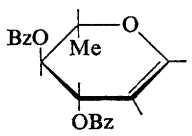
Scheme 54

[402] T. Kondo and T. Goto, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 912.
[403] A. Jordaan and G. J. Lourens, *Chem. Comm.*, 1971, 581.

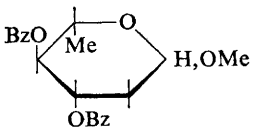


Reagents: i, HF–C_6H_6; ii, HF; iii, H_2O; iv, BzCl–py; v, MeOH

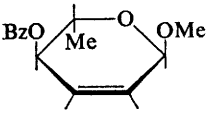
Scheme 55



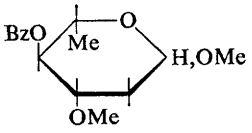
(222)



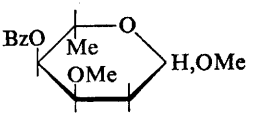
(223)



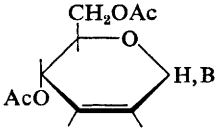
(224)



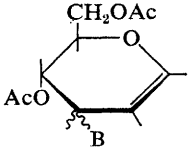
(225)



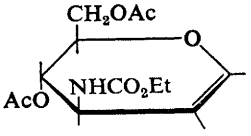
(226)



(227)



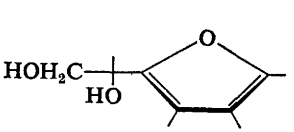
(228)



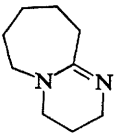
(229)

3,6-Anhydro-D-glucal has been prepared in crystalline form and its conversion into 3,6-anhydro-2-deoxy-D-*arabino*-hexose and the furan (230) on treatment with acid have been discussed.[404]

Appreciable attention has also been given to 2-substituted glycals. 1,5-Diazabicyclo[5,4,0]undec-5-ene (231), a strong base but a poor nucleophile, has been recommended for use in elimination reactions leading to the formation of substituted 2-hydroxyglycals. When acetobromogalactose in DMF was used, 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-galactal was obtained in 85% yield.[405]

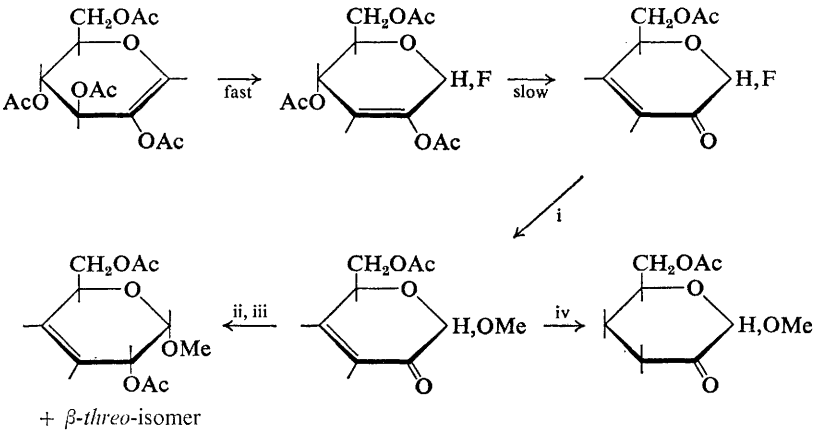


(230)



(231)

Further studies on the formation of (3-deoxyhex-2-enosyl)purine nucleosides from 2-hydroxyglycal esters and 3-deoxyhex-2-enopyranose esters have been reported.[406] Earlier work on the reactions undergone by 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal with anhydrous hydrogen fluoride at low temperatures has been amplified, and the results are summarized in Scheme 56. *cis*-Hydroxylation of the 3,4-unsaturated glycosides so produced offers a new route to hexosides of various configurations.[407]



+ β-*threo*-isomer

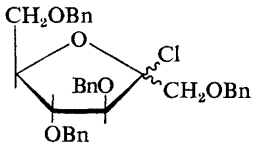
Reagents: i, MeOH; ii, $LiAlH_4$; iii, Ac_2O–py; iv, H_2–Pt

Scheme 56

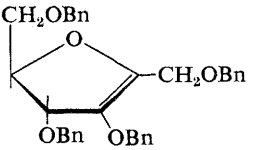
[404] J. S. Brimacombe, I. Da'aboul, and L. C. N. Tucker, *Carbohydrate Res.*, 1971, **19**, 276.
[405] D. R. Rao and L. M. Lerner, *Carbohydrate Res.*, 1971, **19**, 133.
[406] R. J. Ferrier and M. M. Ponpipom, *J. Chem. Soc.* (*C*), 1971, 560.
[407] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1971, **25**, 1021.

1,3,4,6-Tetra-*O*-benzyl-D-fructofuranosyl chloride (232), readily prepared from the tetrabenzylated free sugar, has been dehydrohalogenated to give the substituted glycal (233) and the isomer (234).[408]

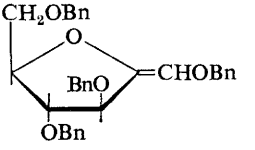
Similar rearrangements to those reported for 2-hydroxyglycal esters occurred when 3,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-1,2-dideoxy-D-*arabino*-hex-1-enopyranose (235) was treated with various carboxylic acids and phenols under acidic conditions. The compounds (236) were thus obtained.[409]



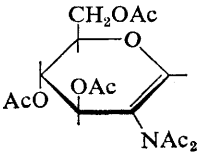
(232)



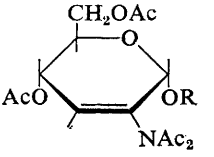
(233)



(234)



(235)



(236)

R = Bz, COBn, or *p*-$O_2NC_6H_4$

Other Unsaturated Compounds

The application of the base (231) in the removal of methanesulphonic acid from mesylates is illustrated in Scheme 57; it can be seen that direct elimination does not always occur.[410]

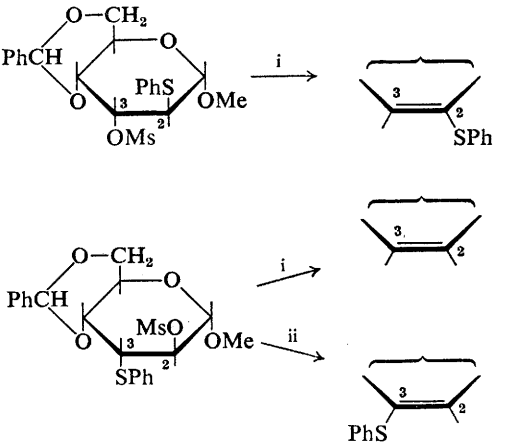
The *cis*-dihydropyran derivative (237) was readily oxidized to the enone with manganese dioxide, whereas the *trans*(β)-isomer was surprisingly inert. It was proposed that the difference is somehow related to the fact that the anomers adopt *H*1 and 1*H* conformations, respectively. An interesting point relating to the large chemical-shift differences of the methylene protons of the ethyl groups in these compounds was discussed.[411]

Several investigations have been reported on the synthesis of saturated racemic carbohydrates from 2,3-unsaturated pyranoid precursors; some of these were referred to in Chapters 4 and 13. Addition of bromine in either ether or methanol to the dihydropyrans (238) has been studied,[412] and

[408] E. Zissis, R. K. Ness, and H. G. Fletcher, jun., *Carbohydrate Res.*, 1971, **20**, 9.
[409] N. Pravdic, B. Zidovec, and H. G. Fletcher, jun., *Croat. Chem. Acta*, 1970, **42**, 523.
[410] S. Hanessian and A. P. A. Staub, *Carbohydrate Res.*, 1971, **16**, 419.
[411] B. Fraser-Reid, B. J. Carthy, N. L. Holder, and M. Yunker, *Canad. J. Chem.*, 1971, **49**, 3038.
[412] M. Chmielewski and A. Zamojski, *Roczniki Chem.*, 1971, **45**, 1689.

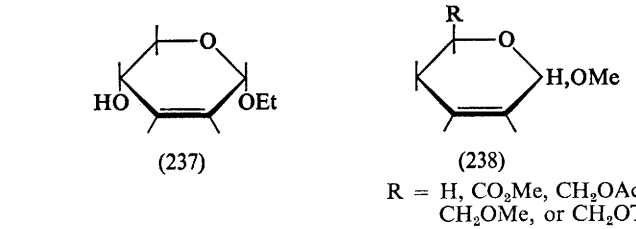
related hydroxylations have given routes to free and deoxy-sugars (Scheme 58).[413]

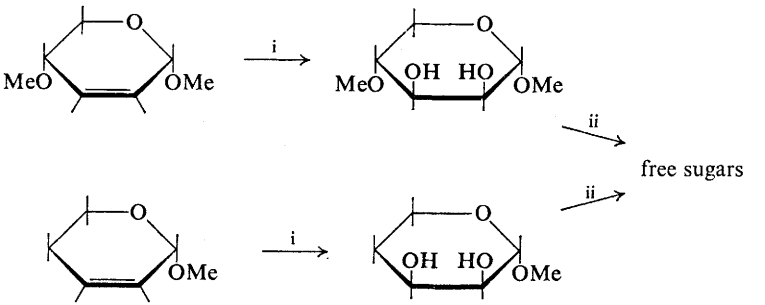
An unusual ring closure undergone by a 2,3-unsaturated 6-trityl-hexopyranoside to give a 1,6-anhydrohexose derivative has been referred



Reagents: i, (231); ii, $NaH–MeOCH_2CH_2OMe$

Scheme 57





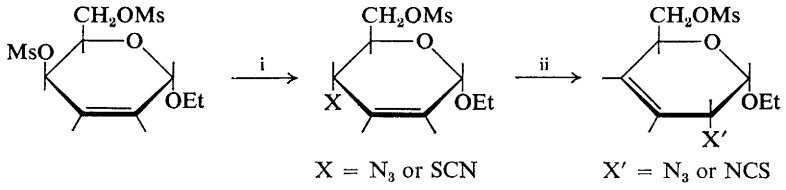
Reagents: i, OsO_4; ii, H_3O^+

Scheme 58

[413] R. M. Srivastava and R. K. Brown, *Canad. J. Chem.*, 1971, **49**, 1339.

to in Chapter 4, and epoxidations of 2,3-unsaturated nitro-sugars are discussed in Chapter 10.

Allylic rearrangements of azido- and thiocyanato-derivatives have been examined. Thus, treatment of ethyl 2,3-dideoxy-4,6-di-*O*-methylsulphonyl-α-D-*erythro*- and -*threo*-hex-2-enopyranoside with azide and thiocyanate ions caused specific nucleophilic displacements at the allylic positions with Walden inversion. Heating of these products caused isomerizations as illustrated in Scheme 59 for the D-*erythro*-series. The azides gave equili-

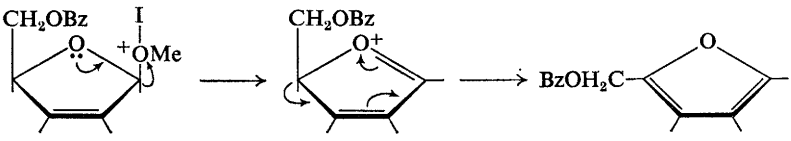


Reagents: i, NaN_3 or KSCN–DMF; ii, heat

Scheme 59

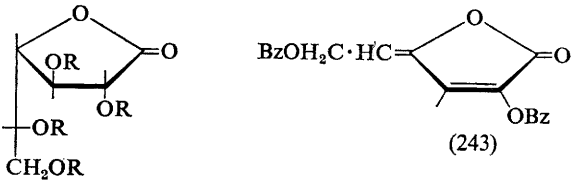
brated mixtures, whereas the thiocyanates were converted completely into the 3,4-unsaturated isothiocyanates, which were readily transformed into corresponding 2-acetamido-2-deoxy-derivatives.[414]

Reports on 2,3-unsaturated furanoid compounds have also appeared. The glycoside (239) was converted into (240) on treatment with iodine in dry ether at room temperature, the proposed route for the conversion being shown in Scheme 60.[415] Reaction of D-galactono-1,4-lactone (241) with



(239) (240)

Scheme 60



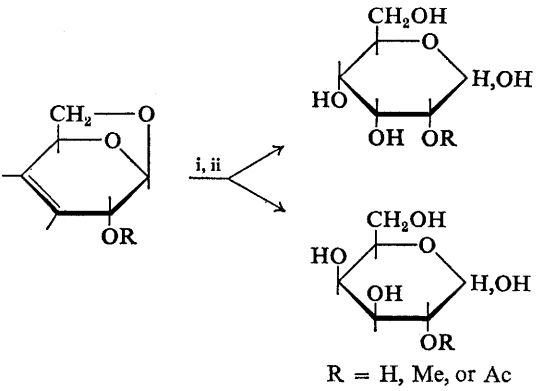
(241) R = H
(242) R = Bz

(243)

[414] R. J. Ferrier and N. Vethaviyaser, *J. Chem. Soc.* (*C*), 1971, 1907.
[415] R. G. S. Ritchie and W. A. Szarek, *Carbohydrate Res.*, 1971, **18**, 443

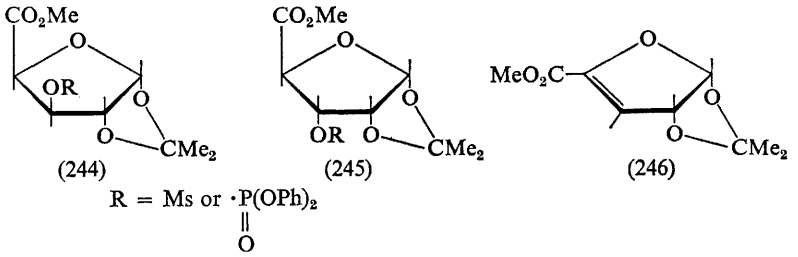
benzoyl chloride in pyridine afforded initially the tetrabenzoate (242), but on prolonged reaction the unsaturated product (243) was obtained.[416] Related 2-acetamido-2-deoxy-compounds have been produced during studies of 2-acetamido-2-deoxyaldono-1,4-lactones (Chapter 17).

Syntheses of saturated compounds from 3,4-unsaturated precursors have already been referred to, and others are illustrated in Scheme 61.[417] The



Reagents: i, OsO_4; ii, H_3O^+

Scheme 61

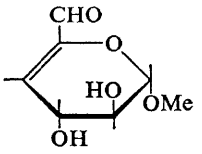


mesylates and the diphenyl phosphates (244) and (245) underwent rapid elimination of the 3-substituent under basic conditions to give the unsaturated derivative (246), which polymerized slowly on standing.[418] 3,4-Unsaturated hexofuranosides were formed as by-products during nucleophilic displacement reactions.[243]

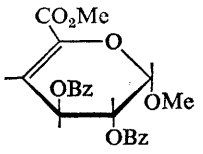
In the area of 4,5-unsaturated compounds, the enal (247) has been prepared from methyl α-D-altropyranoside, which, following conversion into the 2,3,4-triacetate, was oxidized with DMSO–sulphur trioxide in triethylamine and deacetylated with methanolic ammonia.[418a] In related

[416] R. M. De Lederkremer and M. I. Litter, *Carbohydrate Res.*, 1971, **20**, 442.
[417] U. P. Singh and R. K. Brown, *Canad. J. Chem.*, 1971, **49**, 1179.
[418] J. Kiss and K. Noack, *Carbohydrate Res.*, 1971, **16**, 245.
[418a] N. Bourguignon-Zylber and J. Polonsky, *Biochimie*, 1971, **53**, 263.

work, bimolar benzoylation of methyl (methyl α-D-galactopyranosid)-uronate gave the 2,3-dibenzoate, in 60% yield, and treatment of the derived 4-mesylate with sodium azide in HMPT gave the unsaturated ester (248).[419] Analogous eliminations carried out on methyl ethers of uronates have also been studied (Chapter 17).



(247)

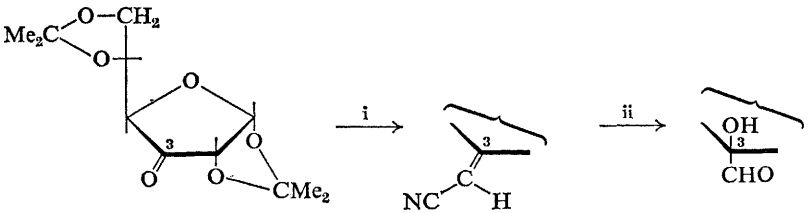


(248)

Hydroformylation of 5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enose [396] and formation of the unusual unsaturated compound 2,3,5-tri-*O*-acetyl-1,6-anhydro-β-L-*arabino*-hex-5-enofuranose [176] have been referred to already.

5,6-Enopyranoses have been used in the synthesis of 6-deoxy-L-idopyranose derivatives.[293]

A modified application of the Wittig reaction to the synthesis of branched-chain sugars involved the use of cyanomethylene triphenylphosphorane and hydroxylation of the products. This procedure gave isomers with the alternative stereochemistry at the branch-point to that obtained by usual Grignard syntheses (see Scheme 62).[420] In a subsequent



(249)

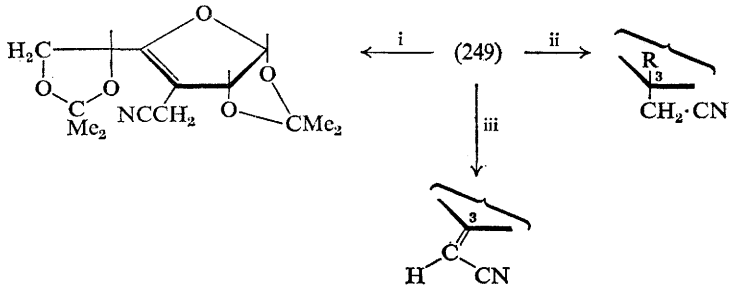
Reagents: i, $Ph_3P{=}CHCN$; ii, $KMnO_4$

Scheme 62

paper, the authors examined the reactions of intermediate (249) with various nucleophiles (Scheme 63).[421]

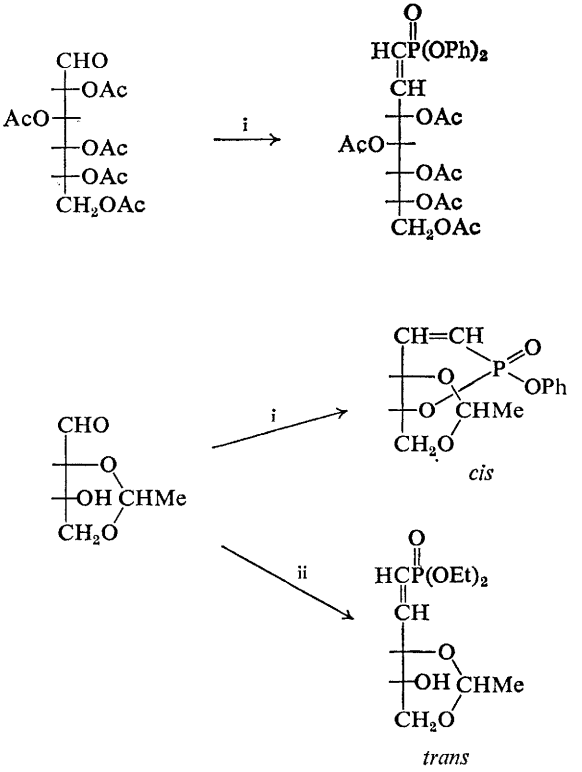
Studies on unsaturated derivatives of sugar phosphonates are illustrated in Scheme 64.[422] In related work, 2,3:5,6-di-*O*-isopropylidene- and

[419] P. L. Gill, M. W. Horner, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1971, **17**, 213.
[420] J. M. J. Tronchet, J.-M. Bourgeois, J.-M. Chalet, R. Graf, R. Gurny, and J. Tronchet, *Helv. Chim. Acta*, 1971, **54**, 687.
[421] J. M. J. Tronchet and J. M. Bourgeois, *Helv. Chim. Acta*, 1971, **54**, 1718.
[422] H. Paulsen, W. Bartsch, and J. Thiem, *Chem. Ber.*, 1971, **104**, 2545.



Reagents: i, $BnNH_2$; ii, CN^-, MeO^-, EtO^-, or PhS^- (R^-); iii, $MeS\bar{C}H\overset{+}{P}Ph_3$

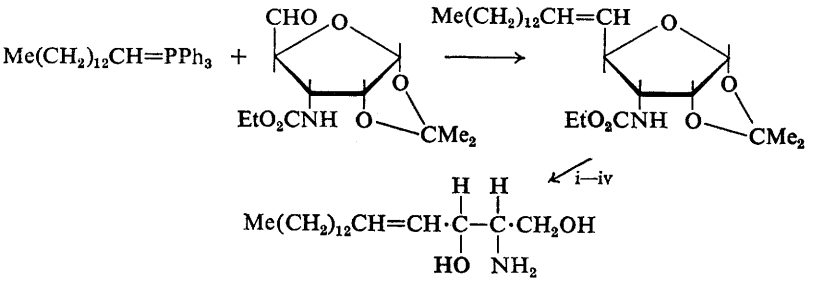
Scheme 63



Reagents: i, $Ph_3P{=}CHP(OPh)_2$; ii, $(EtO)_2P{\cdot}\bar{C}H{\cdot}P(OEt)_2$

Scheme 64

-cyclohexylidene-D-mannofuranose were converted into α,β-unsaturated octonic acid derivatives with alkoxycarbonylmethylene triphenylphosphoranes.[423] *cis*- and *trans*-Isomers of sphingosine have been synthesized as shown in Scheme 65.[424]



Reagents: i, H_3O^+; ii, IO_4^-; iii, $NaBH_4$; iv, HO^-

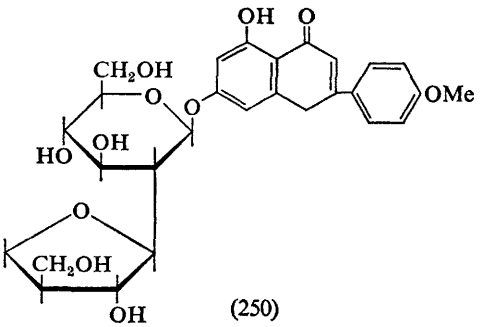
Scheme 65

[423] Yu. A. Zhdanev and L. A. Uzlova, *Zhur. obshchei Khim.*, 1971, **41**, 1396.
[424] E. J. Reist and P. H. Christie, *J. Org. Chem.*, 1970, **35**, 4127

15
Branched-chain Sugars

Compounds with an $R^1—C—OR^2$ Branch

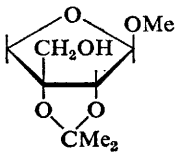
The synthesis of branched-chain sugars related to apiose continues to be of interest. 4′-*O*-Methylapiin (250) has been synthesized by a route that provided confirmation of its structure.[425] Both α-D-apio-D-furanosyl and



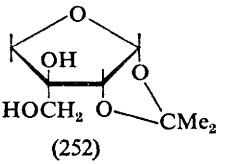
(250)

α-D-apio-L-furanosyl 1-phosphate esters have been prepared, in 20% yield, from a mixture of β-D-apiose tetra-acetates. The two phosphates were examined in detail by n.m.r. spectroscopy.[426] Methyl 2,3-*O*-isopropylidene-α-D-apio-L-furanoside (251) was obtained when 1,2-*O*-isopropylidene-α-D-apio-L-furanose (252) was treated with acidified anhydrous methanol.[427] It was shown that the rearrangement involved (*i*) opening of the furanose ring and ring closure at the other primary hydroxy-group, (*ii*) intramolecular migration of the isopropylidene group, and (*iii*) formation of the methyl glycoside. Syntheses of 3-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-erythrofuranose (253) and 3-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-apio-L-furanose (252) were accomplished when the spiroepoxides resulting from addition of diazomethane to the ketone (254) were separated chromatographically and hydrolysed under alkaline conditions. The *erythro*-isomer was the major product, and there was no evidence of significant ring

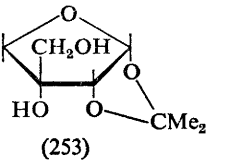
[425] A. D. Ezekiel, W. G. Overend, and N. R. Williams, *J. Chem. Soc.* (*C*), 1971, 2907.
[426] J. Mendicino and R. Hanna, *J. Biol. Chem.*, 1970, **245**, 6113.
[427] D. H. Ball, F. H. Bissett, I. L. Klundt, and L. Long, jun., *Carbohydrate Res.*, 1971, **17**, 165.



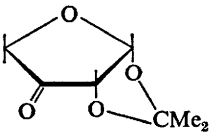
(251)



(252)



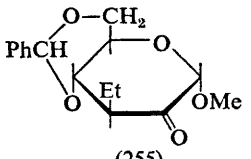
(253)



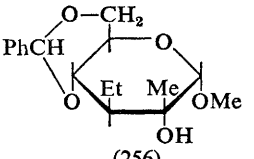
(254)

expansion by methylene insertion[428] (*cf.* Vol. 3, p. 123). A nucleoside containing D-apiose as the carbohydrate component has been prepared.[429]

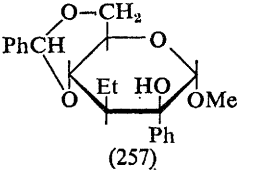
Comparisons have been made of the steric course of addition reactions of Grignard reagents, lithium aluminium hydride, and diazomethane with pyranosiduloses. It was demonstrated that Grignard reagents can give products of different configurations when added to pyranosid-2-uloses.[429a] Thus, whereas (255) reacted with methylmagnesium iodide to give mainly the D-*gluco*-derivative (256), the preponderant product from the reaction between (255) and phenylmagnesium bromide was the D-*manno*-isomer (257). Use has been made of stereodependent elimination reactions in-



(255)



(256)

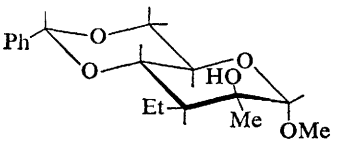


(257)

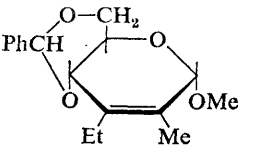
duced by thionyl chloride in pyridine to establish the configuration at CH_3—C—OH branch-points. For example, endocyclic dehydration occurred in alkyl 3-deoxy-3-*C*-ethyl-2-*C*-methyl-α-D-hexopyranosides where H-3 and HO-2 were antiparallel [*i.e.* (258) → (259)], whereas exocyclic dehydration occurred when H-3 and HO-2 had a *gauche* relation [*i.e.* (260) → (261)]. Studies of addition reactions to 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranosid-4-ulose (262) have also been reported. The configuration at the branch-point of the products (263) and (264), formed by addition of diazomethane and Grignard reagents, respectively, was shown to be the same. A hydroxymethyl sugar (265) with

[428] A. D. Ezekiel, W. G. Overend, and N. R. Williams, *Carbohydrate Res.*, 1971, **20**, 251.
[429] J. M. J. Tronchet and J. Tronchet, *Helv. Chim. Acta*, 1971, **54**, 1466.
[429a] T. D. Inch, G. J. Lewis, and N. E. Williams, *Carbohydrate Res.*, 1971, **19**, 17.

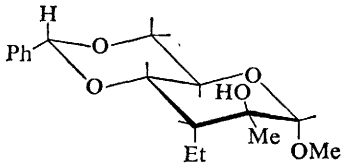
a branch-point at C-3 was formed when (262) was treated with paraformaldehyde in methanolic potassium carbonate, and a dimer (266) resulted when (262) was treated with ethyl formate under basic conditions.[430]



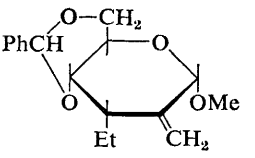
(258)



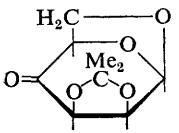
(259)



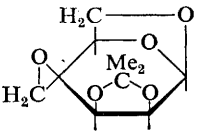
(260)



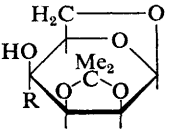
(261)



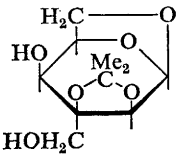
(262)



(263)



(264) R = Me, $-CH{=}CH_2$, or $-C{\equiv}CH$



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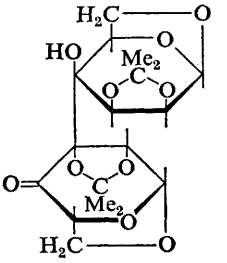


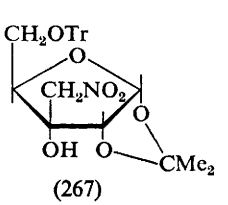
(266)

The transformation (260) → (261) allows the conversion of branched-chain sugars of type $R^1{-}C{-}OR^2$ into type $R{-}C{-}H$. A number of other such examples have been reported. Full details have been published of the formation of branched-chain sugars by treatment of uloses with nitromethane [431] (see Vol. 3, p. 122). Thus, compound (267) was prepared from

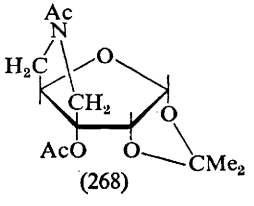
[430] D. Horton and E. K. Just, *Carbohydrate Res.*, 1971, **18**, 81.
[431] G. L. Lourens, *Carbohydrate Res.*, 1971, **17**, 35.

1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose, and thereafter, by suitable reduction and elimination procedures, was converted into (268), (269), and (270).

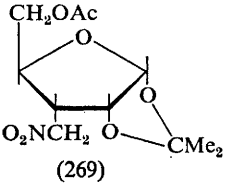
The 3-*C*-methylene derivative (271) has been obtained from (272) by ring opening with sodium iodide to give the 3-iodomethyl derivative, which was then treated with zinc powder in DMF.[432] Hydroboronation of (271) afforded a mixture of methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-3-*C*-methyl-α-D-glucopyranoside and a 3-*C*-hydroxymethyl derivative of unassigned configuration at C-3. A modification of the Wittig reaction in the synthesis of branched-chain sugars involved treatment of a dicarbonyl sugar with cyanomethylene triphenylphosphorane (see Scheme 62).[420]



(267)



(268)



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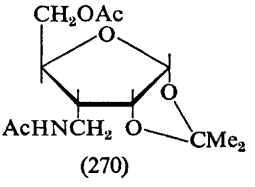

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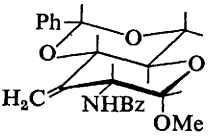

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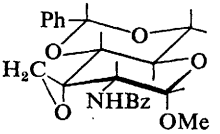


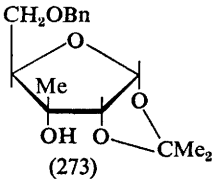
(272)

Compounds (273), (274), and (275) were obtained in the ratio 5 : 7 : 88 when (276) was submitted to a hydroboronation–oxidation sequence.[433]

Treatment of 3-deoxy-2-*C*-hydroxymethyl-di-*O*-*p*-tolylsulphonyl-D-*erythro*-pentono-1,4-lactone (277) with potassium thiolacetate did not give the expected dithiolacetate, but instead the spiroepoxide (278) was obtained.[434] The spiroepoxide (279) was obtained, in 64% yield, by treating (280) with dimethyloxosulphonium methylide. Compound (279) afforded (281) on treatment with sodium acetate in hot DMF, and (281) was subsequently oxidized to the 3-*C*-formyl derivative (282) with phosphorus pentoxide in DMSO.[435]

[432] J. H. Jordaan and S. Smedley, *Carbohydrate Res.*, 1971, **18**, 303.
[433] A. Rosenthal and M. Sprinzl, *Carbohydrate Res.*, 1971, **16**, 337.
[434] D. R. Strobach, *Carbohydrate Res.*, 1971, **17**, 457.
[435] J. H. Jordaan and S. Smedley, *Carbohydrate Res.*, 1971, **16**, 177.

Nogalose, the carbohydrate component of nogalamycin, has been shown largely by n.m.r. spectroscopy and *X*-ray methods to be 6-deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-L-mannose, and appears to be the first fully methylated sugar to be isolated from a natural source. Various aspects of its chemical reactivity were discussed.[436]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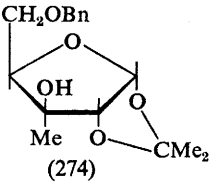
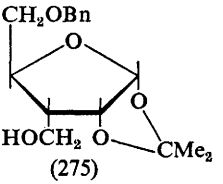

(274)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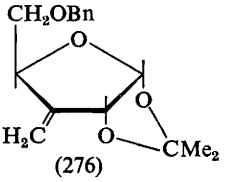
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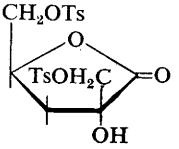
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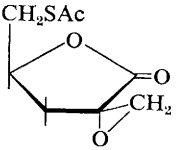
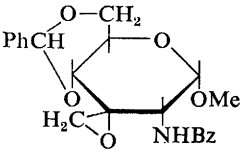

(278)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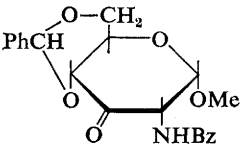
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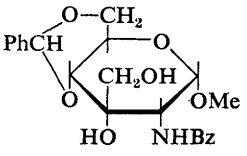
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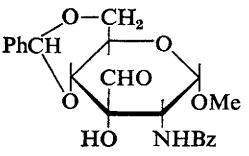
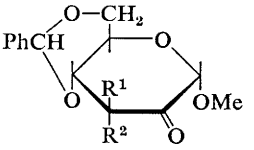

(282)

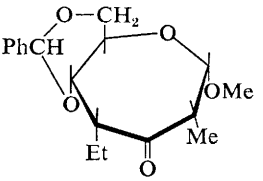
[436] P. F. Wiley, D. J. Duchamp, V. Hsiung, and C. C. Chidester, *J. Org. Chem.*, 1971, **36**, 2670.

Compounds with an R—C—H Branch

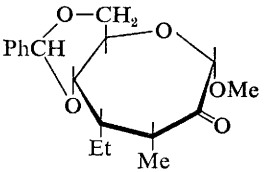
Full details have been published of reactions between diazoalkanes and pyranosid-2-uloses[437] (see Vol. 4, p. 106). The nature of the products (spiro-epoxides and ring-expanded derivatives) from the reaction of (283) with diazoalkanes could be predicted from the stereochemistry of the charge-separated intermediates. However, it was not easy to predict the steric preference of similar reactions with (284), although it was concluded that, whereas diazoalkanes attack (283) equatorially, axial attack on (284) preponderated. Thus, the major products from the reaction of (284) with diazoethane were the *allo*-heptoseptanosid-3-ulose (285) and the *allo*-heptoseptanosid-2-ulose, (286).



(283) R^1 = Et, R^2 = H
(284) R^1 = H, R^2 = Et



(285)



(286)

Lanthanide-shift reagents have been used to simplify the n.m.r. spectra of methyl 2,3,4,6-tetradeoxy-4-*C*-ethynylhexopyranosides.[438]

The reactions of (249) with various nucleophiles have been studied;[421] the reactions investigated are shown in Scheme 63.

[437] T. D. Inch, G. J. Lewis, and R. P. Peel, *Carbohydrate Res.*, 1971, **19**, 29.
[438] D. Horton and J. K. Thomson, *Chem. Comm.*, 1971, 1389.

16

Aldehydo-sugars, Alduloses, Dialduloses, and Diuloses

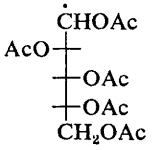
Compounds of general structure $(R^1O)_2P{:}OCHOHR^2$ (where R^1 = alkyl, R^2 = sugar derivative) have been prepared by treating the protected *aldehydo*-form of monosaccharides with dialkylphosphites.[439]

The hydration of acetylated *aldehydo*-aldoses in tetrahydrofuran–D_2O (7 : 3) has been examined by n.m.r. spectroscopy. In the ribose, arabinose, xylose, and lyxose derivatives, the percentage of *aldehydo*-form observed was less than 10%, demonstrating that the aldehydrol form is favoured overwhelmingly over the *aldehydo*-form in hydroxylic solvents.[440]

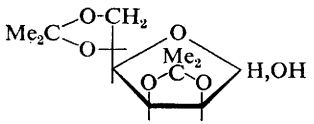
U.v. irradiation of *aldehydo*-D-glucose penta-acetate in benzene solution afforded D-arabinitol penta-acetate, 1-*C*-phenyl-D-*manno*-pentitol penta-acetate, and a decitol deca-acetate[441] (tentatively allocated the D-*gluco*-L-*gulo*-configuration on the basis of the results of periodate oxidation). All derivatives were derived from the radical (287). Yields of the foregoing products improved as the temperature was increased and, at 60 °C, the yields were 17, 6, and 2%, respectively. 2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (288) afforded (289) when treated with ethynylmagnesium bromide. Mercury-catalysed hydration and hydrolysis of (289) gave 1-deoxy-D-*glycero*-D-*galacto*-octulose (290).[26]

Dimerization (*i.e.* intermolecular acetal formation) and/or intramolecular acetal formation in ulose derivatives have been reported by a number of authors. For example, (291) crystallized as the 2,3-*cis*-dimer (292), (293) crystallized as the 3,4-*cis*-dimer (294), and (295) dimerized to a mixture of the 2,3-*cis*- and 3,4-*cis*-forms.[442] Further studies[443] have shown that, whereas (296) and (297), having the carbonyl group vicinal to an axial hydroxy-group, may be isolated as dimeric hemiacetals, compound (298) having the carbonyl group vicinal to equatorial hydroxy-groups does not afford dimeric hemiacetals. 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*- and -*xylo*-hexofuranos-3-ulose readily lose the 5,6-acetal ring when heated in

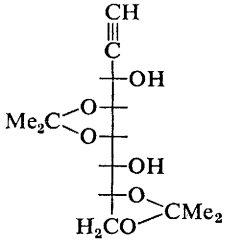
[439] Yu. A. Zhdanov, L. A. Uzlova, and Z. I. Glebova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **197**, 1331.
[440] D. Horton and J. D. Wander, *Carbohydrate Res.*, 1971, **16**, 477.
[441] R. L. Whistler and K.-S. Ong, *J. Org. Chem.*, 1971, **36**, 2575.
[442] K. Heyns, P. Köll, and H. Paulsen, *Chem. Ber.*, 1971, **104**, 2553.
[443] K. Heyns and P. Köll, *Chem. Ber.*, 1971, **104**, 3835.



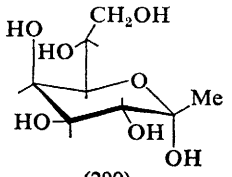
(287)



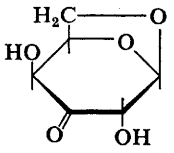
(288)



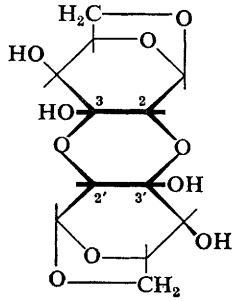
(289)



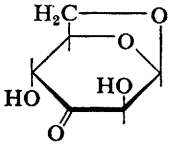
(290)



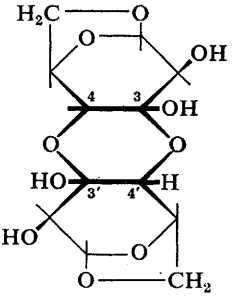
(291)



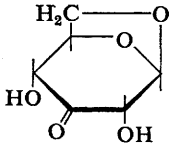
(292)



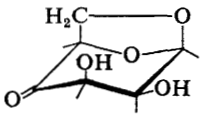
(293)



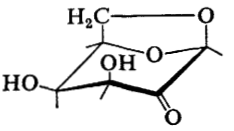
(294)



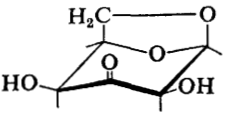
(295)



(296)

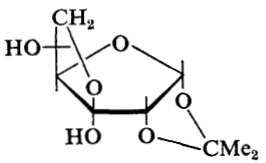


(297)

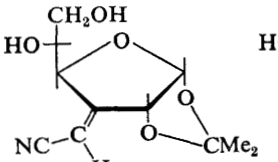


(298)

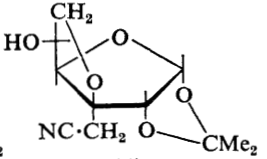
water to afford hemiacetal derivatives [*e.g.* (299)]. Similarly, the 3-cyanomethylene analogues [*e.g.* (300)] cyclized under basic conditions, providing the primary hydroxy-group was free, to give such products as (301).[444] The main products from hydroformylation of the epoxide (302) with carbon monoxide and hydrogen in the presence of dicobalt octacarbonyl were (303) (7%) and (304), which was isolated as the hemiacetal (305) (78%). The dialdose derivative (305) was used in the synthesis of nucleoside analogues.[445] D-*ribo*-Hexos-3-ulose (306) on treatment with methanolic hydrogen chloride afforded (307) and (308) as major products. Similar



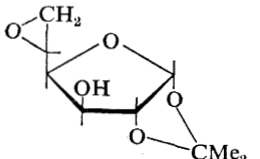
(299)



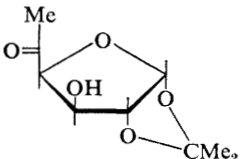
(300)



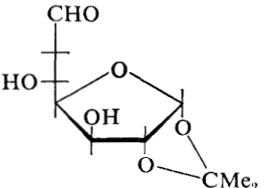
(301)



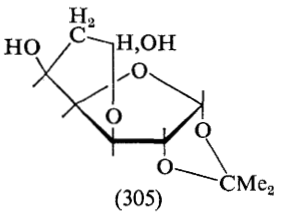
(302)



(303)

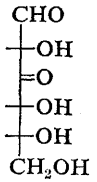


(304)

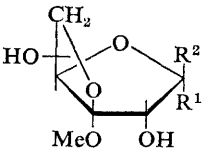


(305)

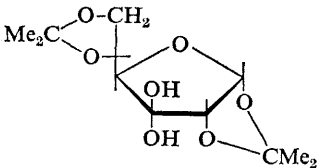
[444] J. M. J. Tronchet and J. M. Bourgeois, *Helv. Chim. Acta*, 1971, **54**, 1580.
[445] A. Rosenthal and G. Kan, *Carbohydrate Res.*, 1971, **19**, 145.



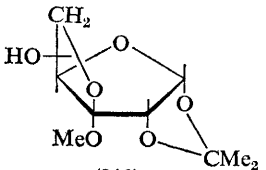
(306)



(307) R^1 = H, R^2 = OMe
(308) R^1 = OMe, R^2 = H

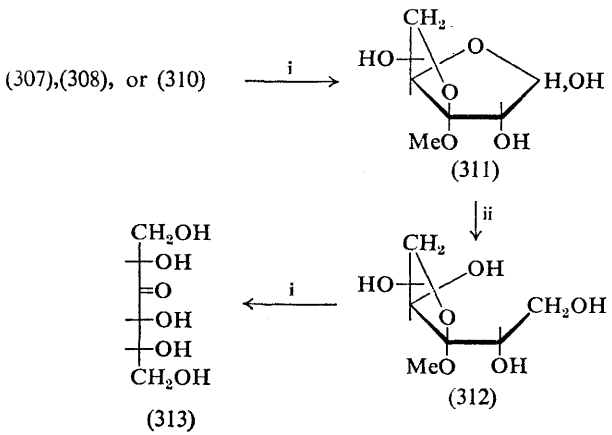


(309)



(310)

treatment of (309) afforded (310) as well as (307) and (308). Compounds (307), (308), and (310) were converted into D-*ribo*-hex-3-ulose (313) by way of (311) and (312) as illustrated in Scheme 66. The preparation of (313) by partial reduction of (306) was not practicable.[446]



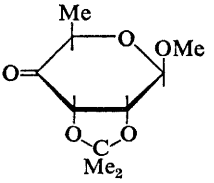
Reagents: i, H^+; ii, Pt–H_2

Scheme 66

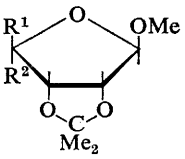
Full details of the photolysis of pyranosid-4-uloses have been published [447] (*cf.* Vol. 3, p. 131), and further studies have been described.[448] Photolysis of (314) in pentane afforded the diastereoisomeric pentofuranosides (315) and (316) in approximately the same ratio as obtained from photolysis of the α-L-*lyxo* analogue of (314); this result was considered to be evidence in

[446] H. P. Humphries and O. Theander, *Carbohydrate Res.*, 1971, **16**, 317.
[447] P. M. Collins, *J. Chem. Soc.* (*C*), 1971, 1960.
[448] P. M. Collins and P. Gupta, *J. Chem. Soc.* (*C*), 1971, 1965.

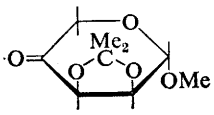
favour of a biradical intermediate. As expected, photolysis of (317) caused decarbonylation and formation of (318). Photolysis procedures have also been used to convert 6-azido-6-deoxy-derivatives of amylose and whole starch into the corresponding 6-aldehydo-derivatives.[449] The aldehydo-derivatives were characterized (*i*) as 2,4-dinitrophenylhydrazones, (*ii*) by reduction with sodium borodeuteride, hydrolysis, acetonation, and mass spectrometry of the resulting [6-^{2}H]1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, and (*iii*) by complete hydrolysis and characterization of the free sugar as the crystalline tetra-*O*-acetyl-D-*gluco*-hexodialdose tetraethyl bis(dithioacetal).



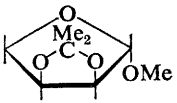
(314)



(315) R^1 = Me, R^2 = H
(316) R^1 = H, R^2 = Me



(317)

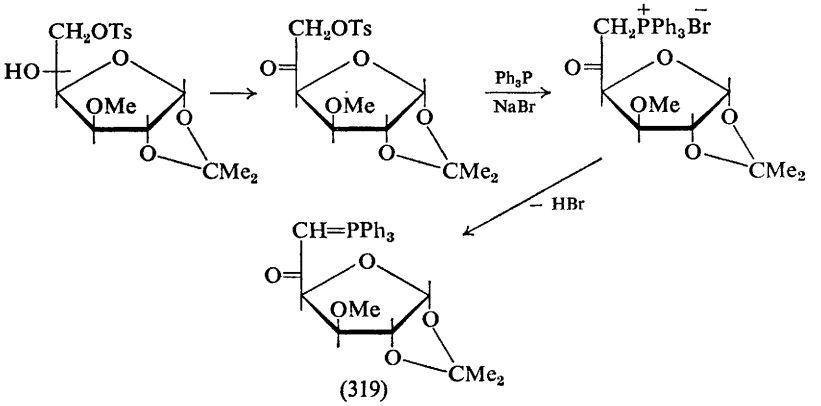


(318)

The preparation and properties of some 3-*O*-methyl-D-*arabino*-hexos-2-ulose derivatives have been described.[450] The utility of the reaction between monosaccharides and benzoylhydrazine for the synthesis of 3-deoxyaldosuloses has been extended[451] (see Vol. 4, p. 110). Thus, 3-deoxy-D-*erythro*- and 3-deoxy-D-*threo*-hexos-2-uloses, 3-deoxy-D-*glycero*-pentos-2-ulose, and 4-hydroxy-2-oxobutyraldehyde were obtained, in about 20% net yields, by heating aqueous solutions of D-glucose, D-galactose, D-xylose, or D-erythrose, respectively, with benzoylhydrazine and *p*-toluidine, followed by decomposition of the resultant 3-deoxyaldos-2-ulose bis(benzoylhydrazones) with benzaldehyde.

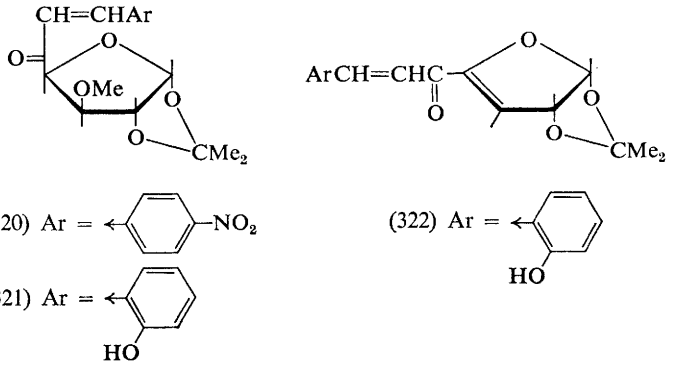
A sugar phosphorane (319) has been prepared by the sequence of reactions illustrated in Scheme 67. Compound (319) reacted with reactive aldehydes such as 4-nitro- and 2-hydroxy-benzaldehyde to afford (320) and (322) [formed from (321)], respectively, but it did not react with 4-dimethylamino-, 4-hydroxy-, or 2,4-dihydroxy-benzaldehyde.[452]

[449] D. M. Clode and D. Horton, *Carbohydrate Res.*, 1971, **17**, 365.
[450] K. Antonakis and M. J. Arvor, *Bull. Soc. chim. France*, 1970, 3010.
[451] H. El Khadem, D. Horton, M. H. Meshreki, and M. A. Nashed, *Carbohydrate Res.*, 1971, **17**, 183.
[452] Yu. A. Zhdanov and V. A. Polenov, *Carbohydrate Res.*, 1971, **16**, 465.



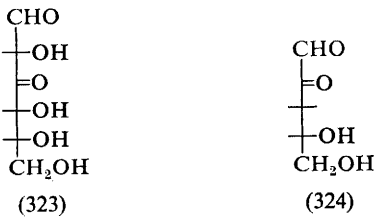
(319)

Scheme 67



The pathway postulated for the formation of saccharinic acids from free sugars was apparently followed when (323) was degraded to (324) in aqueous sodium hydroxide.[453] On prolonged reaction, the products were mainly those resulting from benzylic acid rearrangement.

Some reactions of ulose derivatives, which resulted in the formation of branched-chain sugars, have been described in Chapter 15. In some of the

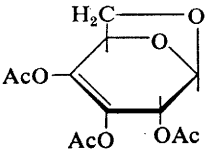


(323) (324)

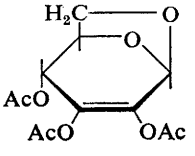
[453] H. P. Humphries and O. Theander, *Acta Chem. Scand.*, 1971, **25**, 883.

examples described, the effect of the stereochemistry of the ulose on the reaction pathway was discussed in detail.[429a, 430, 437]

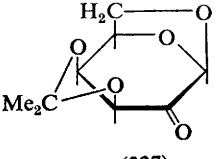
1,6-Anhydro-β-D-hexopyranos-3-uloses [*e.g.* (291) and (293)] have been converted into the corresponding *erythro*-enediol acetates (325) and (326) by treatment with acetic anhydride in pyridine.[454] Similarly, (327) was converted into (328).



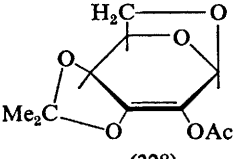
(325)



(326)



(327)



(328)

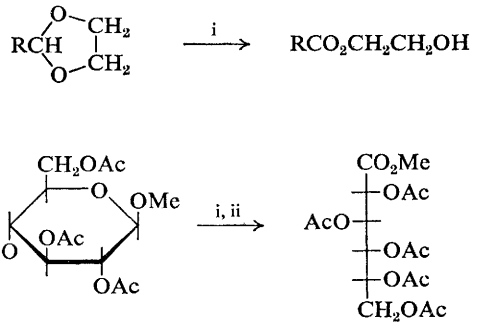
[454] K. Heyns, P. Köll, and H. Paulsen, *Chem. Ber.*, 1971, **104**, 3096.

17
Sugar Acids and Lactones

Aldonic Acids

Refluxing of methylated aldoses in benzene with silver carbonate–Celite caused smooth oxidation to the corresponding aldonolactone ethers. It was tentatively suggested that C-2 substituents are necessary to prevent degradation of the products.[455]

It has been demonstrated that acetals react with ozone to give esters rapidly and quantitatively (Scheme 68), and the suggestion was made that for such compounds as methyl 4,6-*O*-alkylidene-α-D-glucopyranoside, ozonolysis can be used to cleave the acetal ring. In the case of β-glycosides, oxidation to aldonic acid esters occurred, whereas α-analogues were quite inert.[456]



Reagents: i, O_3; ii, Ac_2O–NaOAc

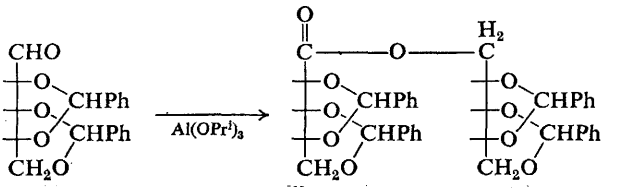
Scheme 68

Di-*O*-benzylidene-*aldehydo*-pentoses have been shown to take part in the Claisen–Tischenko reaction to give 1-deoxypentitol-1-yl pentonate derivatives (Scheme 69).[457]

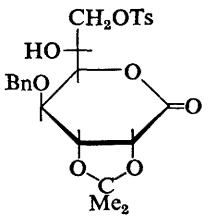
D-Glucono-1,5-lactone has been converted into ethyl 2,3,4,6-tetra-*O*-acetyl-D-gluconate on acetylation with acetic anhydride and pyridine, followed by recrystallization from ethanol.[458] A related reaction has been

[455] S. Morgenlie, *Acta Chem. Scand.*, 1971, **25**, 1154.
[456] P. Deslongchamps and C. Moreau, *Canad. J. Chem.*, 1971, **49**, 2465.
[457] H. Zinner and R. Reck, *Carbohydrate Res.*, 1971, **16**, 459.
[458] A. M. Dempsey and L. Hough, *Carbohydrate Res.*, 1971, **16**, 449.

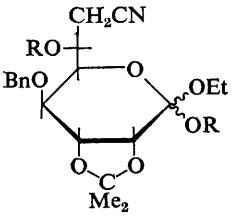
reported for the lactone (329); treatment with potassium cyanide in ethanol, as well as causing direct displacement at C-7, resulted in addition of ethanol at the lactone centre with formation of the orthoacid derivative (330), which was isolated as its acetate (331). Mild deacylation of the acetate (331) gave the hydroxy-compound (330), but more vigorous reaction led to isolation of the aldonate ester (332), which, on the basis of the previous result, would be expected to exist in alcoholic solution, at least to some degree, in the orthoacid form.[459]



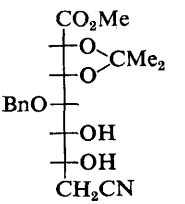
Scheme 69



(329)



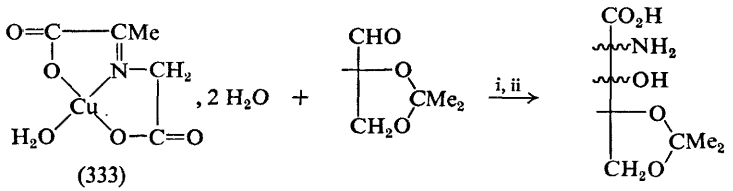
(330) R = H
(331) R = Ac



(332)

The polarography of the Eu^{III} complex of D-gluconic acid has been studied.[460]

2-Amino-2-deoxyaldonic acids have been synthesized by means of a base-catalysed condensation between the glycine derivative (333) and 2,3-*O*-isopropylidene-glyceraldehyde (Scheme 70).[461]



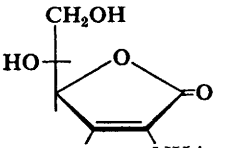
(333)

Reagents: i, pH 9.5; ii, Na_2S

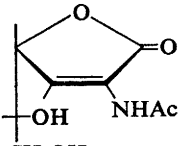
Scheme 70

[459] I. Dijong and U. Wittkötter, *Chem. Ber.*, 1971, **104**, 2806.
[460] Y. Masuda and S. Misumi, *J. Chem. Soc. Japan*, 1971, **92**, 710.
[461] T. Ichikawa, T. Okamoto, S. Maeda, S. Ohdan, Y. Araki, and Y. Ishido, *Tetrahedron Letters*, 1971, 79.

Whereas oxidation of 2-acetamido-2-deoxy-D-mannose and -galactose with bromine gave the expected 1,4-lactones, a comparable product was not obtained in the D-glucose series.[462] Instead, a mixture of 2-amino-2-deoxy-D-gluconic acid, 2-acetamido-2-deoxy-D-glucono-1,5-lactone, 2-acetamido-2-deoxy-D-gluconic acid, and 2-acetamido-2-deoxy-D-mannono-1,4-lactone was formed. Treatment of the 1,4-lactones obtained during these studies with methanolic potassium hydroxide gave equilibrium mixtures of the lactones (334) and (335).



(334)



(335)

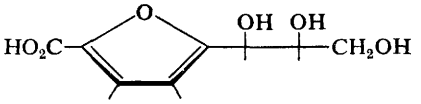
The crystal structures of D-glucono-1,5-lactone and D-gulono-1,4-lactone have been solved (see Chapter 24).

Ulosonic Acids

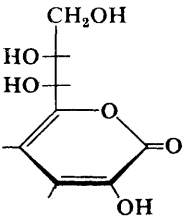
The reaction undergone by methyl 2,3,4,5-tetra-*O*-acetyl-α-L-*xylo*-hex-2-ulopyranosonate in benzene in the presence of boron trifluoride etherate has been examined. Several unsaturated degradation products *en route* to furan derivatives were detected, and the results were correlated with the degradation undergone by L-ascorbic acid under acid conditions.[463] D-*arabino*-Hexulosonic acid has been found as a constituent of a polysaccharide isolated from the fungus *Cyttaria harioti*. Conditions normally used for the complete hydrolysis of polysaccharides caused decarboxylation of this acid with the formation of D-arabinose.[464]

Electrolytic oxidation of di-*O*-isopropylidene-L-sorbose in solutions of alkali metals in the presence of nickel and cobalt salts resulted in its conversion into the L-*xylo*-hexulosonic acid acetal.[465] The behaviour of 3-deoxyaldulosonic acids in acid and alkaline conditions has been examined. Octose compounds underwent retroaldol degradation to give pyruvic acid and pentoses under both sets of conditions and, in addition, acid treatment gave the heterocyclic derivatives (336) and (337).[466]

[462] N. Pravdić and H. G. Fletcher, jun., *Carbohydrate Res.*, 1971, **19**, 339.
[463] K. Goshima, N. Maezono, and K. Tokuyama, *Carbohydrate Res.*, 1971, **17**, 245.
[464] A. F. Cirelli and R. M. De Lederkremer, *Chem. and Ind.*, 1971, 1139.
[465] A. I. Borisov, I. A. Avrutskaya, and M. Ya. Floshin, *Electrokhimiya*, 1970, **6**, 1397.
[466] B. A. Dmitriev, L. V. Backinowsky, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1970, **193**, 1304.



(336)

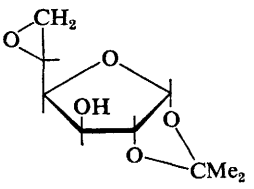


(337)

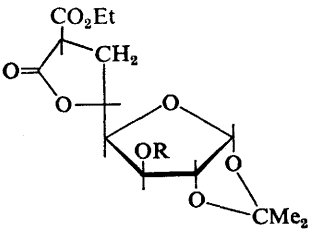
Uronic Acids

Treatment of the oxiran (338) with three molar equivalents of sodioethyl malonate afforded the ester (339) by ring-opening followed by trans-esterification. The reaction illustrates another method by which the carbon chain of sugars can be lengthened and opens a new route to extended-chain uronic acid derivatives.[467]

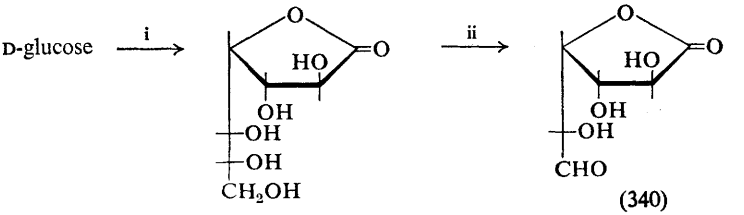
A new method for synthesizing D-idurone (340), required as a reference compound for work on polysaccharides containing iduronic acid, is illustrated in Scheme 71.[468]



(338)



(339) R = OC·CH_2CO_2Et



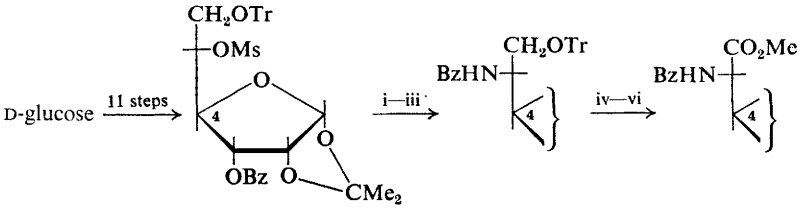
(340)

Reagents: i, HCN; ii, IO_4^-

Scheme 71

Derivatives of 5-amino-5-deoxy-α-D-allofuranuronic acid, the sugar component of the fungal-nucleoside antibiotics (the polyoxins), have been synthesized by the route shown in Scheme 72.[469]

[467] S. Hanessian and P. Dextrase, *Chem. and Ind.*, 1971, 958.
[468] W. Sowa, *Canad. J. Chem.*, 1971, **49**, 1176.
[469] T. Naka, T. Hashizume, and M. Nishimura, *Tetrahedron Letters*, 1971, 95.



Reagents: i, NaN_3–DMF; ii, H_2–Pt; iii, BzCl; iv, H^+–Me_2CO; v, $KMnO_4$; vi, CH_2N_2

Scheme 72

A kinetic study of the esterification and glycosylation of D-galacturonic acid and its α-(1 → 4)-linked di- and tri-saccharide analogues with methanol has been reported. The monomer esterified much more rapidly than the larger molecules.[470]

The 2- and 3-*O*-methyl ethers of methyl (methyl α-D-glucopyranosid)-uronate have been prepared and, together with the 4-*O*-methyl ether and the triether, have been subjected to reactions leading to 4,5-unsaturated derivatives.[471] The conformations and intramolecular hydrogen-bonding patterns of various D-glucuronolactone derivatives have been studied by n.m.r. and i.r. spectroscopy.[472]

Various unsaturated uronic acid derivatives are mentioned in Chapter 14.

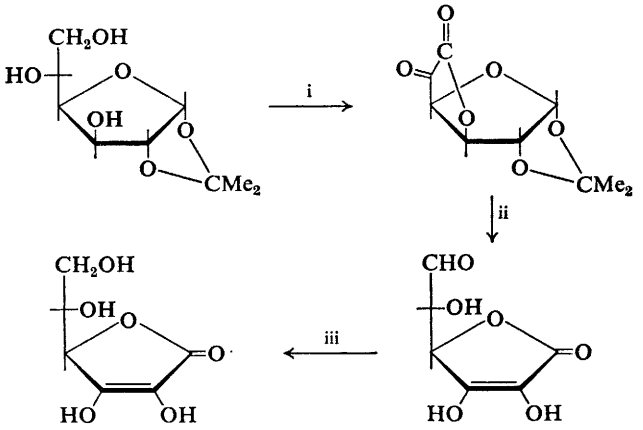
Ascorbic Acid

A shorter and more convenient synthesis of L-ascorbic acid has been described, and represents a significant development in this area (see Scheme 73).[473] The pathway proposed for the acid-catalysed degradation of L-ascorbic acid is outlined in Scheme 74.[474]

The biosynthesis of L-ascorbic acid from *myo*-inositol and other substrates in hen-kidney and rat-liver slices and homogenates has been investigated.[475]

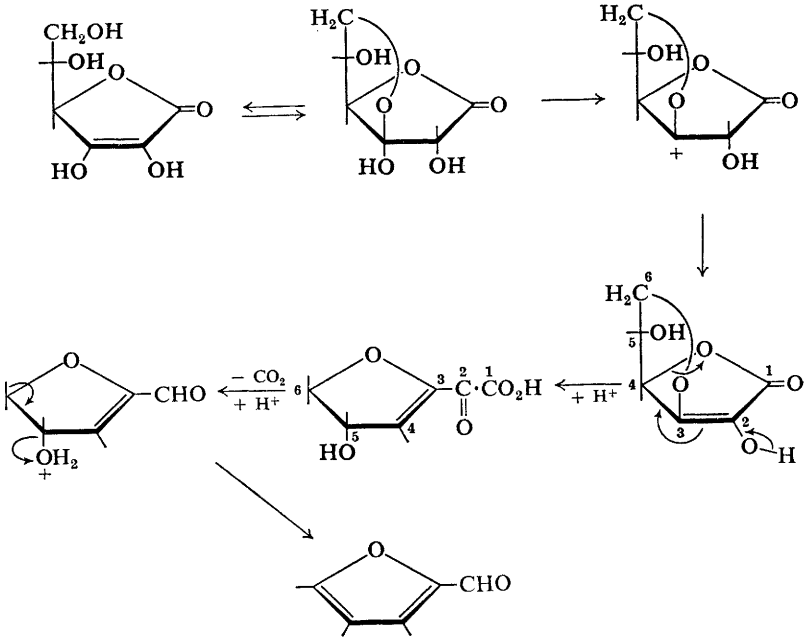
Phosphorylation of L-ascorbic acid or its 5,6-*O*-isopropylidene derivative with phosphoryl chloride in aqueous pyridine gave the 3-phosphates selectively and in high yield.[475a] A detailed kinetic study has been made of the hydrolysis of L-ascorbic acid 3-phosphate under various conditions.[476] It has been proposed that the corresponding 3-sulphate is an important

[470] C. W. Nagel, *Carbohydrate Res.*, 1971, **18**, 453.
[471] H. Hashimoto, T. Sekiyama, H. Sakai, and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1971, **44**, 235.
[472] H. Weidmann and K. Dax, *Monatsh.*, 1971, **102**, 877.
[473] J. Bakke and O. Theander, *Chem. Comm.*, 1971, 175.
[474] K. Tokuyama, K. Goshima, N. Maezono, and T. Maeda, *Tetrahedron Letters*, 1971, 2503.
[475] O. Hänninen, R. Raunio, and J. Marniemi, *Carbohydrate Res.*, 1971, **16**, 343.
[475a] H. Nomura, M. Shimomura, and S. Morimoto, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 1433.
[476] H. Nomura, M. Kuwayama, T. Ishiguro, and S. Morimoto, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 341.



Reagents: i, Pt–O_2 at pH 3—4.5; ii, H_3O^+; iii, $NaBH_4$

Scheme 73



Scheme 74

intermediate in metabolic sulphation. The 3-sulphate may be conveniently prepared by treating 5,6-*O*-isopropylidene-L-ascorbic acid with pyridine sulphate ([^{35}S]-labelled if required) and acetic anhydride, followed by appropriate hydrolysis.[477]

Free radicals produced on oxidation of L-ascorbic acid with Ti^{III}–hydrogen peroxide have been studied by e.s.r. spectroscopy.[478]

The complexes formed between L-ascorbic acid and bivalent metal ions under different conditions have been examined in detail.[479, 480]

I.r. and g.l.c.–mass spectrometric investigations of L-ascorbic acid are noted in Chapter 24.

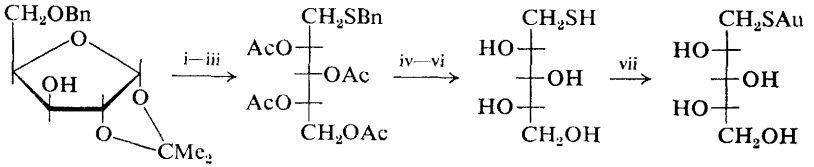
[477] R. O. Mumma, A. J. Verlangieri, and W. W. Weber, sec., *Carbohydrate Res.*, 1971, **19**, 127.
[478] Y. Kirino and T. Kwan, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 718.
[479] O. Wahlberg, *Acta Chem. Scand.*, 1971, **25**, 1045.
[480] P. Ulmgren and O. Wahlberg, *Acta Chem. Scand.*, 1971, **25**, 1000.

18
Inorganic Derivatives

Carbon-bonded Compounds

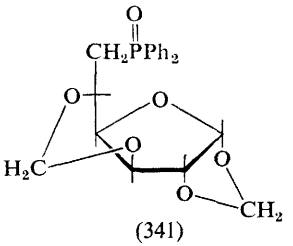
A novel development was the synthesis of a carbohydrate–gold compound (Scheme 75).[481]

Several carbohydrates containing a carbon–phosphorus bond have been synthesized. It has been shown that treatment of sulphonates or oxirans with lithium diphenylphosphine afforded such compounds as (341) and (342).[482] Sugar phosphonates have been mentioned already,[422] and others

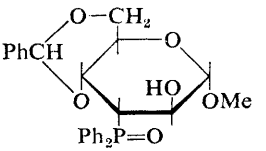


Reagents: i, H_3O^+; ii, $NaBH_4$; iii, Ac_2O–py; iv, Na–NH_3; v, Ac_2O–py; vi, NaOMe; vii, Au^{III}–HBr

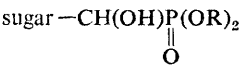
Scheme 75



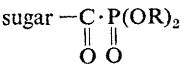
(341)



(342)

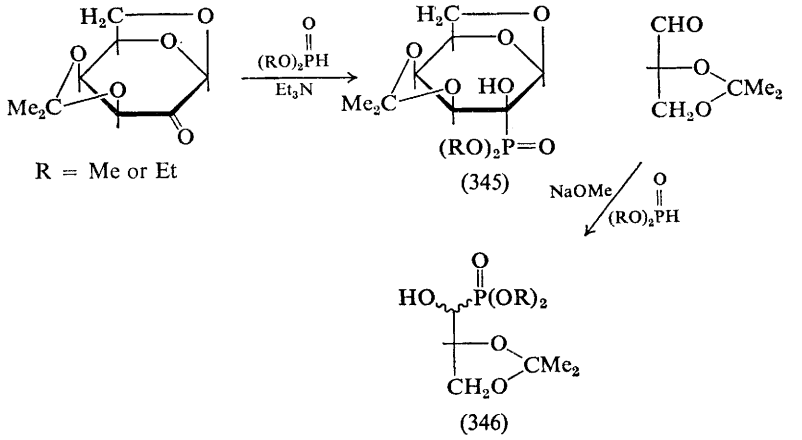


(343)

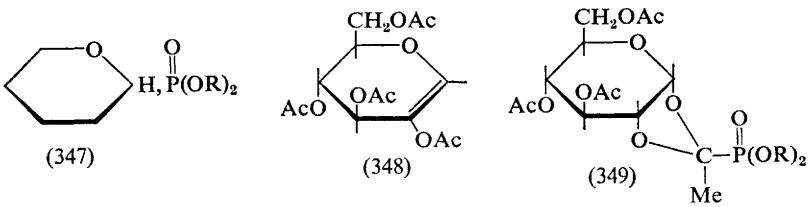


(344)

[481] P. Wirz, J. Staněk, jun., and E. Hardegger, *Helv. Chim. Acta*, 1971, **54**, 2027.
[482] L. D. Hall and P. R. Steiner, *Chem. Comm.*, 1971, 84.



of the types (343) and (344) have been prepared by Russian workers.[483, 484] A series of sugar phosphonates [*e.g.* (345) and (346)] has been obtained by application of the Arabramov reaction.[485] However, attempts to prepare sugar 1-phosphonates of general type (347) were unsuccessful.[486] Treatment of acetobromoglucose with alkylphosphites under the conditions of the Michaelis–Arbuzov reaction gave either the acetylated 2-hydroxyglycal (348) or 1,2-*O*-(1-dialkylphosphonoalkylidene) derivatives (349).



Oxygen-bonded Compounds

D-*glycero*-D-*ido*-Heptonic acid can be conveniently isolated and purified as the double salt cadmium D-*glycero*-D-*ido*-heptonate cadmium chloride monohydrate.[487] The complexes formed between L-ascorbic acid and bivalent metal ions,[480] between cyclitols, sugars, and glycosides and Cd^{II} ions,[66] and between D-gluconic acid and europium(III) compounds [460] have been investigated.

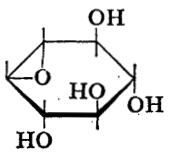
References to molybdate and diphenylborinate derivatives are made in Chapters 25 and 26, respectively.

[483] Yu. A. Zhdanov, L. A. Uzlova, and Z. Glebova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **197**, 1331.
[484] Yu. A. Zhdanov and L. A. Uzlova, *J. Gen. Chem., U.S.S.R.*, 1970, **40**, 2124 (*Zhur. obshchei Khim.*, 1970, **40**, 2138).
[485] H. Paulsen, W. Greve, and H. Kuhne, *Tetrahedron Letters*, 1971, 2109.
[486] H. Paulsen, J. Thiem, and M. Moner, *Tetrahedron Letters*, 1971, 2105.
[487] H. S. Isbell and H. L. Frush, *Carbohydrate Res.*, 1971, **20**, 176.

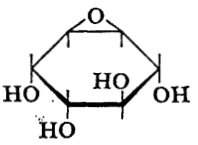
19
Cyclitols

1-*O*-(β-D-Glucopyranosyl)-*scyllo*-inositol and other cyclitol derivatives have been isolated from leaves and branches of *Quercus stenophylla* Makino.[488] *neo*-Inositol has been isolated from calf brain and was identified both as its trimethylsilyl ether and its acetate by g.l.c. and by combined g.l.c.–mass spectroscopy. L-*neo*-Inositol 1-phosphate has been prepared from D-mannose 6-phosphate using an enzyme preparation that converted D-glucose 6-phosphate into L-*myo*-inositol 1-phosphate.[489]

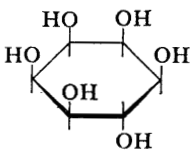
Syntheses of 1-*O*-[^{14}C]methyl-DL-*myo*-inositol and of 5-*O*-[^{14}C]methyl-*myo*-inositol have been described [490] and 1-L-1,2-anhydro-*myo*-inositol (350) and 1-L-1,2-anhydro-*chiro*-inositol (351) have been prepared.[491] *epi*-Inositol (352) has been converted into *cis*-inositol (359) in seven steps in an overall yield of 25%. The key conversion of (355) into (357) was accomplished by reduction of the ketone (356).[492]



(350)

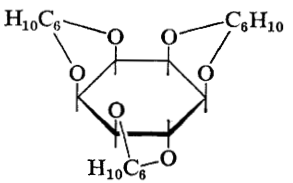


(351)

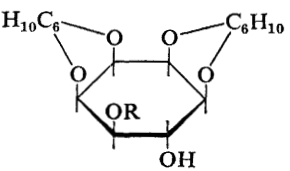


(352)

C_6H_{10} = cyclohexylidenyl

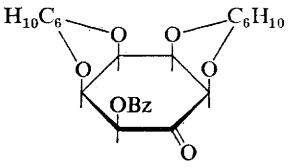


(353)

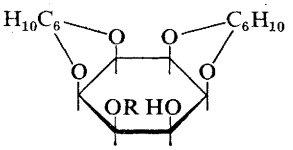


(354) R = H
(355) R = Bz

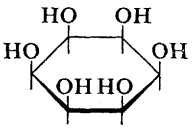
[488] Y. Kamano, Y. Tachi, T. Otake, and M. Komatsu, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 1113.
[489] W. R. Sherman, S. L. Goodwin, and K. D. Gunnell, *Biochemistry*, 1971, **10**, 3491.
[490] R. H. Shah and F. Loewus, *J. Labelled Compounds*, 1970, **6**, 333.
[491] D. Mercier, A. Olesker, S. D. Gero, and J. E. G. Barnett, *Carbohydrate Res.*, 1971, **18**, 227.
[492] S. J. Angyal and R. J. Hickman, *Carbohydrate Res.*, 1971, **20**, 97.



(356)



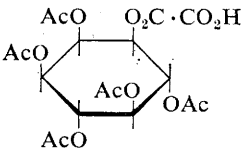
(357) R = Bz
(358) R = H



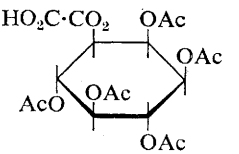
(359)

For the oxidation of *myo*-inositol to various inosose stereoisomers, electrochemical oxidation was more effective than was chemical oxidation. Electrolysis of aqueous solutions of *myo*-inositol using Pt and PbO_2 anodes gave *myo*-inosose, in 5.1—6.3% yield.[493] Results from spectrophotometric and e.s.r. studies of the mechanism of oxidation of 4-inososes with the Somoyi reagent (*J. Biol. Chem.*, 1926, **70**, 599) at 25—55 °C were compatible with a one-electron transfer process. At 90—100 °C, extensive degradation of inososes by this reagent was caused by the generation of transient radicals.[494]

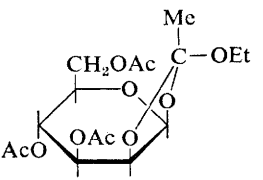
Racemic 1,2,4,5,6-penta-*O*-acetyl-*myo*-inositol has been resolved as the acid oxalate derivatives (360) and (361) using quinidine and (−)α-phenethylamine as resolving agents,[495] and racemic 1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol has been resolved as the diastereoisomeric orthoacetates of D-mannose following transesterification with (362).[496] In connection with the latter study, diastereoisomeric orthoesters were obtained by



(360)



(361)

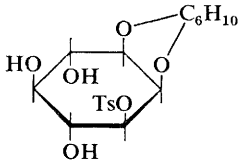


(362)

[493] J. H. Sohn, C. W. Nam, and Y. U. Kim, *Daehan Hwahak Awoejee*, 1971, **15**, 127 (*Chem. Abs.*, 1971, **23**, 141 087).
[494] A. J. Fatiadi, *Carbohydrate Res.*, 1971, **17**, 419.
[495] J. G. Molotkovsky and L. D. Bergelson, *Tetrahedron Letters*, 1971, 4791.
[496] B. A. Klyashchitskii, A. K. Starostina, L. F. Linberg, V. I. Shvets, and R. P. Evstigneeva, *J. Org. Chem. U.S.S.R.*, 1971, **7**, 498 (*Zhur. org. Khim.*, 1971, **7**, 492).

transesterification of the 1,2-*t*-butylorthoacetate of 3,4,6-tri-*O*-acetyl-D-glucopyranose and racemic 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol.[497] Phosphorylation of racemic 1,4,5,6-tetra-*O*-benzyl-*myo*-inositol by phenyl phosphodichloridate in pyridine at 20 °C afforded the 1-*O*-phenyl-, 2-*O*-phenyl-, and 1,2-di-*O*-phenyl-phosphonates.[498] Natural 1-*O*-(1,2-di-*O*-palmitoyl-*sn*-glycerylphosphoryl)-*sn*-*myo*-inositol was also synthesized.

Several studies of selective tosylation of inositol derivatives have been reported. Selective tosylation of 2,3-*O*-cyclohexylidene-*myo*-inositol afforded a monotosylate (363) and three ditosylates (1,4; 1,5; and 1,6).



(363)

More drastic conditions gave three triesters (1,4,5; 1,4,6; and 1,5,6) and a tetratosylate. Thus, it was shown that the most reactive hydroxy-group was at C-1.[499] 4,5,6-Tri-*O*-acetyl-1-*O*-*p*-tolylsulphonyl-*myo*-inositol with tosyl chloride in pyridine afforded 85% of 4,5,6-tri-*O*-acetyl-1,3-di-*O*-*p*-tolylsulphonyl-*myo*-inositol; that is equatorial sulphonylation was preferred to axial sulphonylation. Similarly, 5,6-di-*O*-acetyl-1,4-di-*O*-*p*-tolylsulphonyl-*myo*-inositol afforded 5,6-di-*O*-acetyl-1,3,4-tri-*O*-*p*-tolylsulphonyl-*myo*-inositol. Related selective tosylations were described.[500] Selective tosylations of some partially esterified *myo*-inositols are summarized in Scheme 76,[501] and it was concluded that the order of reactivity is 1-OH, 3-OH > 4-OH, 6-OH > 5-OH > 2-OH.

The crystal structure of *epi*-inositol has been determined.[502]

The complexing of cyclitols with metal ions, particularly Ca^{II}, has been studied. The essential stereochemistry for coupling was shown to be an *ax.*, *eq.*, *ax.* arrangement. Thus, *allo*-, *cis*-, and *epi*-inositol complexed with calcium chloride, whereas *myo*-inositol did not. N.m.r. spectroscopy was used to study complex formation.[66]

Specifically labelled 2-deuterio-derivatives of 3-dehydroquinic acid have been prepared in connection with a study of the shikimate pathway.[503] The preferred conformations of many (−)quinic acid derivatives have been

[497] B. A. Klyashchitskii, A. E. Stepanov, V. I. Shvets, R. P. Evstigneeva, and N. A. Preobrazhenskii, *J. Org. Chem. U.S.S.R.*, 1971, **7**, 494 (*Zhur. org. Khim.*, 1971, **7**, 487).

[498] B. A. Klyashchitskii, E. G. Zhelvakova, V. V. Pimenova, V. I. Shvets, R. P. Evstigneeva, and N. A. Preobrazhensky, *Zhur. obshchei Khim.*, 1971, **41**, 1386.

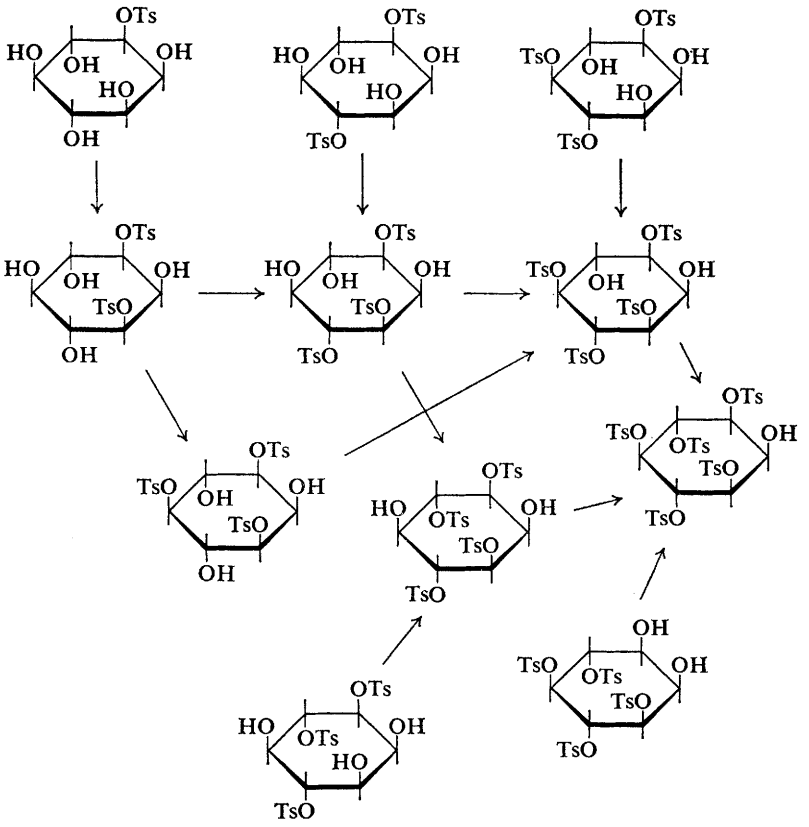
[499] T. Suami, S. Ogawa, T. Tanaka, and T. Otake, *Bull. Chem. Soc. Japan*, 1971, **44**, 835.

[500] T. Suami, S. Ogawa, and S. Oki, *Bull. Chem. Soc. Japan*, 1971, **44**, 2820.

[501] T. Suami, S. Ogawa, and S. Oki, *Bull. Chem. Soc. Japan*, 1971, **44**, 2824.

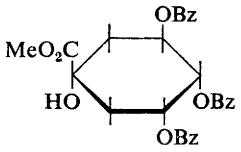
[502] G. A. Jeffrey and H. S. Kim, *Acta Cryst.*, 1971, **27B**, 1812.

[503] E. Haslam, M. J. Turner, D. Sargent, and R. S. Thompson, *J. Chem. Soc.* (*C*), 1971, 1489.

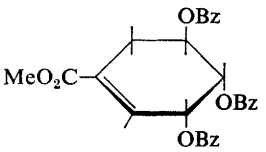


Scheme 76

determined by n.m.r. spectroscopy.[504] A stereospecific conversion of (−)methyl (3,4,5-tri-*O*-benzoyl)quinate (364) into the corresponding (−)shikimate (365) has been accomplished using thionyl chloride in pyridine.[505] Optically pure shikimic acid and quinic acid have been obtained from D-arabinitol by the route illustrated in Scheme 77.[506]

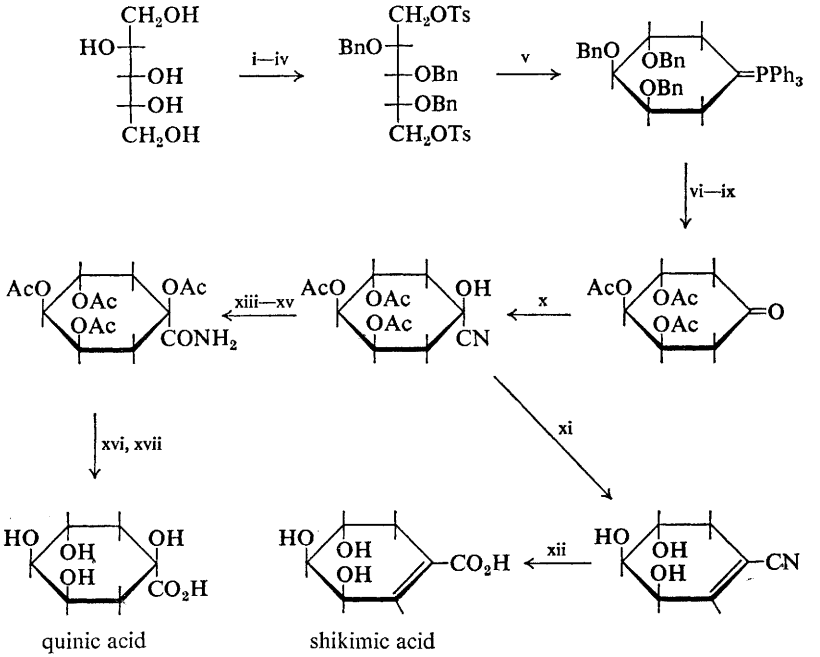


(364)



(365)

[504] E. Haslam and M. J. Turner, *J. Chem. Soc.* (*C*), 1971, 1496.
[505] J. Cléophax, D. Mercier, and S. D. Gero, *Angew. Chem. Internat. Edn.*, 1971, **10**, 652.
[506] H. J. Bestmann and H. A. Heid, *Angew. Chem. Internat. Edn.*, 1971, **10**, 336.



Reagents: i, TrCl–py; ii, BnCl–KOH; iii, 70% AcOH; iv, TsCl–py; v, $Ph_3P{=}CH_2$; vi, HCHO; vii, $Na–NH_3$; viii, Ac_2O–py; ix, OsO_4–$NaIO_4$; x, HCN; xi, $POCl_3$–py; xii, aq. NaOH; xiii, Ac_2O–py; xiv, HBr–AcOH; xv, H_2O; xvi, N_2O_3; xvii, aq. NaOH

Scheme 77

The formation of inositol *p*-bromophenylosotriazoles from inositol phenylosazones has been described.[329]

All the *cis*-cyclopentane-tetraols and -pentaols have been prepared by opening of epoxide rings and from α-bromobenzyloxy-derivatives.[506a] Acyloxonium ions of glycerol and 1,2- and 1,3-cyclopentanetriol underwent rearrangement by fast valence isomerism.[27]

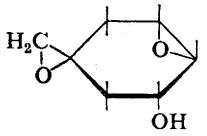
Nitrogen-containing Derivatives

Full details of methods for blocking vicinal hydroxy-amino-groups in aminocyclitols have been given [301a] (see Vol. 4, p. 64).

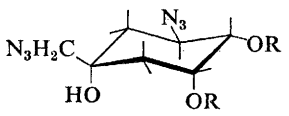
The diepoxide 1-D-1,2:5,7-dianhydro-5-hydroxymethylcyclohexane-1,2,3,5/O-tetraol (366), obtained from (−)quinic acid, is a useful intermediate for the synthesis of nitrogen-containing cyclitols. Treatment of (366) with sodium azide afforded (367) and (369), whose structures were

[506a] F. G. Cocu and T. Posternak, *Helv. Chim. Acta*, 1971, **54**, 1676.

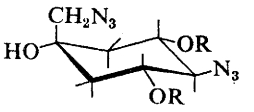
established by n.m.r. analysis of the respective dibenzoates (368) and (370).[507] *scyllo*-, *chiro*-1-, *myo*-2-, *myo*-4-, *allo*-5-, *neo*-1-, and *chiro*-3-inosamines have been prepared by treating the appropriate bromodeoxyinositols with sodium azide and reduction of the resulting azido-derivatives.[508] The bromodeoxyinositols were prepared by reaction of the appropriate inositols with bromine in acetic anhydride.[509]



(366)



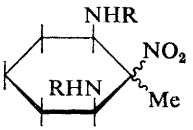
(367) R = H
(368) R = Bz



(369) R = H
(370) R = Bz

Further details have been given of the conversion of 1,3-di-*O*-acetyl- and 1,3-di-*O*-methylsulphonyl-2-nitroinositol derivatives into 1,3-diamino-2-nitroinositol derivatives [510] (see Vol. 3, p. 148). Treatment of glutaraldehyde with nitroethane in the presence of amines also afforded such products as (371).

A series of 9-adenyl-deoxyinositols have been prepared from inosamines as illustrated in Scheme 78.[511]

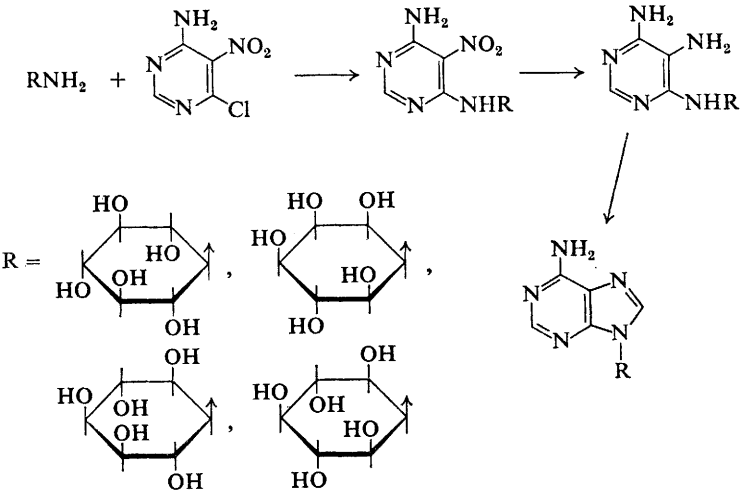


(371)

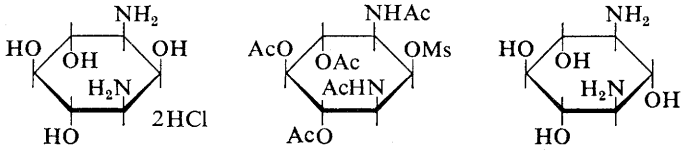
Synthesis of the hexa-acetate of streptamine (374) from (372) was accomplished by selective acetylation of amino- and equatorial hydroxy-groups, mesylation to give (373), followed by configurational inversion with sodium acetate in 2-methoxyethanol.[512] Compound (373) was also converted into triaminoinositol derivatives. Such glucopyranosyl-deoxystreptamines as 4- and 6-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystreptamine have been prepared;[513] the former compound showed antibacterial activity whereas the latter did not.

[507] D. Mercier, J. Leboul, J. Cléophax, and S. D. Gero, *Carbohydrate Res.*, 1971, **20**, 299.
[508] T. Suami, S. Ogawa, and M. Uchida, *Bull. Chem. Soc. Japan*, 1970, **43**, 3577.
[509] T. Suami, S. Ogawa, K. Yabe, and M. Uchida, *Bull. Chem. Soc. Japan*, 1971, **44**, 2804.
[510] T. Nakagawa, T. Sakakibara, and F. W. Lichtenthaler, *Bull. Chem. Soc. Japan*, 1970, **43**, 3861.
[511] T. Suami, Y. Fukai, Y. Sakota, M. Karimoto, N. Takoi, and Y. Tsukamoto, *Bull. Chem. Soc. Japan*, 1971, **44**, 1695.
[512] T. Suami, S. Ogawa, H. Sano, and N. Kato, *Bull. Chem. Soc. Japan*, 1971, **44**, 1992.
[513] Y. Nishimura, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1971, **44**, 2521.

Several aminocyclopentanetetraols have been synthesized from the known 2,3-*O*-cyclohexylidene-5-nitro-1,2,3,4-cyclopentanetetraol by hydrogenation and appropriate configurational inversions.[514]



Scheme 78



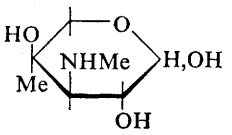
(372) (373) (374)

[514] T. Suami, Y. Sakota, K. Tadano, and S. Nishiyama, *Bull. Chem. Soc. Japan*, 1971, **44**, 2222.

20
Antibiotics

A selective review of sugars found in antibiotics has been published.[515]

The majority of the work reported this year has been in the area of aminoglycoside antibiotics, and several novel structures have been elucidated. Garosamine, the common monosaccharide component of the gentamicin C complex, has been shown to be 3-deoxy-4-*C*-methyl-3-methylamino-L-arabinose (375).[516, 517]



(375)

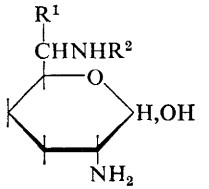
Mercaptolysis of *N*-acetylated gentamicin C components, namely C_1, C_2, and C_{1a}, gave, as their diethyl dithioacetals, three new sugars (purpurosamines A, B, and C, respectively), which form a new class of naturally occurring 2,6-diamino-2,3,4,6-tetradeoxy-sugars (376), (377), and (378).[518] Synthetic studies have shown that compound (378) [and by inference (376) and (377)] has the D-*erythro* configuration.[315] Methanolysis of gentamicin C components gave, in addition to methyl garosaminide, three pseudodisaccharides named gentamines C_1, C_2, and C_{1a}, respectively, which were shown to be derivatives of (376), (377), and (378) linked to the 4-position of 2-deoxystreptamine. Exhaustive methylation of per-*N*-acetylated gentamicins gave (379) after hydrolysis, thereby establishing a 4,6-linkage to the two sugars in the complete antibiotic.[519]

A new aminoglycoside antibiotic, originally named[520] '6640', but

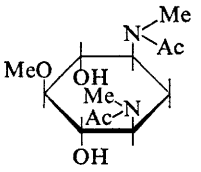
[515] J. S. Brimacombe, *Angew. Chem. Internat. Edn.*, 1971, **10**, 236.
[516] D. J. Cooper, M. D. Yudis, R. D. Guthrie, and A. M. Prior, *J. Chem. Soc.* (*C*), 1971, 960.
[517] W. Meyer zu Reckendorf and E. Bischof, *Angew. Chem. Internat. Edn.*, 1971, **10**, 660.
[518] D. J. Cooper, M. D. Yudis, H. M. Marigliano, and T. Traubel, *J. Chem. Soc.* (*C*), 1971, 2876.
[519] D. J. Cooper, P. J. L. Daniels, M. D. Yudis, H. M. Marigliano, R. D. Guthrie, and S. T. K. Bukhari, *J. Chem. Soc.* (*C*), 1971, 3126.
[520] M. J. Weinstein, J. A. Marquez, R. T. Testa, G. H. Wagman, E. M. Oden, and J. A. Waitz, *J. Antibiotics*, 1970, **23**, 551, 555.

subsequently re-named sisomicin, has been shown to have the partial structure (380).[521, 522]

Several deoxykanamycins and related compounds have been described, including 3′-deoxy-[523] and 3′,4′-dideoxy-kanamycin B.[524] 3′,4′-Dideoxy-



(376) R^1 = Me, R^2 = Me
(377) R^1 = Me, R^2 = H
(378) R^1 = H, R^2 = H

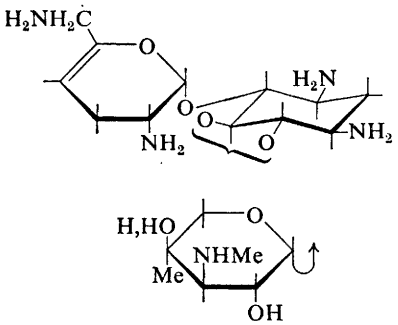


(379)

neamine has also been prepared by means of a Tipson–Cohen reaction;[525] this compound is in fact gentamine C_{1a} (see above). Other kanamycin analogues have been synthesized.[513]

Two other new aminoglycoside antibiotics, butirosins A and B, have been shown to have structures (381) and (382), respectively.[526–528]

Stretomyces lividus gave rise to four aminoglycoside antibiotics 2230-C, 2230-D (identical to paramomycin), and lividomycins A and B.[529] Livido-

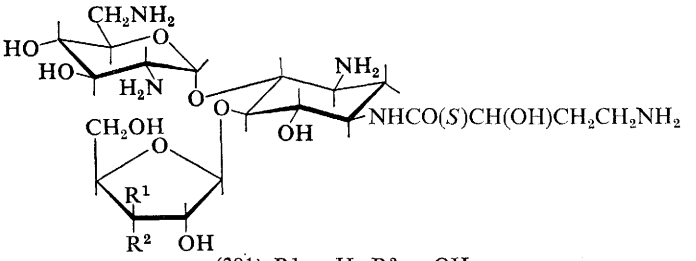


(380)

[521] D. J. Cooper, R. S. Jaret, and H. Reimann, *Chem. Comm.*, 1971, 285.
[522] H. Reimann, R. S. Jaret, and D. J. Cooper, *Chem. Comm.*, 1971, 924.
[523] S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 274.
[524] H. Umezawa, S. Umezawa, T. Tsuchiya, and Y. Okazaki, *J. Antibiotics*, 1971, **24**, 485.
[525] S. Umezawa, T. Tsuchiya, T. Jikihara, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 711.
[526] P. W. K. Woo, H. W. Dion, and Q. R. Bartz, *Tetrahedron Letters*, 1971, 2617.
[527] P. W. K. Woo, *Tetrahedron Letters*, 1971, 2621.
[528] P. W. K. Woo, H. W. Dion, and Q. R. Bartz, *Tetrahedron Letters*, 1971, 2625.
[529] T. Mori, T. Ichiyanagi, H. Kondo, K. Tokunaga, T. Oda, and K. Munakata, *J. Antibiotics*, 1971, **24**, 339.

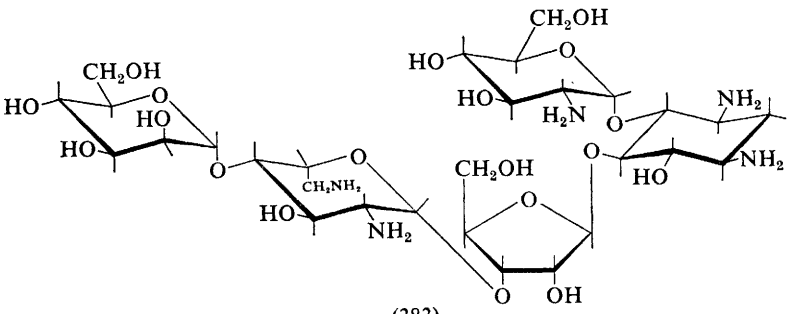
mycin A has been shown to have structure (383),[530, 531] *i.e.* 'α-D-mannosyl-deoxyparamomycin'.

A new family of antibiotics, the validamycins, has been shown to contain the novel cyclitols validatol (384), deoxyvalidatol (385), hydroxyvalidamine (386), and validamine (387).[532] The structure of the latter compound has been confirmed by *X*-ray analysis.[533]

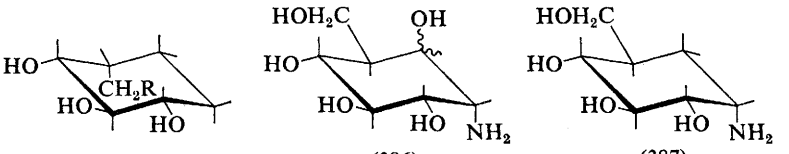


(381) R^1 = H, R^2 = OH
(382) R^1 = OH, R^2 = H

(*S*) refers to the configuration at the carbon centre



(383)



(384) R = OH
(385) R = H

(386)

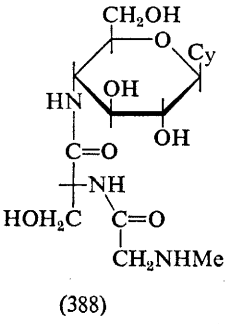
(387)

[530] T. Oda, T. Mori, and Y. Kyōtani, *J. Antibiotics*, 1971, **24**, 503.
[531] T. Oda, T. Mori, Y. Kyōtani, and M. Nakayama, *J. Antibiotics*, 1971, **24**, 511.
[532] S. Horii, T. Iwasa, E. Mizuta, and Y. Kameda, *J. Antibiotics*, 1971, **24**, 61.
[533] K. Kamiya, Y. Wada, S. Horii, and M. Nishikawa, *J. Antibiotics*, 1971, **24**, 317.

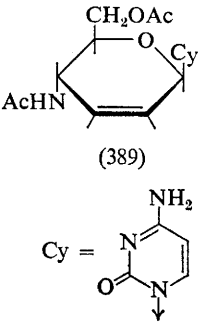
Mycosamine has been found as a component of candidin, a polyene macrolide antibiotic.[534] Hikizimycin has been shown to contain cytosine and 3-amino-3-deoxy-D-glucose (kanosamine).[535] The antiviral antibiotic tunicamycin was shown to contain 2-amino-2-deoxy-D-glucose as a component.[536] Methyl sibirosaminide, isolated after methanolysis of sibiromycin, is a new branched-chain 2-deoxy-2-methylamino-sugar of undisclosed structure.[537]

Pertrimethylsilyl or per-*N*-trifluoroacetyltrimethylsilyl derivatives of eighteen aminoglycoside antibiotics were separable by g.l.c.,[537a] and mass-spectral studies have also been made on aminoglycoside antibiotics.[538]

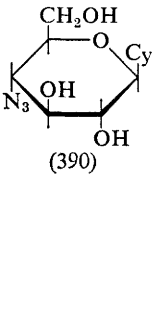
The gougerotin analogue (388) has been synthesized.[539] The degradation product (389) of blasticidin S has been prepared from (390) by standard sequences.[540]



(388)



(389)



(390)

The nucleoside antibiotic formycin B (391) has been synthesized[541] and cordycepin has been prepared by the route outlined in Scheme 79.[542]

The structures (392) of the macrolide antibiotics SF-837A_2, A_3, and A_4 have been elucidated; each contains mycaminose and *O*-acylmycarose residues.[543–545]

[534] E. Borowski, L. Falkowski, J. Golik, J. Zielinski, T. Ziminski, W. Mechlinski, E. Jereczek, P. Kolodziejczyk, H. Adlercreutz, C. P. Schaffner, and S. Neelakantan, *Tetrahedron Letters*, 1971, 1987.

[535] K. Uchida, T. Ichikawa, Y. Shimauchi, T. Ishikura, and A. Ozaki, *J. Antibiotics*, 1971, **24**, 259.

[536] A. Takatsuki, K. Arima, and G. Tamura, *J. Antibiotics*, 1971, **24**, 215.

[537] A. S. Mezentsev, V. V. Kulyaeva, and L. M. Rubasheva, *Antibiotikii*, 1971, **16**, 867.

[537a] S. Omoto, S. Inouye, and T. Niida, *J. Antibiotics*, 1971, **24**, 430.

[538] P. J. L. Daniels, M. Kugelman, A. K. Mallams, R. W. Tkach, H. F. Vernay, J. Weinstein, and A. Yehaskel, *Chem. Comm.*, 1971, 1629.

[539] F. W. Lichtenthaler, G. Trummlitz, G. Bambach, and I. Rychlik, *Angew. Chem. Internat. Edn.*, 1971, **10**, 334.

[540] K. A. Watanabe, I. Wempen, and J. Fox, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 2368.

[541] E. M. Acton, K. J. Ryan, D. W. Henry, and L. Goodman, *Chem. Comm.*, 1971, 986.

[542] K. L. Nagpal and J. P. Horwitz, *J. Org. Chem.*, 1971, **36**, 3743.

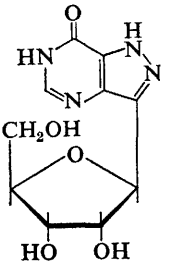
[543] T. Niida, T. Tsuruoka, N. Ezaki, T. Shomura, E. Akita, and S. Inouye, *J. Antibiotics*, 1971, **24**, 319.

[544] T. Tsuruoka, S. Inouye, T. Shomura, N. Ezaki, and T. Niida, *J. Antibiotics*, 1971, **24**, 526.

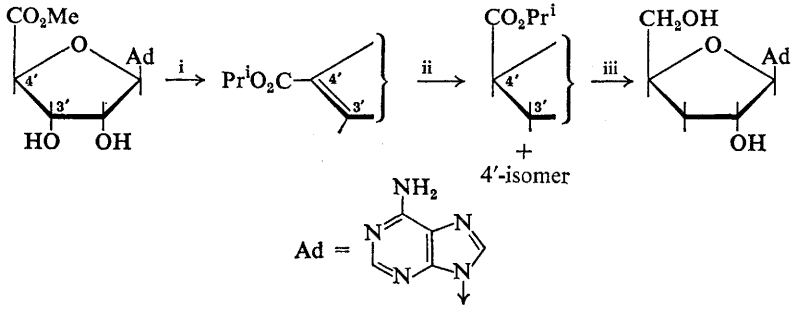
[545] S. Inouye, T. Tsuruoka, T. Shomura, S. Omoto, and T. Niida, *J. Antibiotics*, 1971, **24**, 460.

Evertetrose and everninonitrose, the degradation products of everninomicin, have been shown to have structures (393) and (394), respectively.[546]

Clindamycin has been converted into the 3-phosphate, and into 3-phosphate diesters linking the antibiotic to 5′-ribosylnucleotides by several *Streptomyces* species; the products had no antibacterial activity.[547] Experiments aimed at the synthesis of *N*-methyl and *NN*-dimethyl derivatives of methyl α-thiolincosaminide have been described.[548]



(391)



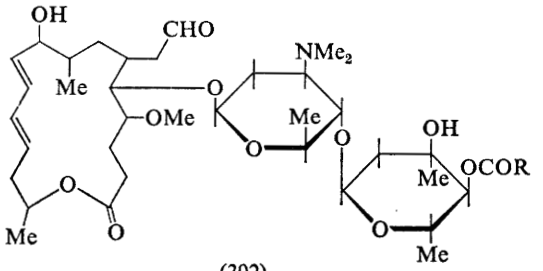
Reagents: i, Pr^iOH–Pr^iONa; ii, Pd–C/H_2; iii, $(MeOCH_2CH_2O)_2LiAlH_2$

Scheme 79

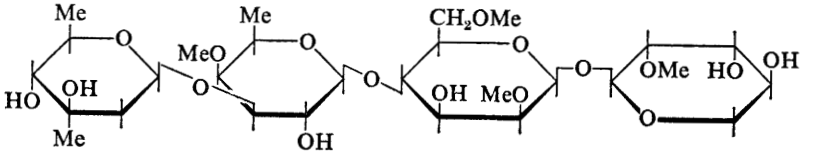
6-Deoxy-2-*O*-methyl-L-mannose was found as a component of scopamycin A.[549] The sugar components of SF-666A and SF-666B have been shown to be 7-deoxy-D-*glycero*-D-*gluco*-heptose and 7-deoxy-D-*altro*-heptulose, respectively.[16] Degradative studies on A.396.I suggested that it is the de-*N*-methyl analogue of hygromycin B.[550]

The structure of curacin, the carbohydrate ester isolated from such antibiotics as curamycin and everninomicins B and D, has been confirmed. Condensation of (395) and (396) gave (397), after acid treatment, which was formed also on methylation and glycosidation of curacin.[551]

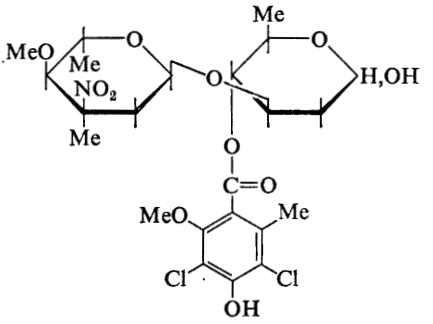
[546] A. K. Ganguly, O. Z. Sarre, and S. Szmulewicz, *Chem. Comm.*, 1971, 746.
[547] A. D. Argoudelis and J. H. Coates, *J. Amer. Chem. Soc.*, 1971, **93**, 534.
[548] B. J. Magerlein, *J. Org. Chem.*, 1971, **36**, 596.
[549] J. B. McAlpine, J. W. Corcoran, and R. S. Egan, *J. Antibiotics*, 1971, **24**, 51.
[550] J. Shoji and Y. Nakagawa, *J. Antibiotics*, 1970, **23**, 569.
[551] E. G. Gros and E. M. Gruneiro, *J. Org. Chem.*, 1971, **36**, 1166.



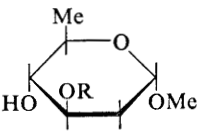
(392)



(393)

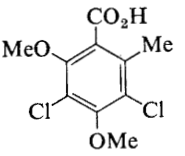


(394)

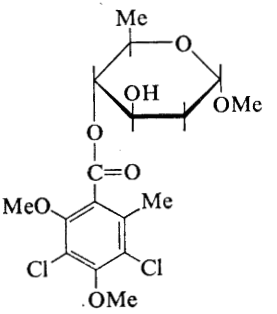


(395)

R = tetrahydropyranyl



(396)



(397)

The structure of nystatin A, an antibiotic containing mycaminose, has been elucidated.[552] Tetrin A and B were found to contain mycosamine as a component,[553] and this amino-sugar, in its β-pyranose form, also occurs in amphotericin B.[284]

[552] E. Borowski, J. Zielinski, L. Falkowski, T. Ziminski, J. Golik, P. Kolodziejczyk, E. Jereczek, M. Gdulewicz, Y. Shenin, and T. Kotienko, *Tetrahedron Letters*, 1971, 685.

[553] (*a*) R. C. Pandey, V. F. German, Y. Nishikawa, and K. L. Rinehart, *J. Amer. Chem. Soc.*, 1971, **93**, 3738; (*b*) R. C. Pandey, V. F. German, Y. Nishikawa, and K. L. Rinehart, *J. Amer. Chem. Soc.*, 1971, **93**, 3747.

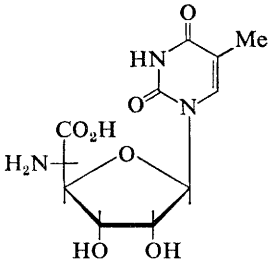
21
Nucleosides

A review (in Japanese) of the chemistry of nucleosides has been published.[554] 9-α-D-Ribofuranosyladenine (*i.e.* α-adenosine) has been isolated from a natural source, *Propionibacterium shermanii*.[555]

Synthesis

Amongst compounds which have been synthesized by conventional procedures are: 1-β-D-allopyranosyl-uracil and -cytosine,[556] 1-(2-deoxy-α-D-*ribo*-hexofuranosyl)uracil,[557] 1-(5-amino-5-deoxy-β-D-allofuranuronosyl)thymine[558] (398; thymine polyoxin C), 3-cyano-2-(β-D-ribofuranosyl)-imidazole,[559] 5-formyl-, 5-hydroxymethyl-, and 5-benzoyloxymethyl-uracil,[560] pyrazolopyrimidine nucleosides related to formycin[561] and tubercidin,[562] and a new tricyclic nucleoside 6-amino-4-methyl-8-(β-D-ribofuranosyl)-(4-*H*,8-*H*)pyrrolo-[4,3,2-*de*]pyrimido-[4,5-*c*]pyridazine.[563] The β-D-ribosides of *N*-(purin-6-ylcarbamoyl)-L-threonine and *N*-(purin-6-ylcarbamoyl)glycine,[564] 1-β-L-arabinofuranosylcytosine and O^2,$O^{2'}$-anhydro-1-(β-L-arabinofuranosyl)cytosine,[565] 6-selenoguanosine, 6-methylselenoguanine, 6-methylselenoguanosine, and 6-methylselenoinosine,[566] L-lysyl and L-glutamyl derivatives of 1-(3-amino-3-deoxy-β-D-glucopyranosyl)uracil,[567] the isomeric *N*-ribosyl-8-azahypoxanthines,[568] the nucleoside antibiotic formycin B,[541] and blasticidin S[540] have also been prepared.

[554] Y. Mizuno, T. Nomura, and M. Ito, *Kagaku No Ryoiki, Zokan*, 1971, **94**, 179 (*Chem. Abs.*, 1971, **75**, 20 808w).
[555] F. Dinglinger and P. Renz, *Z. physiol. Chem.*, 1971, **352**, 1157.
[556] D. H. Warnock, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1971, **18**, 127.
[557] S. David and J. Eustache, *Carbohydrate Res.*, 1971, **16**, 469.
[558] H. Ohrui, H. Kuzuhara, and S. Emoto, *Tetrahedron Letters*, 1971, 4267.
[559] G. R. Revankar and L. B. Townsend, *J. Heterocyclic Chem.*, 1970, **7**, 1329.
[560] M. P. Mertes and M. T. Shipchandler, *J. Heterocyclic Chem.*, 1971, **8**, 133.
[561] R. A. Long, A. F. Lewis, R. K. Robins, and L. B. Townsend, *J. Chem. Soc.* (*C*), 1971, 2443.
[562] G. R. Revankar and L. B. Townsend, *J. Chem. Soc.* (*C*), 1971, 2440.
[563] K. H. Schram and L. B. Townsend, *Tetrahedron Letters*, 1971, 4757.
[564] G. B. Chheda and C. I. Hong, *J. Medicin. Chem.*, 1971, **14**, 748.
[565] D. T. Gish, G. L. Neil, and W. J. Wechter, *J. Medicin. Chem.*, 1971, **14**, 882.
[566] S.-H. Chu, *J. Medicin. Chem.*, 1971, **14**, 254.
[567] H. A. Friedman, *J. Medicin. Chem.*, 1971, **14**, 174.
[568] J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 1971, **36**, 1962.



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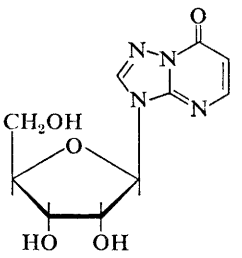


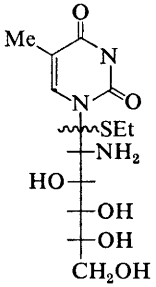
(399)

Other nucleoside analogues that have been prepared include the inosine analogue (399) (the first example of a nucleoside with a bridgehead nitrogen atom),[569] some *N*-glycofuranosyluracils,[335] 3-deaza-analogues of orotidine,[570] and homoadenosine (which was obtained starting from 1-amino-2,5-anhydro-1-deoxy-D-allitol).[571] 5′-Amido-analogues of cyclic adenosine monophosphate have been reported,[572] and 9-[2-deoxy-5-*O*-(2-hydroxyethyl)-α-D-*erythro*-pentofuranosyl]adenine and its β-anomer,[150] 9-β-D-xylofuranosylthioguanine, and 9-α- and 9-β-D-arabinofuranosylthioguanine[573] have been prepared.

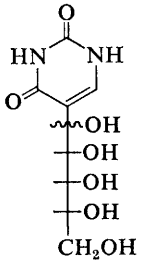
The first thiazolo[5,4-*d*]pyrimidine nucleosides have been reported.[574] Among syntheses utilizing trifluoroacetyl protecting groups were the preparation of pyrimidine nucleosides of the furanose forms of 2-amino-2-deoxy-D-xylose[575] and 2-amino-2-deoxy-D-glucose.[576] Pyrimidine nucleosides of 2-amino-2-deoxy-D-galactopyranose were obtained by condensation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-D-galactopyranose with bis(trimethylsilyl)-cytosine and -thymine, followed by removal of the protecting groups.[577] Epimeric C-1 acyclic nucleoside analogues [*e.g.* (400)] have been isolated as their hydrochlorides following syntheses in which trifluoroacetate was used as the N-protecting group.[578] Other acyclic nucleosides such as (401) have been prepared.[130]

9-α- and 9-β-Fucopyranosyladenine have been prepared by coupling 1,2,3,4-tetra-*O*-acetyl-α- and -β-D-fucopyranose with 6-benzamidochloromercuripurine by the titanium chloride procedure, followed by removal of protecting groups and separation of the anomers.[579] Arabinosylcytosine

[569] M. W. Winkley, G. F. Judd, and R. K. Robins, *J. Heterocyclic Chem.*, 1971, **8**, 237.
[570] B. L. Currie, M. J. Robins, and R. K. Robins, *J. Heterocyclic Chem.*, 1971, **8**, 221.
[571] J. Farkaš, *Coll. Czech. Chem. Comm.*, 1971, **36**, 3043.
[572] A. Murayama, B. Jastorff, F. Cramer, and H. Hettler, *J. Org. Chem.*, 1971, **36**, 3029.
[573] W. W. Lee, A. P. Martinez, R. W. Blackford, V. J. Bastuska, E. J. Reist, and L. Goodman, *J. Medicin. Chem.*, 1971, **14**, 819.
[574] C. L. Schmidt, W. J. Rusho, and L. B. Townsend, *Chem. Comm.*, 1971, 1515.
[575] M. L. Wolfrom and P. J. Conigliaro, *Carbohydrate Res.*, 1971, **20**, 391.
[576] M. L. Wolfrom, P. J. Conigliaro, and H. B. Bhat, *Carbohydrate Res.*, 1971, **20**, 383.
[577] M. L. Wolfrom, H. B. Bhat, and P. J. Conigliaro, *Carbohydrate Res.*, 1971, **20**, 375.
[578] M. L. Wolfrom and P. J. Conigliaro, *Carbohydrate Res.*, 1971, **20**, 369.
[579] L. M. Lerner, *Carbohydrate Res.*, 1971, **19**, 255.



(400)



(401)

3-*N*-oxide resulted from oxidation of the nucleoside with 3-chloroperbenzoic acid.[580] 5′-Deoxy-5′-halogeno-cytidine and -adenosine have been obtained by treating the parent nucleoside with a solution of thionyl chloride or thionyl bromide in HMPT.[581] Condensation of 2,3:5,6-di-*O*-isopropylidene-D-allofuranosyl chloride with 6-benzamidochloromercuripurine afforded 9-α- and 9-β-allofuranosyladenine.[582] A number of papers have described syntheses of nucleoside analogues by methods which involved coupling of suitably protected glycosyl halides with trimethylsilylated pyrimidines and related compounds.[386, 583–585] A detailed analysis of the aluminium trichloride-catalysed reaction of D-xylo-, D-arabino-, and D-ribo-furanosyl penta-acetates with N^2-nonanyl- and palmitoyl-guanine has been reported.[586] 9-Substituted products preponderated and the anomers with 1′,2′-*trans* relationships were usually formed in the greatest amounts, but appreciable proportions of the 1′,2′-*cis*-isomers were also obtained. Acylated D-ribose, D-glucose, and acylated methyl glycosides of D-ribofuranose, 2-deoxy-D-*erythro*-pentofuranose, and L-arabinofuranose on treatment with 4-amino-5-nitropyrimidines in the presence of acid catalysts gave the corresponding 5-nitro-4-(acylglycosylamino)pyrimidines. The effects of catalysts on the yields of anomers were investigated.[587]

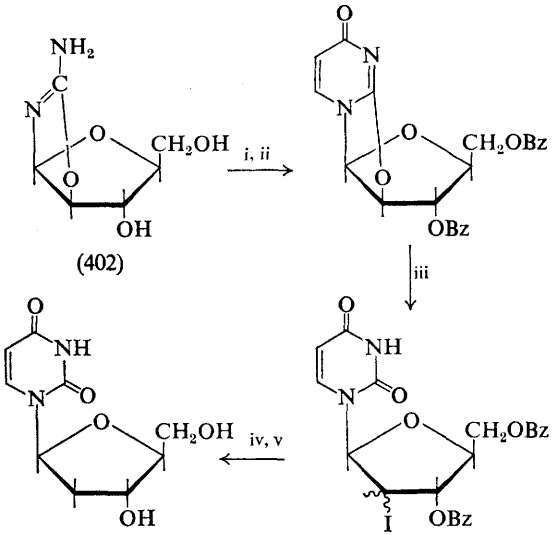
3-Hydroxypyridazine derivatives of L-arabinofuranose have been prepared and were shown to possess mainly the α-configuration. The major product was the *O*-glycoside, which was isomerized to the *N*-glycoside by silver bromide in toluene.[588]

Treatment of pyrimidine nucleosides with purines in the presence of such catalysts as mercuric bromide provided a useful application of a

[580] R. P. Panzica, R. K. Robins, and L. B. Townsend, *J. Medicin. Chem.*, 1971, **14**, 259.
[581] K. Kikugawa and M. Ichino, *Tetrahedron Letters*, 1971, 87.
[582] B. Kohn, P. Kohn, and A. Dubin, *Carbohydrate Res.*, 1971, **18**, 349.
[583] R. P. Panzica and L. B. Townsend, *J. Org. Chem.*, 1971, **36**, 1594.
[584] W. Pfleiderer and M. Schranner, *Chem. Ber.*, 1971, **104**, 1915.
[585] V. S. Gupta and G. L. Bubbar, *Canad. J. Chem.*, 1971, **49**, 719.
[586] W. W. Lee, A. P. Martinez, and L. Goodman, *J. Org. Chem.*, 1971, **36**, 842.
[587] H. Rokos and W. Pfleiderer, *Chem. Ber.*, 1971, **104**, 748.
[588] D. Heller and G. Wagner, *Z. chem.*, 1971, **11**, 105.

transglycosylation method for the synthesis of purine nucleosides.[589] The preparation and $S \rightarrow N$-migration of 2-deoxy-D-*erythro*-pentofuranose derivatives of 3-mercaptopyridazine have been studied.[590] 2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl cyanide has been converted into *C*-imidazole nucleosides and, thence, into purine derivatives.[591]

A new synthesis of 2′-deoxy-L-uridine has been described[592] and is illustrated in Scheme 80. 1-β-L-Arabinofuranosylcytosine (403) has been synthesized.[593] Condensation of L-arabinose with cyanamide afforded (402), which on treatment with cyanoacetylene and direct hydrolysis with ammonium hydroxide gave (403).

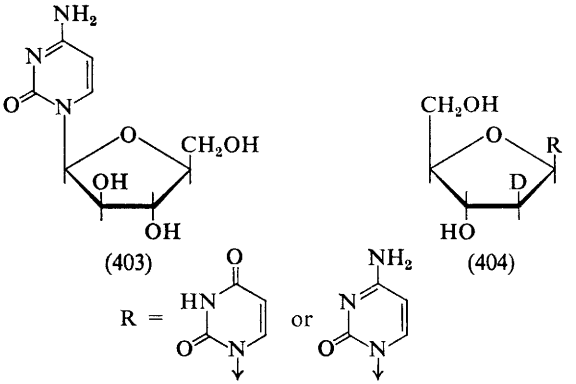


Reagents: i, $HC{\equiv}CCO_2Me$; ii, BzCN; iii, LiI–H^+; iv, Bu^n_3SnH; v, OH^-

Scheme 80

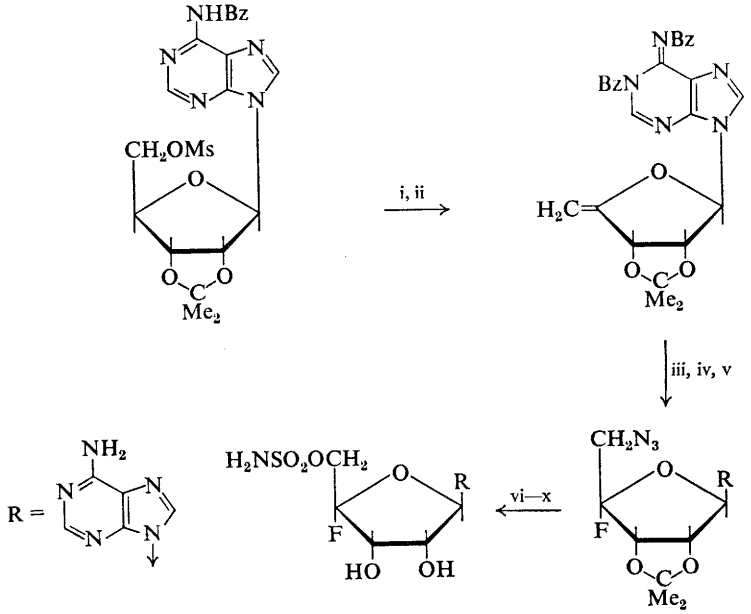
Deuteriated derivatives [*e.g.* (404)] of pyrimidine deoxyribonucleosides have been prepared. Comparison of the n.m.r. spectra of these compounds with that of a deuteriated deoxycytidine, obtained by enzymic reduction of cytidine 5′-pyrophosphate with *Escherichia coli* in the presence of deuterium oxide, confirmed that substitution of a hydroxy-group by a hydrogen atom in the enzymic reaction proceeded with retention of configuration.[594] Similar studies have been reported by other workers.[595]

[589] M. Miyaki, A. Saito, and B. Shimizu, *Chem. and Pharm. Bull.* (*Japan*), 1970, **18**, 2459.
[590] D. Heller and G. Wagner, *Z. chem.*, 1971, **11**, 385.
[591] J. Igolen and Tam Huyhn Dinh, *Chem. Comm.*, 1971, 1267.
[592] A. Holý, *Tetrahedron Letters*, 1971, 189.
[593] R. L. Tolman and R. K. Robins, *J. Medicin. Chem.*, 1971, **14**, 1112.
[594] S. David and J. Eustache, *Carbohydrate Res.*, 1971, **20**, 319.
[595] B. Fraser-Reid and B. Radatus, *J. Amer. Chem. Soc.*, 1971, **93**, 6342.



Uridine has been converted into 2′-deoxyuridine by oxidation of 2′,3′-*O*-benzylideneuridine with *N*-bromosuccinimide and subsequent reduction and debenzoylation. The mechanistic implications of these and other reactions in the synthesis of reactive intermediates for pyrimidine nucleosides have been discussed in a preliminary communication.[596]

A synthesis (Scheme 81) of nucleocidin has provided confirmation of the antibiotic's structure.[273] The antibiotic cordycepin (3′-deoxyadenosine)



Reagents: i, Bu^{t}OK; ii, BzCl–py; iii, I_2–AgF; iv, LiN_3–DMF; vi, NH_3–MeOH; vi, u.v. light; vii, H^+; viii, $NaBH_4$; ix, NH_2SO_2Cl; x, CF_3CO_2H

Scheme 81

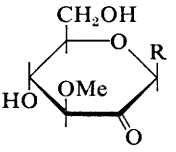
[596] M. M. Ponpipom and S. Hanessian, *Carbohydrate Res.*, 1971, **17**, 248.

and its 4′-epimer have been synthesized,[542] and gougerotin analogues have been prepared [539] (see Chapter 20).

Esters and Other Derivatives

Dinucleoside carbonates and trinucleoside dicarbonates have been prepared as nucleotide analogues by transesterification.[257] Mono- and dimethacrylates of nucleosides have been copolymerized to give cross-linked polymers,[597] and 5′-*O*-acryloyl esters of cytidine, guanosine, and adenosine have been copolymerized with acrylamide.[598]

5′-Thiophosphates of inosine have been prepared,[599] and a synthesis of 9-β-D-arabinofuranosyladenine 3′,5′-cyclic phosphate has been described.[600] Compound (405) has been prepared by oxidation of (406) with the Pfitzner–Moffatt reagent and removal of the benzylidene group.[601]



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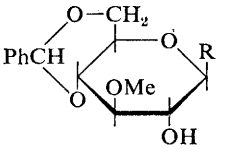
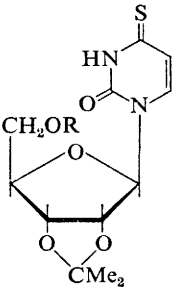
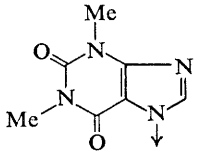


(406)



(407) R = H
(408) R = CH_2CO_2H

R =



A direct method for the conversion of such nucleoside derivatives as (407) into (408) has been reported.[602]

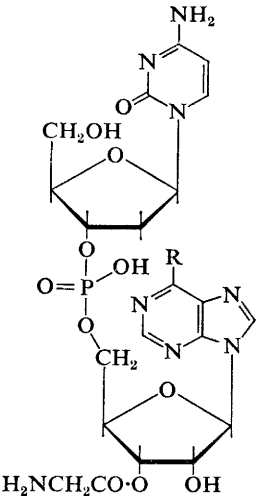
Treatment of adenosine, cytidine, and guanosine with 4-nitrobenzaldehyde and ethyl orthoformate in the presence of trifluoroacetic acid afforded both N- and 2′,3′-O-protected products in high yield. Treatment of these products with benzoyl chloride in pyridine caused both *O*-benzoylation and conversion of the amino-function into a Schiff base.[603] N^6-Benzoyl-2′-*O*-tetrahydropyranyladenosine, N^4-benzoyl-2′-*O*-tetrahydropyranylcytidine, and related compounds have been prepared as new

[597] H. Schott and G. Greber, *Makromol. Chem.*, 1971, **145**, 11.
[598] M. J. Cooper, R. S. Goody, A. S. Jones, J. R. Tittensor, and R. T. Walker, *J. Chem. Soc.* (*C*), 1971, 3183.
[599] K. Haga, M. Kainosho, and M. Yoshikawa, *Bull. Chem. Soc. Japan*, 1971, **44**, 460.
[600] W. W. Lee, L. V. Fisher, and L. Goodman, *J. Heterocyclic Chem.*, 1971, **8**, 179.
[601] K. Antonakis and F. Leclercq, *Bull. Soc. chim. France*, 1971, 2142.
[602] S. David, J. P. Coat, and J. P. Guilbert, *Carbohydrate Res.*, 1971, **19**, 281.
[603] J. Žemlička and J. P. Horwitz, *J. Org. Chem.*, 1971, **36**, 2809.

derivatives for the synthesis of oligoribonucleotides.[604] Syntheses of (409) and (410) have been described.[605]

A series of thymidine nucleotides containing 4-nitrophenylphosphate groups have been synthesized as useful substrates for nucleases.[606]

2′,3′-Cyclic phosphates of pyrimidine nucleosides have been prepared by way of the 2′(3′)-phosphites.[231]

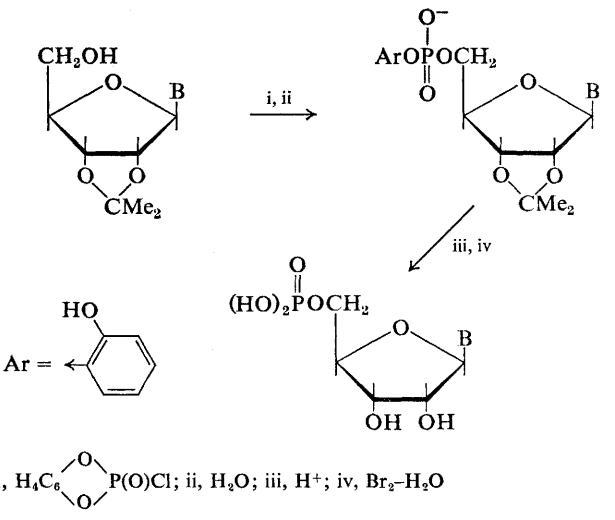


(409) R = OH
(410) R = NH_2

Convenient syntheses of 2′,3′,5′-tri-*O*-acetyl-adenosine and -uridine have been accomplished using acetic anhydride–boron trifluoride as the acylating agent.[607]

The use of benzoic anhydride and acetic anhydride for *O*-acylation of nucleotides in aqueous solution has been reported,[608] and the utility of benzoyl cyanide as a benzoylating reagent in nucleoside and nucleotide chemistry has been emphasized;[212] mild reaction conditions and short reaction times are characteristic of the latter reagent. The use of *O*-phenylene phosphorochloridate for phosphorylation of nucleosides, as illustrated in Scheme 82, has been described, and stability studies of the intermediate 5′-*O*-hydroxyphenylphosphates have been reported.[609] 2-Methylthio-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (81) has been described as a potentially useful phosphorylating agent [232] (see Scheme 27).

[604] T. Neilson and E. S. Werstiuk, *Canad. J. Chem.*, 1971, **49**, 493.
[605] J. Žemlička and S. Chladex, *Biochemistry*, 1971, **10**, 1521.
[606] R. P. Glinski, A. B. Ash, C. L. Stevens, M. B. Sporn, and H. M. Lazarus, *J. Org. Chem.*, 1971, **36**, 245.
[607] F. J. M. Rajabalee, *Angew. Chem. Internat. Edn.*, 1971, **10**, 75.
[608] R. J. Cedergren, B. Larue, and P. Laporte, *Canad. J. Biochem.*, 1971, **49**, 730.
[609] T. A. Khwaja and C. B. Reese, *Tetrahedron*, 1971, **27**, 6189.



Scheme 82

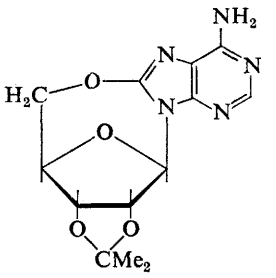
Syntheses of thiophosphate analogues of nucleoside di- and triphosphates have been reported,[610] and methyl and ethyl phosphotriester derivatives of some dinucleosides have been prepared as mixtures of diastereoisomers.[611]

Anhydronucleosides

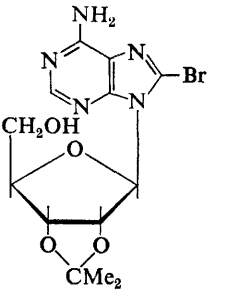
The 8,5′-anhydroadenosine derivative (411) was formed by treating (412) with sodium hydride in *p*-dioxan at room temperature. Anhydro ring-opening reactions of (411) were described.[612] The synthesis and some properties of 8,5′-anhydro-8-mercaptoadenosine (413) have been reported.[613] Treatment of the cyclouridine (414) with dimethyloxosulphonium methylide afforded (415), which on treatment with Raney nickel gave (416); u.v. irradiation of (415) converted it into the novel cyclonucleoside (417). Related studies were carried out on other cyclonucleosides.[614]

3′-Deoxy-3′-fluoro- (418) and 3′-chloro-3′-deoxy-thymidine (419) have been prepared by reaction of (420) with hydrogen fluoride or hydrogen chloride, respectively. Compound (422) could not be formed from (421). In alkali, both (418) and (419) were converted into (423).[615]

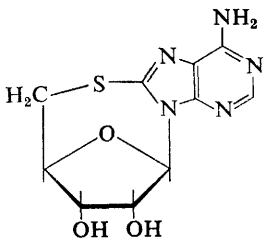
[610] R. S. Goody and F. Eckstein, *J. Amer. Chem. Soc.*, 1971, **93**, 6252.
[611] P. S. Miller, K. N. Fang, N. S. Kondo, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, 1971, **93**, 6657.
[612] M. Ikehara, M. Kaneko, and R. Okano, *Tetrahedron*, 1970, **26**, 5675.
[613] M. Ikehara, M. Kaneko, and M. Sagai, *Tetrahedron*, 1970, **26**, 5757.
[614] T. Kunieda and B. Witkop, *J. Amer. Chem. Soc.*, 1971, **93**, 3487.
[615] G. Etzold, R. Hintsche, G. Kowollik, and P. Langen, *Tetrahedron*, 1971, **27**, 2463.



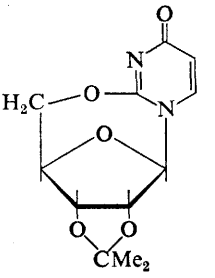
(411)



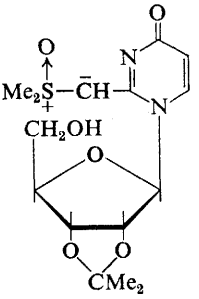
(412)



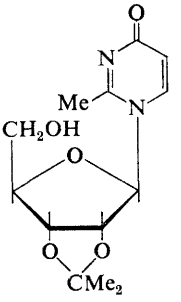
(413)



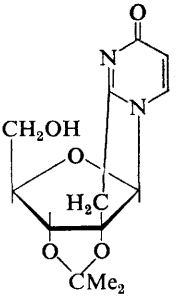
(414)



(415)



(416)



(417)

Thymidine, on treatment with *N*-iodosuccinimide in DMSO containing trifluoroacetic acid, gave an inseparable mixture of (424) and (425).[616] However, u.v. irradiation of the mixture caused (425) to epimerize to (424), which on treatment with base afforded O^6,5′-cyclothymidine.

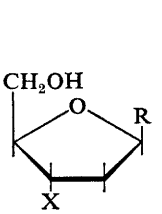
A novel rearrangement of the *S*-cyclonucleoside (426) to the *O*-cyclonucleoside (428) *via* the intermediate sulphoxide (427) has been reported. Compound (428) was not isolated, but evidence for its formation was adduced from its rearrangement products.[617]

Several 8-hydroxypurine nucleoside 2′,3′-carbonates have been prepared as potential precursors of 8,2′-anhydronucleosides. However, treatment of

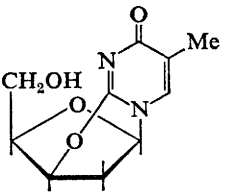
[616] D. Lipkin and J. A. Rabi, *J. Amer. Chem. Soc.*, 1971, **93**, 3309.
[617] M. Ikehara, Y. Ogiso, Y. Matsuda, and T. Morii, *Tetrahedron Letters*, 1971, 2965.

the carbonates under various basic conditions caused hydrolysis of the carbonate esters without formation of anhydronucleosides.[618]

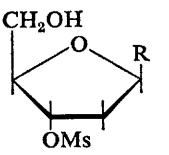
The synthesis and properties of some 8,2′-*N*-cycloadenosines (the first purine 8-*N*-cyclonucleosides) such as (429) have been reported.[619] Treat-



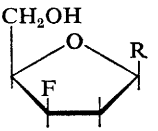
(418) X = F
(419) X = Cl



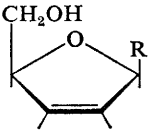
(420)



(421)



(422)



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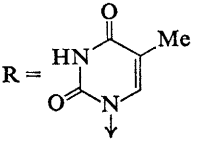

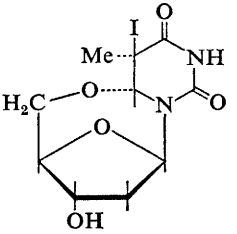

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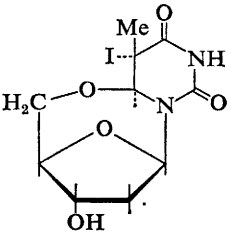
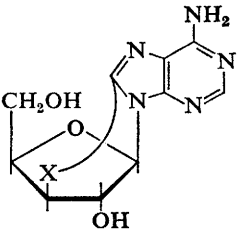
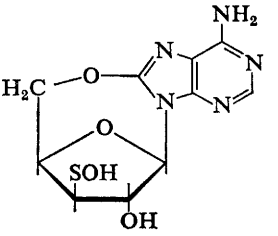


(425)



(426) X = S
(427) X = S → O

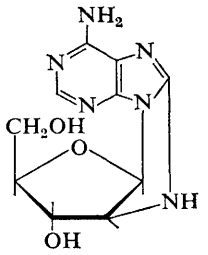


(428)

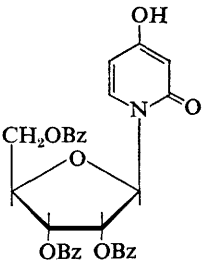
[618] K. K. Ogilvie and L. Slotin, *J. Org. Chem.*, 1971, **36**, 2556.
[619] M. Kaneko, B. Shimizu, and M. Ikehara, *Tetrahedron Letters*, 1971, 3113.

ment of (430) with phosphorus pentasulphide in moist β-picoline and subsequent debenzoylation afforded (431).[620]

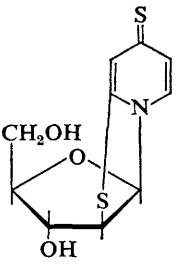
A useful bibliography referring to amino-sugar nucleosides has been given in a paper in which the formation of azido-sugar nucleosides by treatment of anhydro-sugars with lithium azide in HMPT is described.[621]



(429)



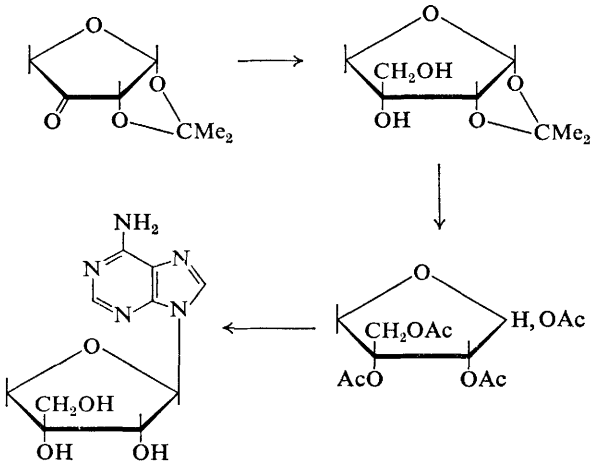
(430)



(431)

Nucleosides containing Branched-chain Sugar Components

The conversion of 4-*C*-cyclopropyl-αβ-D-*xylo*-tetrofuranose (Vol. 2, p. 145) into 9-[4-*C*-cyclopropyl-α(and β)-D-*xylo*-tetrofuranosyl]adenine has been reported,[622] and standard procedures have been employed for the synthesis of 9-β-D-apiosyladenine (Scheme 83).[429]

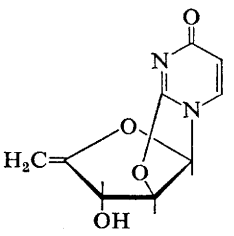


Scheme 83

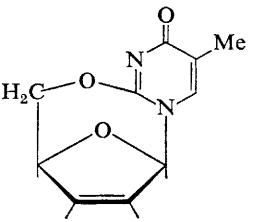
[620] M. J. Robins, B. L. Currie, R. K. Robins, and A. D. Broom, *Canad. J. Chem.*, 1971, **49**, 3067.
[621] J. P. H. Verheyden, D. Wagner, and J. G. Moffatt, *J. Org. Chem.*, 1971, **36**, 250.
[622] D. Horton and C. G. Tindall, jun., *Carbohydrate Res.*, 1971, **17**, 240.

Nucleosides containing Unsaturated Carbohydrate Components

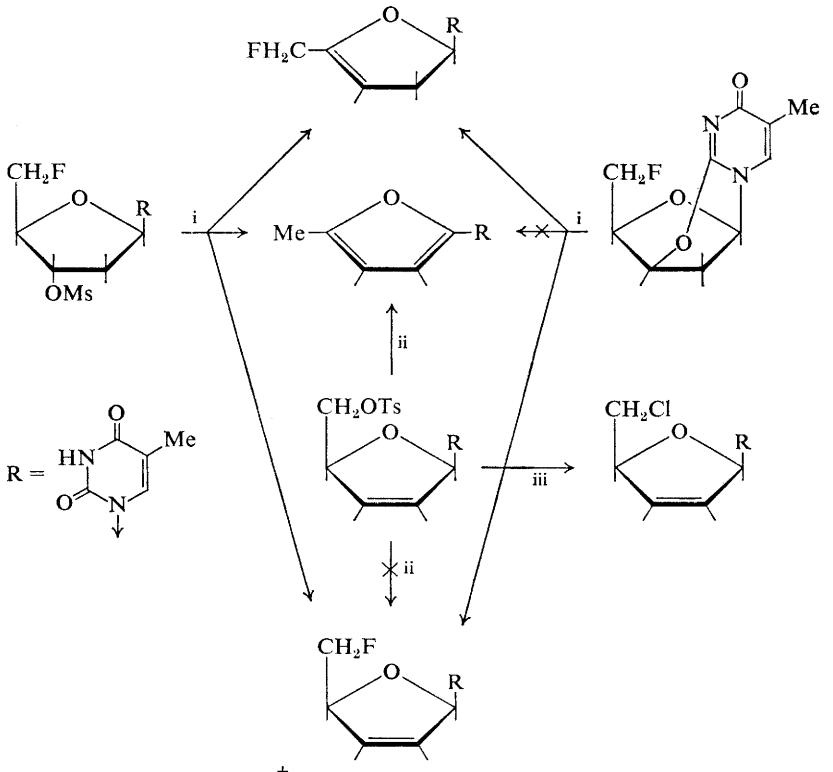
Unsaturated anhydronucleosides such as (432) have been prepared, but attempts to form unsaturated compounds such as (433) were unsuccessful.[623] Unsaturated derivatives of thymidine have been prepared by the reactions shown in Scheme 84.[624]



(432)



(433)



Reagents: i, Bu^tOK; ii, $Bu^n_4\overset{+}{N}\overset{-}{F}$; iii, LiCl

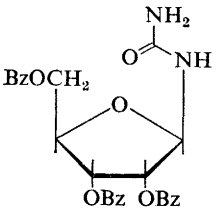
Scheme 84

[623] M. W. Winkley, *Carbohydrate Res.*, 1971, **16**, 462.
[624] G. Kowollik, K. Gaertner, and P. Langen, *Tetrahedron Letters*, 1971, 1737.

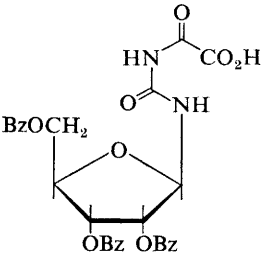
Unsaturated nucleoside derivatives, prepared by treatment of D-glucal triacetate with purine bases,[401, 406] are described in Chapter 14.

Reactions

Compounds (434) and (435) have been identified as products of oxidation of 2′,3′,5′-tri-*O*-benzoylcytidine with potassium permanganate in bicarbonate buffer.[625]



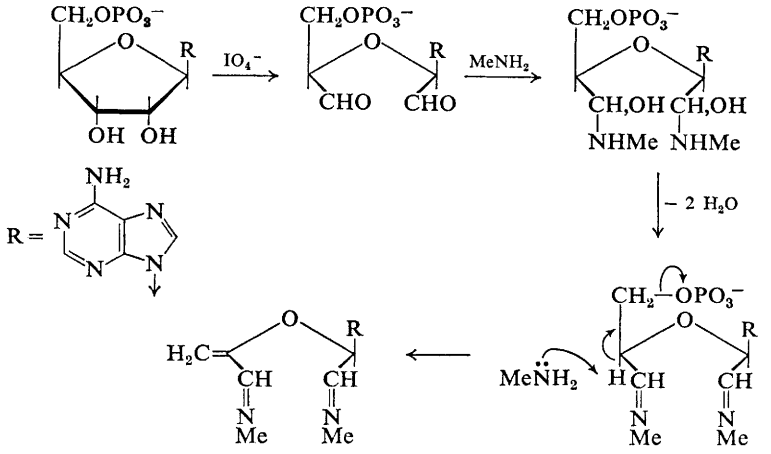
(434)



(435)

3-Methylpyrimidine nucleosides underwent rupture of the heterocyclic ring when treated with alkali. 5′-Substituents appeared to inhibit this reaction, whereas 2′,3′-*O*-isopropylidene groups appeared to accelerate it.[626]

The reactions occurring when adenosine 5′-phosphate was oxidized with periodate and was then treated with methylamine have been summarized (Scheme 85).[627]

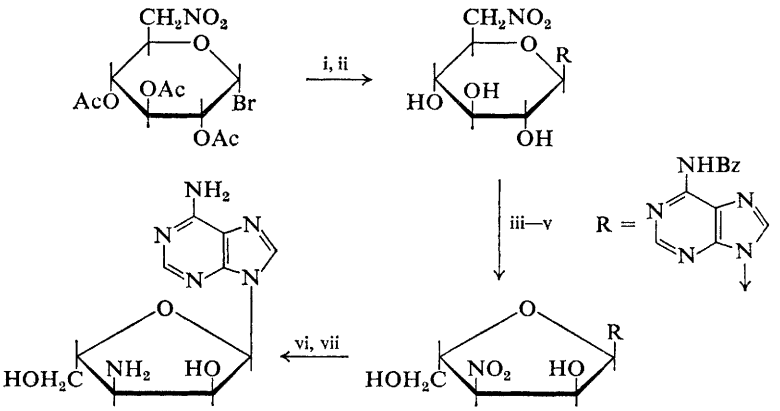


Scheme 85

[625] R. S. Goody, A. S. Jones, and R. T. Walker, *Tetrahedron Letters*, 1971, **27**, 65.
[626] Y. Kondo, J.-L. Fourrey, and B. Witkop, *J. Amer. Chem. Soc.*, 1971, **93**, 3527.
[627] A. Steinschneider, *Biochemistry*, 1971, **10**, 173.

3′-Aminonucleoside analogues have been prepared as illustrated in Scheme 86.[628] The syntheses of various 5′-*N*-aminoacyl-5′-amino-5′-deoxynucleosides and 2′-deoxy-analogues, a new class of peptide nucleoside, have been described, and a good survey of this class of compounds has been provided. These compounds were found to have significant effects on protein synthesis.[629]

Nucleoside derivatives have been converted into 5′-halogenonucleosides [276] (see Chapter 7).



Reagents: i, RHgCl; ii, MeONa; iii, IO_4^-; iv, MeONa; v, $NaBH_4$; vi, Pt–H_2; vii, picric acid

Scheme 86

Physical Measurements

Conformational aspects of nucleosides are dealt with in Chapter 23.

The optical rotations of nucleotides have been found to be affected markedly by urea. This observation was interpreted as evidence for a change from an ordered conformation into a random set of conformations.[630]

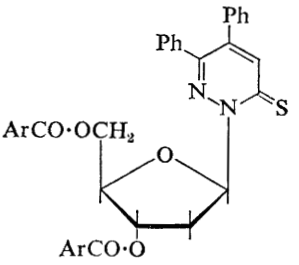
Molecular orbital calculations have been carried out to compare the conformational energies of nucleosides containing sugars having the *exo*-C-3′ or *exo*-C-2′ conformations.[631] The rotational strengths of the two longer-wavelength transitions (B_{2u} and B_{1u}) of four mononucleosides as a function of the glycosidic rotational angles have been investigated theoretically.[632]

[628] H. H. Baer and M. Bayer, *Canad. J. Chem.*, 1971, **49**, 568.
[629] M. J. Robins, L. N. Simon, M. G. Stout, G. A. Ivanovics, M. P. Schweizer, R. J. Rousseau, and R. K. Robins, *J. Amer. Chem. Soc.*, 1971, **93**, 1474.
[630] S. Hirano, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 1219.
[631] H. Berthod and B. Pullman, *Biochim. Biophys. Acta*, 1971, **246**, 359.
[632] N. N. H. Teng, M. S. Itzkowitz, and I. Tinoco, *J. Amer. Chem. Soc.*, 1971, **93**, 6257.

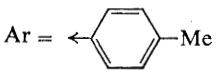
N.m.r. and c.d. characteristics of 2′,3′-*O*-[4-*N*-(2-chloromethyl)-*N*-methylamino]benzylidene derivatives of uridine, adenosine, and their 5′-methylphosphate esters have been discussed.[633] The o.r.d. and c.d. spectra of pyrimidine and azapyrimidine nucleosides have been studied, and the B_{2u} Cotton effect has been related to conformation.[634] C.d. conformational studies of guanine nucleosides have also been reported.[635]

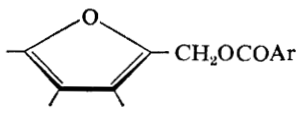
The n.m.r. spectrum of β-cyanuric acid ribofuranoside has been analysed. Since a carbonyl group must be orientated above the sugar ring in this compound, the observed chemical shifts are useful for comparison with other nucleosides containing α-keto-groups to test for *anti* or *syn* conformations.[636]

The anomeric configurations of 3-D-glycosyl-6-methyluracils, prepared by condensation of acetylated glycosyl chlorides with 6-methyluracil, have been determined by physical methods and by synthesis of the appropriate compounds for comparison.[637] The anomerization of the

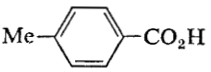


(436)





(437)



(438)

N^2-thiopyridazone glycoside of the 2-deoxy-D-ribofuranose (436) has also been studied.[638] In benzene containing mercuric bromide, as well as α ⇋ β equilibration, decomposition of (436) to (437) and (438) occurred. After 4 h, the extent of decomposition was 25% and the α : β ratio was 0.3. After 8 h, the extent of decomposition was 35% and the α : β ratio was 0.8, whereas after 20 h, with 80% decomposition, the α : β ratio was 2.5.

[633] A. M. Belikova, N. I. Grineva, V. F. Zarytova, G. N. Kabasheva, and D. G. Knorre, *Doklady Akad. Nauk. S.S.S.R.*, 1970, **195**, 1337.
[634] G. T. Rogers and T. L. V. Ulbricht, *European J. Biochem.*, 1971, **22**, 457.
[635] D. W. Miles, L. B. Townsend, M. J. Robins, R. K. Robins, W. H. Inskeep, and H. Eyring, *J. Amer. Chem. Soc.*, 1971, **93**, 1600.
[636] H. Dugas, B. J. Blackburn, R. K. Robins, R. Deslauriers, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1971, **93**, 3468.
[637] N. Yamaoka, E. Takahashi, and K. Tuzimura, *Carbohydrate Res.*, 1971, **19**, 262.
[638] G. Wagner and D. Göbel, *Z. chem.*, 1971, **11**, 253.

Crystal structures have been determined for 2,4-dithiouridine,[639, 640] dihydrouridine hemihydrate,[641] deoxyuridine,[642] 6-chloro-9-(3,4-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentopyranosyl)purine,[643] 2-thiocytidine dihydrate,[644] deoxycytidine 5′-phosphate,[645] 5-chlorouridine,[646] 3′-*O*-acetyl-4-thiothymidine,[647] and 5′-deoxy-5′-methylammoniumadenosine iodide.[648]

Mass spectrometric investigations of nucleosides are described in Chapter 24.

[639] W. Saenger and D. Suck, *Acta Cryst.*, 1971, **27B**, 1178.
[640] G. H.-Y. Lin and M. Sundaralingam, *Acta Cryst.*, 1971, **27B**, 961.
[641] M. Sundaralingam, S. T. Rao, and J. Abola, *J. Amer. Chem. Soc.*, 1971, **93**, 7055.
[642] A. Rahman and H. R. Wilson, *Nature*, 1971, **232**, 333.
[643] D. J. Abraham, R. D. Rosenstein, T. G. Cochran, E. E. Leutzinger, and L. B. Townsend, *Tetrahedron Letters*, 1971, 2353.
[644] G. H.-Y. Lin, M. Sundaralingam, and S. K. Arora, *J. Amer. Chem. Soc.*, 1971, **93**, 1235.
[645] M. A. Viswamitra, B. Swaminatha Reddy, G. H.-Y. Lin, and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1971, **93**, 4565.
[646] S. W. Hawkinson and C. L. Coulter, *Acta Cryst.*, 1971, **27B**, 34.
[647] W. Saenger and D. Suck, *Acta Cryst.*, 1971, **27B**, 2105.
[648] W. Saenger, *J. Amer. Chem. Soc.*, 1971, **93**, 3035.

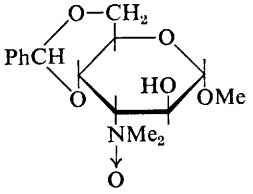
22
Oxidation and Reduction

Oxidation methods of general applicability in carbohydrate chemistry have been reviewed.[649]

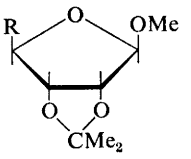
Periodate Oxidation

Periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and -altropyranoside derivatives containing vicinal hydroxy- and *N*-methylamino- or *NN*-dimethylamino-substituents at C-2 and C-3 has been reported.[650] Oxidation of *NN*-dimethylamino-groups gave *N*-oxides [*e.g.* (439)], whereas the oxidations followed a pattern analogous to $CHOH \cdot CHNH_2$ systems when *N*-methylamino-groups were present (see Vol. 4, p. 145). Slow non-Malapradian oxidation was observed in compounds containing vicinal $CHOMe \cdot CHNH_2$ groups.

The reactions occurring when adenosine 5′-phosphate was oxidized with periodate and the product treated with methylamine have been described.[627]



(439)



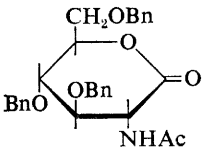
(440) R = CH_2OH
(441) R = CHO
(442) R = CH_2OAc
(443) R = CH_2OCH_2SMe

DMSO-based Oxidations

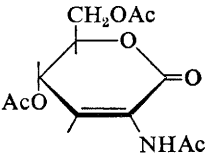
It has been observed that, whereas (440) was oxidized to (441) by the DMSO–DCC (Pfizner–Moffatt) reagent, the acetate (442) and the methylthiomethyl ether (443) were the preponderant products when (440) was treated with DMSO–acetic anhydride[651] (*cf.* Vol. 3, p. 167). Oxidation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose by DMSO–acetic

[649] R. F. Butterworth and S. Hanessian, *Synthesis*, 1971, 70.
[650] R. D. Guthrie and A. M. Prior, *Carbohydrate Res.*, 1971, **18**, 373.
[651] R. F. Butterworth and S. Hanessian, *Canad. J. Chem.*, 1971, **49**, 2755.

anhydride afforded 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucono-1,5-lactone (444).[652] By contrast, similar oxidation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose and the corresponding D-mannopyranose derivative afforded the unsaturated derivative (445) as well as the expected 1,5-lactones.



(444)

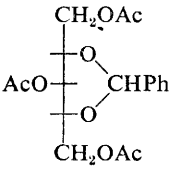


(445)

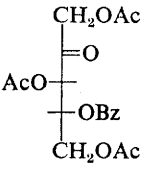
The use of DMSO–phosphorus pentoxide for oxidizing 3-*C*-hydroxymethyl substituents to 3-*C*-formyl substituents has been described earlier.[435] Oxidation of methyl 2,3,4-tri-*O*-acetyl-α-D-altropyranoside with sulphur trioxide and triethylamine in DMSO followed by deacetylation afforded (247).[418a]

Other Oxidations

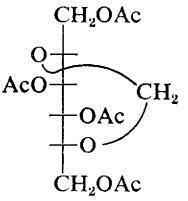
Chromium trioxide in acetic acid has been shown to oxidize acetals of acyclic sugars as well as glycosidic hemiacetals [653] (see Vol. 4, p. 32). Typical of the reactions reported were the conversions of (446) into (447) (84% yield), (448) into (449) (87%), and (450) into (451) (50%). The mechanisms of these oxidations have been discussed and differences in the oxidation pathways of methyl α- and β-D-glucopyranoside tetra-acetates (see Vol. 3,



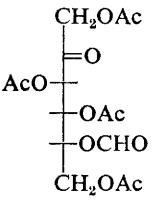
(446)



(447)



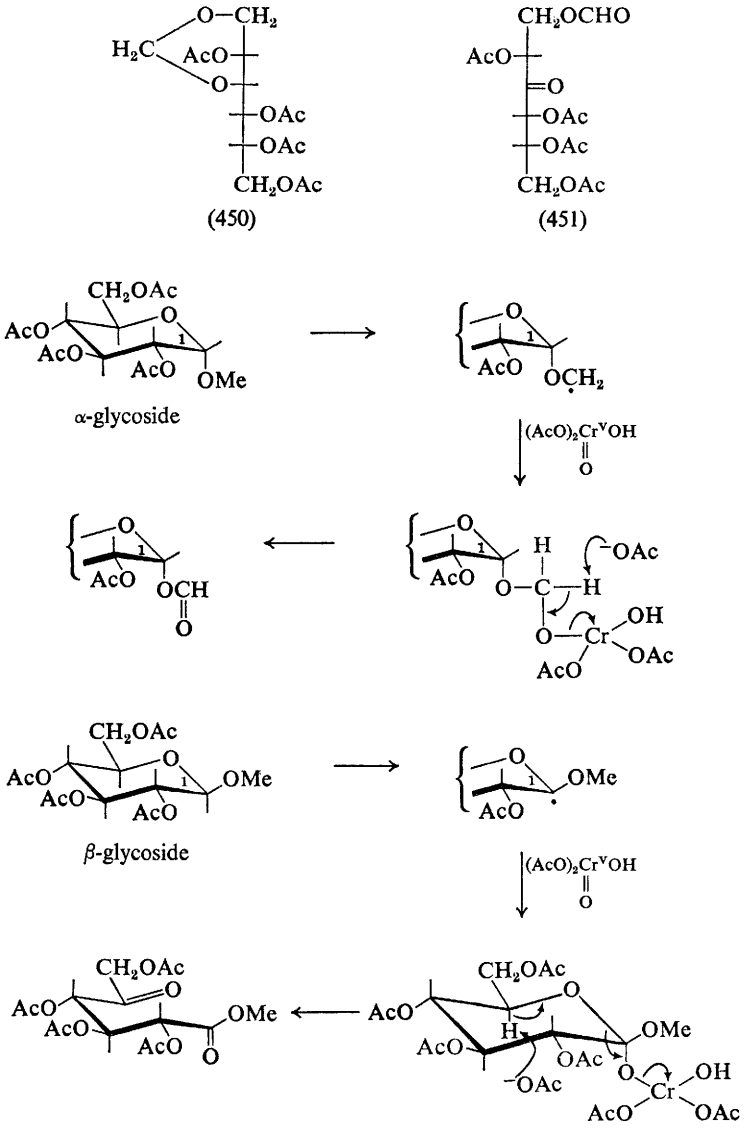
(448)



(449)

[652] N. Pravdić and H. G. Fletcher, jun., *Carbohydrate Res.*, 1971, **19**, 353.
[653] S. J. Angyal and K. James, *Austral. J. Chem.*, 1971, **24**, 1219.

p. 169) have been rationalized.[654] The oxidation pathways illustrated in Scheme 87 are considered to depend on the relative steric accessibility of H-1.



Scheme 87

[654] M. Bertolini and C. P. J. Glaudemans, *Carbohydrate Res.*, 1971, **17**, 449.

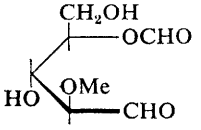
Conditions for the oxidation of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose by sodium hypochlorite have been studied in detail with a view to obtaining reproducible yields of products.[655] The mechanism of the oxidation of α-glycols with nickel peroxide has been examined.[656] Oxidation of *O*-acetylaldose dialkyl monothioacetals with potassium permanganate afforded *O*-acetyl-1-(alkylsulphonyl)-1-deoxyaldose hemiacetals, which were saponified to 1-(alkylsulphonyl)-1-deoxyaldose hemiacetals.[657]

Studies of the oxidation of D-glucose by a mixture of chromic and perchloric acids have been reported.[53]

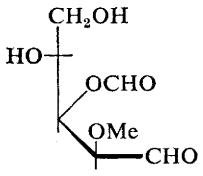
An example of the use of manganese dioxide for the oxidation of unsaturated sugar derivatives to enones [411] is described in Chapter 14.

3-*O*-Methyl-D-glucose has been converted into 2-*O*-methyl-D-arabinose by oxidative degradation with silver carbonate–Celite in methanol.[658] The initial products of oxidation ultimately transformed into 2-*O*-methyl-D-arabinose were (452) and (453).

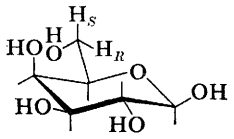
Dehydrogenation of the primary hydroxy-group in D-galactose by D-galactose oxidase has been shown to involve removal of the *pro-S*-6-hydrogen atom (454). This result was obtained using D-[6-^{2}H]galactose and methyl β-D-[6-^{2}H]galactopyranoside as substrates.[659]



(452)



(453)



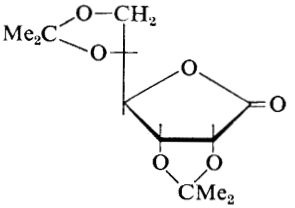
(454)

Reduction

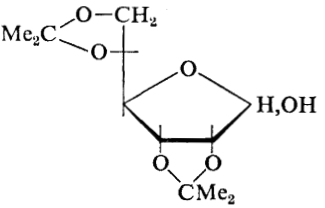
Reduction of the lactone (455) with bis-(2-butyl-3-methyl)borane afforded 2,3:5,6-di-*O*-isopropylidene-D-allofuranose (456).[582]

Stereoselective reductions have been utilized in syntheses of methyl β-D-rhodinoside (461) and methyl β-D-amicetoside (462).[660] Thus, reduction of (457) with hydrogen over palladium afforded (458). Reduction of (458) with Raney nickel afforded (459), the immediate precursor of (461), whereas reduction with sodium borohydride afforded (460), the immediate precursor of (462).

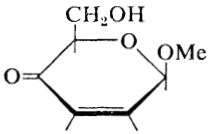
[655] S. Iacobescu Cilianu, C. Banciu, M. Blasnic, and I. Rogozea, *Ann. pharm. franç.*, 1970, **28**, 545.
[656] R. Konaka and K. Kuruma, *J. Org. Chem.*, 1971, **36**, 1703.
[657] H. Zinner, R. Kleeschätzky, and M. Schlutt, *Carbohydrate Res.*, 1971, **19**, 71.
[658] S. Morgenlie, *Acta Chem. Scand.*, 1971, **25**, 2773.
[659] A. Maradufu, G. M. Cree, and A. S. Perlin, *Canad. J. Chem.*, 1971, **49**, 3429.
[660] E. H. Williams, W. A. Szarek, and J. N. K. Jones, *Carbohydrate Res.*, 1971, **20**, 49.



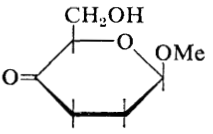
(455)



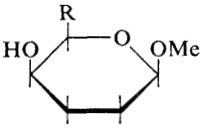
(456)



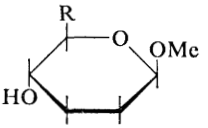
(457)



(458)

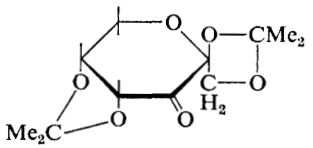


(459) R = CH_2OH
(461) R = Me

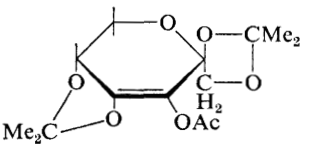


(460) R = CH_2OH
(462) R = Me

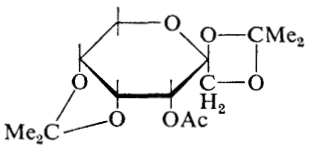
The use of oxidation–reduction sequences for inverting the configuration at C-3 in 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose, to give the corresponding D-psicose derivative,[18] and at C-4 in a suitably protected D-fructofuranose derivative, to give the corresponding D-tagatose derivative,[19] are described in Chapter 2. The use of an oxidation–reduction sequence for the synthesis of D-[2-^{3}H]ribose is also reported in Chapter 2.[23]



(463)



(464)



(465)

A novel application of an oxidation–reduction sequence was investigated unsuccessfully for a synthesis of 1,2:4,5-di-*O*-isopropylidene-D-tagatopyranose.[661] Thus, (463) was acetylated in its enolic form to give (464), which was then reduced. The only product was the β-D-psicopyranose derivative (465), which differs in configuration from the required D-tagatopyranose derivative at both C-3 and C-4.

[661] R. F. Brady, jun., *Carbohydrate Res.*, 1971, **20**, 170.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

As in previous volumes, n.m.r. spectroscopy and stereochemical aspects of carbohydrate chemistry will be treated together. A selected number of papers on studies of model compounds relevant to carbohydrates are also described. This year there has been increasing use of lanthanide shift reagents, and the potential of pulse Fourier-transform ^{13}C n.m.r. techniques in carbohydrate chemistry is more and more apparent.

Two detailed papers have clearly demonstrated to the carbohydrate chemist the refinements that are now possible with n.m.r. techniques. 6-Deoxy-1,2:3,5-di-*O*-[$^2H_2$]isopropylidene-α-D-glucofuranose has been prepared and used for an investigation of internuclear double-resonance (INDOR) techniques.[662] The various types of homonuclear INDOR experiments were shown to have potential for aiding assignments of transitions in computerized iterative analysis of spectra. Detection of unresolved couplings is a useful bonus. Many of the more sophisticated n.m.r. techniques have been applied in the analysis of the ^{13}C, ^{19}F, ^1H, and ^{15}N spectra of ^{15}N-labelled 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose and its trifluoroacetyl and other derivatives.[663] Many features of n.m.r. spectra not normally considered by carbohydrate chemists were discussed. The practical value of the highly sophisticated approach remains to be demonstrated, although its promise was clearly shown.

A preliminary account of chlorine quadrupolar-resonance studies of three pairs of acetylated glycosyl chlorides has been published.[664]

Vicinal H—N—C—H couplings of *N*-acetylglycosylamines, 2-acetamido-2-deoxyaldoses, and 1,1-bis(acetamido)-1-deoxyalditols have been measured using DMSO as solvent.[665] In this solvent, the doublets from the NH protons were broadened only to a relatively slight degree by the quadrupolar effects of ^{14}N. For the cyclic compounds, coupling constants of 9—11 Hz were considered to be indicative of preferred rotamer conformations in which the C—H and C=O bonds are eclipsed. As is to be

[662] B. Coxon, *Carbohydrate Res.*, 1971, **18**, 427.
[663] B. Coxon and L. F. Johnson, *Carbohydrate Res.*, 1971, **20**, 105.
[664] S. David and L. Guibé, *Carbohydrate Res.*, 1971, **20**, 440.
[665] A. S. Cerezo, *Chem. and Ind.*, 1971, 96.

expected, smaller couplings (7—9 Hz) were observed for the acyclic derivatives.

A detailed n.m.r. study has been made of the conformations of the seven-membered 1,3-dioxepan ring in 2,5-*O*-methylene-D-mannitol and some related compounds.[666] Twist-chair conformations preponderate, but evidence was found to suggest that, for 1,3-dioxepan fused *trans* to a 1,3-dioxolan ring, the 1,3-dioxepan ring adopts a twist-boat conformation.

Lemieux and his co-workers[667] have published a study of the mutarotation of 2-deoxy-β-D-*erythro*-pentose, which illustrates the value of combining structural information based on n.m.r. spectroscopy with that derived from measurements of optical rotations and from empirical rules for calculating the rotations of saturated carbohydrates. 2-Deoxy-β-*erythro*-pentopyranose in aqueous solution was shown by n.m.r. spectroscopy to retain the same $1C$ conformation as in the solid. N.m.r. studies also showed that, following mutarotation, the percentages of β-pyranose, α-pyranose, β-furanose, and α-furanose forms in the equilibrium mixture were 43 : 42 : 10 : 5 at 0 °C and 30 : 30 : 18 : 22 at 90 °C. Rate measurements of the mutarotation, both by n.m.r. spectroscopy and by polarimetry, showed that the mutarotation is kinetically complex, with rate constants for the approach of the furanoses to their equilibrium level substantially greater than those for the pyranoses. In contrast to the pyranose ⇋ pyranose equilibrium, the furanose ⇋ pyranose and furanose ⇋ furanose equilibria were highly temperature dependent. The effects of unshared pairs of electrons and their solvation on conformational equilibria have been reviewed.[668]

General Observations on Model Compounds

An empirical equation has been proposed, which enables prediction of $^2J(XCH_2Y)$ couplings and which depends on three main contributions: (i) the Pauling electronegativities of X and Y, (ii) the bond distances C—X and C—Y, and (iii) the mutual orientation of the free orbitals and σ-CH bonds.[669]

A detailed, fundamental n.m.r. study of the anomeric effect has been reported in which both oxygen and nitrogen heterocycles were used as model compounds.[670] The anomeric effect has also been reviewed.[671]

The eight racemic isomers of 2-t-butyl-5-methyl-cyclohexane-1,4-diol have been prepared, and n.m.r. and i.r. evidence has been obtained to show that some of the isomers exist to a considerable extent in non-chair

[666] J. F. Stoddart and W. A. Szarek, *J. Chem. Soc.* (*B*), 1971, 437.
[667] R. U. Lemieux, L. Anderson, and A. H. Conner, *Carbohydrate Res.*, 1971, **20**, 59.
[668] R. U. Lemieux, *Pure and Appl. Chem.*, 1971, **25**, 527.
[669] M. Anteunis, G. Swaelens, and J. Gelan, *Tetrahedron*, 1971, **27**, 1917.
[670] H. Booth and R. U. Lemieux, *Canad. J. Chem.*, 1971, **49**, 777.
[671] N. S. Zefirov and N. M. Shekhtman, *Russian Chem. Rev.*, 1971, 315 (*Uspekhi Khim.*, 1971, 593).

conformations.[672] Equilibration studies of *cis*-2-*r*-4-*trans*-6- and *trans*-2-*r*-4-*trans*-6-trimethyl-1,3-oxathians have been reported, and differences in the conformational energies of axial methyl groups in positions 4 and 6 have been calculated.[673] The conformational free energies of $NHSiMe_3$ and $OSiMe_3$ groups attached to the cyclohexane ring have been found to be *ca.* 1.2 and 0.9 kcal/mol, respectively.[674]

Detailed conformational studies of substituted cyclopentanes, cyclopentenes, and cyclopentene oxides have shown that cyclopentanes easily tolerate 1,3-*syn*-diaxial interactions and that 1,2-*trans*-substituents are generally disposed diaxially rather than diequatorially. Many other aspects of the n.m.r. spectra and conformational properties of cyclopentane derivatives were discussed.[675]

Pyranoid Compounds

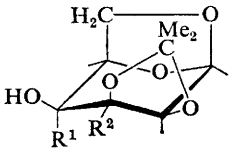
Durette and Horton have continued to report exceptionally detailed studies on the effects of substituent changes on the conformations and n.m.r. parameters of a wide range of pyranoid derivatives (*cf.* Vol. 3, p. 173; Vol. 4, p. 152). The conformational equilibria adopted by all isomers of D-aldopentopyranose tetra-acetates and tetrabenzoates,[676] by 2,3,4-tri-*O*-acetyl-D-aldopyranosyl benzoates having the *β-ribo*, *α-arabino*, *α-lyxo*, and *β-xylo* configurations, and by 2,3,4-tri-*O*-benzoyl-D-aldopyranosyl acetates having corresponding configurations have been determined.[677] Conformational equilibria of tribenzoylated and triacetylated methyl, ethyl, isopropyl, and t-butyl β-D-ribopyranosides[678] and of eight methyl D-aldopentopyranoside triacetates and tribenzoates having the *β-ribo*, *β-arabino*, α- and *β-xylo*, and *α-lyxo* configurations have also been measured.[679] Two further papers have described studies of the conformational equilibria of 1-thio-D-aldopentopyranose tetra-acetates having *β-ribo*, *α-arabino*, *β-xylo*, and *α-lyxo* configurations[680] and of peracetylated aldopentopyranosyl halides.[681] Procedures for establishing conformational equilibria were compared in these studies, and attempts were made to correlate conformational results with those calculated on the basis of the summation of steric and polar factors. The following salient observations were made. (i) The axial-directing influence of a 1-acetylthio-group is weaker than that of 1-methoxy-, 1-acetoxy-, or 1-benzoyloxy-groups in those configurations where an axial 1-substituent does not have a *syn*-axial-group at C-3 (*β-ribo*, *α-lyxo*). Where there is a 3-axial substituent, the

[672] R. D. Stolow and J. L. Marini, *Tetrahedron Letters*, 1971, 1449.
[673] P. Pasanen and K. Pihlaja, *Tetrahedron Letters*, 1971, 4515.
[674] J. P. Hardy and W. D. Cumming, *J. Amer. Chem. Soc.*, 1971, **93**, 928.
[675] R. Steyn and H. Z. Sable, *Tetrahedron*, 1971, **27**, 4429.
[676] P. L. Durette and D. Horton, *J. Org. Chem.*, 1971, **36**, 2658.
[677] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 389.
[678] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 303.
[679] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 403.
[680] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 419.
[681] P. L. Durette and D. Horton, *Carbohydrate Res.*, 1971, **18**, 57.

axial-directing effect of the acetylthio-group is stronger.[680] (ii) The axial-directing effects of acetoxy- and benzoyloxy-groups at C-1 are dependent both on total stereochemistry and on the nature of the acyloxy-groups at C-2, C-3, and C-4.[677] (iii) Benzoylated alkyl β-D-ribopyranosides favour the ^1C(D) conformation to a greater extent than the corresponding acetylated glycosides, whereas t-butyl β-D-ribopyranosides favour the ^1C(D) conformation to a lesser extent than methyl and ethyl glycosides.[678] (iv) The axial-directing effect of a methoxy-group at C-1 is greater than that of acetoxy- or benzoyloxy-substituents, excepting where it is *syn*-axial to a 3-substituent. The axial-directing effect of a methoxy-group at C-1 is enhanced by replacing acetoxy-groups at C-2, C-3, and C-4 by benzoyloxy-groups.[679] (v) For methyl 2,3,4-tri-*O*-benzoyl-β-D-xylopyranoside, the proportion of the all-axial chair conformer was found to decrease with increasing polarity of the solvent. This behaviour contrasts with that observed with some aldopyranose tetra-acetates and 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl chloride, for which the polarity of the solvent has little effect on conformational populations.[679] Readers are recommended to this series of papers for an authoritative and highly detailed treatment of the subject of quantitative conformational analysis applied to substituted tetrahydropyran systems.

The potential energies of sixteen aldopyranoses in ^1C(D) or ^1C(D) conformations have been calculated and minimized by suitably tilting the axial substituents.[682] The calculated energy values not only explain the conformations known to be favoured from n.m.r. studies, but are also in fairly good agreement with energy values assigned by Angyal. The agreement between the two sets of values seems to indicate that experimental observations in aqueous solution can be explained if both non-bonded and electrostatic interactions are taken into account.

N.m.r. studies of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-talopyranose (466) and the deuteriated analogues (467), (468), and (469) have been reported. The parameters obtained from a total analysis of (466) and its analogues were discussed in conformational terms.[177] Syntheses of a



(466) $R^1 = R^2 = H$
(467) $R^1 = R^2 = D$
(468) $R^1 = H$, $R^2 = D$
(469) $R^1 = D$, $R^2 = H$

[682] V. S. R. Rao, K. S. Vijayalakshmi, and P. R. Sundararajan, *Carbohydrate Res.*, 1971, **17**, 341.

number of substituted methyl 6-chloro(bromo and iodo)-6-deoxy-α-D-glucopyranosides have been described, and their n.m.r. spectra have been measured. Specific shielding effects were discussed, with particular reference to the anisotropic effects of carbonyl groups and aromatic rings.[683] Attention was also drawn to various distinguishing features in the spectra of α- and β-anomers of acetylated D-glucopyranosides.[684]

Molecular motions displayed by 1,6-anhydro-β-D-glucopyranose on heating have been studied by n.m.r. spectroscopy.[685]

The conformations of α-nucleoside derivatives of D-mannose and 6-deoxy-L-mannose have been examined by n.m.r. spectroscopy.[686] The axial aglycone in the α-nucleosides destabilized the 'normal' conformation, and it was also found that DMSO, DMF, and pyridine strongly solvate the axial hydroxy-groups of the pyranose derivatives, so that an alternative conformation is favoured.

Methyl 1-thio-α-D-ribopyranoside and methyl 1,5-dithio-α-D-ribopyranoside have been shown by *X*-ray crystallography to exist in $^1C_4$ conformations. Intramolecular hydrogen-bonding between the 2- and 4-hydroxy-groups was demonstrated.[687]

Furanoid Systems

A number of conformational studies of nucleoside derivatives have been reported, including those of orotidine[688] (6-carboxyuridine), α-pseudouridine,[689] and some pyrimidine nucleosides.[690] The *anti*-form of α-pseudouridine is favoured and is also the preferred conformation of many pyrimidine nucleosides, as evidenced by $^5J_{5,1'}$ values, although the absence of this coupling in some pyrimidine derivatives suggested that they adopt the *syn* conformation. The 100 and 200 MHz spectra of β-4-thiouridine suggested that an *anti* conformation is adopted in aqueous solution,[691] whereas the *syn* conformation is adopted in the crystalline form.

Nuclear Overhauser effects, and c.d. and u.v. spectra of cytidine, uridine, and their 2′, 3′-*O*-isopropylidene derivatives were collated from measurements in various solvents. In hydrogen-bonding solvents, all four compounds were shown to favour the *syn*-form.[692]

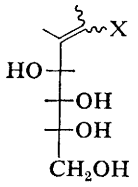
A correlation has been established between the mode of puckering of the furanoid ring in nucleosides and the preferred orientation about the

[683] H. B. Sinclair and L. W. Tjarks, *Carbohydrate Res.*, 1971, **19**, 402.
[684] M. Matsui and M. Okada, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 2129.
[685] G. W. Smith and F. Shafizadeh, *J. Chem. Soc. (B)*, 1971, 908.
[686] K. Onodera, 'Conformational Analysis', Paper Industry Symposium, 1969, ed. G. Chiurdoglu, Academic Press, 1971, p. 191.
[687] R. L. Girling and G. A. Jeffrey, *Carbohydrate Res.*, 1971, **18**, 339.
[688] F. E. Hruska, *J. Amer. Chem. Soc.*, 1971, **93**, 1795.
[689] A. A. Grey, I. C. P. Smith, and F. E. Hruska, *J. Amer. Chem. Soc.*, 1971, **93**, 1765.
[690] F. E. Hruska, *Canad. J. Chem.*, 1971, **49**, 2111.
[691] F. E. Hruska, K. K. Ogilvie, A. A. Smith, and H. Wayborn, *Canad. J. Chem.*, 1971, **49**, 2449.
[692] P. A. Hart and J. P. Davis, *J. Amer. Chem. Soc.*, 1971, **93**, 753.

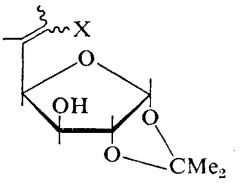
C(4′)—C(5′) bond.[693] A gauche–gauche orientation, with O-5 projecting over the ring, is favoured strongly if C-3′ is displaced in an *endo* direction from the plane of the furanoid ring, but if C-2′ is *endo*, the gauche–gauche orientation is less favoured.

Deoxyadenyl-(3′ → 5′)-deoxyadenosine has been investigated by n.m.r. spectroscopy.[694] It was suggested that the deoxyadenyl 3′-phosphate residue of the dinucleoside adopts a C-2′-*endo* (envelope) or C-2′-*endo*, C-3′-*exo* (twist) conformation, whereas the furanose ring of the deoxyadenyl 5′-phosphate residue is a rapidly equilibrating mixture of C-2′-*endo* and C-3′-*endo* forms. In general, the preferred conformations of α-nucleosides in the *solid state* are opposite (enantiomeric) to those of β-nucleosides.[695] The furanose ring-puckerings that preponderate in α-nucleosides are C-2′-*exo*, C-3′-*exo*, and C-4′-*endo*, in contrast to the C-3′-*endo* and C-2′-*endo* puckerings of β-nucleosides. An abbreviated system of nomenclature for the conformations of furanoid rings was introduced.

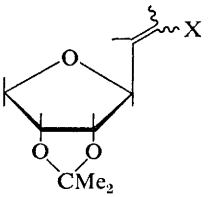
The rotamer states about the glycosyl–vinyl bonds of compounds of types (470), (471), and (472) have been determined by n.m.r. spectroscopy, and the populations of the various rotamers were evaluated in terms of electronic and steric factors.[696]



(470)



(471)



(472)

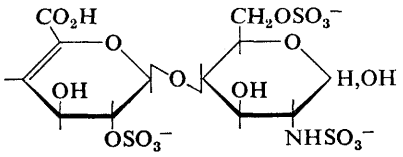
X = OMe, SMe, CN, Ph, or SO_2Me

Disaccharides

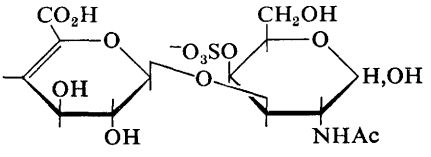
Configurational assignments of α- and β-linked D-glucosyl and D-galactosyl derivatives have been made by n.m.r. studies of trimethylsilylated disaccharides.[697] In the case of some oligosaccharides, glycosidic configurations have been determined by n.m.r. spectroscopy following reduction of the reducing, terminal sugar.[698] 3,6:3′,6′-Dianhydro-αα-trehalose tetra-acetate and tetrabenzoate were shown to have the expected $1C(D)$ conformations by n.m.r. spectroscopy.[699]

[693] F. E. Hruska, A. A. Smith, and J. G. Dalton, *J. Amer. Chem. Soc.*, 1971, **93**, 4334.
[694] K. N. Fang, N. S. Kondo, P. S. Miller, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, 1971, **93**, 6647.
[695] M. Sundaralingam, *J. Amer. Chem. Soc.*, 1971, **93**, 6644.
[696] J. M. J. Tronchet and B. Baehler, *Helv. Chim. Acta*, 1971, **54**, 546.
[697] C. G. Hellerquist, O. Larm, and B. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 743.
[698] J. N. C. Whyte, *Analyt. Biochem.*, 1971, **42**, 476.
[699] G. Birch, C. K. Lee, and A. C. Richardson, *Carbohydrate Res.*, 1971, **16**, 235.

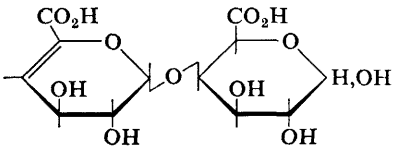
N.m.r. structural studies of the unsaturated disaccharide (473), produced from heparin by enzymes from *Flavobacterium heparinium*, have provided further evidence that heparin is composed largely of a repeating sequence of (1 → 4)-linked 4-*O*-(α-L-idopyranosyluronic acid 2-sulphate)-(2-deoxy-2-sulphamino-α-D-glucopyranosyl 6-sulphate)biose residues.[700] The anomeric configuration of the unsaturated uronic acid residue of (473) was deduced primarily from the fact that the olefinic proton H-4 experiences long-range coupling with H-2 of the same residue. From such model compounds as (474), (475), (476), and (477), it was established that H(4)—H(2) coupling



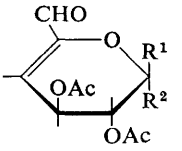
(473)



(474)



(475)



(476) R^1 = H, R^2 = OMe
(477) R^1 = OMe, R^2 = H

appears to be general for 4-deoxy-α-L-*threo*-hex-4-enopyranose derivatives but does not occur in β-L-anomers.

Use of 1,3-diaxial shielding effects as a means of distinguishing between α- and β-D-glycosides has been extended to include trehaloses and reduced disaccharides.[701]

N.M.R. Studies for Establishing the Positions of Substituents

The n.m.r. parameters of six monomethyl-D-glucose derivatives, five monomethyl-D-galactose derivatives, and three monomethyl-D-mannose derivatives have been described,[702] and spectral data for the monomethyl ethers of D-galactopyranose, methyl α- and β-D-galactopyranoside, and galactitol have also been presented.[135] Deuteriomethylated derivatives were used to facilitate positional assignments, and it was found that the most convenient method for identifying a methyl ether of D-galactopyranose was by reduction to the corresponding galactitol derivative, which gave a much simpler spectrum.[703]

[700] A. S. Perlin, D. M. Mackie, and C. P. Dietrich, *Carbohydrate Res.*, 1971, **18**, 185.
[701] M. Matsui and M. Okada, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 395.
[702] E. G. Gros, I. O. Mastronardi, and A. R. Frasca, *Carbohydrate Res.*, 1971, **16**, 232.
[703] E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydrate Res.*, 1971, **20**, 141.

A correlation has been established between the chemical shift of methoxy-substituents and the configuration of adjacent carbon atoms in di- and tri-*O*-methyl derivatives of D-galactose and D-mannose.[704]

Acyclic Derivatives

The tetra-acetates of all four D-pentononitriles have been prepared and examined by n.m.r. spectroscopy. It was found that, as with other acyclic sugar derivatives, 1,3-interactions play an important part in determining the conformation adopted. A noteworthy feature of these compounds is that rotation occurs primarily about the C(3)—C(4) bond of D-xylonitrile tetra-acetate, but about the C(2)—C(3) bond of the D-*ribo* and D-*lyxo* analogues.[705]

The preferred rotamer states about the C(1)—C(2) bonds of various *aldehydo*-sugar hydrazones have been determined by n.m.r. methods, and factors controlling the relative stabilities of possible conformations have been discussed.[706]

Lanthanide Shift Reagents

Late in 1970, a second paper appeared which further demonstrated the utility of lanthanide shift reagents in carbohydrate chemistry (see Vol. 4, p. 149). The uses of paramagnetic complexes of europium were shown by n.m.r. studies with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, benzyl 2,3-*O*-isopropylidene-β-D-ribofuranoside, ethyl 1,2:3,4-di-*O*-isopropylidene-α-D-galacturonate, and other carbohydrates. Displacements of protons to low field were observed, whereas analogous complexes of praseodymium and samarium caused displacements towards higher field.[707] More detailed studies have been reported subsequently.[708] Studies of the effect of lanthanide shift reagents on the ^{1}H n.m.r. spectra of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and -allofuranose and their acetates have also shown that, whereas europium and thulium caused shifts to low field, praseodymium produced shifts to high field.[709]

The n.m.r. shift reagents Eu(dpm)$_3$ and Pr(dpm)$_3$ have been used in assigning specific resonances in a variety of carbohydrate derivatives.[710] According to the magnitude of the observed shifts, the positions of complexation were deduced. It was suggested that complexation occurs predominantly at the primary position with D-glucose penta-acetate; in acetamido-derivatives, however, the amide function complexed more

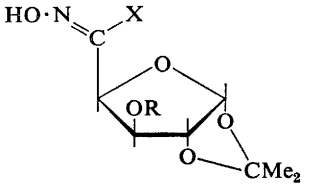
[704] A. R. Frasca, I. O. Mastronardi, and E. G. Gros, *Anales Asoc. quím. argentina*, 1971, **59**, 87 (*Chem. Abs.*, 1971, **75**, 49 456r).
[705] W. W. Binkley, D. R. Diehl, and R. W. Binkley, *Carbohydrate Res.*, 1971, **18**, 459.
[706] J. M. J. Tronchet, B. Baehler, A. Jotterand, and F. Perret, *Helv. Chim. Acta*, 1971, **54**, 1660.
[707] P. Girard, H. Kagan, and S. David, *Bull. Soc. chim. France*, 1970, 4515.
[708] P. Girard, H. Kagan, and S. David, *Tetrahedron*, 1971, **27**, 5911.
[709] I. Armitage and L. D. Hall, *Canad. J. Chem.*, 1971, **49**, 2770.
[710] R. F. Butterworth, A. G. Pernet, and S. Hanessian, *Canad. J. Chem.*, 1971, **49**, 981.

efficiently than any of the ester groups. Unresolved signals in the spectra of acetylated nucleosides were readily resolved by addition of $Eu(dpm)_3$, the anomeric proton resonances experiencing the largest shifts. With methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside, the observed shifts decreased as follows: OH > $H_{5,5'}$ > H_4, H_3 > H_1; this result indicated that complex formation occurs at the primary hydroxy-group.

The $Eu(dpm)_3$ reagent has allowed analysis of the n.m.r. spectrum of methyl α-amicetoside, and has permitted configurational assignments of two methyl 2,3,4,6-tetradeoxy-4-*C*-ethynyl-hexopyranosides.[438]

The effects on chemical shift of $Eu(dpm)_3$ with furanose derivatives have been described.[711]

Lanthanide shift reagents have also been used in studies of the *syn*- and *anti*-oximes of such compounds as (478).[712] It was found that the *syn*-isomers exist exclusively as eclipsed rotamers, whereas the *anti*-isomers assume conformations in which the rotamers are significantly staggered. The α-proton of the *anti*-isomers was more deshielded than the corresponding proton of the *syn*-isomers in the presence of a shift reagent.



(478) R = Bn or Me; X = H or Cl

^{19}F N.M.R. Spectroscopy

Further joint papers from Hall's and Foster's groups have illustrated important relations between the signs and magnitudes of $^{19}F—^{1}H$ and $^{19}F—^{19}F$ coupling constants and stereochemistry. In 3-deoxy-3-fluoro-D-glucose derivatives, $^4J_{F,H}$ values of 4.0 and − 1.5 Hz were determined for the *eq.,eq.* and *eq.,ax.* orientations, respectively.[713] In the anomeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-D-gluco- and -manno-pyranosyl fluorides, all $^{19}F—^{19}F$ coupling constants were found to be negative with values in the range 13.5—20 Hz. The largest value was for the *ax.,ax.* α-*manno*-isomer. Long-range $^{19}F—^{1}H$ couplings were also studied in these series.[714] Long-range $^{19}F—^{19}F$ couplings were studied using the anomeric 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-D-glucopyranosyl fluorides and 2,4-di-*O*-acetyl-3-deoxy-3-fluoro-β-D-xylopyranosyl fluoride;[715] $^4J_{F,H}$ values obtained were *ax.,ax.*, + 10.4; *ax.,eq.* + 1.0; and *eq.,eq.* − 3.0 Hz.

[711] S. B. Tyan and F. R. Visser, *Tetrahedron Letters*, 1971, 2833.
[712] J. M. J. Tronchet, F. Barbalat-Rey, and N. Le-Hong, *Helv. Chim. Acta*, 1971, **54**, 2615.
[713] A. B. Foster, R. Hems, and L. D. Hall, *Canad. J. Chem.*, 1970, **48**, 3937.
[714] L. D. Hall, R. N. Johnson, J. Adamson, and A. B. Foster, *Canad. J. Chem.*, 1971, **49**, 118.
[715] L. D. Hall, R. N. Johnson, A. B. Foster, and J. H. Westwood, *Canad. J. Chem.*, 1971, **49**, 236.

The ^{19}F n.m.r. spectra of anomeric pairs of all possible monodeoxymonofluoro-D-glucopyranoses have been described in detail.[716] ^{19}F Chemical shifts and coupling constants have been summarized and some structural dependencies were noted.[717, 718] It was suggested that chemical-shift information can be related to the structures of fluorinated carbohydrates and will open the way for the use of fluorine as a general probe for structural work in macromolecules. The ^{19}F resonance of 2-deoxy-2-fluoroacetamido-D-glucose showed a 13 Hz downfield shift on addition of hen-egg lysozyme, and this was attributed to a fast exchange between free and bound forms of the sugar.[719]

The n.m.r. spectra of a number of polyfluoro-1,4-dioxans and -1,4-oxathians have been described, and attempts were made to assign conformations to the compounds. The results make an interesting comparison with those obtained with fluorinated carbohydrates.[720]

^{13}C N.M.R. Spectroscopy

The increasing use of pulse Fourier-transform procedures in ^{13}C n.m.r. spectroscopy has been reflected in carbohydrate chemistry, and the general principles have been reviewed with some examples being taken from carbohydrate chemistry.[721] The application of Fourier-transform and partially relaxed Fourier methods for the assignment of all the resonances in stachyose has been described, and it was suggested that the procedure is a useful addition to other methods of assigning ^{13}C resonances.[722] Examination of an aqueous solution of D-fructose showed that, at equilibrium, the solute had the following composition: α-pyranose 3 ± 1%, β-pyranose 57 ± 6%, α-furanose 9 ± 1%, and β-furanose 31 ± 3%. Clearly, this information is difficult to obtain by ^{1}H n.m.r. methods.[723] The disaccharide 3-*O*-α-D-glucopyranosyl-D-fructose was similarly examined, and was found to have corresponding components in the ratio 4 : 39 : 20 : 41, providing additional evidence that substitution markedly favours formation of furanoses.

The pulse Fourier-transform ^{13}C n.m.r. spectra of twenty methyl and aryl glycosides have been measured, and a valuable table of chemical shifts was compiled from which configurational and conformational correlations can be made.[724] In anomeric glycosides, C-1 was observed to resonate at a relatively higher field when the substituent it carried was axially orientated,

[716] L. Phillips and V. Wray, *J. Chem. Soc.* (*B*), 1971, 1618.
[717] P. W. Kent, R. A. Dwek, and N. F. Taylor, *Tetrahedron*, 1971, **27**, 3887.
[718] P. W. Kent, R. A. Dwek, and N. F. Taylor, *Biochem. J.*, 1971, **121**, 10P.
[719] P. W. Kent and R. A. Dwek, *Biochem. J.*, 1971, **121**, 11P.
[720] J. Burdon and I. W. Parsons, *Tetrahedron*, 1971, **27**, 4553.
[721] E. Breitmaier, G. Jung, and W. Voelter, *Angew. Chem. Internat. Edn.*, 1971, **10**, 673.
[722] A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, 1971, **93**, 2777.
[723] D. Doddrell and A. Allerhand, *J. Amer. Chem. Soc.*, 1971, **93**, 2779.
[724] E. Breitmaier, W. Voelter, G. Jung, and C. Tänzer, *Chem. Ber.*, 1971, **104**, 1147.

and it was also observed that axial $O^{13}CH_3$ resonances were at 1.5—2.0 p.p.m. higher field than corresponding resonances from equatorial groups.[725] The latter result is not in agreement with other studies. For example, the $^1H—^{13}C$ INDOR technique has been applied to the determination of the chemical shifts of acetoxy, methoxy, and methyl substituents on pyranose derivatives using ^{13}C-enriched samples.[726] Values obtained for $\delta\,^{13}C$ were: *ax.* anomeric OMe, 55.12—56.63; *eq.* anomeric OMe, 56.63—58.69; *O*Ac, 20.54—20.72; *N*Ac, 22.98—23.08 p.p.m. from TMS.

^{13}C Chemical shifts for carbons carrying hydroxy-groups have been correlated with electron densities. The rates of oxidation of aldopyranoses with bromine water and of alicyclic alcohols with chromate have been correlated with measured electron densities, and oxidation mechanisms have been considered in the light of the findings.[727]

^{13}C N.m.r. spectra of a number of D-glucobioses have been measured in the hope that the C-1 resonance would be characteristic of the type of glycosidic linkage.[728] The following results (^{13}C shifts in p.p.m. from $^{13}CS_2$ in water) were obtained: methyl α-D-glucopyranoside, 93.4; kojibiose (α-1,2) 96.1; nigerose (α-1,3), 93.4; maltose (α-1,4), 92.4; methyl β-D-glucopyranoside, 89.3; sophorose (β-1,2), 88.2; laminaribose (β-1,3), 89.7; and cellobiose (β-1,4), 89.7.

An important paper has appeared on the ^{13}C n.m.r. spectra of di- and poly-saccharides. The fact that the latter spectra were measurable has been claimed as indicating that an important analytical method for these compounds is now available.[729] The effect of pH on the anomeric composition of sugars was also studied.

[725] W. Voelter, E. Breitmaier, R. Price, and G. Jung, *Chimia* (*Switz.*), 1971, **25**, 168.
[726] R. Burton, L. D. Hall, and P. R. Steiner, *Canad. J. Chem.*, 1971, **49**, 588.
[727] A. S. Perlin, *Canad. J. Chem.*, 1971, **49**, 1972.
[728] N. Yamaoka, T. Usui, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *Tetrahedron Letters*, 1971, 2047.
[729] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 4463.

24
Other Physical Methods

I.R. Spectroscopy

Absorptions in the region 750—950 cm^{-1} for a large number of tetra-acetylated α- and β-D-glucopyranosides have been reported and compared with values previously obtained for unacetylated free sugars and glycosides. With the acetates, as with other compounds, bands at 845 ± 15 cm^{-1} and 890 ± 10 cm^{-1} were found to be characteristic of α- and β-compounds, respectively.[730] Aryl D-glucopyranosides have been similarly examined; in these cases, α-anomers were characterized by absorptions in the 871—865 cm^{-1} region.[731] The spectra of crystalline L-ascorbic acid, deuteriated L-ascorbic acid, and sodium L-ascorbate have been recorded over the range 200—4000 cm^{-1}; Raman spectra of the samples and of their saturated aqueous solutions were also obtained. Observed O—H and O—D stretching frequencies have been correlated with O—O distances and with hydrogen bonding.[732] Intramolecular hydrogen-bonding in 1,6-anhydro-β-D-glucopyranose[133] and D-glucuronolactone derivatives[472] has been studied in detail.

The spectra of 2,4-*O*-methylenexylitol and some of its derivatives have been discussed,[733] and information on methyl L-rhamnopyranosides has already been noted.[112] Studies on ^{15}N compounds are referred to in Chapter 8.

U.V. Spectroscopy

The far-u.v. absorption spectra of D-glucose phosphates in aqueous solution have been measured and discussed. Absorptions were observed down to 200 nm, and temperature and solvent effects were studied. As was to be expected, no specific absorption maxima were detected.[734] Studies have been reported on a wide range of carbohydrate thio-carbonates.[382]

[730] S. H. Doss and W. M. Müller, *Austral. J. Chem.*, 1971, **24**, 2711.
[731] T. D. Audichya, T. R. Ingle, and J. L. Bose, *Indian J. Chem.*, 1971, **9**, 78.
[732] J. Hvoslef and P. Klaeboe, *Acta Chem. Scand.*, 1971, **25**, 3043.
[733] S. I. Bagaev, N. B. Bagaeva, and L. G. Soboleva, *J. Gen. Chem. U.S.S.R.*, 1970, **40**, 1638 (*Zhur. obshchei Khim.*, 1970, **40**, 1651).
[734] M. Trachtman and M. Halmann, *Carbohydrate Res.*, 1971, **19**, 245.

Mass Spectrometry

A practical note on the transfer of samples from t.l.c. plates into the mass spectrometer, which could be applied to carbohydrate samples, has appeared.[735]

A significant review containing over a hundred references has been published on the mass spectrometry of natural products containing sugars. A wide range of derivatives of free sugars and of more complex compounds, such as nucleosides and antibiotics, was discussed.[736] Trimethylsilyl (TMS) ethers have been treated exclusively in another review (in English).[737]

The mass spectrometry of fluoro-sugar acetates has been studied in detail,[738] and results on acetylated fluoroglucitols have been rationalized in terms of the position of the fluorine atom in the chain.[739] 4-Thioxylose derivatives have been compared with their xylose analogues.[381]

G.l.c.–mass spectrometry has been used to study the TMS ethers of sorbose, L-*xylo*-hexulosonic acid, and L-ascorbic acid.[740] Several reports have appeared on oligosaccharide derivatives, which make it clear that such structural details as the presence and location of ketohexofuranosyl residues,[741] the positions of linkages in trisaccharides [742, 743] and 2-acetamido-2-deoxyaldohexosyl-aldohexoses,[744] the anomeric configurations at glycosidic and reducing centres,[745] and sequences [339, 746, 747] can be elucidated by this technique. The peak at *m/e* 583, which is characteristic of TMS ethers of 1,6-linked disaccharides, has been assigned to an ion formed not from a furanose form, but from a rearrangement of the pyranose ring.[748] Oligosaccharides containing D-fructose have received particular attention,[749] and

[735] R. L. Clarke, *Chem. and Ind.*, 1971, 1434.
[736] S. Hanessian, *Methods of Biochemical Analysis*, 1971, **19**, 105.
[737] H. G. J. De Wilt and T. Tsuchiya, *Shitsuryo Bunseki*, 1970, **18**, 2914 (*Chem. Abs.*, 1971, **74**, 88 245v).
[738] O. S. Chizov, V. I. Kadentsev, B. M. Zolotarev, A. B. Foster, M. Jarman, and J. H. Westwood, *Org. Mass Spectrometry*, 1971, **5**, 437.
[739] J. Adamson, A. D. Barford, A. B. Bessell, A. B. Foster, M. Jarman, and J. H. Westwood, *Org. Mass Spectrometry*, 1971, **5**, 865.
[740] H. G. J. De Wilt, *J. Chromatog.*, 1971, **63**, 379.
[741] W. W. Binkley, R. C. Dougherty, D. Horton, and J. D. Wander, *Carbohydrate Res.*, 1971, **17**, 127.
[742] J. Kärkkäinen, *Carbohydrate Res.*, 1971, **17**, 11.
[743] J. Kärkkäinen, *Carbohydrate Res.*, 1971, **17**, 1.
[744] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. de Ridder, *Tetrahedron*, 1971, **27**, 4749.
[745] J. Vink, J. J. de Ridder, J. P. Kamerling, and J. F. G. Vliegenthart, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 1050.
[746] O. S. Chizhov, N. K. Kochetkov, N. N. Malysheva, A. I. Shiyonok, and V. L. Chashin, *Org. Mass Spectrometry*, 1971, **5**, 1145.
[747] N. K. Kochetkov, O. S. Chizhov, N. N. Malysheva, A. I. Shiyonok, and V. L. Chashin, *Org. Mass Spectrometry*, 1971, **5**, 1157.
[748] O. S. Chizhov, N. N. Malysheva, V. I. Kadentsev, and G. F. Fridlyanskii, *Bull. Acad. Sci. U.S.S.R.*, 1971, 184 (*Izvest Akad. Nauk. S.S.S.R., Ser. khim.*, 1971, 196).
[749] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. de Ridder, *Tetrahedron Letters*, 1971, 2367.

further investigations have been made of aldononitrile acetates of methylated sugars [750] and TMS ethers of *O*-methyloximes.[751]

A study of peracetates of glycosides has shown that alkyl pyranosides and furanosides can be distinguished, but the same does not apply to the aryl counterparts.[752] Introduction of the aromatic ring in aryl glycosides significantly protected the sugar molecule from damage during field-ionization mass spectrometry and during exposure to ionizing radiation.[753] Combined g.l.c.–mass spectrometry was used to identify stigmasteryl and sitosteryl D-glucosides in an extract from *Phaseolus aureus*.[754] Other glycosides to be so studied were derivatives of 1-phenylflavazoles,[755] including those derived from tri-, tetra-, and penta-saccharides.[756]

Other carbohydrate-containing compounds of biochemical interest to be investigated (as substituted derivatives) were glycosyl serine and threonine methyl esters,[757] 5′-nucleotides,[758] and aminoglycoside antibiotics (gentamicins, kanamycins, *etc.*).[538] Various methyl 4-azido-4-deoxypentoside [345] and 6-amino-6-deoxy-D-glucose [296] derivatives have also been examined.

X-Ray Crystallography

A useful review of crystal structure data for simple carbohydrates and their derivatives has appeared.[759]

So many compounds have been analysed by *X*-ray methods that a detailed description of each is no longer possible. The continued acceleration in the use of the method is exemplified by the following compounds and complexes that have been analysed: *free sugars* – α-D-glucopyranose–urea complex,[40] α-L-rhamnose,[39] α-D-xylopyranose,[38] and α-lactose [36, 37] (7% was anomerized in the solid); *glycosides* – methyl α-D-galactopyranoside,[760] methyl α-D-altropyranoside,[761] ethyl 2,3:4,5-di-*O*-isopropylidene-1-thio-β-D-glucoseptanoside, methyl 2,3,4,5-tetra-*O*-acetyl-β-D-glucoseptanoside,[762] and methyl 6-deoxy-6-methylsulphinyl-α-D-

[750] B. A. Dmitriev, L. V. Backinowsky, O. S. Chizhov, B. M. Zolotarev, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **19**, 432.
[751] R. A. Laine and C. C. Sweeley, *Analyt. Biochem.*, 1971, **43**, 533.
[752] Y. Ida, T. Komori, T. Kawasaki, K. Yoshida, and K. Kato, *J. Pharm. Soc.* (*Japan*), 1971, **91**, 119.
[753] G. O. Phillips, W. G. Filby, and W. L. Mead, *Carbohydrate Res.*, 1971, **18**, 165.
[754] R. A. Laine and A. D. Elbein, *Biochemistry*, 1971, **10**, 2547.
[755] G. S. Johnson, W. S. Ruliffson, and R. G. Cooks, *Carbohydrate Res.*, 1971, **18**, 233.
[756] G. S. Johnson, W. S. Ruliffson, and R. G. Cooks, *Carbohydrate Res.*, 1971, **18**, 243.
[757] O. S. Chizhov, V. A. Derevitskaya, B. M. Zolotarev, L. M. Likhosherstov, O. S. Novikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **20**, 275.
[758] A. M. Lawson, R. N. Stillwell, M. M. Tacker, K. Tsuboyama, and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1971, **93**, 1014.
[759] G. Strahs, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 53.
[760] B. M. Gatehouse and B. J. Poppleton, *Acta Cryst.*, 1971, **B27**, 654.
[761] B. M. Gatehouse and B. J. Poppleton, *Acta Cryst.*, 1971, **B27**, 871.
[762] J. P. Beale, N. C. Stephenson, and J. D. Stevens, *Chem. Comm.*, 1971, 484.

glucopyranoside;[763] *acid derivatives* – D-glucono-1,5-lactone,[764] D-gulono-1,4-lactone,[765] and cadmium D-glycerate 3-phosphate;[766] *anhydrides* – 1,6-anhydro-β-D-glucopyranose,[767] and 1,6:2,3-dianhydro-β-D-gulopyranose;[768] *alditols* and *cyclitols* – D-glucitol,[769] D-glucitol–pyridine (1 : 1 complex),[770] 1,6-dibromo- and 1,6-dichloro-1,6-dideoxygalactitols,[771] and epi-inositol;[502] *nucleosides* – 2′-*O*-tetrahydropyranyladenosine,[772] 5′-deoxy-5′-methylammoniumadenosine iodide,[648] 5-chlorouridine,[646] 2,4-dithiouridine,[639, 640] dihydrouridine,[641] deoxyuridine,[642] 3′-*O*-acetyl-4-thiothymidine,[647] deoxycytidine 5′-phosphate,[645] 2-thiocytidine,[644] and 6-chloro-9-(3,4-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentopyranosyl)purine.[643]

[763] B. Lindberg and P. Kierkegaard, *Acta Chem. Scand.*, 1971, **25**, 1139.
[764] M. L. Hackert and R. A. Jacobson, *Acta Cryst.*, 1971, **B27**, 203.
[765] M. M. Berman, R. D. Rosenstein, and J. Southwick, *Acta Cryst.*, 1971, **B27**, 7.
[766] A. Mostad and E. Rosenquist, *Acta Chem. Scand.*, 1971, **25**, 147.
[767] Y. J. Park, H. S. Kim, and G. A. Jeffrey, *Acta Cryst.*, 1971, **B27**, 220.
[768] B. Berking and N. C. Seeman, *Acta Cryst.*, 1971, **B27**, 1752.
[769] Y. J. Park, G. A. Jeffrey, and W. C. Hamilton, *Acta Cryst.*, 1971, **B27**, 2393.
[770] H. S. Kim, G. A. Jeffrey, and R. D. Rosenstein, *Acta Cryst.*, 1971, **B27**, 307.
[771] K. Simon and K. Sasvári, *Acta Cryst.*, 1971, **B27**, 806.
[772] O. Kennard, W. D. S. Motherwell, J. C. Coppola, B. E. Griffin, C. B. Reese, and A. C. Larson, *J. Chem. Soc.* (*B*), 1971, 1940.

25
Polarimetry

Specific rotations of aromatic glycosides and their substituted derivatives were found to increase with the polarity of the solvents used, and the solvent dependencies have been shown to be much greater for the α-anomers.[773] Added urea had a pronounced effect on the optical rotations of solutions of nucleotides, and this observation suggested that a breakdown of an ordered conformation into a random set of conformations was taking place.[630]

As in recent years, much more emphasis has been put on o.r.d. and c.d. measurements. Two new methods have been reported for determining the chirality of α-diols. One method involves examination of the c.d. spectra obtained after addition of either of the lanthanide shift reagents $Pr(dpm)_3$ or $Eu(dpm)_3$. This technique requires only very small samples of diols, which may contain tertiary hydroxy-groups.[774] The second method is based on examination of the Cotton effects exhibited by cyclic thionocarbonates.[260] Two further reports indicate that c.d. spectra of disaccharide–molybdate complexes can be used to characterize (1 → 6)-linked compounds.[775, 776]

Other o.r.d. and/or c.d. examinations have been carried out on aryl β-D-glucopyranosides having various carbonyl-containing groups attached at *o*- or *p*-positions of the aryl rings,[777] on *N*-aryl-β-D-galactopyranosyl amines during mutarotation,[338] on α- and β-anomers of methyl 2-acetamido-2-deoxy-D-mannosides,[778] on methyl 2-*O*-acetyl-3,6-dideoxy-α- and -β-D-*xylo*-hexopyranosides (the spectra were used to confirm the α-configuration of the anomeric linkage of 2-*O*-acetylabequose in a *Salmonella* lipopolysaccharide),[779] and on guanine nucleosides.[635] In the last case, it was shown that *anti* conformations are preferred in aqueous solution, whereas *syn* conformations are preferred in alcohols. This was

[773] A. Liptak and R. Bognar, *Magyar Kém. Folyoirat.*, 1971, **77**, 204 (*Chem. Abs.*, 1971, **75**, 88 857s).
[774] K. Nakanishi and J. Dillon, *J. Amer. Chem. Soc.*, 1971, **93**, 4058.
[775] W. Voelter, G. Kuhfittig, O. Oster, and E. Bayer, *Chem. Ber.*, 1971, **104**, 1234.
[776] W. Voelter, G. Kuhfittig, and E. Bayer, *Angew. Chem. Internat. Edn.*, 1970, **9**, 964.
[777] Y. Tsuzuki, S. Kataoka, M. Funayama, and K. Satsumabayashi, *Bull. Chem. Soc. Japan*, 1971, **44**, 526.
[778] S. Beychok, G. Ashwell, and E. A. Kabat, *Carbohydrate Res.*, 1971, **17**, 19.
[779] H. S. Borén, P. J. Garegg, and S. Svensson, *Acta Chem. Scand.*, 1970, **24**, 3084.

also shown to be the case in aqueous acidic media when position 8 carried a large substituent.

O.r.d. studies on triazole derivatives have been reported,[325] and studies of pyrimidine and azapyrimidine nucleosides (using o.r.d. and c.d.) have related the B_{2u} Cotton effect to conformation.[634]

26
Separatory and Analytical Methods

Chromatographic Methods

Gas–Liquid Chromatography.—Several references have been made in Chapter 24 to the combined application of g.l.c. and mass spectrometry in carbohydrate chemistry.

Cyclic butaneboronic esters have been recommended as suitable aldose and alditol derivatives for gas-chromatographic separations,[250] but most of the papers published in 1971 have described the use of more usual derivatives. Relative retention times of twenty-three disaccharide TMS ethers on three stationary phases have been reported,[780] and a related paper has described the reduction of disaccharides prior to conversion into TMS ethers or trifluoroacetyl esters.[781] Other TMS ethers examined were those of free sugars, alditols, saccharinic acids and their lactones,[782] and methyl ethers of amino-sugars.[783, 784] D-Glucose and D-fructose have been determined in syrups by a g.l.c. procedure using the same derivatives.[785]

Alditol acetates continue to be used for the analysis of sugars by g.l.c. and have been applied with methyl ethers of 2-acetamido-2-deoxy-D-glucose and -galactose,[784] with free aldoses,[786] and with forty-three methylated aldoses.[787]

Methyl furanosides of D-xylose methyl ethers, which may be formed on methanolysis of methylated polysaccharides containing D-xylose, have also been examined by g.l.c.[788]

Column and Ion-exchange Chromatography.—Gel chromatography of carbohydrates has been reviewed [789] and has been used for the separation of maltodextrins up to maltoheptaose.[790] A method for the calibration of cross-linked, gel-filtration media has been developed based on the elution characteristics of selected sugars and alcohols.[791]

[780] J. Haverkamp, J. P. Kamerling, and J. F. G. Vliegenthart, *J. Chromatog.*, 1971, **59**, 281.
[781] H. Nakamura and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 2314.
[782] A. A. El-Dash and J. E. Hodge, *Carbohydrate Res.*, 1971, **18**, 259.
[783] P. A. J. Gorin, *Carbohydrate Res.*, 1971, **18**, 281.
[784] P. A. J. Gorin and R. J. Magus, *Canad. J. Chem.*, 1971, **49**, 2583.
[785] L. T. Sennello, *J. Chromatog.*, 1971, **56**, 121.
[786] E. Sjöström and S. Juslin, *J. Chromatog.*, 1971, **54**, 9.
[787] J. Lönngren and A. Pilotti, *Acta Chem. Scand.*, 1971, **25**, 1144.
[788] D. Anderle, M. Petríková, and P. Kováč, *J. Chromatog.*, 1971, **58**, 209.
[789] S. C. Churms, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 13.
[790] H. Dellweg, M. John, and G. Trenel, *J. Chromatog.*, 1971, **57**, 89.
[791] J. M. Goodson, V. Distefano, and J. C. Smith, *J. Chromatog.*, 1971, **54**, 43.

Chromatography of carbohydrates on ion-exchange resins has been reviewed,[792] and the following compounds have been separated on resins: D- and L-*erythro*-pentulose, xylitol, L-gulonic acid, D-glucuronic acid, L-ascorbic acid, D-glucaric acid,[793] and the anomeric methyl glycopyranosides of common hexoses and 2-acetamido-2-deoxyhexoses.[794] It was suggested that the retention times of hexosides and aminohexosides depend upon the acidities of the C-2 and C-3 hydroxy-groups, respectively. Higher retention volumes were found for C-1—C-2 *trans*-isomers, and the relative acidities were rationalized in terms of the anomeric effect and other forms of dipolar interactions. Refractometry has been used in the routine analysis of sugars eluted from resin columns with borate buffers.[795]

Horizontal cellulose column chromatography of sugars has been described [796] and so has centrifugal chromatography on columns of microparticulate silica gel.[797] Under the influence of a centrifugal force, sugars migrated through the gel in narrow bands at speeds that depended on their molecular size and structure, the strength of the applied force, and the solvent; the method offers advantages in speed of separation and simplicity.

Paper Chromatography and Electrophoresis.—Thorin [1-(*o*-arsonophenylazo)-2-naphthol-3,6-disodium sulphonate] can be used as a non-destructive indicator with paper chromatography of some carbohydrates.[798]

Extensive data on the chromatographic mobilities of carbohydrates have been compiled from earlier issues of *J. Chromatog.*[799]

Various reports have appeared on the separation of methyl ethers of 2-amino-2-deoxy-D-glucose [800] and other amino-sugar methyl ethers.[783] The sugars in untreated urine have also been separated by a paper chromatographic method.[801]

The electrophoretic behaviour of several free and methylated sugars, glycosides, glycitols, and cyclitols in diphenylborinate buffer at pH 10 was found to parallel closely their behaviour in the more frequently used electrolyte of borate at the same pH.[802]

Thin-layer Chromatography.—Microcrystalline cellulose has been used for t.l.c. separations of methylated D-xyloses,[803] various free sugars,[804, 805] and oligosaccharides.[804] Kieselguhr has been used as the stationary phase in

[792] Yu. S. Ovodov, *Russian Chem. Rev.*, 1971, 406 (*Uspekhi Khim.*, 1971, 764).
[793] K. Fujita and T. Asakura, *Carbohydrate Res.*, 1971, **19**, 412.
[794] A. Neuberger and B. M. Wilson, *Carbohydrate Res.*, 1971, **17**, 89.
[795] J. J. Liljamaa and A. A. Hallén, *J. Chromatog.*, 1971, **57**, 153.
[796] J. R. Nunn, H. Paroli, and M. J. Van Der Linde, *Lab. Pract.*, 1971, **20**, 421.
[797] R. L. Anacker, J. H. Simmons, and E. Ribi, *J. Chromatog.*, 1971, **62**, 93.
[798] W. M. Pasika and A. C. West, *J. Chromatog.*, 1971, **63**, 357.
[799] *J. Chromatog.*, 1971, **62**, 173.
[800] Y. C. Lee and J. Scocca, *Analyt. Biochem.*, 1971, **39**, 24.
[801] A. S. Saini, *J. Chromatog.*, 1971, **61**, 378.
[802] P. J. Garegg and K. Lindström, *Acta Chem. Scand.*, 1971, **25**, 1559.
[803] A. Kramer and A. Ebringerova, *Chem. Zvesti*, 1970, **24**, 63.
[804] R. Spitschan, *J. Chromatog.*, 1971, **61**, 169.
[805] C. W. Raadsveld and H. Klomp, *J. Chromatog.*, 1971, **57**, 99.

the t.l.c. of monosaccharides,[806] D-fructose-oligosaccharides,[807] and, in particular, in an assay of L-fucose.[808] Silica gel impregnated with molybdic acid or phosphotungstic acid has been reported to be useful in the separation of oligosaccharides [809] and other methods have been applied to highly methylated sugars.[810]

Other Analytical Methods

The mechanism of the colour reactions of carbohydrates with cysteine and sulphuric acid has been investigated [811] and a new titrimetric analysis of aldoses involved the use of *N*-bromosuccinimide in alkali as oxidant.[812]

Methods continue to appear for the analysis of D-glucose;[813–816] some are specific, involving the use of enzymes,[813, 814] and one procedure [814] enables the anomers to be assayed.

Other methods have appeared for the analysis of free sugars and sucrose in food products,[817] for maltose (enzymically),[818] and for 2-deoxyhexoses;[819] methods for the analysis of 6-deoxyhexoses have been surveyed.[820] Methyl ethers of D-glucose,[821] amino-sugars,[822] and L-ascorbic acid 3-phosphate [823] have been assayed by newly developed procedures.

[806] T. Kartnig and O. Wegschaider, *J. Chromatog.*, 1971, **61**, 375.
[807] F. W. Collins and K. R. Chandorkar, *J. Chromatog.*, 1971, **56**, 163.
[808] M. Q.-K. Talukder, *J. Chromatog.*, 1971, **57**, 391.
[809] T. Mezzetti, M. Lato, S. Rufini, and G. Ciuffini, *J. Chromatog.*, 1971, **63**, 329.
[810] M. M. Shabana and P. Bite, *Acta Chim. Acad. Sci. Hung.*, 1971, **70**, 67.
[811] G. Kunovits, *Analyt. Chim. Acta*, 1971, **55**, 221.
[812] A. Mazzuchin, R. J. Thibert, R. J. Walton, and E. C. Pedley, *Mikrochim. Acta*, 1971, 285.
[813] J. Okuda, G. Okuda, and I. Miwa, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1945.
[814] J. Okuda and I. Miwa, *Analyt. Biochem.*, 1971, **39**, 387.
[815] J. F. Stevens, *Clin. Chim. Acta*, 1971, **32**, 199.
[816] P. L. M. Winckers and P. Jacobs, *Clin. Chim. Acta*, 1971, **34**, 401.
[817] R. E. Oborn, R. A. Libby, J. M. Ernst, and J. C. Henderson, *Cereal Chem.*, 1971, **48**, 270.
[818] J. R. Moody and W. C. Purdy, *Analyt. Chim. Acta*, 1971, **53**, 239.
[819] R. J. Doyle and M. A. Pfeifer, *Microchem. J.*, 1971, **16**, 273.
[820] G. Ya. Vidershain and L. G. Kolibaba, *Voprosy med. Khim.*, 1971, **17**, 428.
[821] A. Lipták and I. N. Jodál, *Acta Chim. Acad. Sci. Hung.*, 1971, **69**, 103.
[822] P. A. Sandford, A. J. Nafziger, and A. Jeanes, *Analyt. Biochem.*, 1971, **42**, 422.
[823] H. Nomura and S. Morimoto, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 335.

27
Alditols

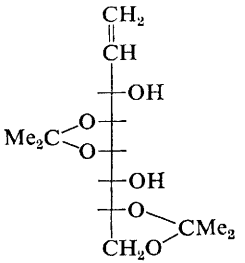
Small quantities of erythritol, arabinitol, and mannitol have been isolated from saké,[125] and D-arabinitol has been isolated from the lichen *Lecanora rupicola* (*L.*) Zahlbr.[824]

The addition of ethynylmagnesium bromide to 2,3:5,6-di-*O*-isopropylidene-D-mannose has been referred to already in Chapter 16. Reduction of the product (289) of this reaction with lithium aluminium hydride gave the octene derivative (479), which yielded 7,8-dideoxy-L-*glycero*-D-*manno*-octitol (480) following catalytic hydrogenation and hydrolysis with acid.[825] Oxidative dimerization of the octyne derivative (481) afforded the C_{16}-diyne (482), which was converted into the diene (483) on reduction with lithium aluminium hydride and reacetylation. Epoxidation of the diene (483) and subsequent hydrolysis with dilute acetic acid gave a hexadecitol, presumably as a mixture of stereoisomers.

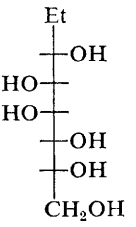
Synthetic approaches to aminodeoxyalditols have been reviewed [826] and a review has also appeared on alditol anhydrides [167] (see also Chapter 4). Phosphorylation of xylitol with tervalent phosphorus compounds also caused dehydration to give 1,4-anhydro-derivatives.[827] For example, the reaction between n-hexylphosphorous acid and xylitol afforded 1,4-anhydroxylitol 5-hexylphosphonite. 5,6-Anhydro-2,4-*O*-benzylidene-1-bromo-1-deoxy-D-mannitol has been used as an intermediate in syntheses of 1-bromo-6-chloro-1,6-dideoxy-D-mannitol, 1-bromo-1,6-dideoxy-6-iodo-D-mannitol, and 1-bromo-1-deoxy-6-*O*-methylsulphonyl-D-mannitol.[279] 1,2:5,6-Diepithio-D-mannitol and -glucitol have been obtained by way of diepoxide precursors, but the epithio-compounds are less active than the corresponding epoxides as antitumour agents.[828]

1-*O*-β-D-Galactopyranosyl-D-mannitol has been prepared following Koenigs–Knorr glycosylation of either 1,2,3,4-tetra-*O*-acetyl-β-D-manno-pyranose or its 6-trityl ether.[83]

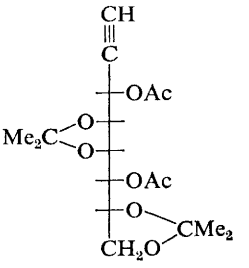
[824] J. P. Devlin, C. P. Falshaw, W. D. Ollis, and R. E. Wheeler, *J. Chem. Soc.* (*C*), 1971, 1318.
[825] R. B. Roy and W. S. Chilton, *J. Org. Chem.*, 1971, **36**, 3242.
[826] Z. F. Fialkiewicz and S. Kolka, *Wiad. Chem.*, 1970, **24**, 823.
[827] E. E. Nifantev, L. T. Elepina, and V. N. Balakhontseva, *J. Gen. Chem. U.S.S.R.*, 1971, **41**, 707 (*Zhur. obshchei Khim.*, 1971, **41**, 707).
[828] J. Kuszmann and L. Vargha, *Acta Chim. Acad. Sci. Hung.*, 1970, **66**, 307.



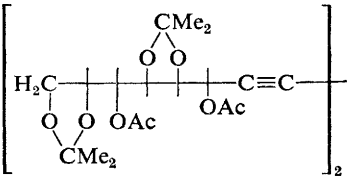
(479)



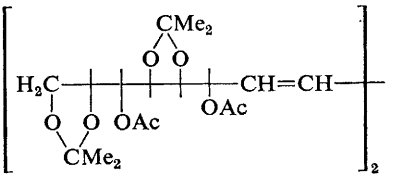
(480)



(481)



(482)



(483)

The products of acid-catalysed acetalation of xylitol with acetylsalicylaldehyde were assigned 2,4:3,5- and 2,4-arrangements for the bis- and mono-acetals, respectively.[195, 829] The i.r. spectra of 2,4-*O*-methylene-xylitol and its derivatives have been discussed.[733] Acetalation of 1-deoxy-D-glucitol with n-butyraldehyde has demonstrated that the 2,3-acetal is the product of kinetic control and that the 2,4-acetal is the thermodynamically favoured product. '2-Deoxy-D-glucitol' gave the 1,3-acetal followed by the 3,4-acetal, whereas the 3-deoxy-isomer gave the 2,4-acetal throughout.[196]

The preparation of a novel gold derivative of L-xylitol has been mentioned already [481] (see Scheme 75).

Alditol acetates continue to be used for the analysis of mixtures of sugars by g.l.c.[786] and the retention times of forty-three methylated alditol acetates have been reported.[787]

The crystal structures of D-glucitol,[769] a 1 : 1 D-glucitol–pyridine complex,[770] and 1,6-dibromo- and 1,6-dichloro-1,6-dideoxy-galactitol [771] have been determined during the past year.

[829] V. V. Rusanova, A. N. Anikeeva, and S. N. Danilov, *J. Gen. Chem. U.S.S.R.*, 1971, **41**, 200 (*Zhur. obshchei Khim.*, 1971, **41**, 204).

Part II

MACROMOLECULES

By

J. F. Kennedy

R. J. Sturgeon

1
Introduction

The primary objectives of this Part have followed the lines of previous Reports in the series. Coverage of journals has again been widened, particularly to include new journals, and some two hundred journals dealing with chemical and life sciences have been scanned regularly.

The chapter and section headings introduced for Volume 4 have largely been followed, but additional subsections have been introduced to avoid confusion in the sections on miscellaneous glycoproteins and on modified polysaccharides, glycoproteins, and enzymes.

Plant and microbial glycoproteins and phytohaemagglutinins have again been placed in the chapter on glycoproteins, whereas references to plant and microbial polysaccharides, muramic acids, and lipopolysaccharides have been placed in other chapters, depending on their source. References dealing with polysaccharide sulphates and other polysaccharides from seaweeds have been incorporated as a subsection in the chapter on plant polysaccharides.

Physicochemical studies of macromolecules have not been placed in a separate chapter, but have been mentioned alongside references to the chemical studies, since the two aspects are often inseparable.

Although the occurrence of lactoferrin is not confined to milk, all references to it have been included under the section on milk glycoproteins. Molecules related to α-lactalbumin are also included in this section, whereas α-lactalbumin itself is referred to in the chapter on enzymes, for the reasons given in Volume 4.

The term 'amylase' has been used either when the type of amylase was not specified or where the combined α- and β-amylase activity was measured.

Notification of any omissions will be welcomed.

2
General Methods

Analysis

A number of methods have been reported for the analysis of carbohydrates in macromolecules. The use of an automatic amino-acid analyser for the simultaneous determination of amino-acids, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose has been described.[1] The optimum conditions for the non-specific, continuous analysis of glycopeptides and glycoproteins using the phenol–sulphuric acid procedure have been reported.[2] Glycoproteins, glycopeptides, and certain amino-acids have been determined using an automated spectrofluorimetric method, which was based on oxidation with periodate.[3] The localization of glycoproteins in disc electrophoresis gels has been described, using different techniques for the identification of tyrosine, tryptophan, and carbohydrate.[4] A method suitable for continuous monitoring of column chromatographic effluents of polysaccharides containing hexosamines used a detection system based on the reaction with sodium hypochlorite.[5] Studies carried out on the reaction of sodium hypochlorite with glycosaminoglycans indicated that the colour yield is influenced by the nature of the amino-sugars present as well as by the position and anomeric type of linkages present.[6] A colorimetric estimation of lipopolysaccharides was based on the measurement of the spectral shift of an acidic solution of carbocyanine-dyed lipopolysaccharide.[7] T.l.c. separations of glycolipids[8] and gangliosides[9] have been reported. Cerebral cerebrosides and sulphatides have been determined after separation by t.l.c. systems,[10] and gangliosides have been determined spectrophotometrically using the sulphophosphovanillin reaction.[11]

[1] T. A. Mashburn and P. Hoffman, *Analyt. Biochem.*, 1970, **36**, 213.
[2] M. C. Brummel, H. E. Mayer, and R. Montgomery, *Analyt. Biochem.*, 1970, **33**, 16.
[3] H. Cho Tun, J. F. Kennedy, M. Stacey, and R. R. Woodbury, *Carbohydrate Res.*, 1969, **11**, 225.
[4] K. Felgenhauer, A. Weis, and G. G. Glenner, *J. Chromatog.*, 1970, **46**, 116.
[5] J. A. Bietz and P. A. Sandford, *Analyt. Biochem.*, 1971, **44**, 122.
[6] P. A. Sanford, A. J. Nafziger, and A. Jeanes, *Analyt. Biochem.*, 1971, **44**, 111.
[7] J. Jand and E. Work, *F.E.B.S. Letters*, 1971, **16**, 343.
[8] N. M. Neskovic, J. L. Nussbaum, and P. Mandel, *J. Chromatog.*, 1970, **49**, 255.
[9] D. H. van den Eijenden, *Z. physiol. Chem.*, 1971, **352**, 1601.
[10] E. Mesdjian, *Compt. rend. Soc. Biol.*, 1970, **164**, 1082.
[11] A. Saifer and N. I. Feldman, *J. Lipid Res.*, 1971, **12**, 112.

A study has been made of procedures used for the acidic hydrolysis and methanolysis of sugar polymers. Individual neutral and amino-sugars in the products of hydrolysis were examined by colorimetric methods and by g.l.c. of TMS derivatives. Methanolysis was found to be preferable for the release of neutral sugars from glycoproteins, whereas acidic hydrolysis was advantageous for the release of amino-sugars.[12]

A novel spectrophotometric method for the determination of 2-deoxy-2-sulphamidohexose and 2-acetamido-2-deoxyhexose residues in heparin has used a 3-methyl-2-benzothiazolone reagent.[13] Hexosamines have been determined by a fluorophotometric method involving formation of the Zn^{2+} chelate of the *N*-pyridoxylidene amino-sugar.[14] The reaction of sodium hypochlorite with amines, amides, and amino-sugars has led to the development of a method for the spectrophotometric determination of free and *N*-acetylated amino-sugars.[15]

The estimation of hexosamine utilizing the Elson–Morgan reaction has been evaluated after a study of the effects of several variables on the reproducibility, colour stability, and factors influencing the acetylation reaction.[16] In glycoproteins containing 2-amino-2-deoxy-D-mannose as a constituent monosaccharide, it was necessary to use two methods for the differential determination of the amino-sugar in the presence of 2-amino-2-deoxy-D-galactose and 2-amino-2-deoxy-D-glucose.[17] Hexosamines have been determined, after t.l.c. separation, as their *N*-(2,4-dinitrophenylhydrazone) derivatives.[18] Condensation of ninhydrin with phenylacetaldehyde and amino-sugars has been shown to produce highly fluorescent ternary products,[19] and the method has been applied to the detection and assay of amino-sugars in solution and for their detection on paper and thin-layer systems.[20]

An automated analytical system for the simultaneous determination of amino-acids and 2-amino-2-deoxyhexoses (by the ninhydrin method) and hexosamines (by a modified Elson–Morgan method) was suitable for column monitoring.[21] The amino-acid and amino-sugar components of bacterial cell-wall peptidoglycans have been determined using a single-column ion-exchange procedure.[22] A g.l.c. procedure has been used to separate two amino-sugars, as their methyl 3,4,6-tri-*O*-(trifluoroacetyl)-2-

[12] G. A. Levvy, A. J. Hay, J. Conchie, and I. Strachan, *Biochim. Biophys. Acta*, 1970, **222**, 333.
[13] A. Tsuji, T. Kinoshita, M. Hoshino, and M. Takeda, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 2544.
[14] M. Maeda, T. Kinoshita, and A. Tsuji, *Analyt. Biochem.*, 1970, **38**, 121.
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[16] A. R. Johnson, *Analyt. Biochem.*, 1971, **44**, 628.
[17] E. G. Brunngraber, A. Aro, and B. D. Brown, *Clinica Chim. Acta*, 1970, **29**, 333.
[18] H. J. Haas and A. Weigerding, *Carbohydrate Res.*, 1970, **12**, 211.
[19] K. Samejima, W. Dairman, and S. Udenfriend, *Analyt. Biochem.*, 1971, **42**, 222.
[20] K. Samejima, W. Dairman, J. Stone, and S. Udenfriend, *Analyt. Biochem.*, 1971, **42**, 237.
[21] M. Monsigny, *Bull. Soc. Chim. biol.*, 1969, **51**, 1263.
[22] P. Guire, *Analyt. Biochem.*, 1971, **42**, 1.

(trifluoroacetyl)amino-2-deoxyhexose derivatives, from thirty-five amino-acids.[23]

Improved resolution of D-glucosaminitol from D-galactosaminitol and the parent amino-sugars has been achieved by high-temperature chromatography on an amino-acid analyser.[24] Seven 2-amino-2-deoxy-D-hexoses have been separated by ion-exchange chromatography.[25] The separation of hexosamines has been performed on copper-impregnated sheets by ligand-exchange chromatography.[26] Ligand exchange of positively charged zinc complexes of amino-sugars with cationic resins has been used to resolve 2-amino-2-deoxy-D-glucose and either 2-amino-2-deoxy-D-mannose or 2-amino-2-deoxy-D-galactose from basic amino-acids.[27] The separation and detection of *O*-methyl derivatives of 2-amino-2-deoxy-D-glucose using an amino-acid analyser have been described.[28] Ornithine, diaminopimelic acid, and muramic acid have been determined by g.l.c. of their (*N*-heptafluorobutyl)-n-propyl ester derivatives.[29] A periodate–resorcinol method for the quantitative estimation of free sialic acids and their glycosides has been reported to be more sensitive than the resorcinol procedure and was not affected by lipids, amino-acids, or sugars; it can be used to detect free or glycosidically bound sialic acids on paper chromatograms.[30]

Hydrolysates of biological fluids (*e.g.* saliva, gastric juice, pancreatic juice, bile, and urine) have been examined for carbohydrate constituents by g.l.c. of their TMS ethers,[31] and a rapid, isothermal g.l.c. method for the analysis of glycoproteins has been reported.[32] Good correlation between colorimetric and g.l.c. analyses was shown in the identification of neutral and amino-sugars from mucins.[33] Neutral and amino-sugars have been determined by g.l.c. of their alditol acetates[34, 35] and trifluoroacetyl derivatives.[36] Procedures have been described for the g.l.c. separation and identification of a large number of biologically important monosaccharides, including hexuronic acids and oligosaccharides up to pentasaccharides.[37]

Further methods have been reported for the determination of glucose

[23] D. J. Casagrande, *J. Chromatog.*, 1970, **49**, 537.
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[26] M. D. Martz and A. F. Krivis, *Analyt. Chem.*, 1971, **43**, 790.
[27] F. W. Wagner and S. L. Shepherd, *Analyt. Biochem.*, 1971, **41**, 314.
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[31] T. Gheorghiu and K. Oette, *J. Chromatog.*, 1970, **48**, 430.
[32] P. E. Reid, B. Donaldson, D. W. Secret, and B. Bradford, *J. Chromatog.*, 1970, **47**, 199.
[33] L. J. Griggs, A. Post, E. R. White, J. A. Finkelstein, W. E. Moeckel, K. G. Holden, J. E. Zarembo, and J. A. Weisbach, *Analyt. Biochem.*, 1971, **43**, 369.
[34] W. Niedermeier, *Analyt. Biochem.*, 1971, **40**, 465.
[35] J. Metz, W. Ebert, and H. Weicker, *Chromatographia*, 1971, **4**, 345.
[36] S. Ando and T. Yamakawa, *J. Biochem.*, 1971, **70**, 335.
[37] T. Bhatti, R. E. Chambers, and J. R. Clamp, *Biochim. Biophys. Acta*, 1970, **222**, 339.

using a modified manual or automated *O*-toluidine system.[38] Attention has been drawn to the interference by dextrans in the determination of glucose by the *O*-toluidine method.[39] A complexometric determination of glucose has used Fehling's solution and H_4edta.[40] Trace amounts of D-glucose and aldosaccharides in urine were measured by the *O*-toluidine procedure, before and after removal of D-glucose with glucose oxidase,[41] and a modified automated enzymic procedure has been reported.[42] Polarographic methods for the determination of D-glucose with glucose oxidase have been described.[43–46] Results from analyses of D-glucose by a variety of spectrophotometric, chromatographic, and polarimetric techniques have been evaluated.[47] Multiple peaks produced in the silylation and subsequent g.l.c. of D-fructose and D-glucose were eliminated by changing the silylation reagent to *N*-(trimethylsilyl)imidazole and trimethylsilyl chloride.[48] The separation of mono-*O*-methyl-per-*O*-trifluoroacetyl-D-glucitols has been reported.[49]

Increased sensitivity has been achieved in a modified determination of galactose with the cysteine–sulphuric acid reagents.[50] D-Galactose has been estimated in the presence of galactolipids by a spectrophotometric method using galactose dehydrogenase.[51] A rapid, quantitative t.l.c. separation of fucose from other neutral monosaccharides has been described,[52] and the fucose content of gastric mucoprotein has been determined by g.l.c. of the TMS ethers of the methyl glycosides.[53] A double-elution method was recommended for the separation of sorbose from other common monosaccharides and for use in its quantitative determination.[54] D-Glucitol and D-mannitol have been assayed by oxidation with periodate and determination of the liberated formaldehyde with 3-methylbenzoylthiazolin-2-one hydrazone.[55] A comparison of gas chromatographic and enzymiec kinetic methods for the determination of D-glucitol and xylitol has been reported.[56]

[38] H. Y. Lee, E. S. Jenest, and F. R. Bowies, *Clinical Chem.*, 1971, **17**, 103.
[39] C. S. Frings, *Clinical Chem.*, 1970, **16**, 618.
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[46] J. Okuda and I. Miwa, *Analyt. Biochem.*, 1971, **39**, 387.
[47] B. Coxon and R. Schaffer, *Analyt. Chem.*, 1971, **43**, 1565.
[48] L. T. Sennello, *J. Chromatog.*, 1971, **56**, 121.
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[51] S. Gatt, *Biochim. Biophys. Acta*, 1970, **218**, 173.
[52] M. Q.-K. Talukder, *J. Chromatog.*, 1971, **57**, 391.
[53] R. Verga and A. Gnocchi, *J. Chromatog.*, 1970, **49**, 46.
[54] M. Iglóy and A. Mizsei, *J. Chromatog.*, 1970, **46**, 207.
[55] M. Pays and M. Beljean, *Ann. pharm. franc.*, 1970, **28**, 241.
[56] F. D. Gauchel, G. Wagner, and K. H. Bässler, *Z. Klinische Chem. Klinische Biochem.*, 1971, **9**, 25.

Selective detection of pentoses has been achieved after separation by t.l.c. and quantitative analysis by g.l.c. of the TMS ethers.[57] Quantitative t.l.c. separating arabinose, ribose, and xylose from all other sugars (except fucose) which are normally found in biological fluids has been described.[58] Small quantities of methyl furanosides of *O*-methyl-D-xyloses, which may be formed on methanolysis of methylated polysaccharides containing D-xylopyranosyl units, have been separated by g.l.c.[59]

Determinations of hexoses with 5-hydroxy-1-tetralone, using copper(II) sulphate as an accelerator of the fluorescence reaction, were not affected by the presence of aldopentoses.[60] Conditions for the g.l.c. separation of hexoses as the TMS ethers on a variety of silicone stationary phases have been studied.[61] Cyclic butaneboronic acid esters of hexitols have been used for the rapid separation of carbohydrates by g.l.c.,[62] and similar derivatives have been used in the qualitative analysis of monosaccharide mixtures.[63] A more stable stationary phase has been described for use in the g.l.c. separation of some partially methylated alditols as their acetates.[64]

Further developments on t.l.c. analyses of sugar mixtures have been reported.[65–68] Clinically important carbohydrates in plasma and urine have been detected by means of t.l.c.[69] Quantitative densitometry of chromatographic and electrophoretic systems using polaroid projection film has been applied to the analysis of monosaccharides.[70] A small, automated, high-resolution analyser has been used in the determination of carbohydrates in body fluids,[71] giving a limit of detection of 0.4—3 μg ml^{-1}. The detection of sugars, after chromatographic separation, has been achieved by direct combustion in a flame-ionization detector.[72] Perchloric acid proved a useful reagent for the detection of sugars and their derivatives on cellulose t.l.c. plates.[73] In an automated determination of reducing sugar and sucrose, the classical copper reduction methods have been compared with the reaction with sodium 2,4-dinitrophenolate.[74]

[57] J. F. Kennedy, *Chromatographia*, 1970, **3**, 316.
[58] D. J. Bell and M. Q.-K. Talukder, *J. Chromatog.*, 1970, **49**, 469.
[59] D. Anderle, M. Petríková, and P. Kováč, *J. Chromatog.*, 1971, **58**, 209.
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[71] S. Katz, S. R. Dinsmore, and W. W. Pitt, *Clinical Chem.*, 1971, **17**, 731.
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[73] K. Nagasawa, A. Ogamo, H. Harada, K. Kumagai, *Analyt. Chem.*, 1970, **42**, 1436.
[74] R. E. Oborn, R. A. Libby, J. M. Ernst, and J. C. Henderson, *Cereal Chem.*, 1971, **48**, 270.

Ion-exchange chromatography of the borate complexes of monosaccharides [75] and oligosaccharides [76] has been reported. In the separation of monosaccharides in aqueous ethanol on ion-exchange resins in their lithium and sulphate forms, elution normally followed in order of increasing number of hydroxy-groups. A linear relationship existed between the logarithm of the distribution coefficient and the number of monomeric units.[77] A comparison of the retention times obtained on g.l.c. of the TMS derivatives of twenty-three disaccharides on three liquid phases of varying polarity has indicated that they are systematically influenced by each of the structural elements of the disaccharide.[78] TMS ethers of the aldoximes formed from mono-, di-, and tri-saccharides have been separated by g.l.c.[79]

In the simultaneous determination of urinary uronic acids and saccharic acids by g.l.c., the uronic acids were converted into the corresponding stable aldonic acids by treatment with sodium borohydride. After silylation of the carboxy- and hydroxy-groups, the acids were separated by g.l.c.[80] A simple and rapid electrophoretic method for separating hexuronic acids in electrolytes of the acetates of various metals has been described.[81] Hydroxy-acids have been separated by anion-exchange chromatography followed by automatic analysis of the eluate by chromic-acid oxidation, the carbazole reaction, and periodate oxidation, with subsequent determination of formaldehyde and periodate consumption.[82]

Analytical and preparative separations of a number of polyols and methyl glycosides have been assessed from the results of g.l.c. analysis of their trifluoroacetates.[83]

In the purification of maltose from related oligosaccharides by paper chromatography, improved detection of the oligomers was obtained by spraying the papers with amyloglucosidase before staining with conventional reagents.[84] A coulometric determination of maltose has been described. In a coupled enzymic reaction, the disaccharide was first converted into D-glucose by the action of a maltase, and then into D-gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The hydrogen peroxide was then measured by iodide–thiosulphate titration.[85]

A densitometric method for determining maltosaccharides on t.l.c. plates

[75] Y. C. Lee, G. S. Johnson, B. White, and J. Scocca, *Analyt. Biochem.*, 1971, **43**, 641.
[76] A. Floridi, *J. Chromatog.*, 1971, **59**, 61.
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[81] M. J. St. Cyr, *J. Chromatog.*, 1970, **47**, 284.
[82] B. Carlsson and O. Samuelson, *Analyt. Chim. Acta*, 1970, **49**, 247.
[83] G. Jung, H. Pauschmann, W. Voelter, E. Breitmaier, and E. Bayer, *Chromatographia*, 1970, **3**, 26.
[84] J. Lehrfeld, *Carbohydrate Res.*, 1971, **19**, 400.
[85] J. R. Moody and W. C. Purdy, *Analyt. Chim. Acta*, 1971, **53**, 239.

involved detection with a double-beam densitometer.[86] The same oligosaccharides have also been separated by t.l.c. on a cellulose support,[87] by liquid-partition chromatography on cellulose columns with quantitative monitoring using an autoanalyser,[88] and by chromatography on cation-exchange resins[89] in which the detection system employed a carbon analyser. Fructo-oligosaccharides up to a DP of 20 have been separated by t.l.c. on Kieselguhr plates.[90]

Gas chromatographic determinations of TMS ethers of mono- and di-saccharides from sugar-cane juice[91] and of the alditol acetates from cellulosic materials[92] have been reported. Techniques for the separation and identification of carbohydrates by g.l.c. have been reviewed.[93]

Micro-methods for determination of the sulphate content of glycosaminoglycans have been reported,[94, 95] and small amounts of sulphate in cellulose nitrate have been determined by a water-digestion procedure.[96] Phosphate has been measured spectrophotometrically as the phosphomolybdate complex[97] and by an automatic procedure, together with ammoniacal nitrogen and total carbohydrate, in fermentation media.[98] Pyruvic acid, liberated from agar and other algal polysaccharides on acidic hydrolysis, has been determined by lactate dehydrogenase.[99]

A method for the assay of methanol has been developed and applied to the measurement of pectin ester content and pectin esterase activity.[100]

The terminal methyl protons of hydroxypropyl groups in a modified starch appear as a doublet in the n.m.r. spectrum and these signals have been utilized as the basis of a quantitative assay.[101] Acetic acid, produced on oxidation of *O*-ethylcellulose, has been determined by g.l.c.[102]

Micro-electrophoretic fractionation of polysaccharides on cellulose acetate membranes has been developed, together with a formula for calculating optimal conditions for the separation.[103] An immuno-electrophoretic assay of β-lipoprotein and albumin in human aortic intima has been achieved by direct electrophoresis of the tissue sample in an antibody-containing gel.[104]

[86] C. T. Mansfield and H. G. McElroy, *Analyt. Chem.*, 1971, **43**, 586.
[87] R. Spitschan, *J. Chromatog.*, 1971, **61**, 169.
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[94] T. T. Terho and K. Hartiala, *Analyt. Biochem.*, 1971, **41**, 471.
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[99] M. Duckworth and W. Yaphe, *Chem. and Ind.*, 1970, 747.
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[101] H. Stahl and R. P. McNaught, *Cereal Chem.*, 1970, **47**, 345.
[102] H. Jacin and J. M. Slanski, *Analyt. Chem.*, 1970, **42**, 801.
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Polyacrylamide-gel electrophoresis and gel-filtration techniques have been applied to the separation of dyed polysaccharides.[105] Aged samples of Blue Dextran have the capacity to bind proteins applied to gel columns, the binding being inhibited with increased salt concentration.[106] In the separation of oligosaccharides on polyacrylamide gels at different temperatures, a negative temperature dependence on the elution volume was established, so that distribution coefficients decreased with increasing temperature.[107] Correlation was found for the separations of xylodextrins on dextran gels and by t.l.c.[108] Separation of cellodextrins on polyacrylamide gel depended on molecular size, but interactions between the gel matrix and solute became more important with increasing molecular weight.[109] When ^3H_2O was used as a reference solute on dextran gels, a correction was made in calculating K_d values since ^3H_2O gave an overestimate of the water space in the column and, hence, an underestimate of the K_d value.[110]

A simplified procedure for preparing dextran gels used in the separation of serum immunoglobulins G and M has been reported.[111] The interaction of phenols, anilines, and benzoic acids with dextran gels has been studied.[112]

Cellulose and starch cross-linked with epichlorohydrin have been formed into gels suitable for gel-permeation chromatography of oligosaccharides.[113] Alginic acid and carboxymethylcellulose columns have been used for the separation of primary aromatic amines, and the relationship between data obtained on t.l.c. and on columns was examined.[114]

Structural Methods

The modern methodology of structural polysaccharide chemistry has been reviewed with material devoted to purification, analysis, and sequence determination of polysaccharides.[115]

G.l.c. and mass spectrometry (m.s.) have been used in combination for the analysis of simple sugars and oligosaccharides. The fragmentation patterns of partially methylated alditol acetates on electron impact have been studied using a technique of deuterium labelling, and detailed fragmentation mechanisms have been postulated.[116] The retention times of a large number of partially methylated alditol acetates and their frag-

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[106] J. J. Marshall, *J. Chromatog.*, 1970, **53**, 379.
[107] H. Dellweg, M. J. Trenel, and G. Trenel, *J. Chromatog.*, 1971, **57**, 89.
[108] W. Brown and O. Andersson, *J. Chromatog.*, 1971, **57**, 255.
[109] W. Brown, *J. Chromatog.*, 1970, **53**, 572.
[110] N. V. B. Marsden, *J. Chromatog.*, 1971, **58**, 304.
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[115] M. Stacey, *Chem. in Britain*, 1970, **6**, 113.
[116] H. Björndal, B. Lindberg, Å. Pilotti, and S. Svensson, *Carbohydrate Res.*, 1970, **15**, 339.

mentation patterns on mass spectrometry have been recorded.[117, 118] The properties of aldononitrile acetates and of partially methylated aldononitrile acetates have been studied by g.l.c.–m.s.[119] A number of disaccharides containing simple sugars and amino-sugars have been reduced to the alditols, with sodium borodeuteride, and the methylated products were separated by g.l.c. The molecular weights of monosaccharide units and the position of the glycoside linkage were determined on the basis of the mass spectra.[120] The mass spectra of six (2-acetamido-2-deoxyaldohexosyl)-aldohexoses with 1 → 2, 1 → 3, 1 → 4, and 1 → 6 glycosidic linkages could be divided into two main groups on the basis of the ratio of intensities of peaks at *m/e* 217 and *m/e* 204. This ratio was greater than unity for disaccharides with 1 → 2 or 1 → 3 linkages and was less than unity for disaccharides with 1 → 4 or 1 → 6 linkages. In all cases, the sequence of monomers was determined from the sum of intensities of the two related peaks.[121] The mass spectra of various 3,6-anhydro-D-galactose derivatives, including derivatives of carrabiose, have been studied. The presence of the 3,6-anhydro-ring was unequivocally confirmed, as well as the nature of the C-1 functional group and the nature and position of substituents at C-2 and C-4.[122] In an approach to oligosaccharide sequencing, the mass spectra of some di-, tri-, tetra-, and penta-saccharides as their 1-phenylflavazole peracetates were measured and the relationship between the spectra and structures was established.[123] The fragmentation of the acetates of oligosaccharide *N*-phenyltriazoles under electron impact has been studied. Mass spectra of the derivatives were used in determining the sequence of oligosaccharide units, the molecular weights, and for location of the interglycosidic bond in disaccharides.[124] Similar information was obtained from a study of the mass spectra of *N*-arylglycosylamine oligosaccharide acetates.[125] Hexosyl oligosaccharides containing D-fructose were shown to behave differently in the mass spectrometer from those consisting of aldohexoses only, thereby enabling characterization of fourteen D-fructose-containing oligosaccharides.[126] A new method for identification of some

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[120] J. Kärkkäinen, *Carbohydrate Res.*, 1970, **14**, 27.
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[124] O. S. Chizhov, N. K. Kochetkov, N. N. Malysheva, A. I. Shiyonok, and V. L. Chashchin, *Org. Mass Spectrometry*, 1971, **5**, 1145.
[125] O. S. Chizhov, N. K. Kochetkov, N. N. Malysheva, A. I. Shiyonok, and V. L. Chashchin, *Org. Mass Spectrometry*, 1971, **5**, 1157.
[126] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. de Ridder, *Tetrahedron Letters*, 1971, 2367.

stereoisomeric disaccharides has been developed from an investigation of the mass spectra of the TMS ether derivatives.[127] Methods based on g.l.c.–m.s. have been reported for structural analyses of disaccharides as their TMS ethers,[128] and trisaccharides as their permethylated methyl glycosides[129] and permethylated alditols.[130]

A study of the mass spectra of acetates of the glycosides of *N*-benzyloxycarbonyl-L-serine and -threonine methyl esters has revealed characteristic features which allowed identification of the amino-acid and sugar moieties and of the type of linkage between them.[131]

An improved one-step method has been developed for permethylation of polysaccharides using methyl iodide and DMSO in the presence of the methylsulphinyl methyl carbanion.[132]

The *O*-methyl ethers formed on methylation and cleavage of polymers containing 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose have been identified by g.l.c. of their alditol acetates.[133]

A procedure has been developed for establishing the type of linkage of internal 2-acetamido-2-deoxy-D-glucose residues in oligosaccharides by g.l.c. following methylation, methanolysis, and *O*-acetylation.[134]

Alkaline hydrolysis followed by deamination with nitrous acid has been applied to human-plasma α-acid glycoprotein. This procedure, which specifically cleaves glycosaminidic bonds, yielded well-defined oligosaccharides.[135]

Cleavage of glycosidic linkages occurred during the decomposition of *N*-nitroso-derivatives of two 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosides in aqueous media producing 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose and the aglycone. In a non-aqueous medium, extensive denitrosation occurred and the aglycone was retained in the products of rearrangement.[136] Alkaline degradation of 2-acetamido-2-deoxy-D-hexoses has been shown to proceed with the formation of 2-acetamido-3,6-anhydro-2-deoxy-D-hexoses; the resulting D-*galacto*-derivative was chromatographically different from the D-*gluco*- and D-*manno*-analogues. This procedure can be applied to the identification of 2-acetamido-2-deoxyhexoses in glycoproteins.[137]

[127] J. Vink, J. J. de Ridder, J. P. Kamerling, and J. F. G. Vliegenthart, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 1024.
[128] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. de Ridder, *Tetrahedron*, 1971, **27**, 4275.
[129] J. Kärkkäinen, *Carbohydrate Res.*, 1971, **17**, 1.
[130] J. Kärkkäinen, *Carbohydrate Res.*, 1971, **17**, 11.
[131] O. S. Chizhov, V. A. Derevitskaya, B. M. Zolotarev, L. M. Likhosherstov, O. S. Novikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **20**, 275.
[132] G. Keilich, P. Salminen, and E. Husemann, *Makromol. Chem.*, 1971, **141**, 117.
[133] P. A. J. Gorin and R. J. Magnus, *Canad. J. Chem.*, 1971, **49**, 2583.
[134] B. Anderson, E. A. Kabat, S. Beychok, and F. Gruezo, *Arch. Biochem. Biophys.*, 1971, **145**, 490.
[135] M. Isemura and K. Schmid, *Biochem. J.*, 1971, **124**, 591.
[136] J. W. Llewellyn and J. M. Williams, *Chem. Comm.*, 1971, 1386.
[137] V. A. Derevitskaya, L. M. Likhosherstov, V. A. Schennikov, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **20**, 285.

The reaction of alkaline sulphite with glycoproteins was potentially useful in differentiating between substitution at C-4 and at C-3 and C-6 of an *O*-(2-amino-2-deoxyhexopyranosyl) residue of a glycoprotein.[138]

The selective cleavage of glycuronosidic linkages has been applied to a xylan and resulted in removal of 94% of the terminal uronic acid residues without effective hydrolysis of other glycosidic linkages. The procedure involved Hofmann degradation of hexopyranuronamide residues followed by mild hydrolysis of the resulting 5-aminopentopyranose residues.[139]

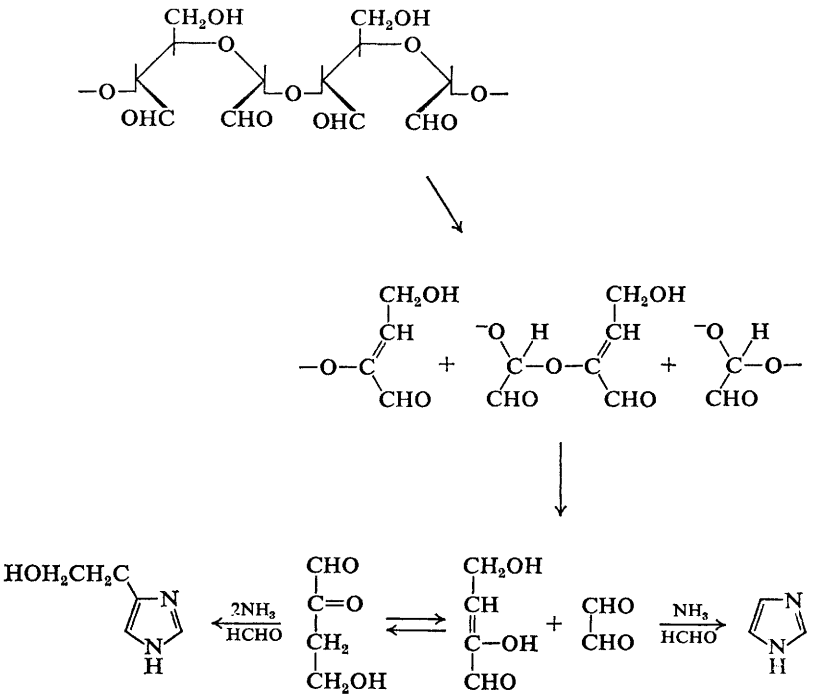
The c.d. spectra of gentiobiose, isomaltose, and melibiose in molybdate solutions showed characteristic negative Cotton effects in contrast to the corresponding spectra of 1- and 4-*O*-pyranosylpyranoses.[140]

Periodate-oxidized amylose reacted with aqueous ammonia to give imidazole and 4(5)-(2-hydroxyethyl)imidazole (Scheme 1), whereas periodate-oxidized dextran gave imidazole and 4(5)-methylimidazole (Scheme 2). Laminarin, after periodate oxidation and treatment with ammonia, gave only traces of imidazoles. Hence, the nature of the imidazoles formed is specific for the linkage in the parent polysaccharide.[141]

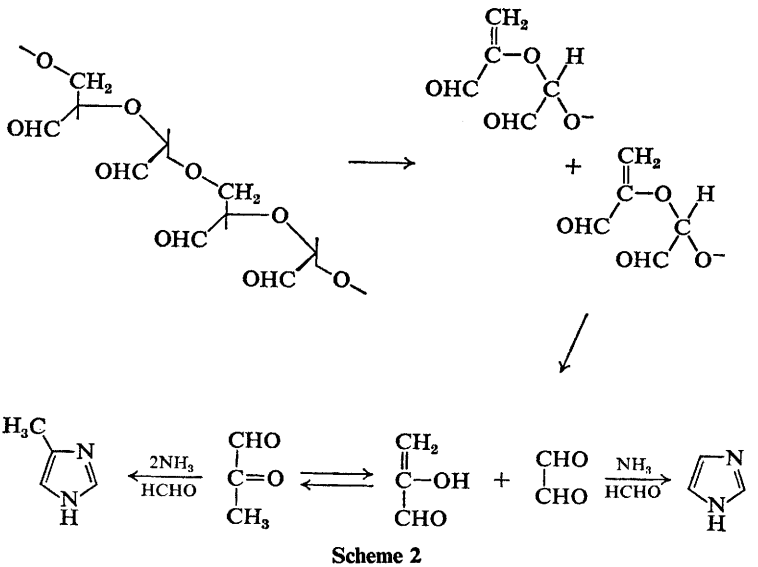
An improved method of separation of the products of Smith degradation of oligosaccharides has been reported.[142] Erythritol, arising from Smith degradation of a number of D-glucose-containing oligo- and polysaccharides, has been determined enzymically, thus giving a measure of the 1,4-linked units in the molecules.[143]

The o.r.d. spectra of D-glucose disaccharides showed that the α-linked disaccharides have a much greater positive rotation than the corresponding β-linked molecules in the u.v. region.[144] A new method for determining the configuration of glycosidic linkages in oligosaccharides has been developed from a study of the ^{1}H n.m.r. spectra of the TMS derivatives of a number of disaccharides.[145] The chemical shifts and coupling constants for the anomeric protons of some TMS oligosaccharides and their alditols have been determined.[146] By reducing oligosaccharides to the corresponding alditols, n.m.r. spectra were obtained with low-field signals derived solely from the anomeric protons at the glycosidic linkages. The chemical shifts, coupling constants, and relative intensities permitted the anomeric configurations and degrees of polymerization (DP) to be determined.[147]

[138] P. Weber and R. J. Winzler, *Arch. Biochem. Biophys.*, 1970, **137**, 421.
[139] N. K. Kochetkov, O. S. Chizhov, and A. F. Sviridov, *Carbohydrate Res.*, 1970, **14**, 277.
[140] W. Voelter, G. Kuhfittig, O. Oster, and E. Bayer, *Chem. Ber.*, 1971, **104**, 1234.
[141] E. L. Richards, *Austral. J. Chem.*, 1970, **23**, 1033.
[142] H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem.*, 1970, **68**, 253.
[143] R. J. Sturgeon, *Carbohydrate Res.*, 1971, **17**, 115.
[144] H. Shiraishi, N. Yamaoka, K. Matsuda, and K. Tuzimura, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1463.
[145] J. P. Kamerling, D. Rosenberg, and J. F. G. Vliegenthart, *Biochim. Biophys. Res. Comm.*, 1970, **38**, 794.
[146] C. G. Hellerqvist, O. Larm, and B. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 743.
[147] J. N. C. Whyte, *Analyt. Biochem.*, 1971, **42**, 476.



Scheme 1



Scheme 2

An enzymic determination of the unit chain length ($\overline{CL}$) of glycogen and related polysaccharides measured the copper reducing power of the reducing ends set free by isoamylase.[148] In a different method, the $\overline{CL}$ of glycogens and amylopectins was determined by hydrolysis of the polysaccharides to maltose and D-glucose by the action of a yeast glucosidetransferase and sweet-potato β-amylase.[149] A method has been described for the determination of $\overline{CL}$ of hexosans containing 1,3- or 1,4-linkages based on the enzymic assay of glycerol released after periodate oxidation, reduction with sodium borohydride, and total acidic hydrolysis.[150]

The $\overline{DP}$ of a number of *N*-acetylchito-oligosaccharides was determined by reduction of the reducing end-groups, acidic hydrolysis, and measurement (by g.l.c.) of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucitol as the TMS ethers.[151] A model study on the g.l.c. estimation of glycitols and reducing sugars as their TMS derivatives has been applied to the estimation of $\overline{DP}$ of polysaccharides after reduction and hydrolysis.[152] Reduction and hydrolysis of glucans, followed by determination of the released D-glucitol by sorbitol dehydrogenase, has been used to measure the $\overline{DP}$ of these polymers.[153] An automated gel-filtration system has been developed to measure the molecular weights of polysaccharides having $\overline{M}_w$ 4000—100 000.[154]

The effect of sample concentration on the chromatography of polysaccharides on polyacrylamide gels has indicated that the V_e and K_d values of polysaccharides can be considered to be independent of sample concentration on Biogel P300. With Biogel P10, however, there was a marked dependence of V_e and K_d on concentration for a dextran of $\overline{M}_w$ 10 000, indicating that the molecular weight values obtained will be meaningful only if the concentrations of the test substance and calibrating solutes are the same.[155]

Estimation of molecular weights of acidic mucopolysaccharides by electrophoresis on polyacrylamide gel was based on the measurement of relative mobilities.[156]

Homopolymers of glucopyranose, galactopyranose, mannopyranose, xylopyranose, and arabinopyranose, with various positions and configurations of linkage, have been compared by model building in a computer in an attempt to formulate rules for conformational analysis. Each polymer had one of four characteristic shapes: extended ribbon-like, flexible and

[148] Z. Gunja-Smith, J. J. Marshall, and E. E. Smith, *F.E.B.S. Letters*, 1971, **13**, 309.
[149] J. H. Carter and E. Y. C. Lee, *Analyt. Biochem.*, 1971, **39**, 373.
[150] D. W. Noble and R. J. Sturgeon, *Carbohydrate Res.*, 1970, **12**, 448.
[151] C. S. Tsai, *Analyt. Biochem.*, 1970, **36**, 114.
[152] G. G. S. Dutton, P. E. Reid, J. J. M. Rowe, and K. L. Rowe, *J. Chromatog.*, 1970, **47**, 195.
[153] D. J. Manners, A. J. Masson, and R. J. Sturgeon, *Carbohydrate Res.*, 1971, **17**, 109.
[154] G. N. Bathgate, *J. Chromatog.*, 1970, **47**, 92.
[155] S. C. Churms, A. M. Stephen, and P. van der Bijl, *J. Chromatog.*, 1970, **47**, 97.
[156] J. C. Hilborn and P. A. Anastassiadis, *Analyt. Biochem.*, 1971, **39**, 88.

helical, rigid and crumpled, or very flexible and extended. Chain-branching was shown to lead to increased steric restriction in many examples that involved two secondary positions.[157] The root-mean-square end-to-end distance was calculated for a model, allowing for free rotation about glycosidic bonds, for the general case of polysaccharides having a disaccharide repeating unit.[158]

The ^{13}C n.m.r. spectra of four disaccharides have been measured, and the ^{13}C n.m.r. spectra of amylose and cellulose acetate have been examined with a view to studying polysaccharide conformation.[159] A review has reported n.m.r. studies on biopolymers.[160] 2-Deoxy-2-trifluoroacetamido-α-D-glucose has been used as a molecular probe of lysozyme structure using ^{19}F n.m.r. techniques.[161]

[157] D. A. Rees and W. Scott, *J. Chem. Soc.* (*B*), 1971, 469.
[158] R. L. Cleland, *Biopolymers*, 1970, **9**, 811.
[159] D. E. Dorman and J. D. Roberts, *J. Amer. Chem. Soc.*, 1971, **93**, 4463.
[160] J. J. M. Rowe, J. Hinton, and K. L. Rowe, *Chem. Rev.*, 1970, **70**, 1.
[161] P. W. Kent and R. A. Dwek, *Biochem. J.*, 1971, **121**, 11P.

3
Plant and Algal Polysaccharides

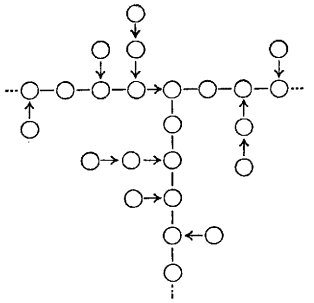
Gums and mucilages have been the subjects of a recent review.[162] Sections were devoted to methods of structural investigation and to the structures of galactan, glucuronomannan, galacturonorhamnan, xylan, and xyloglucan groups of polysaccharides.

The major component of the gum from *Khaya ivorensis* has been shown to be a polysaccharide containing a lower proportion of hexuronic acid residues than the major polysaccharide components of other *Khaya* gums; it is, however, of the same general structural type. From partial acid hydrolysis, the following oligosaccharides were isolated: 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(galactopyranosyluronic acid)-galacturonic acid, 4-*O*-(4-*O*-methylglucopyranosyluronic acid)-galactose, and *O*-(galactopyranosyluronic acid)-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-*O*-(galactopyranosyluronic acid)-(1 → 2)-rhamnose.[163] The fractionation of deacetylated *K. senegalensis* gum has been re-examined. The main component, polysaccharide A, was converted into the carboxy-reduced derivative which, after hydrolysis, allowed characterization of 4-*O*-rhamnopyranosyl-galactose, 4-*O*-galactopyranosyl-galactose, 2-*O*-galactopyranosyl-rhamnose, 4-*O*-(4-*O*-methylglucopyranosyl)-galactose, and *O*-galactopyranosyl-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-galactose. The results confirmed that this polysaccharide differs in composition from, but is similar in structure to, polysaccharide A from *K. ivorensis* gum.[164]

An analytical study of *Acacia* gum exudates of the series *Botryocephalae* indicated the presence of at least two chemically distinct types. Group A, in contrast to Group B, had low galactose : arabinose ratios (2 : 1), large negative rotations, and high intrinsic viscosity, molecular weight, and uronic anhydride and rhamnose contents.[165] Studies of the molecular-weight distribution of the polysaccharide gum from *A. podalyriaefolia* have demonstrated that, on acid hydrolysis, there was rapid cleavage of arabinofuranosyl linkages and subsequent preferential removal of galactopyranose end groups. The persistence of certain peaks in the elution patterns of the hydrolysate could be explained on the basis of a galactan framework,

[162] G. O. Aspinall, *Adv. Carbohydrate Chem. and Biochem.*, 1969, **24**, 333.
[163] G. O. Aspinall and A. K. Bhattacharjee, *J. Chem. Soc.* (*C*), 1970, 361.
[164] G. O. Aspinall and A. K. Bhattacharjee, *J. Chem. Soc.* (*C*), 1970, 365.
[165] D. M. W. Anderson, P. C. Bell, and C. G. A. McNab, *Carbohydrate Res.*, 1971, **20**, 269.

which could equally well be essentially linear or dendritically branched.[166] The polysaccharides from the gum of *A. campylacantha* have been investigated by standard procedures. The results suggested that the polymer is based on a branched galactan core (1) to which are attached uronic acid,



○ = D-galactopyranose, — = β-D-(1 → 3)-linkage, → = β-D-(1 → 6)-linkage

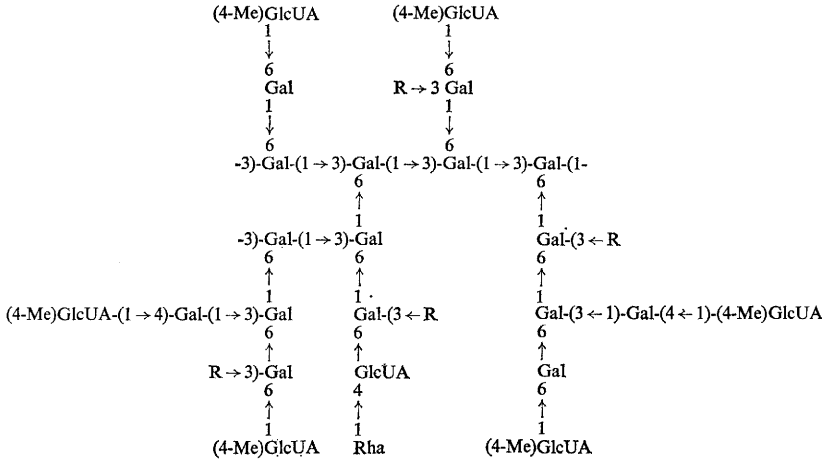
(1)

rhamnose, and unusually short chains containing not more than three arabinose residues. The branched galactan framework of *A. campylacantha* gum appears to be similar to that of *A. senegal* and *A. laeta* gums, the main difference being the lower relative proportion of β-D-(1 → 6)- to β-D-(1 → 3)-linkages in the latter.[167]

A re-examination of the 4-*O*-methylglucuronogalactan core of mesquite gum has shown that partial acid hydrolysis furnishes a mixture of acidic oligosaccharides, including 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, *O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-(1 → 6)-*O*-(β-D-galactopyranosyl)-(1 → 6)-D-galactose, and *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1 → 4)-*O*-β-D-galactose.[168] The results permitted the formulation of the partial structure (2) for the 4-*O*-methylglucuronogalactan framework. Evidence for the sequences and types of linkage between L-arabinofuranose and other sugar residues in the acid-labile outer chains of the gum has been obtained by autohydrolysis studies.[169] It was concluded that the peripheral chains of the gum contain the sugar sequences (3) and (4).

The gum from *Sterculia urens* contained D-galactose, L-rhamnose, D-glucuronic acid, and D-galacturonic acid.[170] Acidic oligosaccharides isolated from partial acid hydrolysates were characterized as 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, *O*-(galactopyranosyluronic

[166] S. C. Churms and A. M. Stephen, *Carbohydrate Res.*, 1970, **15**, 11.
[167] D. M. W. Anderson and A. C. Munro, *Carbohydrate Res.*, 1970, **12**, 9.
[168] G. O. Aspinall and C. C. Whitehead, *Canad. J. Chem.*, 1970, **48**, 3840.
[169] G. O. Aspinall and C. C. Whitehead, *Canad. J. Chem.*, 1970, **48**, 3850.
[170] G. O. Aspinall and G. R. Sanderson, *J. Chem. Soc.* (*C*), 1970, 2256.



(2)

acid)-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-galactose, *O*-(galactopyranosyluronic acid)-(1 → 2)-*O*-rhamnopyranosyl-(1 → 4)-galacturonic acid, and 3-*O*-(glucopyranosyluronic acid)-galacturonic acid. The configuration of the glycosidic linkage in 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose was confirmed by degradation of the aldobiuronic acid to 2-*O*-α-D-galactopyranosylglycerol.

α-L-Ara*f*-(1 → 6)-α-D-Gal*p*-(1 → 3)-α-L-Ara*f*-(1 → 3)-α-L-Ara*f*-(1 →

(3)

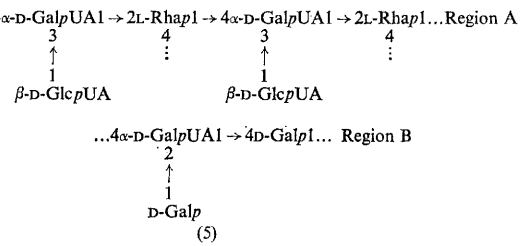
β-L-Ara*f*-(1 → 2)-β-L-Ara*f*-(1 → 2)-β-L-Ara*f*-(1 → 2)-β-L-Ara*f*-(1 → 2)-α-L-Ara*f*-(1 → 4)-β-L-Ara*p*-(1 → 3)-L-Ara*f*-(1 →

(4)

Degradation of the periodate-oxidized and carboxy-reduced gum from *S. urens* yielded a degraded polysaccharide containing essentially linear chains of alternating 4-*O*-substituted D-galactose and 2-*O*-substituted L-rhamnose units.[171] A partial structure (5) was proposed for the gum, containing galacturono-rhamnan blocks with alternating units of the two sugars (region A) and with galactogalacturonan blocks (region B) in the same chain.

The gum in the gum-resin 'ammoniac' contained D-galactose, L-arabinose, L-rhamnose, and D-glucuronic acid, and was shown to be homogeneous by electrophoresis and DEAE–cellulose chromatography.[172] From acid hydrolysates, 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose

[171] G. O. Aspinall and G. R. Sanderson, *J. Chem. Soc.* (*C*), 1970, 2259.
[172] A. Guarnieri and M. Amorosa, *Ann. Chim.* (*Italy*), 1970, **60**, 3.



(5)

and 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose were identified. Methylation analysis indicated that L-arabinose and L-rhamnose are terminal units attached mainly through C-3 to a galactan backbone.[173]

The gum polysaccharide from *Azadiraclta indica* was shown to be a carbohydrate–protein complex containing 28% of uronic acid as well as galactose and arabinose as major components; mannose, xylose, fucose, and rhamnose are minor components.[174]

Characterization of the neutral and acidic oligosaccharides obtained from partial acid hydrolysis of *Araucaria bidwillii* gave clear evidence of (1 → 6)-β-D-galactose linkages and of β-D-glucuronic acid-(1 → 6)-D-galactose linkages in the molecule.[175]

Structural aspects of the gum from *Cussonia spicata* indicated the presence of a molecular core of D-galactose units, mainly linked β-(1 → 3), most of which carry a β-D-glucuronic acid unit attached at C-6.[176] L-Arabinose occurred as short branches with each residue attached to the galactan framework at C-4 and with α-(1 → 5)-linkages between consecutive L-arabinose units.

The mucilage of the Indian fig (*Opuntia ficus-indica*) was shown by methylation to be a highly branched arabinogalactorhamnan.[177]

A mucous polysaccharide, 'plantasan', has been isolated from the seeds of *Plantago major*.[178] The homogeneous polysaccharide contains D-xylose, L-arabinose, D-galacturonic acid, L-rhamnose, and D-galactose.

The composition and preliminary fractionation of the seed mucilage of *Ocimum canum* has indicated that this capsular mucilage is partially *O*-acetylated and contains D-glucose, D-galactose, D-mannose, L-arabinose, D-xylose, and L-rhamnose, together with both D-galacturonic acid and D-mannuronic acid.[179] The material was heterogeneous, containing at least three polysaccharides, and was comparable to the cellulose-containing seed mucilages and acidic bacterial polysaccharides.

[173] A. Guarnieri and M. Amorosa, *Ann. Chim.* (*Italy*), 1970, **60**, 17.
[174] D. M. W. Anderson and A. Hendrie, *Carbohydrate Res.*, 1971, **20**, 259.
[175] G. O. Aspinall, J. A. Molloy, and C. C. Whitehead, *Carbohydrate Res.*, 1970, **12**, 143.
[176] S. C. Churms and A. M. Stephen, *Carbohydrate Res.*, 1971, **19**, 211.
[177] E. S. Amin, O. M. Awad, and M. M. El-Sayed, *Carbohydrate Res.*, 1970, **15**, 159.
[178] M. Tomoda and M. Uno, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 1214.
[179] Y. V. Anjaneyalu and R. N. Tharanathan, *Austral. J. Chem.*, 1971, **24**, 1501.

An arabinoxylan has been isolated from the mucilage of the leaves of 'Tabu' (*Machilus thunbergii*).[180] By application of periodate oxidation, Smith degradation, and methylation analysis, it was concluded that the polymer consists of a backbone of β-(1 → 4)-D-xylopyranose units to which L-arabinofuranose units are attached as non-reducing units at C-2 and C-3.

Further studies on the mucilage of the bark of *Ulmus fulva* (slippery elm) have led to the isolation and characterization of oligosaccharides arising from Smith degradation of the periodate-oxidized polysaccharide. The evidence supported the structure previously postulated.[181]

Two electrophoretically homogeneous pectins have been isolated from dried lemon peel, under conditions of minimum degradation, by extraction with cold water and sodium edta.[182] The results of analytical and electrophoretic studies on the pectic acid fractions, arising from saponification of the pectins, have been interpreted as being typical of those for the fractionation of chemically homogeneous polydisperse systems.[183]

The galactan isolated from the pectic substance of onion (*Allium cepa*) is a highly branched molecule ($\overline{CL}$, 10) comprising (1 → 4)-linked galactose units with branching at C-6.[184]

Digestion with pectinase of zosterine, the pectin substance of Zosteraceae, furnished an apiogalacturonan, an oligosaccharide mixture, and D-galacturonic acid, suggesting a structure containing, in part, galacturonan chains free of glycosidic bonds with neutral sugars.[185] The apiogalacturonan contained D-galacturonic acid and D-apiose (*ca.* 4 : 5); partial acid hydrolysis, periodate oxidation, and methylation indicated a branched structure composed of a linear α-(1 → 4)-linked D-galacturonan chain substituted at the 2-, 3-, or 2,3-positions by D-apiose residues. Apiogalacturonans have been isolated and partially characterized from the cell wall of *Lemna minor*. Purification on DEAE–Sephadex columns indicated the presence of a series of five polysaccharides of one general type.[186] The polymers were resistant to pectinase when apiose was present, but were hydrolysed on removal of the apiose residues. Periodate oxidation suggested that half of the D-apiose residues are substituted at either C-3 or C-3′.

The activity of calcium ions in aqueous solutions of calcium mono-, di-, tri- and tetra-galacturonates has been determined by a spectrophotometric method. The results were interpreted as evidence that an intramolecular chelate bond involving calcium ions and two consecutive galacturonic acid units is not very probable in calcium pectate.[187] Light-scattering studies of

[180] H. Kusunose and T. Oshibuchi, *J. Agric. Chem. Soc. (Japan)*, 1971, **45**, 156.
[181] R. J. Beveridge, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1971, **19**, 107.
[182] G. O. Aspinall and I. W. Cottrell, *Canad. J. Chem.*, 1970, **48**, 1283.
[183] G. O. Aspinall, I. W. Cottrell, J. A. Molloy, and M. Uddin, *Canad. J. Chem.*, 1970, **48**, 1290.
[184] S. K. Sen, B. P. Chatterjee, and C. V. N. Rao, *J. Chem. Soc.* (*C*), 1971, 1788.
[185] L. V. Mkheyskaya, R. G. Ovodova, and I. N. Krasikova, *Carbohydrate Res.*, 1971, **18**, 319.
[186] D. A. Hart and P. K. Kindel, *Biochem. J.*, 1970, **116**, 569.
[187] R. Kohn, *Carbohydrate Res.*, 1971, **20**, 351.

pectic substances in aqueous solution have been reported.[188] The polymers were found to be present as high molecular aggregates having strong intermolecular interactions, and their behaviour depended on the nature of the carbohydrate chain. Additives, such as urea and Tween 20, caused dissociation of the polysaccharide aggregates resulting, in some cases, in particles having similar $\overline{M}_w$ and $\overline{M}_n$.

D-Galacturonic acid residues in α-(1 → 4)-linked chains have been shown by n.m.r. spectroscopy of oligomers in solution and by mathematical model building to have Reeves *C*1 conformations. Multiple helix formation was impossible for α-(1 → 4)-linked galacturonans. L-Rhamnose units in pectic substances are interspersed to form 'kinks', which interrupt any tendency to form an ordered, chain conformation.[189]

The extraction of a flax pectin with a high molecular weight and low degree of esterification has been reported.[190]

Ammonium oxalate was shown to be more efficient than dilute acid in extracting high molecular weight pectin from garlic skins.[191] Thermal degradation of the pectin was accompanied by demethylation, deacetylation, and rupture of glycosidic linkages with the formation of galacturone.

The soybean cell-wall polysaccharides contain galacturonic acid, galactose, glucose, arabinose, xylose, and rhamnose.[192] Fractionation of the polymers indicated the presence of pectic substances (30%), hemicellulose (50%), and cellulose (20%). In the enzymic degradation of soybean cell-wall polysaccharides by *Aspergillus sojae*, a polysaccharide was liberated consisting mainly of galacturonic acid, and which was derived from the pectins of the cell wall.[193]

A water-soluble polysaccharide isolated from the opium poppy (*Papaver somniferum*) contains galacturonic acid (65%) together with galactose, arabinose, xylose, and rhamnose. Its composition and properties suggested that it belongs to the group of pectic substances.[194] Partial hydrolysis of the polysaccharide led to the identification of a series of oligosaccharides (6)—(11).[195]

4-*O*-Me-α-D-Glc*p*UA-(1 → 2)-β-D-Xyl*p*-(1 → 2)-D-Xyl

(6)

α-D-Gal*p*UA-(1 → 2)-L-Rha

(7)

[188] V. D. Sorochan, A. K. Dzizenko, N. S. Bodin, and Y. S. Ovodov, *Carbohydrate Res.*, 1971, **20**, 243.
[189] D. A. Rees and A. W. Wright, *J. Chem. Soc.* (*B*), 1971, 1366.
[190] H. G. Osman and A. F. Abdel-Fattah, *J. Chem. U.A.R.*, 1969, **12**, 551.
[191] A. F. Abdel-Fattah and A. A. Khaireldin, *J. Chem. U.A.R.*, 1970, **13**, 27.
[192] T. Kikuchi, S. Ishii, D. Fukushima, and T. Yokotsuka, *J. Agric. Chem. Soc.* (*Japan*), 1971, **45**, 228.
[193] T. Kikuchi, S. Ishii, D. Fukushima, and T. Yokotsuka, *J. Agric. Chem. Soc.* (*Japan*), 1971, **45**, 235.
[194] J. K. Wold, B. Smestad, R. Winsnes, and D. Resser, *Acta Chem. Scand.*, 1970, **24**, 1202.
[195] J. K. Wold and B. Smestad, *Acta Chem. Scand.*, 1970, **24**, 2472.

4-*O*-Me-α-D-Glc*p*UA-(1 → 4)-D-Gal
(8)

4-*O*-Me-α-D-Glc*p*UA-(1 → 2)-D-Xyl
(9)

β-D-Glc*p*UA-(1 → 4)-L-Fuc
(10)

α-D-Gal*p*UA-(1 → 4)-α-D-Gal*p*UA
(11)

The i.r. spectra of aldobiuronic acids from natural plant gums and mucilages have been reported.[196]

The effect of 2,4-dichlorophenoxyacetic acid on the synthesis of cell-wall polysaccharides in cultured sycamore cells was to facilitate the transfer of arabinose from UDP–arabinose to the pectic arabinogalactan, which is a source of neutral sugars of pectinic acid.[197] The pectic substances and hemicelluloses of pea seedlings were carried and were probably synthesized from D-glucose by the Golgi bodies and their associated vesicles in the root-tip.[198]

The structures of amylopectins and glycogens, as determined by enzymic procedures, have been reviewed.[199, 200] Some industrial aspects of the degradation of starch by amylases and amyloglucosidase have been reported.[201] The fundamental properties of typical vegetable starches, including amylose : amylopectin ratios, changes occurring during growth, and susceptibility to enzyme attack, have been reviewed.[202] A report has been published on the granule structure and the technology of starch.[203]

A direct method of starch analysis is based on dissolution of the polymer in DMSO followed by quantitative hydrolysis with glucamylase.[204] Amylose has been determined by dissolution in DMSO extracts of maize and spectrophotometric measurement of the iodine complex.[205] The amylose contents of starches and flours have been measured by formation of the iodine complex. Results were computed by means of a regression equation.[206] The starch of plant materials has been determined after hydrolysis with amyloglucosidase and subsequent analysis of D-glucose

[196] K. S. Bajrai, V. Chandrasekharan, S. Mukherjee, and A. N. Shrivastava, *Carbohydrate Res.*, 1970, **14**, 259.
[197] P. H. Rubery and D. H. Northcote, *Biochim. Biophys. Acta*, 1970, **222**, 95.
[198] P. J. Harris and D. H. Northcote, *Biochim. Biophys. Acta*, 1971, **237**, 56.
[199] W. J. Whelan, *Biochem. J.*, 1971, **123**, 1P.
[200] W. J. Whelan, *Biochem. J.*, 1971, **122**, 609.
[201] D. J. Manners, *Biochem. J.*, 1971, **123**, 1P.
[202] C. T. Greenwood, *Biochem. J.*, 1971, **123**, 1P.
[203] R. A. Knight and P. Wade, *Chem. and Ind.*, 1971, 568.
[204] R. A. Libby, *Cereal Chem.*, 1970, **47**, 273.
[205] M. J. Wolf, E. H. Melvin, W. J. Garcia, R. J. Dimler, and W. F. Kwolek, *Cereal Chem.*, 1970, **47**, 437.
[206] P. C. Williams, F. D. Kuzina, and I. Hlynka, *Cereal Chem.*, 1970, **47**, 411.

with glucose oxidase.[207] Application of a paper-chromatographic method for the identification of native and modified starches has been reported. The absorption of amylose and its derivatives was measured on the basis of iodine complexes of different intensities.[208]

Studies on starches of high amylose content have indicated that an apparent increase in amylose content is accompanied by an overall reduction in average molecular size. α-Amylolytic activity was a possible cause of fairly large-scale degradation of starch material in an amylomaize starch.[209]

The degree of hydrolysis of starch has been determined by n.m.r. spectroscopy. It was demonstrated that the doublet due to free (unlinked) β-anomeric carbon atoms (β) and to free (unlinked) α-anomeric carbon atoms (α) coincided for an entire maltodextrin series, whereas the remaining ring protons (*R*) occurred as a complex multiplet.[210] The degree of hydrolysis, as determined from the areas under these peaks, was shown to be $600(\alpha + \beta)/R$.

Amylopectin has been reported to undergo sulphation and depolymerization when treated with concentrated sulphuric acid at low temperatures.[211]

The acid hydrolysis of linear (1 → 4)-linked α-D-glucans derived from amylose can be described by a rate constant for the glycosidic bond at one end of the chain and a smaller rate constant for each remaining glycosidic bond. Oligosaccharides of $\overline{DP} > 26$ appeared to protect some of the glycosidic bonds and to decrease the effective concentration of carbohydrate.[212] The rate constants for individual bonds of maltotriose and maltotetraose were determined.[213]

X-Ray-diffraction studies of amylose–aliphatic ketone complexes have shown that the helix-packing diameter of the complex changes with the linear chain-length of the ketone molecule complexed.[214]

Samples of amylose isomerase (E.C. 2.41.18) contained oligonucleotides possessing the property of forming complexes with amylose and starch. Complex formation was strongly inhibited on removal of amylose and the outer branches of amylopectin with β-amylase, but did not take place at all in the presence of glycogen.[215]

A revision of the Meyer–Bernfeld model for amylopectin and glycogen has been proposed. Results from degradation of these polymers with isoamylase, phosphorylase, and β-amylase have indicated that the A and B chains of both amylopectin and glycogen are not arranged in the regularly

[207] R. F. H. Dekker and G. N. Richards, *J. Sci. Food and Agric.*, 1971, **22**, 441.
[208] E. Pertot and M. Blinc, *Chromatographia*, 1971, **4**, 69.
[209] G. K. Adkins, C. T. Greenwood, and D. J. Hourston, *Cereal Chem.*, 1970, **47**, 13.
[210] G. G. Birch and M. S. A. Kheiri, *Carbohydrate Res.*, 1971, **16**, 215.
[211] K. Nagasawa, Y. Tohira, Y. Inoue, and N. Tanoura, *Carbohydrate Res.*, 1971, **18** 95.
[212] M. S. Weintraub and D. French, *Carbohydrate Res.*, 1970, **15**, 241.
[213] M. S. Weintraub and D. French, *Carbohydrate Res.*, 1970, **15**, 251.
[214] K. Takeo and T. Kuge, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 537.
[215] A. N. Petrova, *Biokhimiya*, 1970, **35**, 861.

branched structure as formerly envisaged.[216] The action of purified yeast isoamylase on amylopectin, like that of bacterial pullulanase, resulted in hydrolysis of the outermost inter-chain linkages with liberation of maltosaccharides of $\overline{DP}$ 15. The products of enzymic action were discussed in relation to the probable molecular structure of the polysaccharide.[217] Sweet-potato β-amylase has been shown to act on glycogen and amylopectin in two stages. An initial, rapid action degraded the polysaccharides to a novel limit dextrin in which unbranched side-chains were shortened to three or four units in length. This dextrin was then degraded, in a slower reaction, to the classical β-limit dextrin in which unbranched side-chains were two or three units in length.[218] The rapid action of mammalian muscle phosphorylases on glycogen and amylopectin has been interpreted as being due to the high concentration of chain-ends at the surface of the polysaccharide rather than to a higher affinity for the polysaccharide.[219]

Five varieties of cowpea starch have been reported to have similar viscosities and iodine-affinities.[220] Samples of Triticale starch showed only small differences in physicochemical properties in comparison with those of rye and wheat.[221]

The size of dispersing units in maize amylose has been characterized as a pattern of continuous distribution of molecular weights ranging from a few hundred thousand to more than several million.[222]

In a study of the interaction of linear amylose oligomers of $\overline{DP}$ 22—134, the wavelength of maximum absorption of the iodine complex (λ_{max}) is related to the $\overline{DP}$ by the Langmuir isotherm: $1/\lambda_{max} = 1.558 \times 10^{-3} + 102.5 \times 10^{-4}(1/\overline{DP})$.[223] The results of a kinetic study of the rapid reaction between amylose and iodine have been interpreted on the basis of a chain-initiation mechanism involving the formation of a stable tetramer of iodine molecules inside the cavity of the amylose helix.[224] The conformation of amylose in aqueous solutions has been deduced from a study of the o.r.d. and c.d. spectra of amylose–iodine complexes and from the dependence of chain length on the retrogradation of amylose. Using an enzymically synthesized, homodispersed amylose, a worm-like helical chain seemed to provide the most adequate model for the conformation in neutral solution.[225] Optical rotations of amylose have been measured in mixed solvent systems.[226]

The degree of solvation of amylopectin in DMSO–dioxan was measured

[216] Z. Gunga-Smith, J. J. Marshall, C. Mercier, E. E. Smith, and W. J. Whelan, *F.E.B.S. Letters*, 1970, **12**, 101.
[217] R. B. Evans and D. J. Manners, *Carbohydrate Res.*, 1971, **20**, 339.
[218] E. Y. C. Lee, *Arch. Biochem. Biophys.*, 1971, **146**, 488.
[219] E. E. Smith, *Arch. Biochem. Biophys.*, 1971, **146**, 380.
[220] E. Tolmasquim, A. M. N. Correa, and S. T. Tolmasquin, *Cereal Chem.*, 1971, **48**, 132.
[221] C. P. Berry, B. L. D'Appolonia, and K. A. Gilles, *Cereal Chem.*, 1971, **48**, 415.
[222] S. Oka, S. Shigeta, and S. Sato, *Agric. Biol. Chem. (Japan)*, 1971, **35**, 1216.
[223] W. Banks, C. T. Greenwood, and K. M. Khan, *Carbohydrate Res.*, 1971, **17**, 25.
[224] J. C. Thompson and E. Hamori, *Biopolymers*, 1969, **8**, 689.
[225] B. Pfannemüller, H. Mayerhöfer, and R. C. Schulz, *Biopolymers*, 1971, **10**, 243.
[226] F. R. Dintzis, R. Tobin, and G. E. Babcock, *Biopolymers*, 1971, **10**, 379.

ultracentrifugally. The solvation number was calculated to be approximately three DMSO molecules per D-glucose residue, suggesting that hydroxy-groups take part in the process and that approximately six water molecules per D-glucose residue are involved in the hydration of amylose.[227]

Starch gelatinization has been studied by n.m.r. spectroscopy.[228]

The results of Monte-Carlo investigation of nearest-neighbour autoinhibitory effects in the periodate oxidation of amylose have been interpreted as evidence for the rapid establishment of equilibria between the aldehyde groups of oxidized units and the hemiacetals that they may form with hydroxy-groups on unoxidized neighbouring units.[229]

Changes in the composition of carbohydrate and soluble nucleotides have been determined during the development of sweet-potato roots. Starch rapidly accummulated at the stage of active development, but biosynthesis ceased on exposure of the roots to sunlight.[230]

Spinach ADP-D-glucose: α-1,4-glucan-4-glucosyl transferase was capable of synthesizing a glucan with principally α-1,4- and some α-1,6-linkages in the absence of amylose, amylopectin, or glycogen as primer.[231]

A novel phosphorylase, which had no requirement for primer, was isolated from *Solanum tuberosum*. The properties were found to differ from classical potato phosphorylase. The polymer formed was a branched α-glucan.[232]

The plastids in Mullerian body cells of *Cecropia peltata* contain a polysaccharide with properties resembling those of glycogen rather than those of starch.[233]

The characteristic ratio of unperturbed cellulose chain has been computed as a function of the $\overline{DP}$ and of the angle τ at the bridge oxygen atom. The high values obtained indicate that the cellulose chains are highly extended.[234]

The internal pore structures of cotton cellulose and formaldehyde-modified cellulose were characterized by applying the principles of gel-permeation chromatography. A linear relationship between molecular weights of 200—700 and elution volumes was obtained.[235]

Further evidence has been obtained for the thermal decomposition of cellulose by two competitive reactions. The exotherm appearing in thermograms did not result from decomposition of anhydrocellulose, but rather from breakdown of the product, presumably levoglucosan, of the depolymerization reaction.[236]

[227] S. Tomita and K. Terajina, *J. Agric. Chem. Soc.* (*Japan*), 1970, **44**, 111.
[228] E. Jaska, *Cereal Chem.*, 1971, **48**, 437.
[229] O. Smidsrød, B. Larsen, and T. Painter, *Acta Chem. Scand.*, 1970, **24**, 3201.
[230] T. Murata, *J. Agric. Chem. Soc.* (*Japan*), 1970, **44**, 412.
[231] J. L. Ozbun, J. S. Hawker, and J. Preiss, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 631.
[232] E. Slabnik and R. B. Frydman, *Biochem. Biophys. Res. Comm.*, 1970, **38**, 709.
[233] F. R. Rickson, *Science*, 1971, **173**, 344.
[234] N. Yathindra and V. S. R. Rao, *Biopolymers*, 1970, **9**, 783.
[235] L. F. Martin, F. A. Blouin, and S. P. Rowland, *Separation Sci.*, 1971, **6**, 287.
[236] D. F. Arseneau, *Canad. J. Chem.*, 1971, **49**, 632.

D-Glucose 6-sulphate was isolated in small amounts after treatment of cellulose with sulphuric acid under hydrolytic conditions similar to those normally used for the quantitative determination of the carbohydrate contents of wood and pulp. Possible losses during hydrolysis and, in particular, those caused by incomplete hydrolysis of sulphate esters were considered.[237] Hydrolysis of cellulose by small amounts of concentrated sulphuric acid progressed according to successive first-order reversible reactions of the type cellulose $\rightleftharpoons$ insoluble D-glucose polymer $\rightleftharpoons$ soluble D-glucose polymer, with reference to cellulose, if the reaction between cellulose and sulphuric acid was ignored.[238]

The aldehyde end-groups in cellulose have been determined by ion-exchange chromatography of D-glucitol derived after reduction with sodium borohydride and hydrolysis.[239]

Degradation of cotton cellulose with alkali in the absence of an aqueous phase occurred with rapid chain-splitting and with little peeling of end-groups. During the degradation, the log $\overline{DP}$ values and reaction times exhibited linearity.[240]

In a study of the degradation of carbohydrates during oxygen bleaching, it was shown that magnesium complexes protected cellobiitol from attack by alkali in the presence of oxygen, and that an excess of chelating agents resulted in decreased protective action.[241] When cotton cellulose was treated with alkali in the presence of oxygen at elevated temperatures, oxidation of hydroxy-groups along the cellulose chains occurred and, after cleavage by β-alkoxy-elimination, D-glucose end-groups were further oxidized, fragmented, and rearranged to produce D-erythronic acid and D-arabinonic acid.[242]

Chlorine dioxide acted on cellulose by arresting the drop in viscosity during chlorination due to retardation of the rate at which chlorine reacted with cellulose. The findings indicated that oxidation of cellulose by chlorine is partly a radical process and that chlorine dioxide and oxygen function as radical scavengers.[243]

The kinetics of cellulose destruction under the action of aluminium trichloride in non-aqueous solvents have been reported. Glucosidic bonds were cleaved in amorphous zones, but no changes appeared to occur at ordered zones due to the formation of carbonium ions resulting from the action of aluminium trichloride with one or two acetal oxygen atoms.[244]

Chromatography of the culture filtrates of *Trichoderma koningii* gave

[237] H. L. Hardell and O. Theander, *Svensk Papperstidn.*, 1970, **73**, 291.
[238] K. Goto, Y. Sakai, Y. Kamiyama, and T. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 111.
[239] E. Päärt and O. Samuelson, *Carbohydrate Res.*, 1970, **15**, 111.
[240] T. N. Kleinert, *Monatsch.*, 1971, **102**, 83.
[241] O. Samuelson and L. Stolpe, *Svensk Papperstidn.*, 1971, **74**, 545.
[242] H. Kolmodin and O. Samuelson, *Svensk Papperstidn.*, 1970, **73**, 93.
[243] P. S. Fredericks, B. O. Lindgren, and O. Theander, *Tappi*, 1971, **54**, 87.
[244] V. I. Ivanov, V. A. Afanasiev, and R. I. Sarybaeva, *Doklady Akad. Nauk S.S.S.R.*, 1970, **192**, 1043.

two carboxymethylcellulase components, one of which has the ability to form short fibres from cotton cellulose.[245]

A study of tri-*O*-methylcelluloses by n.m.r. spectroscopy has been reported.[246]

Because of conflicting observations in published methods for the compositional analysis of polysaccharides by g.l.c. of alditol acetates, an investigation was made of the problems of analysis of acidic polysaccharides. After acid hydrolysis, further hydrolysis of lactones and ion-exchange chromatography of acidic components were incorporated into the scheme of analysis. Methods used for the reduction of uronic acids were also examined.[247] A study has been made of the effectiveness and reproducibility of the fractionation of hemicelluloses by fractional precipitation with ethanol and by ion-exchange chromatography on cellulose, dextran, and polystyrene derivatives.[248] A method combining dialysis and ion-exchange chromatography has been used in the fractionation of hemicelluloses. The conventional separation of hemicelluloses by acidification of alkaline extracts has been shown to be subject to unpredictable variability, which can be avoided by inclusion of a heat treatment during the fractionation procedure.[249] Aqueous solutions of hemicelluloses from a number of plant sources have been examined for time- and temperature-dependent effects. The results indicated the existence of a dynamic system of aggregating polysaccharide molecules.[250]

Cellulose and 'apparent' hemicellulose in plant tissue have been determined by g.l.c.[251] DEAE–cellulose in the carbonate form has proved to be a useful medium for the fractionation of acidic and neutral polysaccharides from rape seed.[252]

A method for the determination of linkages of β-glucans by digestion with an *exo*-β-1,3-glucanase has shown that the polysaccharide from *Lupinus albus* is a β-(1 → 3)-D-glucan, whereas that from *Avena sativa* contains both β-(1 → 3)- and β-(1 → 4)-linkages.[253]

Barley β-glucan on being heated above 85 °C gave rise to solutions of greatly reduced viscosity and apparently lower molecular weight.[254]

The hemicellulosic glucan from oat leaf has been shown to have a high $\overline{DP}$ and to contain β-(1 → 3)- and β-(1 → 4)-linkages in the ratio 1 : 1.65.[255] Quantitative studies on the polysaccharides in the non-endospermic tissues

[245] G. Haliwell and M. Riaz, *Biochem. J.*, 1970, **116**, 35.
[246] D. Gagnaire, N. Heran, R. L. Fur, L. Pouit, and M. Vincendon, *Bull. Soc. chim. France*, 1970, 4326.
[247] J. D. Blake and G. N. Richards, *Carbohydrate Res.*, 1970, **14**, 375.
[248] J. D. Blake and G. N. Richards, *Carbohydrate Res.*, 1971, **17**, 253.
[249] J. D. Blake, P. T. Murphy, and G. N. Richards, *Carbohydrate Res.*, 1971, **16**, 49.
[250] J. D. Blake and G. N. Richards, *Carbohydrate Res.*, 1971, **18**, 11.
[251] J. H. Sloneker, *Analyt. Biochem.*, 1971, **43**, 539.
[252] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1971, **16**, 452.
[253] K. K. Batra and W. Z. Hassid, *Plant Physiol.*, 1970, **45**, 233.
[254] K. Morgan, *J. Int. Brew.*, 1971, **77**, 509.
[255] C. G. Fraser and K. C. B. Wilkie, *Phytochemistry*, 1971, **10**, 199.

of the oat plant in relation to growth have been reported.[256] Non-cellulosic β-glucans were isolated from oat-leaf tissues at different stages of maturity. Selective fractionation was inadequate for isolating polysaccharides for comparative purposes, but comparison of the D-glucose residues in various total hemicellulose fractions was recommended, since there was evidence for D-glucose being present in β-glucans only.[257] A fall in the ratio of β-(1 → 3)- to β-(1 → 4)-D-glucosidic linkages was observed in the β-glucans in the total hemicelluloses isolated from the stem, leaf, and hull tissues of oat plants of increasing maturity.[258] β-Glucans have been isolated from the stem tissues of barley, rye, and wheat, and the composition of β-(1 → 3)- and β-(1 → 4)-linkages has been measured.[259]

Laricinan, a glucan from the compression wood of *Larix laricina*, gave 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,4,6-tri-*O*-methyl-D-glucose on methylation analysis. Laminaribiose and laminaritriose were isolated from partial acid hydrolysates of the glucan. The polysaccharide was reported to be a β-(1 → 3)-glucan of $\overline{DP}$ 115 with six non-reducing end-groups to five branch-points per molecule.[260]

Wheat seedlings synthesized a glucan with β-(1 → 3)- and β-(1 → 4)-linkages from UDP-D-glucose. The structure of the glucan was dependent on the conditions used for the enzymic reaction and on the concentration of the substrate.[261]

A particulate enzyme from *Phaseolus aureus* incorporated UDP-D-[^{14}C]-glucose into a water-insoluble, alkali-soluble polysaccharide, which gave laminaribiose on hydrolysis. GDP-D-[^{14}C]glucose was incorporated into an alkali-insoluble polymer containing β-(1 → 4)-linkages.[262]

An autolytically solubilized glucan isolated from cell-wall fragments of corn (*Zea Mays*) coleoptiles has been partially characterized as a lichenin-type having (1 → 3)- and (1 → 4)-linkages.[263] The cell walls of oat coleoptiles contain dextran-like α-glucans, which can be broken down by dextranase.[264]

A D-fructose-rich polymer was synthesized in the presence of dextransucrase and a glycogen-value lowering factor.[265] Polyfructans of the (2 → 1)-linked type, with D-glucose at the non-reducing terminal, have been isolated from bulbs of two *Leucojum* species. Methylation and periodate oxidation indicated branching at C-6 of D-fructofuranoside units with non-reducing terminal D-fructose.[266] A glucofructan from Durum wheat flour

[256] A. J. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1971, **10**, 1285.
[257] C. G. Fraser and K. C. B. Wilkie, *Phytochemistry*, 1971, **10**, 1539.
[258] A. J. Buchala and K. C. B. Wilkie, *Phytochemistry*, 1971, **10**, 2287.
[259] A. J. Buchala and K. C. B. Wilkie, *Naturwiss.*, 1970, **57**, 496.
[260] G. C. Hoffmann and T. E. Timell, *Wood Sci. Tech.*, 1970, **4**, 159.
[261] C. Péaud-Lenoel and M. Axelos, *F.E.B.S. Letters*, 1970, **8**, 224.
[262] J. Chambers and A. D. Elbein, *Arch. Biochem. Biophys.*, 1970, **138**, 620.
[263] A. Kivilaan, R. S. Bandurski, and A. Schulze, *Plant Physiol.*, 1971, **48**, 389.
[264] A. N. J. Heyn, *Biochem. Biophys. Res. Comm.*, 1970, **38**, 831.
[265] S. Ogino, *Agric. Biol. Chem. (Japan)*, 1970, **34**, 1268.
[266] H. Hammer, *Acta Chem. Scand.*, 1970, **24**, 1294.

was shown to contain β-(2 → 6)-D-fructofuranose units with sucrose end-unit.[267]

In a study of the interaction of polysaccharides with iodine in salt solutions, the intensity of the reaction with xylans at a given polymer concentration varied with the salt involved, and was dependent on the nature and concentration of both salt and polysaccharide. No reaction took place with highly branched xylans.[268] Conformational studies on β-(1 → 3)-linked D-xylans indicated that a right-hand triple helix is favoured.[269] The structure of a β-(1 → 4)-xylan hydrate has been investigated using *X*-ray diffraction techniques. The xylan structure corresponded to the $P3_2$ space group, the trigonal symmetry of which explained the retention of a hexagonal unit cell, irrespective of water content. Water and branch units such as 4-*O*-methyl-D-glucuronic acid could be accommodated in the 'empty' lattice site.[270]

Pentosans extracted from Durum wheat gluten contained higher proportions of pentosans, with a greater degree of branching, than the corresponding pentosans extracted from the flour.[271]

Water-soluble polysaccharides have been extracted from Iraqi dates and were fractionated on DEAE–cellulose. Three of the fractions contained xylose, arabinose, and galactose (1 : 3 : 4), but varied in $\overline{M}_n$, whereas a fourth fraction contained glucose, xylose, arabinose, and galactose (1 : 1 : 2 : 3).[272]

The hemicelluloses from the stem tissues of the aquatic moss *Fontinalis antipyretica* have been fractionated, and the isolated arabinogalactan was found to have a structure similar to others found in soft woods. It has a highly branched framework of β-(1 → 3)- and β-(1 → 6)-D-galactopyranose units with some of the arabinopyranose units at non-terminal positions.[273] Coffee-bean arabinoxylan has been degraded by a purified galactanase from *Rhizopus niveus*, liberating arabinose, galactose, and 6-*O*-β-D-galactosyl-D-galactose.[274] The arabinogalactan isolated from tea leaf by extraction with hot DMSO contained L-arabinose and D-galactose (1 : 2). Structural studies have indicated the presence of a backbone of β-(1 → 4)-linked D-galactose units with non-reducing α-L-arabinofuranosyl end-groups linked to C-6.[275]

Leaves of a number of species of deciduous trees have been hydrolysed; apiose, 2-*O*-methylxylose, and 2-*O*-methylfucose have been identified after chromatography of the sugars, and probably arose from a hemicellulose

[267] D. G. Medcalf and P. W. Cheung, *Cereal Chem.*, 1971, **48**, 1.
[268] B. D. E. Gaillard and N. S. Thompson, *Carbohydrate Res.*, 1971, **18**, 137.
[269] B. K. Sathyanarayana and V. S. R. Rao, *Carbohydrate Res.*, 1970, **15**, 137.
[270] I. Nieduszynski and R. H. Marchessault, *Nature*, 1971, **232**, 46.
[271] B. L. D'Appolonia and K. A. Gilles, *Cereal Chem.*, 1971, **48**, 427.
[272] S. Koro, W. Tanimura, and K. Suminoe, *J. Agric. Chem. Soc.* (*Japan*), 1970, **44**, 489.
[273] D. S. Geddes and K. C. B. Wilkie, *Carbohydrate Res.*, 1971, **18**, 333.
[274] Y. Hashimoto, *J. Agric. Chem. Soc.* (*Japan*), 1971, **45**, 147.
[275] T. Mizuno and E. Harashima, *J. Agric. Chem. Soc.* (*Japan*), 1970, **44**, 202.

fraction. A further constituent was tentatively identified as 3-*O*-methyl-galactose and was probably accompanied by a small amount of 4-*O*-methyl-galactose.[276]

The patterns of incorporation of radioactivity from variously labelled D-glucoses and [U-^{14}C]*myo*-inositol into neutral sugars and uronic acids of the polysaccharides synthesized in different regions of the root-tip of maize were determined. The root-cap tissue synthesized a slime in which a polysaccharide containing a high proportion of fucose (32%) and galactose (21%) was found. Part of the meristematic tissue of the root was analysed and incorporation of radioactivity into arabinoxylans was demonstrated.[277]

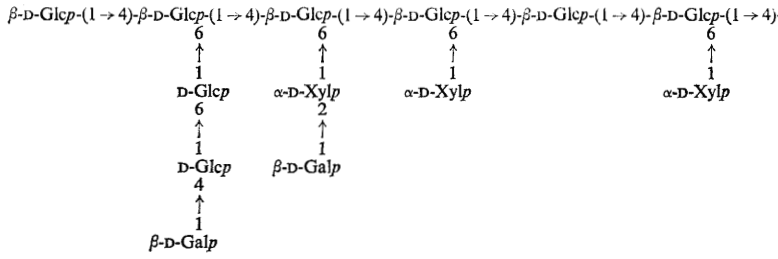
Two xyloglucan fractions were isolated from the cotyledons of resting white mustard seeds, the first by extraction with hot edta and the second by subsequent extraction with alkali. Both polysaccharides appeared to have the 'amyloid' type of structure in which chains of (1 → 4)-linked β-D-glucopyranose residues carry D-xylose-rich side-chains attached through position 6. However, these side-chains are different in structure in the two polysaccharides and one of the xyloglucans has fewer of them. The side-chains in both polysaccharides also differ from those of other seed amyloids in having xylose linked through positions 3 and 4 (instead of through position 2) and in containing fucose residues.[278]

A water-soluble amyloid type of polysaccharide has been isolated from rape seed (*Brassica tempestris*) and was shown from methylation analysis to be a complex branched polymer composed of D-galactose, D-glucose, and D-xylose (12).[279]

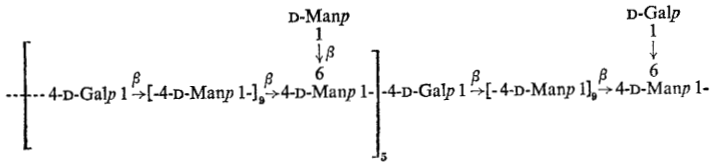
The alkali-soluble polysaccharide from the seeds of *Phoenix dactylifera* contains D-galactose and D-mannose (1 : 10). On the basis of methylation and periodate-oxidation studies, a tentative structure (13) has been assigned to the polysaccharide. The structure is based on a backbone of β-(1 → 4)-linked D-galactopyranose and β-(1 → 4)-linked D-mannopyranose units, to which are attached single units of D-mannose and possibly D-galactose linked β-(1 → 6).[280]

The isolation and characterization of the products of enzymic hydrolysis of konjac mannan have been described.[281] A cellulase from *Trichoderma viride* liberated five oligosaccharides. The structures of the three main oligosaccharides and the molar ratio of D-glucose to D-mannose (2 : 3) suggested that the polysaccharide contains the repeating unit (14). Another group of workers using similar techniques have proposed a modified structure for the polysaccharide in which at least five contiguous β-(1 → 4)-D-mannose units occur in blocks.[282]

[276] J. S. D. Bacon and M. V. Cheshire, *Biochem. J.*, 1971, **124**, 555.
[277] P. J. Harris and D. H. Northcote, *Biochem. J.*, 1970, **120**, 479.
[278] S. E. B. Gould, D. A. Rees, and N. J. Wight, *Biochem. J.*, 1971, **124**, 47.
[279] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1971, **17**, 97.
[280] V. K. Jindal and S. Mukherjee, *Indian J. Chem.*, 1970, **8**, 417.
[281] T. Saton, A. Moriya, J. Mizuguchi, and S. Suzuki, *J. Chem. Soc. Japan*, 1970, **91**, 1071.
[282] K. Kato, T. Watanabe, and K. Matsuda, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 532.



(12)



(13)

Galactomannans from the seeds of *Annona muricata, Arenga saccharifera, Cocos nucifera, Convolvulus tricolor,* and *Sophora japonica* were all shown to be of similar type consisting of main chains of β-(1 → 4)-linked D-mannose units to which are attached, in differing proportions, side-chains of single α-(1 → 6)-D-galactose units.[283]

-4)-β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1-

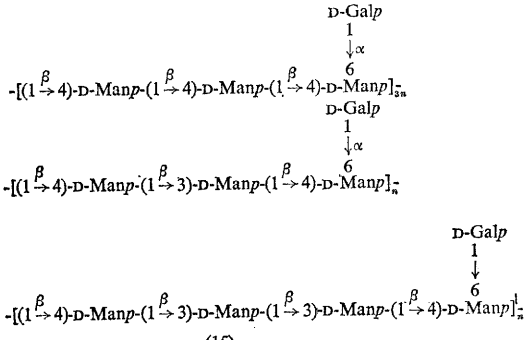
(14)

No change in the proportion of D-galactose to D-mannose residues was observed during simultaneous formation of stachyose and the reserve galactomannan in the seeds of *Trigonella foenum-graecum.* It was considered unlikely that the polysaccharide is formed by random attachment of D-galactosyl residues to a pre-formed mannan chain.[284] Galactomannans present in clover seeds were reported to serve as structural polysaccharides

[283] P. Kooiman, *Carbohydrate Res.*, 1971, **20**, 329.
[284] J. S. G. Reid and H. Meier, *Phytochemistry*, 1970, **9**, 513.

in the regulation of water balance for the seeds rather than as a reserve polysaccharide.[285]

The seeds of *Crotalaria mucronata* contain a galactomannan, which was purified by repeated complexing with copper acetate; the polysaccharide was shown to contain mannose and galactose (3 : 1). Methylation analysis showed that galactose is present only as non-reducing end-groups and that the mannose units are (1 → 3)-, (1 → 4)-, and (1 → 2)-linked. One in every six mannose residues was resistant to periodate oxidation and the occurrence of 2-*O*-β-D-mannopyranosyl-D-erythritol and *O*-β-D-mannopyranosyl-(1 → 3)-*O*-β-D-mannopyranosyl-(1 → 2)-D-erythritol in equimolar proportions indicated a significant frequency of isolated and consecutive (1 → 3)-linkages.[286] These features are accommodated in the proposed structure (15).



(15)

The major structural features of the seed galactomannans from *Caesalpina pulcherima*,[287] *Gleditsia triacanthos*,[288] and *Desmodium pulchellum*[289] have been reported to be (1 → 4)-linked mannose units with galactose linked to the main chains by (1 → 6)-bonds. Purified *endo*-β-mannanases from *Bacillus subtilis*, alfalfa, and lucerne acted on a variety of galactomannans at sections of the polymer chains where mannosyl units did not carry galactose branches.[290]

Particulate preparations from *Phaseolus aureus* have been used in rate studies on polysaccharide biosynthesis. GDP-α-D-[^{14}C]glucose appeared to be a precursor for synthesis of a glucomannan, the mannose units of which were derived from an intermediate existing in the particulate preparation. From the rate results, it appeared that GDP-α-D-[^{14}C]mannose was

[285] R. Sömme, *Acta Chem. Scand.*, 1971, **25**, 759.
[286] A. M. Unrau and Y. M. Choy, *Canad. J. Chem.*, 1970, **48**, 1123.
[287] A. M. Unrau and Y. M. Choy, *Carbohydrate Res.*, 1970, **14**, 151.
[288] C. Leschziner and A. S. Cerezo, *Carbohydrate Res.*, 1970, **15**, 291.
[289] M. P. Sinha and R. D. Tiwari, *Phytochemistry*, 1970, **9**, 1881.
[290] J. E. Courtois and P. Le Dizet, *Bull. Soc. Chim. biol.*, 1970, **52**, 15.

the precursor of at least two polysaccharides, one of which was a glucomannan.[291]

In the presence of a corn root preparation, D-[^{14}C]mannose was incorporated into a polysaccharide, which on hydrolysis yielded L-galactose, D-mannose, and L-fucose. Radioactivity was not detected in the major sugar components (D-glucose, D-galactose, D-xylose, and L-arabinose), indicating that D-mannose was converted into the polysaccharide by a relatively direct route not involving equilibration with the glycolytic hexose pool.[292]

A particulate preparation of *P. aureus* seedlings was shown to contain an acid-labile lipid intermediate, which is necessary for the transfer of D-mannose from GDP-D-mannose to polysaccharide.[293] The preparation used [^{3}H]betulaprenol phosphate as an exogenous acceptor of D-[^{14}C]mannose from GDP-D-[^{14}C]mannose, resulting in the formation of an acid-labile lipid intermediate chromatographically similar to undecaprenol phosphate.[294] In addition to a lipid intermediate for the mediation of mannan biosynthesis, a low-molecular-weight protein derivative of an oligosaccharide has been identified.[295]

GDP-D-glucose has been reported to be a possible *in vivo* precursor of a glucomannan rather than cellulose.[296]

The synthesis of a soluble uridylic acid-containing polysaccharide and of the insoluble pollen tube wall have been studied by incorporation of labelled arabinose. It appeared that the soluble polysaccharide is a large precursor subunit of some component of the cell wall.[297]

Stem tissues of the fern bracken *Pteridium aquilinum* contain a hemicellulose composed of D-mannose, D-glucose, and D-galactose (60 : 15 : 1). Methylation analysis and other evidence suggested the presence of β-(1 $\rightarrow$ 4)-linked D-glucopyranose and D-mannopyranose residues, in the ratio 1 : 4, with D-mannopyranose and D-galactopyranose residues as non-reducing end-groups. Branching appeared to occur at position-6 of the main glucomannan chain.[298]

The alkali-soluble galactomannan of white willow, *Salix alba*, is a glucomannan containing forty-seven β-D-glucopyranose and β-D-mannopyranose units (1 : 1.6) linked (1 $\rightarrow$ 4), with 2.5 branch-points per molecule.[299]

Water-soluble extracts from salep contained a linear or slightly branched β-(1 $\rightarrow$ 4)-glucomannan, as well as a low molecular weight linear α-(1 $\rightarrow$ 4)-linked glucan.[300]

[291] C. L. Villemez, *Biochem. J.*, 1971, **121**, 151.
[292] R. M. Roberts, *Arch. Biochem. Biophys.*, 1971, **145**, 685.
[293] C. L. Villemez and A. F. Clark, *Biochem. Biophys. Res. Comm.*, 1969, **36**, 57.
[294] S. S. Alam and F. W. Hemming, *F.E.B.S. Letters*, 1971, **19**, 60.
[295] C. L. Villemez, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 636.
[296] C. L. Villemez and J. S. Heller, *Nature*, 1970, **227**, 80.
[297] J. P. Mascarenhas, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 142.
[298] I. Bremner and K. C. B. Wilkie, *Carbohydrate Res.*, 1971, **20**, 193.
[299] S. Karacsonyi, *Coll. Czech. Chem. Comm.*, 1969, **34**, 3944.
[300] N. K. Shcherbukhina, B. N. Stepanenko, and V. D. Shcherbukhin, *Rast. Resur.*, 1969, **5**, 398

The major polysaccharide components of spear grass, *Heteropogon contortus*, increased during growth. Hemicelluloses were found to be predominantly of the B-type in the leaf and of both A- and B-types in the stem.[301] A xylan isolated from the leaf tissue was composed of chains of (1 → 4)-linked β-D-xylopyranose units to which are attached single L-arabinofuranose groups at C-3 and 4-*O*-methyl-D-glucuronic acid units at C-2 of the basal residues.[302]

An arabinoxylan containing glucose, galactose, and uronic acid has been purified from the hemicelluloses in milled rice by fractionation on ion-exchange cellulose columns.[303] The alkali-soluble hemicellulose from rice bran has been separated from pectin and protein components, and was shown to contain D-galactose, D-xylose, and L-arabinose. Hydrolysis by an α-L-arabinofuranosidase indicated that 35—50% of the pentose occurs as non-reducing end-groups.[304]

On the basis of periodate oxidation and methylation studies, the structure of the main hemicellulose of bamboo, *Dendrocalamus strictus*, was shown to be a β-(1 → 4)-xylan containing one D-glucuronic acid group attached to C-2 of every ninth D-xylopyranose unit.[305]

The polysaccharides isolated from soybean cotyledon meal contained an arabinan, a previously characterized arabinogalactan, and an acidic complex. Identification of the partially methylated alditol acetates from the methylated arabinan showed that the parent polysaccharide was highly branched and of the same structural type as other arabinans associated with pectins.[306]

The main, water-soluble, non-starchy polysaccharide from the endosperm of blackwheat has a molecular weight of 240 000 and contains xylose, mannose, galactose, and glucuronic acid.[307]

Tea-leaf cell-walls have been reported to contain polysaccharides composed of glucose, galactose, xylose, arabinose, ribose, and galacturonic acid.[308]

The influence of temperature on some of the constituents in alfalfa meal has shown that drying at high temperatures caused an apparent decrease in the hemicellulose content and an increase in the cellulose content.[309]

Byssinosan, an aminopolysaccharide isolated from aqueous extracts of cotton dust, contained D-glucose, D-galactose, D-mannose, and 2-acetamido-2-deoxy-D-glucose. The polymer was shown to be homogeneous by molecular sieve chromatography and by paper electrophoresis.[310]

[301] J. D. Blake and G. N. Richards, *Austral. J. Chem.*, 1970, **23**, 2353.
[302] J. D. Blake and G. N. Richards, *Austral. J. Chem.*, 1970, **23**, 2361.
[303] A. V. Cartaño and B. O. Juliano, *J. Agric. Food Chem.*, 1970, **18**, 40.
[304] H. Gremli and B. O. Juliano, *Carbohydrate Res.*, 1970, **12**, 273.
[305] J. S. Negi, T. R. Ingle, and J. L. Bose, *Indian J. Chem.*, 1970, **8**, 44.
[306] G. O. Aspinall and I. W. Cottrell, *Canad. J. Chem.*, 1971, **49**, 1019.
[307] K. Asano, M. Morita, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1522.
[308] R. R. Selvendran and B. P. M. Perera, *Chem. and Ind.*, 1971, 577.
[309] I. Delic, B. Milic, and M. Vlahovic, *J. Agric. Food Chem.*, 1971, **19**, 254.
[310] Y. S. Mahomed, R. M. El-Gazzar, and K. Adamyova, *Carbohydrate Res.*, 1971, **20**, 431.

Electrophoresis of the xylans from wheat-straw hemicellulose indicated the presence of a number of arabinoglucuronoxylans of differing percentage composition.[311]

The distribution and nature of sialic acids,[312] and their variation during development of vegetable seeds,[313] have been reported.

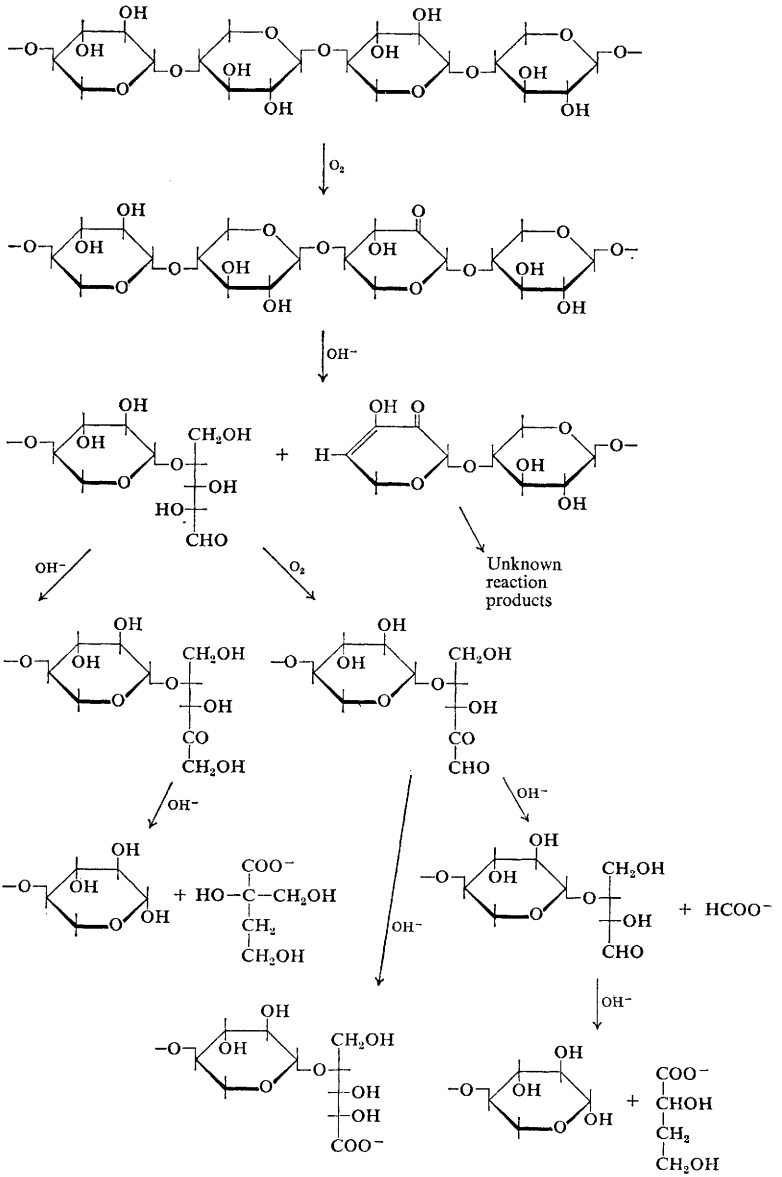
Fractions containing both hemicellulose and lignin have been isolated in different ways from two birch sulphite cooking liquors. Electrophoresis and chromatography confirmed that the hemicellulose and lignin are joined by covalent bonds. Xylose was the only component sugar found in the complex of low lignin content, but a large number of arabinose and galactose residues were detected in complexes of high lignin content.[314] *X*-Ray analysis and u.v. microscopy of the pure xylan–lignin compound supported the existence of covalent linkages. Acid hydrolysis, followed by electrophoresis, indicated that the lignin fragments were distributed along the original polysaccharide chain.[315] The hemicellulose of the lignin–carbohydrate complexes isolated from birch wood could not be separated from the lignin by electrophoresis or by chromatography.[316]

Treatment of corn-cob xylan with oxygenated alkali has been used to simulate the oxygen-bleaching process of hemicelluloses of wood pulp. The main reaction was the oxidation at secondary hydroxy-groups followed by β-elimination to give D-xylose end-groups. After rearrangement to a D-xylulose unit, a β-elimination reaction produced 3-deoxy-2-*C*-hydroxymethyltetronic acid. In a parallel reaction, the terminal units were oxidized to pentuloses, which by a benzilic-type rearrangement gave stable lyxonic acid end-groups. A competing reaction subjected the pentuloses to fragmentation to formic acid and threose end-groups, which were removed by β-elimination to produce 2,4-dihydroxybutyric acid. Other fragmentations produced 2-hydroxypropionic acid, 3-hydroxypropionic acid, and glyceric acids.[317] The main features of these reactions are described in Scheme 3.

Two aldotriuronic acids, eight aldobiuronic acids, and three hexuronic acids were isolated from hydrolysates of a hemicellulose from Scandinavian spruce. The predominant acids were 4-*O*-methyl-D-glucuronic acid and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.[318]

The products of partial acid hydrolysis of a 4-*O*-methylglucuronoxylan were acetylated and then reduced with diborane to give a mixture amenable to routine sugar analysis. The ratio of 4-*O*-methyl-D-glucitol to xylitol

[311] M. S. Dudkin and N. G. Shkantova, *Zhur. priklad. Khim.*, 1970, **43**, 206.
[312] O. Schettino, M. I. La Rotonda, and L. Ferrara, *Boll. Soc. Ital. Biol. Sper.*, 1970, **46**, 497.
[313] O. Schettino, L. Ferrara, and M. I. La Rotonda, *Boll. Soc. Ital. Biol. Sper.*, 1970, **46**, 499,
[314] R. Simonson, *Svensk Papperstidn.*, 1971, **74**, 153.
[315] R. Simonson, *Svensk Papperstidn.*, 1971, **74**, 268.
[316] R. Simonson, *Svensk Papperstidn.*, 1971, **74**, 604.
[317] H. Kolmodin and O. Samuelson, *Svensk Papperstidn.*, 1971, **74**, 301.
[318] B. Carlsson, S. Johnson, and O. Samuelson, *Svensk Papperstidn.*, 1971, **74**, 168.



Scheme 3

indicated the degree of substitution of the xylan with respect to uronic acid. The reduction step proceeded in 80% yield, thus giving a semiquantitative method for uronic acid analysis.[319]

Extracts of the cottonwood, *Populus trichocarpa*, contained lignin, cellulose, 4-*O*-methylglucuronoxylan, and glucomannan. Differential thermal analysis and thermogravimetric analysis showed the relationship between the pyrolytic properties of the wood and its components.[320] The woody tissues of *Artemisia tridentata vaseyana* were shown to contain cellulose, *O*-acetyl-4-*O*-methylglucuronoxylan, and glucomannan.[321]

The structure, conformation, and mechanism of formation of polysaccharide gels and networks have been the subjects of a review.[322]

In a study of the solution properties of alginates, the intrinsic viscosity at zero rate of shear was determined for alginates with molecular weights 1×10^5—2.7×10^6. The intrinsic viscosity was found to be linear with respect to the reciprocal square root of the ionic strength.[323]

Extensive autoxidative degradation of low- and high-molecular-weight alginic acid was achieved using an electrolysis cell and iron–edta as a reductant at neutral pH. Free-radical induced degradation was of the order of 20% after 10 h.[324] The degraded products obtained from sodium alginate, by either γ-irradiation or oxidative–reductive depolymerization, were found to have gross chemical and biological properties similar to those of a derivative of sodium alginate prepared by hydrolytic degradation.[325] Calculation of the nearest-neighbour frequencies in fragments of alginates was made from measurement of individual yields of free monomers after partial acid hydrolysis to a known degree of scission.

The alginate from *Ishige okamurai* was fractionated to give a guluronic acid-rich fraction, a mannuronic acid-rich fraction, and an intermediate fraction. Digestion with either polymannuronide lyase or polyguluronide lyase left alginate-like polyuronides, which were richer in either guluronic acid or mannuronic acid. It was shown that the alginic acid of this alga is heterogeneous in the localization of each kind of uronic acid residue.[326]

X-Ray diffraction studies have been reported on polyuronides of green algae.[327] The alginate produced by *Azotobacter vinelandii* has been characterized as an extracellular, partially acetylated polyuronide; the yield was increased by growth on acetate, and the ratio of uronic acids was dependent on the Ca^{2+}-ion concentration.[328] A polymannuronic acid C-5-epimerase has been isolated from *A. vinelandii*. When incubated with calcium

[319] B. Enström and J. Janson, *Svensk Papperstidn.*, 1970, **73**, 371.
[320] F. Shafizadeh and G. D. McGinnis, *Carbohydrate Res.*, 1971, **16**, 273.
[321] F. Shafizadeh and W. Bukwa, *Phytochemistry*, 1970, **9**, 871.
[322] D. A. Rees, *Adv. Carbohydrate Chem. Biochem.*, 1969, **24**, 267.
[323] O. Smidsrød, *Carbohydrate Res.*, 1970, **13**, 359.
[324] M. Harris, A. Herp, R. Phelps, and W. Pigman, *Biochim. Biophys. Acta*, 1970, **244**, 501.
[325] E. R. Humphreys and G. R. Howells, *Carbohydrate Res.*, 1971, **16**, 65.
[326] S. Fujibayashi, H. Habe, and K. Nisizawa, *J. Biochem. (Tokyo)*, 1970, **67**, 37.
[327] D. R. Kreger, *Nature*, 1970, **227**, 81.
[328] B. Larsen and A. Haug, *Carbohydrate Res.*, 1971, **17**, 287.

alginate, the enzyme epimerized D-mannuronic acid residues to L-guluronic acid residues in the polymer chain.[329] Incubation of polymannuronic acid in tritiated water with this enzyme led to incorporation of tritium into the glycuronan. Hydrolysis of the enzyme-treated polymer and separation of the uronic acids indicated that 92% of the activity was present in L-guluronic acid residues, with a small but significant incorporation into D-mannuronic acid residues, possibly indicating that the reaction was reversible. The mechanism of the reaction is assumed to involve abstraction of the hydrogen atom adjacent to the carboxy-group (*i.e.* H-5).[330]

A semi-quantitative estimation of the composition of alginates is based on i.r. spectroscopy. The areas of the bands at 808 and 787 cm^{-1}, measured directly from the i.r. traces, gave values in agreement with the ratio of D-mannuronic acid and L-guluronic acid determined by other conventional means.[331]

A separation of primary aromatic amines on columns of alginic acid has been reported.[114]

A definitive assay for pyruvic acid in agar and other algal polysaccharides was based on estimation of the pyruvate liberated by lactate dehydrogenase.[99]

Removal of sulphate groups from sulphated mono- and poly-saccharides was achieved by treatment of the pyridinium salts with such solvents as pyridine, dioxan, DMF, or DMSO.[332]

A number of new procedures for the purification of agarose have been reported. Agarose, containing 0.6% sulphate and 0.05% pyruvate, was obtained by modification of the polyethyleneglycol procedure. Further fractionation of this material on columns of DEAE–Sephadex gave an agarose with minimal sulphate and pyruvate contents.[333] Agar has been fractionated by absorption of the sulphated polymers on chitin and chitosan.[334] The combination of extraction (or electrophoresis) with an ion-exchange procedure has been used to produce an agarose of low sulphur content.[335] The isolation of agarose and the granulation of agar and agarose gels have been achieved by treatment with ammonium sulphate and acetone.[336]

Enzymic hydrolysis of agar has been followed by t.l.c. of the neutral and charged sugars, which first had been separated from each other by ion-exchange chromatography.[337]

[329] A. Haug and B. Larsen, *Carbohydrate Res.*, 1971, **17**, 297.
[330] B. Larsen and A. Haug, *Carbohydrate Res.*, 1971, **20**, 225.
[331] W. Mackie, *Carbohydrate Res.*, 1971, **20**, 413.
[332] A. I. Usov, K. S. Adamyants, L. I. Miroshnikova, A. A. Shaposhnikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **18**, 336.
[333] M. Duckworth and W. Yaphe, *Analyt. Biochem.*, 1971, **44**, 636.
[334] G. G. Allan, P. G. Johnson, Y.-Z. Lai, and K. V. Sarkanen, *Carbohydrate Res.*, 1971, **17**, 234.
[335] S. Hjertén, *J. Chromatog.*, 1971, **61**, 73.
[336] A. M. Egorov, A. K. Vakhabov, and V. Y. Chernyak, *J. Chromatog.*, 1970, **46**, 143.
[337] M. Duckworth and W. Yaphe, *J. Chromatog.*, 1970, **49**, 482.

Agarose has been evaluated as a stabilizing agent in gel electrofocusing; twelve commercial preparations of agarose, which were tested for electro-endosmosis, showed electro-osmotic flow. It was possible to reduce electro-osmosis by treatment with an anion-exchange resin.[338]

The water-holding capacity of agarose, isolated from various mucilaginous substances of red seaweeds, decreased with increasing D-galactose and 6-*O*-methyl-D-galactose to 3,6-anhydro-L-galactose ratios, and also with lower liquefying temperatures of the gels.[339] N.m.r. spectroscopic examination of the sol to gel transition in agarose and carrageenans showed that distinct changes in polysaccharide conformation occurred with agarose, whereas most water molecules remained in a highly mobile state. The results were compatible with a coil to double-helix model.[340]

The concept that agar is composed of two polysaccharides (neutral agarose and charged agaropectin) has been considered to be an over-simplification. Fractionation of a commercial agar on DEAE–Sephadex (Cl^-) indicated that agar is a complex mixture of polysaccharides having the same backbone structure, but which are substituted to different degrees with charged groups. The complete agar is thought to consist of chains having alternating α-(1 → 3)- and β-(1 → 4)-linkages with three extremes of structure; *viz.* (*i*) a neutral agarose (Akari) with alternating (1 → 4)-3,6-anhydro-α-L-galactose and (1 → 3)-β-D-galactose residues, (*ii*) a pyruvated agarose with little sulphation; the D-galactose residues are substituted by 4,6-*O*-(1-carboxyethylidene) groups until the degree of substitution reaches 1 in 20, and (*iii*) a non-gelling, sulphated galactan with few or no 3,6-anhydro-L-galactose or 4,6-*O*-(1-carboxyethylidene)-D-galactose residues.[341] A bacterial agarase has been used to elucidate structural features of the charged polysaccharides. Characterization of the products of hydrolysis indicated that masking of the basic repeating unit, with 4,6-*O*-(1-carboxyethylidene)-D-galactose in place of D-galactose, occurred in regions of the molecule of low sulphate content.[342] A variety of agars were analysed for pyruvate and sulphate, and pyruvic acid was shown to be a common feature of agars from different agarophytes.[343] The agar from *Gelidium amansii* consists of a family of polydisperse polysaccharides having similar macromolecular structure with continuously variable proportions of acidic substituents such as sulphate, pyruvate, and uronic acid.[344] In a study of the agar polysaccharides of the *Gracilaria* species, chemical and enzymic analysis as well as ion-exchange chromatography showed differences in the series of related polysaccharides that constitute different agars. Only the agar from *G. debilis* had a high

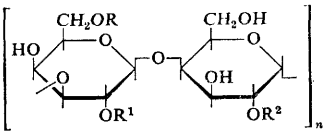
[338] R. Quast, *J. Chromatog.*, 1971, **54**, 405.
[339] T. Fuse and F. Goto, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 799.
[340] T. F. Child, N. G. Pryce, M. J. Tait, and S. Ablett, *Chem. Comm.*, 1970, 1214.
[341] M. Duckworth and W. Yaphe, *Carbohydrate Res.*, 1971, **16**, 189.
[342] M. Duckworth and W. Yaphe, *Carbohydrate Res.*, 1971, **16**, 435.
[343] K. Young, M. Duckworth, and W. Yaphe, *Carbohydrate Res.*, 1971, **16**, 446.
[344] K. Izumi, *Carbohydrate Res.*, 1971, **17**, 227.

gel strength, but other agars gave increased gel strengths on treatment with alkali.[345]

The agar group of polysaccharides described in *Rhodomela larix* had the characteristic property of containing a high percentage of 3,6-anhydro-2-*O*-methyl-L-galactose.[346]

An enzyme, which cleaved agar-like polysaccharides with an action similar to those of bacterial agarases, was found in molluscs.[347]

Methylation analysis of phyllymenan and desulphated phyllymenan indicated that the native polysaccharide is an essentially α-(1 → 3)- and β-(1 → 4)-linked galactan. No unique structure was proposed, but the partial structure (16) was consistent with the evidence accumulated.[348]



R = H and less frequently Me; R^1 = SO_3^- and less frequently H; R^2 = Me and less frequently H

(16)

A highly sulphated polysaccharide from the red seaweed *Aeodes ulvoidea* was shown to contain D-galactose, 4-*O*-methyl-L-galactose, and 2-*O*-methyl-D-galactose as well as traces of 6-*O*-methylgalactose, xylose, and mannose. The results of periodate oxidation indicated the presence of (1 → 3)- and (1 → 4)-glycosidic bonds, with 2-*O*-methyl-D-galactose units either (1 → 4)- and/or (1 → 3)-linked and 4-*O*-methyl-L-galactose probably present only as non-reducing end-groups.[349]

Pachymenia carnosa yielded a sulphated polysaccharide composed of D-galactose, 2-*O*-methyl-D-galactose, 4-*O*-methyl-D-galactose, and 6-*O*-methyl-D-glucose. Although a number of oligosaccharides were isolated and characterized following hydrolysis, no unique structure was postulated for the polymer.[350]

Polysaccharides from aqueous extracts of *Anatheca dentata* were fractionated with cetyltrimethylammonium bromide to give a sulphated polymer containing D- and L-galactose, D-xylose, and traces of 3-*O*-methyl-galactose and uronic acids. From the characterization of the oligosaccharides (17)—(21), it was apparent that a substantial part of the macromolecule is composed of alternating D- and L-galactose residues in an alternating α-(1 → 3)- and β-(1 → 4)-sequence.[351]

[345] M. Duckworth, K. C. Hong, and W. Yaphe, *Carbohydrate Res.*, 1971, **18**, 1.
[346] A. I. Usov, R. A. Lotov, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1971, **41**, 1154.
[347] A. I. Usov, M. D. Martynova, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1970, **194**, 225.
[348] J. R. Nunn and H. Parolis, *Carbohydrate Res.*, 1970, **14**, 145.
[349] A. J. R. Allsobrook, J. R. Nunn, and H. Parolis, *Carbohydrate Res.*, 1971, **16**, 71.
[350] A. J. Farrant, J. R. Nunn, and H. Parolis, *Carbohydrate Res.*, 1971, **19**, 161.
[351] J. R. Nunn, H. Parolis, and I. Russell, *Carbohydrate Res.*, 1971, **20**, 205.

4-*O*-β-D-Gal*p*-L-Gal
(17)

3-*O*-α-L-Gal*p*-D-Gal
(18)

4-*O*-β-D-Gal*p*-D-Gal
(19)

β-D-Gal*p*-(1 → 4)-α-L-Gal*p*-(1 → 3)-D-Gal
(20)

β-D-Gal*p*-(1 → 4)-α-L-Gal*p*-(1 → 3)-D-Gal*p*-(1 → 4)-L-Gal
(21)

Five polysaccharides have been isolated from *Gloiopeltis furcata*. All contained galactose, 3,6-anhydrogalactose, and sulphate residues in similar ratios as well as variable amounts of uronic acid and protein.[352] It has been demonstrated that, in addition to D-galactose, D-xylose, and 2-*O*-methyl-L-galactose,[353] the sulphated polysaccharide from *Laingia pacifica* contains small amounts of 3-, 4-, and 6-*O*-methylgalactose; the proportion of 3,6-anhydro-2-*O*-methyl-L-galactose was greater than that of 3,6-anhydro-L-galactose.[354] A polysaccharide, with similar properties to κ-carrageenan, was isolated from *Tichocarpus crinitus*. It was shown that the carrabiose residue (3,6-anhydro-4-*O*-β-D-galactopyranosyl-D-galactose) was the main repeating unit of the polysaccharide chain.[355] From the acetolysis products of this polysaccharide, di-, tri-, and tetra-saccharides were isolated; the structure of these oligomers provided evidence on the alteration of α-(1 → 3)- and β-(1 → 4)-linkages between D-galactopyranose residues.[356] Mass spectra of various 3,6-anhydrogalactose derivatives have been studied and have confirmed the presence of the anhydro-rings.[122]

Precipitation of some pathological sera in agar gels has been demonstrated. Agaropectin and other sulphated galactans gave this precipitation reaction, but other sulphated polysaccharides did not. The reaction was considered not to be an immunological one, but was likely to be due to the interaction of immunoglobulin G amino-groups with the sulphate groups of the polysaccharide.[357]

An enzyme fraction from the seaweed *Gigartina stellata* effected formation of 3,6-anhydride-units in κ-carrageenan. The enzyme appeared

[352] K. Izumi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 653.
[353] N. K. Kochetkov, A. I. Usov, and L. I. Miroshnikova, *Zhur. obshchei Khim.*, 1970, **40**, 2469.
[354] N. K. Kochetkov, A. I. Usov, and L. I. Miroshnikova, *Zhur. obshchei Khim.*, 1970, **40**, 2473.
[355] A. I. Usov, M. A. Rekhter, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1970, **40**, 2732.
[356] N. K. Kochetkov, A. I. Usov, and M. A. Rekhter, *Zhur. obshchei Khim.*, 1971, **41**, 1160.
[357] P. Burtin and M. C. Gendron, *Immunochemistry*, 1971, **8**, 423.

to be capable of reducing the 'kinks' in the polysaccharide chain, thereby increasing chain-rigidity, and of effecting metabolic control of the polysaccharide's conformation and function.[358]

Optical rotation shifts in ι-carrageenan arose from a coil to double-helix transition, showing that intramolecular cohesion between sugar residues was sufficient to outweigh solvation and polymer-conformational entropy effects in aqueous solution.[359] Evidence was obtained that the double helices in the gel are normally aggregated, but can be kept separate within a narrow temperature range.[360] Good agreement was obtained between calculated and observed rotations for helical and coil forms of ι-carrageenan, and supported the suggestion that optical rotation shifts originate from a coil to double-helix transition.[361]

A glucan isolated from the cell-wall of the red alga *Rhodymenia pertusa* was shown by g.l.c., mass spectral, and n.m.r. studies on the permethylated polysaccharide to consist of β-(1 → 4)-linked D-glucopyranose units.[362] A water-soluble glucan from *R. pertusa* was shown to have a structure resembling that of phytoglycogen from *Zea Mays* in having, on average, twelve or thirteen α-(1 → 4)-linked D-glucopyranose units with a branch point at C-6.[363]

Incorporation of D-[U-^{14}C]glucose indicated that the starch stored by the alga *Platymonas tetrathele* is utilized to form the cell-wall polysaccharides.[364]

X-Ray intensity data have been collected for the cellulose in the cell walls of the filamentous alga *Chaetomorpha melagonium.* Significant differences in the intensities between this cellulose and known data from ramie cellulose indicated structural differences over and above the number of chains within the unit cell.[365] Microtubules were considered to take no part in the biosynthesis of cellulose in the alga *Valonia.*[366]

The light-induced breakdown of the β-(1 → 3)-glucan of *Euglena gracilis* was associated with chloroplast development, since a mutant, which was unable to synthesize pigments, did not break down the glucan when exposed to light. Continuous exposure to light was necessary for degradation of the glucan, and the cells had high activities of β-1,3-glucan phosphorylase and β-1,3-glucan synthetase.[367] It was suggested that the main role of the β-(1 → 3)-glucan is to provide the energy necessary for survival during periods in the dark and in the absence of suitable food sources, and also to provide the energy and carbon sources necessary for transformation from

[358] C. J. Lawson and D. A. Rees, *Nature*, 1970, **227**, 392.
[359] A. A. McKinnon, D. A. Rees, and F. B. Williamson, *Chem. Comm.*, 1969, 701.
[360] D. A. Rees, I. W. Steele, and F. B. Williamson, *J. Polymer. Sci., Part C, Polymer Symposia*, 1969, **28**, 261.
[361] D. A. Rees, W. E. Scott, and F. B. Williamson, *Nature*, 1970, **227**, 390.
[362] J. N. C. Whyte and J. R. Englar, *Canad. J. Chem.*, 1971, **49**, 1302.
[363] J. N. C. Whyte, *Carbohydrate Res.*, 1971, **16**, 220.
[364] G. W. Gooday, *Biochem. J.*, 1971, **123**, 3P.
[365] A. Nieduzynski and E. D. T. Atkins, *Biochim. Biophys. Acta*, 1970, **222**, 109.
[366] M. Marx-Figini, *Biochim. Biophys. Acta*, 1971, **237**, 75.
[367] M. R. Dwyer and R. M. Smillie, *Biochim. Biophys. Acta*, 1970, **216**, 392.

heterotropy to phototropy.[368] The metabolic fate of the glucan and its possible relation to chloroplast formation were investigated by preferentially labelling the glucan of cells adapted to darkness. During the first day of illumination, 25% of the label was recovered in lipid fractions, 11% in protein fractions, and the rest in carbon dioxide. In the dark, the label was released slowly with no net increase into other cell fractions.[369]

Very mild acid conditions for the extraction of fucoidan suggested that, in its native state in *Ascophyllum nodosum* and *Fucus vesiculosus*, the polysaccharide is present mostly as a building element of a much more complex macromolecule, and that isolation of fucoidan entailed chemical degradation as well as physical separation.[370] A cell-wall component of *A. nodosum* was identified as a sulphated glucuronoxylofucan. Methylation analysis suggested a highly branched molecule in which xylose is present as end-groups and in the (1 → 4)-linked form, fucose is present as end-groups and in the (1 → 2)-linked form, and fucose 4-sulphate is linked (1 → 2) and (1 → 3).[371]

Cladophora rupestris metabolized D-glucose, D-fructose, sucrose, and a homologous series of linear oligosaccharides, containing (2 → 1)-linked D-fructose residues attached to the D-fructose moiety of sucrose, to produce an insulin-type of polymer.[372] A study of the Cladophorales showed that sucrose lactate (4-*O*-lactyl-β-D-fructofuranosyl-α-D-glucopyranose) is found only in *C. laetevirens* and a fresh-water *Rhizoclonium* species, but panose, maltotriose, maltotetraose, and 6-*O*-D-glucosylmaltotetraose appeared to be common to all the species examined.[373] Results from autohydrolysis and methylation of a partially desulphated water-soluble polysaccharide of *C. rupestris* were correlated with those from Smith degradation to show that a structural feature of the inner part of the molecule consists of eight (1 → 4)-linked arabinose units joined together by a single 1,3- or 1,3,6-linked galactose unit.[374]

A xylan from the cell wall of *Penicillus dumetosus* had a $\overline{DP} > 10\,000$, as shown by light scattering, osmometry, and viscosity measurements.[375] A xylan from *Chaetangium fastigiatum* contained 75% of (1 → 4)- and 25% of (1 → 3)-linked β-D-xylopyranose units with only a small degree of branching. The polysaccharide had a random-coil conformation in aqueous solution.[376]

[368] M. R. Dwyer, J. Smydzuk, and R. M. Smillie, *Austral. J. Biol. Sci.*, 1970, **23**, 1005.
[369] M. R. Dwyer and R. M. Smillie, *Austral. J. Biol. Sci.*, 1971, **24**, 15.
[370] B. Larsen, A. Haug, and T. Painter, *Acta Chem. Scand.*, 1970, **24**, 3339.
[371] E. Percival, *Carbohydrate Res.*, 1971, **17**, 121.
[372] E. Percival and M. Young, *Phytochemistry*, 1971, **10**, 807.
[373] E. Percival and M. Young, *Carbohydrate Res.*, 1971, **20**, 217.
[374] E. J. Bourne, P. G. Johnson, and E. Percival, *J. Chem. Soc.* (*C*), 1970, 1561.
[375] W. Mackie and D. B. Sellen, *Biopolymers*, 1971, **10**, 1.
[376] A. S. Cerezo, A. Lezerovich, R. Labriola, and D. A. Rees, *Carbohydrate Res.*, 1971, **19**, 289.

4
Microbial Polysaccharides

Bacterial Cell Walls and Membranes

The membrane glycerol teichoic acid of *Lactobacillus fermentii* was extracted by two different procedures. Extraction with phenol gave a complex of teichoic acid associated with glycolipid and phospholipid (lipoteichoic acid), whereas trichloracetic acid treatment liberated a degraded lipoteichoic acid together with free teichoic acid side-chains. The teichoic acid was a (1 → 3)-phosphodiester-linked glycerophosphate polymer substituted with D-alanine, D-galactose, and a disaccharide of D-galactose and D-glucose.[377] The membrane glycerol teichoic acid of group F lactobacilli has been identified as the group antigen. Specificity of the group antigen depended primarily on D-galactose, with D-glucose being a minor contributor.[378] Both wall and membrane teichoic acids isolated from *L. helveticus* were glycerol phosphate polymers partially substituted with α-D-glucose residues. The membrane teichoic acid was isolated as a complex with lipid and was antigenic when injected into rabbits with Freund's adjuvant. The α-D-glucose substituents were primarily responsible for serological specificity of the membrane antigen and accounted for the reaction of the wall teichoic acid with antisera to the membrane teichoic acid.[379]

Two phages, virulent for strains of *L. plantarum*, have been isolated. A cell-wall polysaccharide was responsible for infection by phage 1, whereas the D-glucose moiety of ribitol teichoic acid was an important determinant of infection by phage 2.[380] The identities of the component glycerol-glucosides of the wall teichoic acids of *L. plantarum* have been confirmed by methylation analysis as *O*-α-D-glucopyranosyl-(1 → 1)-L-glycerol, *O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 1)-L-glycerol, and *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 1)-L-glycerol. These units are connected by phosphodiester groups attached to the 3-OH of L-glycerol and to the 6-OH of the non-reducing, terminal D-glucose residue in an adjacent unit. Concanavalin A formed a precipitate

[377] A. J. Wicken and K. W. Knox, *J. Gen. Microbiol.*, 1970, **60**, 293.
[378] K. W. Knox, M. J. Hewett, and A. J. Wicken, *J. Gen. Microbiol.*, 1970, **60**, 303.
[379] K. W. Knox and A. J. Wicken, *J. Gen. Microbiol.*, 1970, **63**, 237.
[380] L. J. Douglas and M. J. Wolin, *Biochemistry*, 1971, **10**, 1551.

with the teichoic acid, but the precipitated material contained only the *O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 1)-L-glycerol component, suggesting that the teichoic acid was a mixture of this and possibly of other homogeneous chains containing the other components.[381]

The teichoic acid of *Staphylococcus aureus*, phage type 187, was shown to contain ribitol, phosphate, 2-acetamido-2-deoxy-D-galactose, and D-alanine. Immunochemical analysis indicated that the amino-sugar was the immunodominant determinant.[382] Preparations of the cell walls of *S. aureus* contained glycerol teichoic acid, but in a strain lacking teichoic acid the only phosphorylated compound to be identified as a general wall component was a polymer giving muramic acid and phosphate on hydrolysis.[383] Sensitized sheep erythrocytes gave high titres in haemagglutination tests against whole-cell antisera, and this was dependent on the immunological specificity of the glycosidic linkage of 2-acetamido-2-deoxy-D-glucose of the teichoic acid of *S. aureus*.[384]

A study has been made of the binding of Mg^{2+} ions to the cell wall of *S. aureus*, which has been reported to contain a ribitol teichoic acid having ester-bound alanine. When 75% of the wall phosphorus was removed by chemical procedures, the Mg^{2+}-binding ability of the cell wall was decreased by 80%. When the organism was grown in a medium supplemented by sodium chloride, the walls contained less alanine ester, but were able to bind substantially greater amounts of Mg^{2+} ions. It was concluded that the phosphodiester residues of the teichoic acid effect the binding of ions to the cell wall.[385]

Concanavalin A has been reported to react with the α-glucosylated teichoic acid from *S. epidermidis* and with the α-linked 2-acetamido-2-deoxy-D-glucose teichoic acid from *S. aureus*.[386] The intracellular or membrane teichoic acid from *Streptococcus faecalis*, which contains kojibiosyl- and kojitriosyl-glycerol residues, gave a precipitation reaction with concanavalin A.[387]

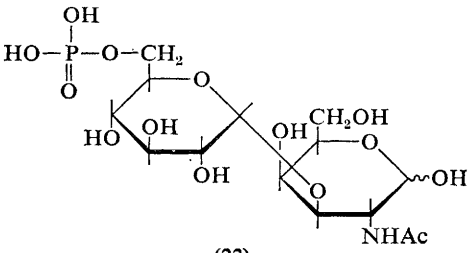
The cell walls of *Bacillus licheniformis* contain a teichuronic acid composed of D-glucuronic acid and 2-acetamido-2-deoxy-D-galactose. After reduction of the carboxy-groups of the uronic acid residues, the polysaccharide contained equal proportions of D-glucose and amino-sugar. Methylation analysis indicated that the D-glucose units are substituted at C-4. Partial acid hydrolysis of the original polysaccharide showed that the uronic acid units are glycosidically linked to C-3 of the amino-sugar units.[388]

After growth of *B. subtilis* in a phosphate-limited medium containing sodium chloride, the cell walls contained both teichoic acid and teichuronic

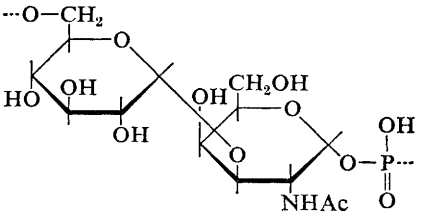
[381] A. R. Archibald and H. E. Coapes, *Biochem. J.*, 1971, **124**, 449.
[382] W. W. Karakawa and J. A. Kane, *J. Immunol.*, 1971, **106**, 900.
[383] D. Mirelman, D. R. D. Shaw, and J. T. Park, *J. Bacteriol.*, 1971, **107**, 239.
[384] J. H. Brock and B. Reiter, *Immunochem.*, 1971, **10**, 933.
[385] S. Heptinstall, A. R. Archibald, and J. Baddiley, *Nature*, 1970, **225**, 519.
[386] W. J. Reeder and R. D. Ekstedt, *J. Immunol.*, 1971, **106**, 334.
[387] A. R. Archibald and H. E. Coapes, *Biochem. J.*, 1971, **123**, 665.
[388] R. C. Hughes and P. F. Thurman, *Biochem. J.*, 1970, **117**, 441.

acid. The teichoic acid level increased as the salt-level was increased and, in 6% sodium chloride, no teichuronic acid was found in the cells.[389] Earlier results had suggested that these polymers are mutually exclusive.[390]

The walls of *Micrococcus* species contain about 43% of a polymer of D-glucose, 2-acetamido-2-deoxy-D-galactose, and phosphate, which was readily hydrolysed under mild acidic conditions to the phosphorylated disaccharide 2-acetamido-2-deoxy-3-*O*-α-D-glucopyranosyl-D-galactose 6′-phosphate (22).[391] Similar degradation of the polymer itself indicated that the repeating structure is the disaccharide (23).



(22)



(23)

A choline-containing teichoic acid from *Diplococcus pneumoniae* is composed of 2-amino-2-deoxy-D-galactose, phosphate, and choline (1 : 1.65 : 0.9). The choline component of the teichoic acid plays a key role in determining the sensitivity to an autolytic enzyme, since preparations from Pneumococci containing ethanolamine in place of choline were resistant to the enzyme.[392] Pneumococci containing the ethanolamine teichoic acid also contained an autolytic enzyme, and could be converted into the choline-type by incubation *in vitro* with cell walls containing choline.[393]

The existence of a membrane-bound enzyme system, which displayed maximum activity in the presence of Mg^{2+} ions bound to the endogenous

[389] D. C. Ellwood, *Biochem. J.*, 1971, **121**, 349.
[390] D. C. Ellwood, *Biochem. J.*, 1970, **118**, 367.
[391] M. D. Partridge, A. L. Davison, and J. Baddiley, *Biochem. J.*, 1971, **121**, 695.
[392] J. L. Mosser and A. Tomasz, *J. Biol. Chem.*, 1970, **245**, 287.
[393] A. Tomasz and M. Westphal, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2627.

teichoic acid directly, demonstrated that the function of teichoic acids is in concentrating Mg^{2+} ions at the cytoplasmic membrane.[394]

Although the primary action of chloramphenicol in inhibiting growth of most microbial cells is a consequence of its effect on protein synthesis, the direct action of inhibiting the transfer of D-glucose to teichoic acid is considered to be a contributory factor in some organisms. The effect was specific for D-glucose and was not observed with the transfer of other residues from nucleotides, *e.g.* glycerol phosphate, 2-acetamido-2-deoxy-D-glucose, or 2-acetamido-2-deoxy-D-galactose. Inhibition appeared to occur at the stage of transfer of D-glucose from the nucleotide precursor to a lipid carrier; in the cases studied, there was no inhibition of transfer where lipid carriers were not involved.[395]

Inhibition of teichoic acid synthesis brought about by addition of both peptidoglycan precursor and antibiotics was the result of undecaprenol phosphate being channelled into the peptidoglycan biosynthetic cycle. It was concluded that the same lipid carriers are used for transporting precursors of both peptidoglycan and teichoic acid.[396]

A study of the turnover of cell walls of Gram-positive bacteria has indicated that the synthesized wall did not become available for turnover until about a half to one generation of cells. Cell-wall mucopeptide and teichoic acid have identical turnover rates, and the products of cell-wall turnover were identified as products of cleavage of the cell wall by an *N*-acetylmuramyl-L-alanine amidase.[397]

The major products of autolysis of the cell walls of *Bacillus stearothermophilus* were peptides, peptidoglycan, and a peptidoglycan–teichoic acid complex.[398]

After removal of teichoic acid from the cell wall of *B. licheniformis*, the insoluble residue was identified as a teichuronic acid–mucopeptide complex.[399] The results of autolysis of isolated cell walls of *B. licheniformis* and *B. subtilis* and ion-exchange chromatography of the soluble products were in agreement with the autolysis proceeding with hydrolysis of amide bonds between L-alanine and *N*-acetylmuramic acid residues in the mucopeptide components. Oligosaccharides originating from the mucopeptide component were shown to contain equal amounts of 2-amino-2-deoxy-D-glucose and muramic acid, and to have CL 10, estimated by release on non-reducing end-groups of 2-acetamido-2-deoxy-D-glucose.[400] Autolysis of *B. cereus* cell walls was accompanied by hydrolysis of the majority of

[394] A. H. Hughes, M. Stow, I. C. Hancock, and J. Baddiley, *Nature New Biol.*, 1971, **229**, 53.
[395] M. Stow, B. J. Starkey, I. C. Hancock, and J. Baddiley, *Nature New Biol.*, 1971, **229**, 56.
[396] R. J. Watkinson, H. Hussey, and J. Baddiley, *Nature New Biol.*, 1971, **229**, 57.
[397] J. Mauck, L. Chan, and L. Glaser, *J. Biol. Chem.*, 1971, **246**, 1820.
[398] W. D. Grant and A. J. Wicken, *Biochem. J.*, 1970, **108**, 859.
[399] R. C. Hughes, *Biochem. J.*, 1970, **117**, 431.
[400] R. C. Hughes, *Biochem. J.*, 1970, **119**, 849.

4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramic acid linkages in the wall mucopeptide, presumably by an *endo*-β-*N*-acetylglucosaminidase. Hydrolysis of *N*-acetylmuramyl-L-alanine linkages by an amidase also occurred. A disaccharide, *N*-acetylmuramyl-2-acetamido-2-deoxy-D-glucose, and two polysaccharides were obtained from the products of autolysed cell walls. A neutral polysaccharide containing 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and D-glucose accounted for about 40% of the wall. The other polysaccharide was acidic and yielded 2-amino-2-deoxy-D-glucose and -galactose and unidentified acidic substances on hydrolysis.[401]

A lytic enzyme isolated from *B. cereus* was characterized as a muraminyl-alanine amidase, since glucosaminopeptides, peptides, and oligosaccharides containing 2-amino-2-deoxy-D-glucose and muramic acid were isolated from cell walls of *B. cereus* and *Micrococcus lysodeikticus* digested with the enzyme. The oligosaccharides isolated from the walls of *B. cereus* contained equimolar quantities of muramic acid and 2-amino-2-deoxy-D-glucose, and the peptides contained alanine, glutamic acid, and DAP (3 : 2 : 2).[402]

Studies on the formation of *N*-unsubstituted 2-amino-2-deoxy-D-glucose residues in *B. cereus* peptidoglycan resulted in the discovery of an enzyme that hydrolyses the acetamido-groups of 2-acetamido-2-deoxy-D-glucose residues. The enzyme appeared to be specific for the undegraded peptidoglycan and was distinguishable from 2-acetamido-2-deoxy-D-glucose 6-phosphate deacetylase.[403] 4-*O*-(2-Amino-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramic acid and 4-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramic acid 6-phosphate were isolated from acid hydrolysates of peptidoglycans of some strains of *B. cereus* in yields which suggested that the majority of the amino-groups of the glucosamine units are unsubstituted.[404]

Three strains of *B. cereus* had walls completely insensitive to lysozyme. The peptidoglycans isolated from these walls contained large quantities of 2-amino-2-deoxy-D-glucose. The lysozyme-resistant walls were converted into a sensitive form by *N*-acetylation, indicating that the resistance to lysozyme is due to the occurrence of unsubstituted amino-residues in the peptidoglycan.[405]

Strains of *B. cereus* differing in penicillinase production were shown to possess cell walls of differing contents of muramic acid.[406]

[401] R. C. Hughes, *Biochem. J.*, 1971, **121**, 791.
[402] S. Csuzi, *Acta Biochem. Biophys. Acad. Sci. Hung.*, 1970, **5**, 375.
[403] Y. Araki, S. Fukuoda, S. Oba, and E. Ito, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 751.
[404] Y. Araki, T. Nakatani, R. Makino, H. Hayashi, and E. Ito, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 684.
[405] Y. Araki, T. Nakatani, H. Hayashi, and E. Ito, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 691.
[406] K. W. Nickerson and R. A. Day, *J. Bacteriol.*, 1971, **105**, 681.

The first known examples of lysine-containing peptidoglycans of chemotype 1 have been reported to be made up of peptide units of L-alanyl-γ-D-glutamyl-L-lysyl-D-alanine in the wall of *Gaffkya homari*, and of L-alanyl-D-isoglutaminyl-L-lysyl-D-alanine in the walls of *Aerococcus viridans*. The results were consistent with a peptidoglycan in which all of the *N*-acetylmuramic acid residues in the glycan strands are substituted with peptide and in which about half of the peptide units occur as monomers, whereas the others form dimers by means of N^{ε}-(D-alanyl)-L-lysine linkages.[407]

A new type of peptide subunit in the murein of an *Arthrobacter* species has been reported from the isolation and characterization of two UDP-activated precursors of the peptidoglycan; the *N*-acetylmuramyl residues are linked to a glycyl-γ-D-glutamyl-L-glutamyl-D-alanyl-D-alanine unit.[408]

The chemistry of the walls of rod mutants of *Bacillus subtilis* did not provide obvious evidence of expression of genetic lesion.[409]

A haemolytic group L *Streptococcus* has been shown to contain a cell-wall heteroglycan composed of L-rhamnose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and D-galactose; the heteroglycan contained a novel type of immunodominant group consisting of a 2-acetamido-2-deoxy-D-glucosylphosphoryl moiety. The immunodominant group of a group D Streptoccal heterogylcan, having similar monosaccharide composition, consisted of a glucosyl-phosphoryl group.[410]

Heat-treated cells of *Proteus vulgaris* have been degraded with a protease preparation, allowing the isolation of a cell-envelope structural entity. An *endo-N*-acetylmuramidase completely solubilized the peptidoglycan by degrading the glycan moiety into peptide-substituted disaccharide units. Carboxypeptidase hydrolysed D-alanyl-*meso*-diaminopimelyl bonds involved in cross-linking. About 50% of the *N*-acetylmuramic acid residues in the glycan moiety were found to be *O*-acetylated, a property compatible with the high resistance to lysozyme exhibited by the peptidoglycan.[411]

Viable log-phase cells of *P. mirabilis* contain a peptidoglycan, which was partially resistant to lysozyme, but which was degraded by an *N*-acetylneuraminidase preparation into known disaccharide-containing peptides.[412]

The peptidoglycan isolated from *Vibrio fetus* is composed of muramic acid, 2-amino-2-deoxy-D-glucose, alanine, glutamic acid, and DAP (1 : 1 : 2 : 1 : 1). Approximately 30% of the DAP molecules are involved in peptide cross-linkages, and analysis of the products of lysozymolytic degradation indicated a structure similar to that of other Gram-negative genera.[413]

[407] M. Nakel, J.-M. Ghuysen, and O. Kandler, *Biochemistry*, 1971, **10**, 2170.
[408] B. Cziharz, K. H. Schleiter, and O. Kandler, *Biochemistry*, 1971, **10**, 3574.
[409] H. J. Rogers, M. McConnell, and R. C. Hughes, *J. Gen. Microbiol.*, 1971, **66**, 297.
[410] J. H. Pazur, A. Cepure, J. A. Kane, and W. W. Karakawa, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 1421.
[411] J. Fleck, M. Mock, R. Minck, and J. M. Ghuysen, *Biochim. Biophys. Acta*, 1971, **233**, 489.
[412] W. Katz, D. Berger, and H. H. Martin, *Biochim. Biophys. Acta*, 1971, **244**, 47.
[413] A. J. Winter, W. Katz, and H. H. Martin, *Biochim. Biophys. Acta*, 1971, **244**, 58.

Two strains of *Corynebacterium bovis* were found to differ in cell-wall composition; one was shown to be composed of lysine, rhamnose, mannose, and glucose units, whereas the other contained *meso*-α,ε-DAP, arabinose, galactose, and mannose. The wall-preparation from *C. nephridii* contained L-DAP and galactose. The results were discussed in relation to the organisms examined.[414]

Inhibition of precipitin reactions between the peptidoglycan of *Staphylococcus epidermidis* and the sera of rabbits immunized with Group A variant Streptococci led to recognition of the pentapeptide L-alanyl-D-glutamyl-γ-L-lysyl-D-alanyl-D-alanine as the antigenic determinant of the peptidoglycan.[415]

The ready dissolution of the peptidoglycan from Staphylococci in dilute alkali has been attributed to the lability of glycyl peptides forming the cross-linkages in the material.[416]

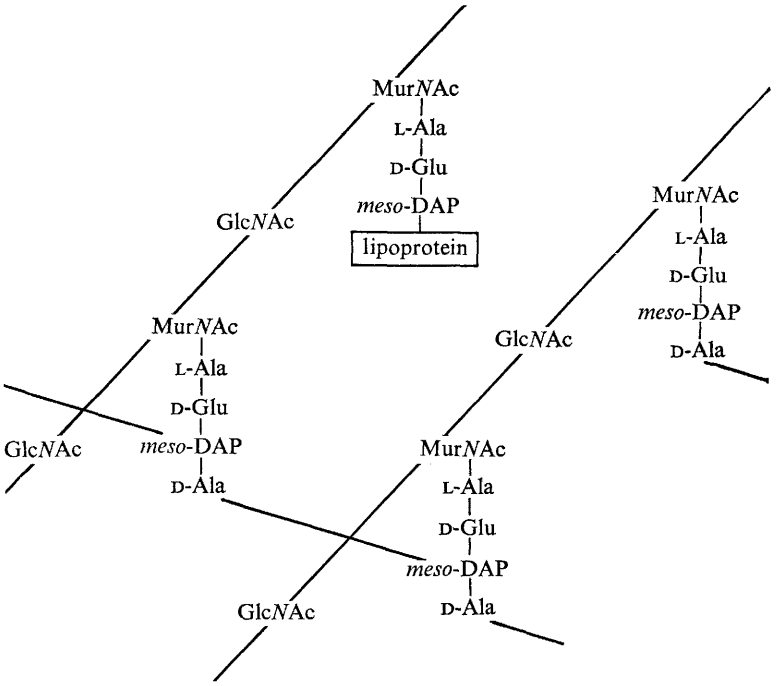
The cell walls of *Lactobacillus fermenti* have been isolated and were shown to have a similar composition to those reported for three other Lactobacilli in containing 2-amino-2-deoxy-D-glucose, muramic acid, L-alanine, D-glutamine, and lysine in equimolar amounts.[417]

Enzymic degradation of the cell-wall peptidoglycan of *Bifidobacterium bifidium* indicated that the tetrapeptide N^{α}-L-alanyl-γ-D-*iso*glutaminyl-L-ornithyl-D-alanine is directly linked to muramic acid. Serylasparagine is involved in cross-linking of adjacent tetrapeptides by forming a bridge between the δ-amino-group of ornithine and the carboxy-group of *C*-terminal D-alanine.[418]

Covalent linkage of the peptidoglycan of *Escherichia coli* to a specific cell-wall envelope lipoprotein has been demonstrated. Treatment of the cell-wall envelope with sodium dodecyl sulphate liberated a peptidoglycan-lipoprotein complex.[419] Digestion of the complex with trypsin cleaved the peptide bond of a lysyl residue linking lipoprotein to approximately every tenth repeating unit of the peptidoglycan-(Glc*p*NAc-Mur*N*Ac-L-Ala-D-Glu-*meso*-DAP-D-Ala) (24). Treatment with pronase also cleaved the lipopolysaccharide–peptidoglycan complex, liberating a peptidoglycan containing lysine and arginine residues.[420] When this product was submitted to partial acidic hydrolysis, three peptides having the structures (25)—(27) were isolated. The results suggested the presence of a covalent linkage between the peptidoglycan and a terminal lysyl-arginine residue of the lipoprotein. Confirmation of the point of linkage of the protein to the peptidoglycan was obtained by characterization of the peptide Glc*p*NAc-Mur*N*Ac-L-Ala-D-Glu-*meso*-DAP-L-Lys-L-Arg resulting from pronase and lysozyme digestion of

[414] C. S. Cummins, *J. Bacteriol.*, 1971, **105**, 1227.
[415] K. H. Schleifer and R. M. Krause, *J. Biol. Chem.*, 1971, **246**, 986.
[416] A. R. Archibald, J. Baddiley, and J. Goundry, *Biochem. J.*, 1970, **116**, 313.
[417] I.-B. Wallinder and H. Y. Neujahr, *J. Bacteriol.*, 1971, **105**, 918.
[418] J. H. Veerkamp, *Arch. Biochem. Biophys.*, 1971, **143**, 204.
[419] V. Braun and K. Rehn, *European J. Biochem.*, 1969, **10**, 426.
[420] V. Braun and U. Sieglin, *European J. Biochem.*, 1970, **13**, 336.

the original complex. Each repeating unit of the peptidoglycan, which serves as a site of attachment to protein, lacks the D-alanine residue normally linked to diaminopimelic acid and this is replaced by the lysyl-arginine dipeptide of the protein.[421]



(24)

meso-DAP-L-Lys-L-Arg

(25)

L-Ala-D-Glu-*meso*-DAP-L-Lys-L-Arg

(26)

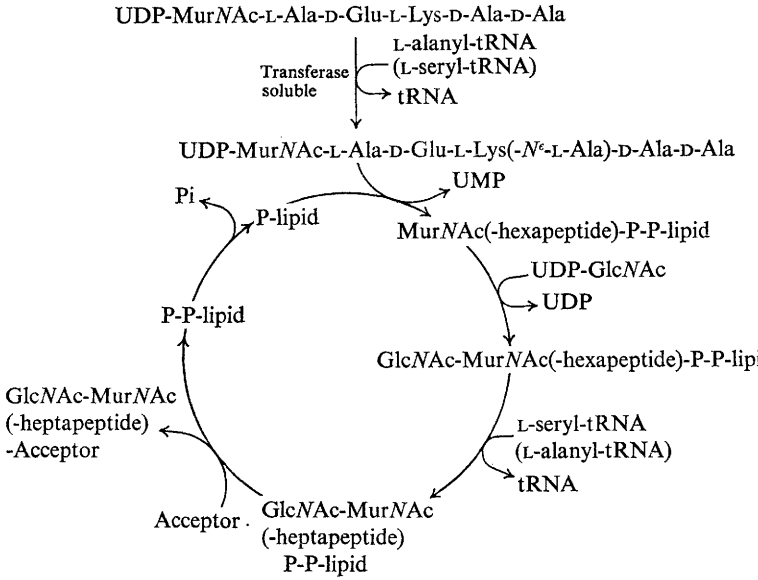
Glc*p*NAc-Mur*N*Ac-L-Ala-D-Glu-*meso*-DAP-L-Lys-L-Arg

(27)

The particulate enzyme obtained from *B. subtilis* was capable of binding radioactive penicillin G; it contained a D-alanine carboxypeptidase, which utilized as a substrate the UDP-muramyl-pentapeptide involved in the biosynthesis of cell-wall peptidoglycan. There were indications that the penicillin is bound in the form of a penicilloyl derivative to a sulphydryl

[421] V. Braun and H. Wolff, *European J. Biochem.*, 1970, **14**, 387.

group in the enzyme.[422] The binding of penicillin G and the inactivation of the carboxypeptidase by penicillin G were reversed by treatment with neutral hydroxylamine; the kinetics of these two processes were identical. The possibility that the particulate D-alanine carboxypeptidase could be an uncoupled transpeptidase was discussed.[423] Mechanisms for the synthesis of interpeptide bridges in the peptidoglycan of *Lactobacillus viridescens*, which has the structure N^{ϵ}-L-Lys-L-Ala-L-Ser(D-Ala), have been investigated. The proposed sequence leading to the formation of peptidoglycan is illustrated in (28).[424] The purification and properties of the L-alanyl transfer



(28)

RNA-UDP-*N*-acetylmuramyl-pentapeptide transferase involved in this sequence have been reported.[425] An ATP-dependent phosphokinase, which catalyses the conversion of C_{55}-isoprenoid alcohols and other related substrates into C_{55}-isoprenoid alcohol phosphates, is present in the membrane fraction of *Staphylococcus aureus*. The product of the reaction served as a substrate for peptidoglycan synthesis. The presence of this enzyme and of C_{55}-isoprenoid alcohol in *S. aureus* accounted for the stimulation by ATP of peptidoglycan synthesis by a particulate enzyme from the organism.[426] Phosphatidylglycerol and cardiolipin have been identified in the phospholipid component of the isoprenoid alcohol phosphokinase; both were effective

[422] P. J. Lawrence and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3653.
[423] P. J. Lawrence and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3660.
[424] R. Plapp and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3667.
[425] R. Plapp and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3675.
[426] Y. Higashi, G. Siewert, and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3683.

in restoring activity to the enzyme.[427] The lipid intermediate involved in peptidoglycan biosynthesis in *S. aureus* has been isolated and recognized as a disaccharide-(pentapeptide)-pyrophosphoryl-lipid.[428]

The cell-wall glycan of *Bacillus stearothermophilus* has been shown to contain alternating residues of 2-amino-2-deoxy-D-glucose and muramic acid in which one half of the muramic acid residues are linked to the tripeptide L-alanyl-D-glutamyl-diaminopimelate and the remainder to the tetrapeptide L-alanyl-D-glutaminyl-diaminopimelyl-D-alanine.[429] By using a double auxotroph of *B. megaterium* requiring both diaminopimelic acid and lysine, it was possible to follow the formation of mucopeptide by measuring the incorporation of radioactive diaminopimelic acid.[430]

The cross-linkage of peptide sub-units in the peptidoglycan of some *Micrococcus* and *Arthrobacter* species by γ-glutamylglutamic acid is considered to involve the γ-carboxy-group of the *C*-terminal glutamic acid and the amino-group of diaminopimelic acid at one end and the carboxy-group of D-alanine and the amino-group of the *N*-terminal glutamine at the other end.[431]

The kinetic behaviour of the reaction of lysozyme with oligosaccharides from bacterial cell walls has been analysed. The model considered the various ways in which oligomers can associate with the enzyme, and assumed that the association constant for any mode depended only on which sub-sites of the enzyme were filled. Non-productive binding was seen to be of major importance in the reactions of small oligomers, which were hydrolysed principally by pathways in which they first reacted as trans-glycosylation acceptors.[432]

Temperature-inactivation of a particulate enzyme preparation from *Bacillus megaterium*, used in *in vitro* studies of peptidoglycan synthesis, involved dephosphorylation of a C_{55}-isoprenoid pyrophosphate.[433] The particulate preparation, obtained by mechanical disintegration of bacilli, had similar characteristics to those of protoplast membrane preparations in peptidoglycan synthesis.[434]

Cell-wall growth in *B. licheniformis*, which was followed by immuno-fluorescence with mucopeptide-specific antiserum, demonstrated that insertion of the new wall during growth occurred discretely and probably at sites of incipient cross-wall formation.[435]

The walls of halophilic cocci lacked muramic acid and, hence, contained none of the peptidoglycan that is characteristic of other Gram-positive

[427] Y. Higashi and J. L. Strominger, *J. Biol. Chem.*, 1970, **245**, 3691.
[428] Y. Higashi, J. L. Strominger, and C. C. Sweeley, *J. Biol. Chem.*, 1970, **245**, 3697.
[429] N. E. Welker, *J. Bacteriol.*, 1971, **107**, 697.
[430] D. W. Pitel and C. Gilvarg, *J. Biol. Chem.*, 1971, **246**, 3720.
[431] D. Bogdanovsky, E. Interschick-Niebler, and K.-H. Schleifer, *European J. Biochem.*, 1971, **22**, 173.
[432] D. M. Chipman, *Biochemistry*, 1971, **10**, 1714.
[433] P. E. Reynolds, *Biochim. Biophys. Acta*, 1971, **237**, 239.
[434] P. E. Reynolds, *Biochim. Biophys. Acta*, 1971, **237**, 255.
[435] R. C. Hughes and E. Stokes, *J. Bacteriol.*, 1971, **106**, 694.

bacteria.[436] Analysis of forty-eight Gram-positive bacteria indicated the presence of only glucomuramic acid in the cell walls.[437]

Whole cell walls of *B. subtilis* and *B. licheniformis* were more resistant to solubilization after treatment with glutaraldehyde owing to the formation of cross-linkages with contiguous side-chains of tripeptides carrying free ε-amino-groups.[438]

The synthesis of 3-*O*-(D-1-carboxyethyl)-2-deoxy-2-glycolamido-D-glucose (*N*-glycolylmuramic acid) has been reported and the product shown to be identical with that isolated from *Mycobacterium smegmatis* cell wall.[439]

The arabinogalactan from *M. bovis* was shown to interact with concanavalin A. Chain-ends of this highly branched polymer are terminated with α-D-arabinofuranosyl residues. Inhibition studies suggested that the hydroxy-groups at C-2, C-3, and C-5 of the pentofuranose are sufficiently similar in their spatial disposition to the C-3, C-4, and C-6 hydroxy-groups of the D-gluco- and D-manno-pyranosyl ring-systems for recognition by the combining sites of the protein.[440]

Analysis of the mycolic acid–arabinogalactan–mucopeptide complex of mycobacterial cell-wall material has suggested that a part of the arabinogalactan may be linked to muramic acid through a phosphodiester linkage, whereas another part may be linked glycosidically through 2-amino-2-deoxy-D-glucose.[441] The arabinogalactan of *M. tuberculosis* was solubilized by extraction of the cell walls with trichloroacetic acid. Further extraction of the insoluble residue with alkali solubilized a (1 → 4)-linked glucan containing a small proportion of (1 → 6)-linkages.[442]

Isoniazid was shown to inhibit a pathway, presumably the synthesis of mycolic acid, involved in formation of the cell envelope of *M. tuberculosis* BCG. This resulted in re-channelling of intermediates into carbohydrate synthesis to produce an increase in trehalose and insoluble glucan.[443]

Alkaline extraction of the cell walls of *M. phlei* removed a polysaccharide containing arabinose and galactose (2.5 : 1), which gave a single precipitation-line on agar against rabbit anti-serum to *M. phlei*. Methylation analysis indicated the existence of a branched structure having side-chains of (1 → 5)- and (1 → 2)-linked D-arabinofuranose units attached to a main chain of (1 → 5)-linked D-arabinose and (1 → 4)-linked D-galactose units at their C-3 and C-6 positions, respectively. A series of (1 → 5)-linked D-arabinose oligosaccharides, isolated from enzymic digests of the polysaccharide, were believed to be derived from the side-chains, since most of the D-galactose residues were found in the high molecular weight fraction.[444]

[436] A. D. Brown and K. Y. Cho, *J. Gen. Microbiol.*, 1970, **62**, 267.
[437] R. W. Wheat and J. M. Ghuysen, *J. Bacteriol.*, 1971, **105**, 1219.
[438] R. C. Hughes and P. F. Thurman, *Biochem. J.*, 1970, **119**, 925.
[439] P. Sinay, *Carbohydrate Res.*, 1971, **16**, 113.
[440] I. J. Goldstein and A. Misaki, *J. Bacteriol.*, 1970, **103**, 422.
[441] F. Kanetsuna and G. San Blas, *Biochim. Biophys. Acta*, 1970, **208**, 434.
[442] C. Amar-Nacasch and E. Vilkas, *Bull. Soc. Chim. biol.*, 1970, **52**, 145.
[443] F. G. Winder and S. A. Rooney, *Biochem. J.*, 1970, **117**, 355.
[444] A. Misaki, N. Ikawa, T. Kato, and S. Kotani, *Biochim. Biophys. Acta*, 1970, **215**, 405.

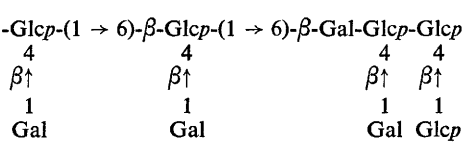
A preliminary survey of the occurrence of *N*-glycolylmuramic acid in bacterial cell walls has shown its presence in three species of Mycobacterium.[445]

Digestion of the cell wall of *Micrococcus lysodeikticus* with lysozyme resulted in the isolation of a glycan composed of D-glucose and 2-acetamido-2-deoxymannopyranosyluronic acid residues. Periodate oxidation indicated that the uronic acid residues are linked through position 4, provided that these residues are situated at internal positions of the polysaccharide chain.[446] Diborane reduced the 2-acetamido-2-deoxymannopyranosyluronic acid units to 2-acetamido-2-deoxymannose; the latter sugar was shown to have the D-configuration by deamination to D-glucose.[447]

A comparative study of the cell-wall composition of the transformable mutants of *Micrococcus lysodeikticus* showed that these strains possess a normal cell wall, which did not differ markedly from that of the parent strain.[448]

Thymidine diphosphate-L-[^{14}C]rhamnose has been incorporated into a rhamnan by membrane fragments from *Streptococcus pyogenes*.[449]

A new diheteroglycan from the walls of *S. faecalis* has been reported. The strain contained two different antigenic glycans, which were shown to be a galactoglucan (*M* 15 000) and a complex polymer (*M* 5000) composed of rhamnose, glucose, galactose, and 2-acetamido-2-deoxygalactose. A tentative structural unit (29) for the galactoglucan was proposed on the basis of chemical and immunochemical investigations.[450]



(29)

Extraction of *S. salivarius* with formamide yielded a mixture of carbohydrate antigens, which was fractionated by precipitation with alcohol into a type antigen, containing rhamnose, glucose, and galactose, and a group-like (Z) antigen. The type antigen, which is related to type III in Group F Streptococci, was submitted to partial acidic hydrolysis and a number of di- and tri-saccharides were isolated and identified. Five of these (30)—(34) were inhibitors in the quantitative precipitation of the type antigen.[451]

[445] I. Azuma, D. W. Thomas, A. Adam, J. M. Ghuysen, R. Bonaly, J. F. Petit, and E. Lederer, *Biochim. Biophys. Acta*, 1970, **208**, 444.
[446] S. Hase and Y. Matsushima, *J. Biochem.*, 1970, **68**, 723.
[447] S. Hase and Y. Matsushima, *J. Biochem.*, 1971, **69**, 559.
[448] R. Kenig-Vakshal, U. Zehavi, and N. Sharon, *Biochim. Biophys. Acta*, 1970, **196**, 107.
[449] M. Cohen and C. Panos, *J. Bacteriol.*, 1971, **106**, 347.
[450] J. H. Pazur, J. S. Anderson, and W. W. Karakawa, *J. Biol. Chem.*, 1971, **246**, 1793.
[451] G. C. Kothari, J. M. N. Willers, and M. F. Michel, *J. Gen. Microbiol.*, 1971, **68**, 77.

β-D-Glc*p*-(1 → 6)-D-Gal
(30)

β-D-Glc*p*-(1 → 4)-D-Gal
(31)

β-D-Glc*p*-(1 → 3)-D-Gal
(32)

β-D-Glc*p*-(1 → 2)-D-Gal
(33)

β-D-Gal*p*-(1 → 6)-D-Gal
(34)

The cell-wall carbohydrate of group L haemolytic Streptococci is composed of 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-galactose, and L-rhamnose, with the antigenic determinant consisting of terminal 2-acetamido-2-deoxy-β-D-glucose residues attached to L-rhamnose side-chains of the polymer. Antigenic cross-reactivity between Group A and Group L Streptococci was attributed to the fact that the antigenic determinants of both are terminal 2-acetamido-2-deoxy-β-D-glucose residues.[452]

A rapid method for the purification of lipopolysaccharides has been reported. The sugar compositions of cell-wall preparations, purified by a gel-filtration method, were identical to those obtained by ultracentrifugation procedures.[453] A procedure has been reported for the colorimetric estimation of dyed lipopolysaccharides.[7] The interaction of concanavalin A with lipopolysaccharides has been used as a 'reagent' for their classification, although not all polysaccharides, presumed to contain α-D-glucopyranosyl side-chains, formed a precipitate with the protein, since the stereochemical environment in which terminal α-D-glucose units are situated may influence the reaction with the protein.[454]

The immunochemistry of *Shigella flexneri* O-antigens has been the subject of a review that included chapters on the general nature and properties of the O-antigens, the structure and biology of the basal region of the polysaccharide, and on the side-chain region of the lipopolysaccharide. Taxonomic aspects, genetic aspects, and the biosynthesis of O-antigens have been reported.[455]

An electron-microscopic study of the lipopolysaccharide of *Sh. flexneri* showed the presence of long filaments (*M* 10—45 × 10^6), which are built up of repeating units (*M* 250 000) linked by hydrophobic interaction, owing to the lipid part of the molecule, and by bivalent cations and carboxy-groups. The unit antigen structures are themselves polymers built up of sub-units linked by weak chemical bonds similar to those binding the units

[452] W. W. Karakawa, J. E. Wagner, and J. H. Pazur, *J. Immunol.*, 1971, **107**, 554.
[453] E. Romanowska, *Analyt. Biochem.*, 1970, **33**, 383.
[454] I. J. Goldstein and A. M. Staub, *Immunochem.*, 1970, **7**, 315.
[455] D. A. R. Simmons, *Bacteriol. Rev.*, 1971, **35**, 117.

in the antigen molecule.[456] The antigen filaments are probably polymerization artefacts of unit molecules which might be the normal biological entities present *in situ*.[457] Complement-fixation inhibition studies with oligosaccharides of known structure have shown that the *Sh. flexneri* group antigens are determined by identical structural sequences in the lipopolysaccharides of different serotypes. The epitopes of group factors 4 and 3,4 contain internal sequences of α-L-rhamnosyl-(1 → 4)-L-rhamnose and α-L-rhamnosyl-(1 → 4)-α-L-rhamnosyl-(1 → 6)-2-acetamido-2-deoxy-D-glucose, respectively.[458] Similarly, a relationship was shown between the epitopes of the O-antigens I—V and 7,8 and the disaccharide sequences (35)—(40), respectively.[459]

I α-D-Glc*p*-(1 → 4)-Glc*N*Ac
(35)

II α-D-Glc*p*-(1 → 4)-L-Rha
(36)

III 2-*O*-Ac-α-D-Glc*p*-(1 → 2)-L-Rha
(37)

IV α-D-Glc*p*-(1 → 6)-Glc*N*Ac
(38)

V α-D-Glc*p*-(1 → 3)-L-Rha
(39)

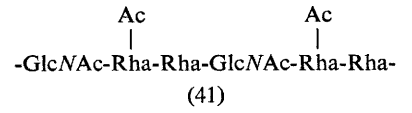
7,8 α-D-Glc*p*-(1 → 2)-L-Rha
(40)

Studies on the O-specific polysaccharide of *Sh. flexneri* Ib(I) and its smooth mutant Z(III) have been reported. From degradation studies on the lipopolysaccharide and on the derived polysaccharide, the probable repeating structure (41) of the O-specific side-chain of mutant Z(III) was proposed, and the structure of the Ib(I) side-chain was suggested to be (42).[460]

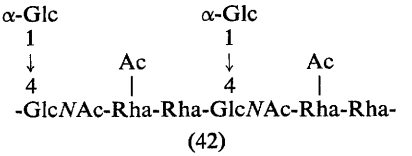
Structural changes occurring during the biosynthesis of *Sh. flexneri* type-specific factors from precursor group antigen have revealed some of the stereochemical factors that are important in determining the antigenic specificity of complex polysaccharide determinants. The spatial configurations of the *Sh. flexneri* O-specific side-chains were presented in the

[456] E. Hannecart-Pokorni, D. Dekegel, F. Depuydt, and J. Dirkx, *Biochim. Biophys. Acta*, 1970, **201**, 155.
[457] E. Hannecart-Pokorni, D. Dekegel, F. Depuydt, and J. Dirkx, *Biochim. Biophys. Acta*, 1970, **201**, 167.
[458] E. Freedlander, R. Manson, and D. A. R. Simmons, *Immunol.*, 1971, **20**, 11.
[459] D. A. R. Simmons, *Immunol.*, 1971, **20**, 17.
[460] E. Romanowska and T. M. Lachowicz, *F.E.B.S. Letters*, 1970, **8**, 293.

form of simple projection formulae, which illustrated the gross steric features of serological importance.[461]

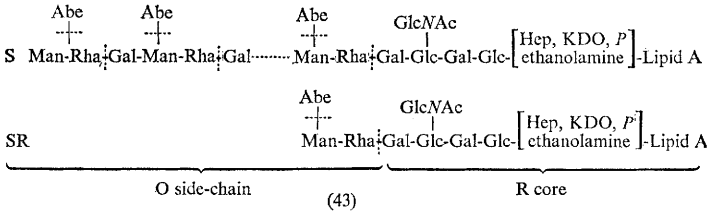


(41)



(42)

A cell-wall polysaccharide from a semi-rough (SR) strain of *Salmonella typhimurium* was studied by partial acidic hydrolysis and methylation. Mutants lacking phosphomannoisomerase were utilized to prepare lipopolysaccharides specifically labelled in the mannose residues, which were found only in the O-side-chain portion of the polymers. After partial acidic hydrolysis, the major oligosaccharide isolated from the SR strain was mannosyl-rhamnose, and the longest mannose-containing oligosaccharide isolated was mannosyl-rhamnosyl-galactose. In contrast, the largest product from the wild strain was galactosyl-mannosyl-rhamnose. Methylation of the SR strain indicated that the structure of the R-core and the O-repeat unit was not altered. This and other work suggested that the SR strains could not polymerize the O-repeat units, but transferred the unpolymerized units to the normal sites of attachment on the R-core (43).[462]

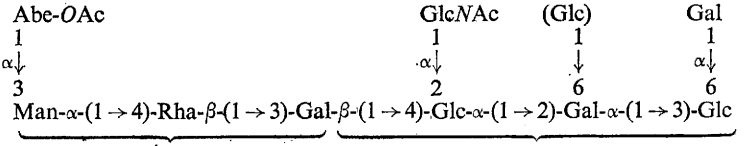


(43)

Structural investigations on the core polysaccharide of *S. typhimurium* and on the mode of attachment to the O-specific side-chains have been reported from a comparative analysis of a lipopolysaccharide derived from an SR mutant and a lipopolysaccharide derived from a corresponding Ra mutant. Partial hydrolysis of these polymers led to the release of a group of oligosaccharides common to both polysaccharides. Structural analysis led to the reconstruction of the hexose region of the core. Four oligosaccharides were identified from the SR lipopolysaccharide which

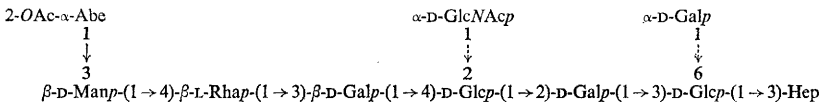
[461] D. A. R. Simmons, *European J. Biochem.*, 1971, **18**, 53.
[462] R. Yuasa, K. Nakane, and H. Nikaido, *European J. Biochem.*, 1970, **15**, 63.

were absent from the Ra lipopolysaccharide: *viz* α-D-Man-(1 → 4)-L-Rha (derived from repeating units), β-D-Gal-(1 → 4)-D-Glc, β-D-Gal-(1 → 4)-α-D-Glc*N*Ac-(1 → 2)-D-Glc, and β-D-Gal-(1 → 4)-α-D-Glc-(1 → 2)-D-Gal. The first three oligosaccharides were derived from the linkage-region between the repeating unit and the core. Methylation studies on both lipopolysaccharides led to the identification of the sugar linkages, thus giving the partial structure (44) for the SR lipopolysaccharide.[463]



(44)

The linkage between the O-side-chains and the core of the cell-wall lipopolysaccharide of *S. typhimurium* has also been studied by methylation analysis coupled with mass spectrometry. The results are consistent with an earlier proposal [464] that the O-side-chains are linked to C-4 of the subterminal D-glucose residue of the core portion and that this residue is also substituted at C-2 by a non-reducing 2-acetamido-2-deoxy-D-glucose unit of the core.[465] Structural studies on the core polysaccharide have indicated the presence of *O*-acetyl groups and/or other alkali-labile groups in some positions.[466] The lipid-free polysaccharides were subjected to methylation analysis, which allowed a partial structure (45) to be proposed

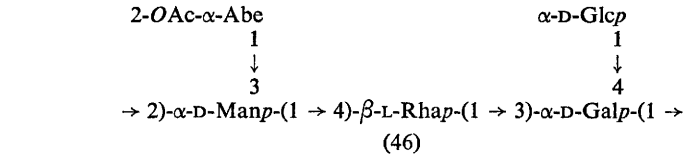


(45)

for the polysaccharide. A trisaccharide alditol, containing intact D-mannose and D-galactose residues and a four-carbon fragment, derived from an L-rhamnose residue, has been isolated and characterized. Both anomeric linkages were assigned α-configurations on the basis of n.m.r. spectroscopy of the trimethylsilyl-trisaccharide alditol, thus allowing the complete structure of the oligosaccharide repeating unit to be formulated as (46).[467]

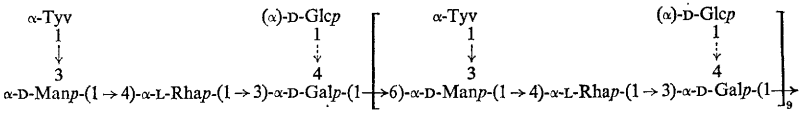
Monosaccharide analysis of the lipopolysaccharide of a strain of *Salmonella strasbourg* showed that the repeating unit of the O-specific side-chains contains one residue each of tyvelose, L-rhamnose, D-mannose,

[463] G. Hämmerling, O. Lüderitz, and O. Westphal, *European J. Biochem.*, 1970, **15**, 48.
[464] H. Nikaido, *J. Biol. Chem.*, 1969, **244**, 2835.
[465] H. Nikaido, *European J. Biochem.*, 1970, **15**, 57.
[466] C. G. Hellerqvist and A. A. Lindberg, *Carbohydrate Res.*, 1971, **16**, 39.
[467] C. G. Hellerqvist, O. Larm, and B. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 744.



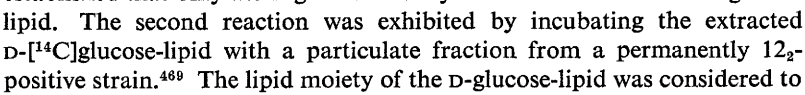
(46)

and D-galactose. Methylation analysis indicated that all the tyvelose residues are terminal, all the L-rhamnose residues are linked in the 4-position, and all the D-galactose residues are linked in the 3-position. Most of the D-mannose residues were found to occur as branch-points linked in the 3- and 6-positions. From the results of methylation analysis of the partially hydrolysed lipopolysaccharide, the biological repeating unit (47) has been proposed.[468]



(47)

The O-antigen 12_2 of *S. typhimurium* has previously been shown to contain short branches of α-D-glucose residues linked to the C-4 position of the D-galactose residues of the main chain (R. Tinelli and A. M. Staub, *Bull. Soc. chim. Biol.*, 1960, **42**, 601). Particulate fractions from permanently 12_2-positive strains of the organism incorporated D-glucose from UDP-D-glucose into endogenous lipopolysaccharide; most of the incorporated D-glucose residues appeared to be linked to D-galactose in the O-side-chain through α-(1 → 4)-linkages. Particulate fractions from permanently 12_2-negative strains incorporated much less D-glucose. Examination of the time course of the reaction showed that two steps are involved:



The first reaction was studied by use of a mutant, which synthesized an incomplete lipopolysaccharide but which could not carry out the second reaction. By use of ^{32}P and ^{3}H doubly labelled UDP-D-glucose, it was established that only the D-glucose moiety is transferred to the endogenous lipid. The second reaction was exhibited by incubating the extracted D-[^{14}C]glucose-lipid with a particulate fraction from a permanently 12_2-positive strain.[469] The lipid moiety of the D-glucose-lipid was considered to be a polyisoprenol phosphate that was either identical or very similar to

[468] C. G. Hellerqvist, B. Lindberg, Å. Pilotti, and A. A. Lindberg, *Acta Chem. Scand.*, 1970, **24**, 1168.
[469] H. Nikaido, K. Nikaido, T. Nakae, and P. H. Mäkelä, *J. Biol. Chem.*, 1971, **246**, 3902.

undecaprenol phosphate.[470] *Salmonella* mutants defective in the biosynthesis of the central R-core portion of the cell-wall lipopolysaccharide accumulated peripheral O-side-chains. When such an O-side-chain polysaccharide was isolated from *S. typhimurium* originally producing O-antigen 12_2, the polysaccharide was found to possess the full determinant group for 12_2 antigenicity. However, the D-galactose residues at the reducing-end of the O-side-chains did not appear to be glucosylated. These results suggested that the transfer of D-glucose residues normally takes place at the level of carrier-linked oligomers of O-side-chain repeating units, rather than at the monomer or polymer levels.[471] A new gene cluster (rfe) concerned with the biosynthesis of *Salmonella* lipopolysaccharides has been described.[472]

The side-chain portion of the cell-wall lipopolysaccharide from TI strains of *Salmonella* was shown to contain D-galactofuranose and D-ribofuranose residues. D-Galactofuranose units are synthesized from D-galactopyranose or its derivatives rather than by direct conversion from other hexopyranoses or their derivatives. Pyranose to furanose conversion did not appear to take place at the level of D-galactose or D-galactose 1-phosphate, but may have occurred at the stage of UDP-D-galactose.[473] Biosynthesis of the TI antigen was studied in a cell-free system and UDP-D-[^{14}C]glucose was found to be incorporated into the D-galactose, D-glucose, and D-ribose residues of the lipopolysaccharide.[474]

The c.d. spectra of a lipid-free polysaccharide from *S. typhimurium* and those of methyl 2-*O*-acetyl-α- and -β-abequosides have been compared. The sign of the Cotton effect of the polysaccharide and the position of the maximum corresponded to those of the α-glycoside. The difference in the magnitude of the signs reflected the attachment of the abequose unit to a mannose unit in the polysaccharide instead of to a methyl group in the glycoside. The results confirmed that 2-*O*-acetylabequose residues are attached by an α-linkage to the backbone of the O-antigen side-chain of the lipopolysaccharide.[475] SR strains of *S. typhimurium* were unable to polymerize the O-repeat unit but, instead, transferred the unpolymerized units to the normal sites of attachment on the R-core.[476]

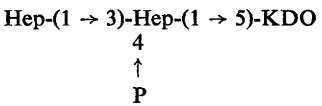
Periodate oxidation of the lipid A backbone of *Salmonella minnesota* lipopolysaccharide indicated the presence of β-(1 → 6)-linked 2-amino-2-deoxy-D-glucose disaccharide units esterified by phosphate at C-4 and 2-keto-3-deoxyoctonoate at either C-2 or C-3 of the non-reducing unit.[477]

Several transfer reactions involved in the biosynthesis of the core part of *Salmonella* lipopolysaccharide have been studied in a cell-free system.

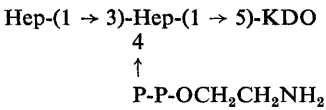
[470] K. Nikaido and H. Nikaido, *J. Biol. Chem.*, 1971, **246**, 3912.
[471] M. Takeshita and P. H. Mäkelä, *J. Biol. Chem.*, 1971, **246**, 3920.
[472] P. H. Mäkelä, M. Jahkola, and O. Lüderitz, *J. Gen. Miocrobiol.*, 1970, **60**, 91.
[473] M. Sarvas and H. Nikaido, *J. Bacteriol.*, 1971, **105**, 1063.
[474] H. Nikaido and M. Sarvas, *J. Bacteriol.*, 1971, **105**, 1073.
[475] H. B. Borén, P. J. Garegg, and S. Svensson, *Acta Chem. Scand.*, 1970, **24**, 3084.
[476] R. Yuasa, K. Nakane, and H. Nikaido, *European J. Biochem.*, 1970, **15**, 63.
[477] J. Gmeiner, M. Simon, and O. Lüderitz, *European J. Biochem.*, 1971, **21**, 355.

The system contained three components: (*i*) enzymes that were solubilized by repeated washings of edta-lysozyme spheroplasts, (*ii*) acceptor, which consisted either of heated cell-wall fractions sedimenting at 20 000 *g* or of heated, partially lysed edta-lysozyme cells, and (*iii*) the labelled precursors [^{32}P]-ATP, UDP-D-[^{14}C]glucose, and UDP-D-[^{14}C]galactose. The results indicated that only the γ-phosphate group of ATP is transferred to the heptose moiety of the core. There was a definite sequence of addition of the core substituents (D-glucose, phosphate, and D-galactose) to the incomplete core stub, *viz* $(Hep)_2$-(KDO, ethanolamine, phosphate, lipid A) → Glc*p*-$(Hep)_2$-(KDO, ethanolamine, phosphate, lipid A) → Glc*p*-$(Hep)_2$P-(KDO, ethanolamine, phosphate, lipid A) → $(Gal p)_2$-Glc*p*-$(Hep)_2$P-(KDO, ethanolamine, phosphate, lipid A).[478]

Mild acidic hydrolysis of the P^+ lipopolysaccharide of *Salmonella minnesota*, a mutant lacking UDP-D-glucose synthetase, led to the identification of five degradation products. Of these, free 2-keto-3-deoxyoctonate (KDO), KDO-7-phosphorylethanolamine, and a trisaccharide containing two heptose residues and one KDO residue [Hep-(1 → 3)-β-Hep-(1 → 5)-β-KDO] have been identified previously as degradation products of a P^- lipopolysaccharide. The two other fragments (48) and (49) isolated from P^+ lipopolysaccharide do not occur in the P^- lipopolysaccharide.[479]



(48)

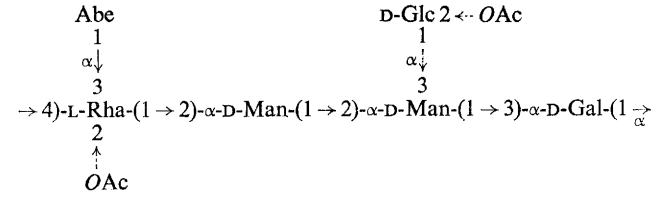


(49)

Fragmentation analysis of the lipopolysaccharide from *Salmonella newport* has been employed to elucidate the structure of the oligosaccharide repeating unit of the O-specific side-chains. The anomeric nature of some of the glycosidic linkages was established by n.m.r. spectroscopy and optical data. A detailed structure (50) has been proposed for the biological repeating unit.[480]

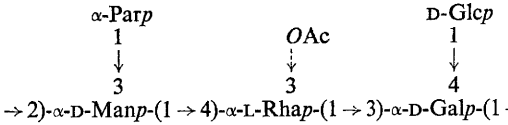
Both sugar and methylation analyses supported the assumption that the O-antigenic side-chains of *S. paratyphi* are composed of tetrasaccharide repeating units of paratose, D-mannose, L-rhamnose, and D-galactose. Methylation analysis revealed that the paratose residue is terminal, and that

[478] P. F. Mühlradt, *European J. Biochem.*, 1971, **18**, 20.
[479] V. Lehmann, O. Lüderitz, and O. Westphal, *European J. Biochem.*, 1971, **21**, 339.
[480] C. G. Hellerqvist, B. Lindberg, J. Lönngren, and A. A. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 601.



(50)

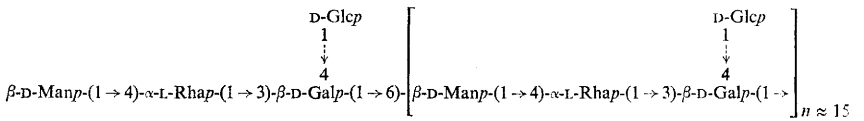
L-rhamnose and D-galactose residues occur preponderantly as chain residues linked at the 4- and 3-positions, respectively; D-mannose is present mainly as a branched residue linked at the 2- and 3-positions. Since most of the D-mannose occurs as chain residues linked through C-2 in the product resulting from mild acidic hydrolysis of the lipopolysaccharide, it was concluded that the terminal paratose residues are linked to D-mannose at the 3-position. The sequence (51) is considered to represent the



(51)

repeating unit of the O-specific side-chains, unless a less-ordered structure is contemplated. Part of the repeating units carry *O*-acetyl groups linked to L-rhamnose at C-3. The structure of the repeating unit resembles those of the serogroups B and D lipopolysaccharides. The main difference is that the α-linked paratose residue, associated with the presence of O-factor 2, replaces abequose or tyvelose at the 3-position of D-mannose.[481]

The structure (52) of the O-specific side-chains of *S. newington* lipopolysaccharide, which contains D-glucose, D-mannose, and D-galactose, was investigated by methylation of the lipopolysaccharide and a chemically modified polysaccharide, and by identification of a trisaccharide obtained on graded hydrolysis.[482]



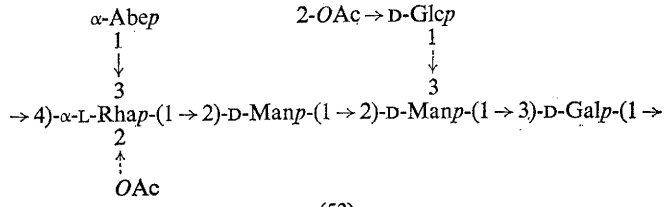
(52)

Structural studies have been reported on the O-specific side-chains of the cell-wall lypopolysaccharides of *Salmonella* serogroups C_2 and C_3 from *S. newport* and *S. kentucky*, respectively. Sugars obtained on acidic

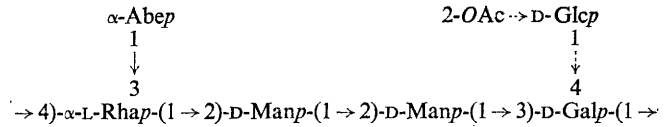
[481] C. G. Hellerqvist, B. Lindberg, K. Samuelsson, and A. A. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 955.

[482] C. G. Hellerqvist, B. Lindberg, J. Lönngren, and A. A. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 939.

hydrolysis of the methylated lipopolysaccharides and partially hydrolysed lipopolysaccharides were analysed by g.l.c.–mass spectrometry, which permitted formulation of the detailed structures (53) and (54) for the



(53)



(54)

repeating units of the side-chains. In most respects, the lipopolysaccharides from these *Salmonella* strains are similar; however, an important difference is that the terminal D-glucose residues are attached to C-4 of D-galactose in *S. kentucky* but to C-3 of D-mannose in *S. newport*. The O-antigen carrying-factor 8 is most likely to be associated with the presence of the α-abequose residue linked (1 → 3) to L-rhamnose. All the L-rhamnose residues are acetylated in the *S. newport* lipopolysaccharide, but are unacetylated in the *S. kentucky* lipopolysaccharide, suggesting serological differences between the two strains.[483] By the use of similar methods, the biological repeating units in the O-specific side-chains of the cell-wall lipopolysaccharides from *S. muenster*[484] and *S. senftenberg*[485] have been established as (55) and (56), respectively.

Immunochemical and immunological studies on the lipopolysaccharides of streptomycin-dependent mutants of *S. enteritidis* have indicated that structural changes in the polysaccharide component of the O-antigen may lead to qualitative changes in antibody production.[486]

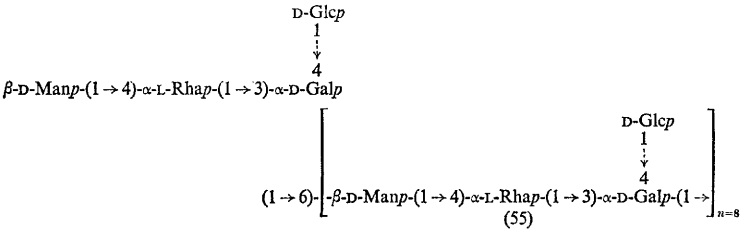
Further structural studies on the O-specific side-chains of the lipopolysaccharide from *S. strasbourg* have confirmed the chemical repeating unit as (57). It was demonstrated that D-galactose is the terminal reducing

[483] C. G. Hellerqvist, B. Lindberg, S. Svensson, T. Holme, and A. A. Lindberg, *Carbohydrate Res.*, 1970, **14**, 17.

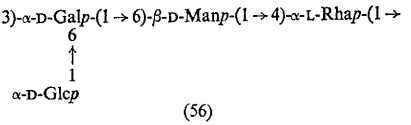
[484] C. G. Hellerqvist, B. Lindberg, J. Lönngren, and A. A. Lindberg, *Carbohydrate Res.*, 1971, **16**, 289.

[485] C. G. Hellerqvist, B. Lindberg, Å. Pilotti, and A. A. Lindberg, *Carbohydrate Res.*, 1971, **16**, 297.

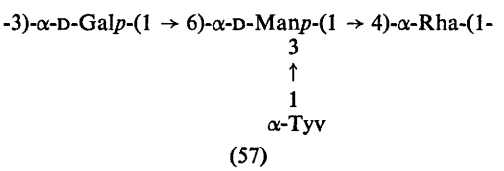
[486] E. S. Stanislavski, L. S. Edvabnaja, V. V. Sergejev, M. I. Zhvaneckaja, and N. A. Rostovceva, *Immunochem.*, 1971, **8**, 49.



(55)



(56)



(57)

sugar of the biological repeating unit, and that tyvelose and L-rhamnose residues are α-linked.[487]

The anomeric nature of the D-mannose residues in lipopolysaccharides was investigated using a new technique that is based on the observation that acetylated β-glycosides are readily oxidized by chromic acid in acetic acid, but that the corresponding α-glycosides are fairly stable. Such treatment of the D_2 polysaccharide from *S. strasbourg* gave rapid oxidation, indicating that the D-mannose residues have the β-configuration, whereas these residues were unaffected in the D_1 polysaccharide from *S. typhi*.[488] A reinvestigation of the anomeric configuration of the D-mannose residues in the antigens of *Salmonella* showed that Groups B and D have α-linked units, whereas those in Group E have the β-configuration.[489]

All transductants formed as hybrids between Groups B and D of *Salmonella*, which did not have antigenic factor 5 and the 2-*O*-acetyl-abequose unit corresponding to it, have 25% of the terminal D-glucose residues in the O-specific side-chains *O*-acetylated at C-4.[490]

H_4edta had a marked bactericidal effect on *Pseudomonas alcaligenes* that was accompanied by solubilization of lipopolysaccharide and release of

[487] C. G. Hellerqvist, B. Lindberg, Å. Pilotti, and A. A. Lindberg, *Acta Chem. Scand.*, 1970, **24**, 1168.

[488] C. G. Hellerqvist, J. Hoffman, B. Lindberg, Å. Pilotti, and A. A. Lindberg, *Acta Chem. Scand.*, 1971, **25**, 1512.

[489] M. Fukuda, F. Egami, G. Hämmerling, O. Lüderitz, G. Bagdian, and A.-M. Staub, *European J. Biochem.*, 1971, **20**, 438.

[490] M. Nurminen, C. G. Hellerqvist, V. V. Valtonen, and P. H. Mälelä, *European J. Biochem.*, 1971, **22**, 500.

intracellular solutes.[491] The lipopolysaccharide was separated from the glycosaminopeptide, and the lipid A portion was shown to contain 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-glucose phosphate, inorganic phosphate, and fatty acids. The sensitivity of the organism to edta may be associated with the high phosphorus content of the lipopolysaccharide.[492]

The low molecular weight solutes released during mild hydrolysis of the lipopolysaccharide of *Pseudomonas aeruginosa* were isolated by gel chromatography and high-voltage electrophoresis. The major components were identified as 2-keto-3-deoxyoctonic acid (KDO), basic amino-acids (free and bound), inorganic phosphate, ethanolamine phosphate, and ethanolamine pyrophosphate. Although the presence of ethanolamine pyrophosphate has been reported in lipopolysaccharides of *Salmonella* species,[479, 493, 494] its presence has not been reported in a pseudomonad. The ease of release of ethanolamine pyrophosphate during hydrolysis revealed an acid lability not suggested by the partial structures proposed for *Salmonella* species.[495] The action of H_4edta on *P. aeruginosa* was apparently the result of extraction of lipopolysaccharide from the cell wall as a complex with protein and loosely bound lipid.[496]

The identification of fucosamine, quinovosamine, and 3-amino-3,6-dideoxy-D-glucose in the cell walls of *Pseudomonas* species has underlined the basic similarity between the lipopolysaccharides from pseudomonads and those from members of the Enterobacteriaceae.[497]

Preparations of *Escherichia coli* 014 lipopolysaccharide contained a common enterobacterial antigen. Mild acidic hydrolysis of the polysaccharide liberated a core fragment (M 2—3 $\times$ 10^3) containing heptose, KDO, glucose, and galactose.[498] A lipopolysaccharide from *E. coli* is required for the stability of L-glycerol-3-phosphate acyl transferase.[499]

Aqueous extraction of *E. coli* released protein and lipopolysaccharide simultaneously as a polymer sedimenting as a single component on analytical ultracentrifugation and giving a coincidental distribution of protein and lipopolysaccharide on ion-exchange chromatography. It was suggested that the complex is derived from the outermost layer of the cell.[500]

A lipopolysaccharide isolated from colicin-sensitive *E. coli* inhibited the activity of colicins. Lipid A and polysaccharide fractions, obtained by

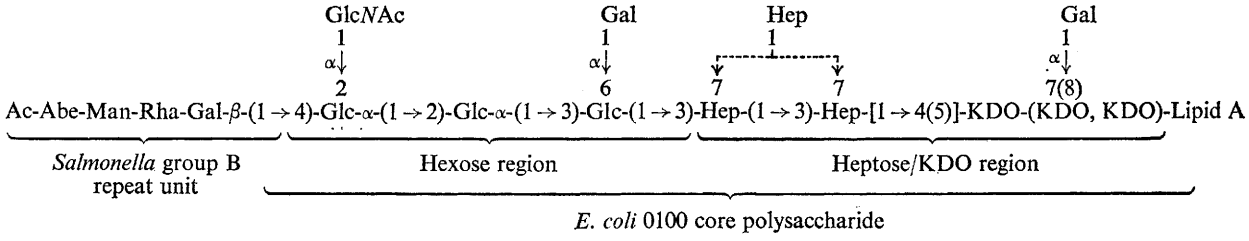
[491] B. A. Key, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1970, **117**, 721.
[492] B. A. Key, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1970, **120**, 559.
[493] M. J. Osborn, *Ann. Rev. Biochem.*, 1969, **38**, 501.
[494] O. Lüderitz, *Angew. Chem. Internat. Edn.*, 1970, **9**, 649.
[495] D. T. Drewry, G. W. Gray, and S. G. Wilkinson, *European J. Biochem.*, 1971, **21**, 400.
[496] N. A. Roberts, G. W. Gray, and S. G. Wilkinson, *Microbios*, 1970, **2**, 189.
[497] S. G. Wilkinson and K. A. Carby, *J. Gen. Microbiol.*, 1971, **66**, 221.
[498] S. Hammarström, H. E. Carlsson, P. Perlmann, and S. Svensson, *J. Exp. Med.*, 1971, **134**, 565.
[499] M. Kito, R. Sasaki, M. Murata, and K. Hasegawa, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 788.
[500] D. Rogers, *Biochim. Biophys. Acta*, 1971, **230**, 72.

mild hydrolysis of the lipopolysaccharide, were inactive either alone or in combination. Periodate oxidation of the polysaccharide destroyed 75% of its inhibitory activity.[501] The lipopolysaccharide and lipopolysaccharide membrane of *E. coli* appear to be composed of sub-structures bound together by both Mg^{2+}-mediated and hydrophobic bonds.[502] Mutants of *E. coli*, which failed to show the characteristic increase in permeability after brief treatment with H_4edta, have been isolated. Analysis of one mutant and its parent showed that the mutant strain released 20—40% less lipopolysaccharide, and that this represented a reduction of a specific lipopolysaccharide fraction.[503]

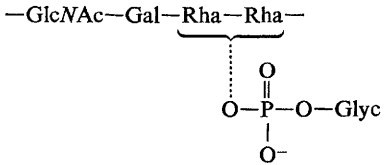
In order to investigate the core structure of the lipopolysaccharide from an *E. coli* 0100 strain, two derivatives were prepared from the strain by introducing genetic material from *Salmonella abony*. One derivative, an SR hybrid, contained a lipopolysaccharide with short side-chains consisting of simple repeating units of *Salmonella* group B specificity attached to the *E. coli* core. The other derivative, an R form, contained only the *E. coli* core without O-side-chains owing to a mutation in the *Salmonella rfb* chromosomal region, which determines the synthesis of the O repeating units. On partial acidic hydrolysis of the SR and R lipopolysaccharides, oligosaccharide fragments were obtained that made it possible to reconstruct the hexose region of the core. Methylation studies performed on the dephosphorylated polysaccharides confirmed and extended these results. It was found that only about 60% of the distal D-glucose units of the core chains are substituted by 2-acetamido-2-deoxy-D-glucose residues. Further, the heptose region was shown to form a branched trisaccharide. On mild acidic hydrolysis of both lipopolysaccharide preparations, an α-D-Gal-(1 → 7)-KDO disaccharide was formed, indicating that a D-galactose unit is linked to the 2-keto-3-deoxyoctonate (KDO) portion of the lipopolysaccharide. After Smith degradation of the SR lipopolysaccharide, the oligosaccharide β-D-Gal-(1 → 4)-D-Glc-glycerol was identified, which appeared to be derived from the linkage region between the *Salmonella*-type repeating unit and the *E. coli* core. Only the core stubs substituted by 2-acetamido-2-deoxy-D-glucose residues carried an O-specific unit. On the basis of these findings, the partial structure (58) of the *E. coli* 0100 core polysaccharide with a *Salmonella* group B-specific unit attached to it is proposed.[504]

The K29 antigen of *E. coli* 09 : K29(A) : H^- was shown to be an acidic polysaccharide, which after alkali treatment had *M* 332 000 and consisted of glucose, mannose, galactose, and glucuronic acid (2 : 2 : 1 : 1). It also contained an acid-labile constituent that was tentatively identified as pyruvate. After partial acidic hydrolysis, seven oligosaccharides were

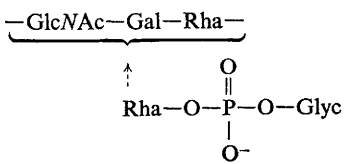
[501] Y.-Y. Chang and L. P. Hager, *J. Bacteriol.*, 1970, **104**, 1106.
[502] M. L. De Pamphilis, *J. Bacteriol.*, 1971, **105**, 1184.
[503] M. J. Voll and L. Leive, *J. Biol. Chem.*, 1970, **245**, 1108.
[504] G. Hämmerling, O. Lüderitz, O. Westphal, and P. H. Mäkelä, *European J. Biochem.*, 1971, **22**, 331.


GlcNAc
Gal
Hep
Gal
1
1
1
1
α↓
α↓
2
6
7
7
7(8)
Ac-Abe-Man-Rha-Gal-β-(1 → 4)-Glc-α-(1 → 2)-Glc-α-(1 → 3)-Glc-(1 → 3)-Hep-(1 → 3)-Hep-[1 → 4(5)]-KDO-(KDO, KDO)-Lipid A
Salmonella group B repeat unit
Hexose region
Heptose/KDO region
E. coli 0100 core polysaccharide

(58)


—GlcNAc—Gal—Rha—Rha—
O
O—P—O—Glyc
O⁻

(59)


—GlcNAc—Gal—Rha—
Rha—O—P—O—Glyc
O
O⁻

(59a)

isolated. Oligosaccharide analysis, together with the results of methylation, periodate oxidation, and carboxy-group reduction indicated that the acidic polysaccharide consists of the hexasaccharide repeating unit, Man*p*-(1 → 3)-Glc*p*-(1 → 6)-Man-(1 → 3)-Glc*p*-(1 → 3)-β-Glc*p*UA-(1 → 3)-β-Gal. The glycosidic bond between the repeating units was an α-galactosyl-(1 → 6)-mannose linkage. The alkali-treated, acidic polysaccharide was indicated to represent a subunit chain with a length of about 300 repeating units. It was suggested that these subunit chains are interlinked by alkali-labile ester bonds between the carboxy-group of glucuronic acid residues and hydroxy-groups in the chains. With the aid of passive haemagglutination and immune precipitation in O-, OK-, and K-sera, and with bacterial agglutination in OK serum before and after absorption with the acidic polysaccharide, it was shown that the acidic polysaccharide represents the K29 antigen. Serological studies with chemically altered polysaccharide preparations indicated that glucuronic acid and mannose are the immunodominant sugar residues. Inhibition of the K29-specific precipitin reaction with oligosaccharides obtained from the K29 polysaccharide and with degraded polysaccharides of *Salmonella anatum* and *S. newington* indicated that the determinant region of the K29 antigen is represented by the trisaccharide Glc*p*UA-β-(1 → 3)-Gal*p*-α-(1 → 6)-Man, which is probably partially esterified by pyruvate.[505]

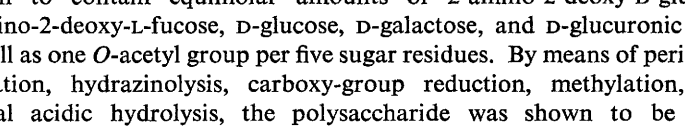
The core lipopolysaccharides of bacteria from different enterobacterial genera were compared by studying the lipopolysaccharides isolated from appropriate R-mutants. The results of phage typing were corroborated by serological and chemical investigations on the lipopolysaccharide. In serological studies, lipopolysaccharides of all R types studied, except *coli* R1, were found to cross-react with each other, indicating structural similarities. In all cross-reacting lipopolysaccharide preparations, the same sugar constituents, namely D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, L-*glycero*-D-*manno*-heptose, and 3-deoxy-D-*manno*-octulosonic acid were present, although quantitative analysis revealed striking differences between them. The results showed that *E. col* 0111 core represents a further core type that is designated *coli* R3. Serological and chemical investigation of the lipopolysaccharide of the *Arizona* R-mutant confirmed its identity with the *Salmonella* core type, thus indicating the close relationship of these bacterial groups.[506]

A lipopolysaccharide and an acidic polysaccharide have been extracted, separated, and purified from *E. coli* 0100 : K?(B) : H_2. Both substances were shown to contain rhamnose, galactose, 2-amino-2-deoxy-D-glucose, glycerol, and phosphate. The lipopolysaccharide released the specific polysaccharide moiety on mild acidic hydrolysis. Comparison of this fraction with the acidic polysaccharide, by means of chemical analysis, periodate oxidation, alkaline hydrolysis, and partial acidic hydrolysis, showed that

[505] L.-B. Nhan, B. Jann, and K. Jann, *European J. Biochem.*, 1971, **21**, 226.
[506] G. Schmidt, I. Fromme, and H. Mayer, *European J. Biochem.*, 1970, **14**, 357.

both substances have the same structure consisting of a main chain composed of rhamnose, 2-amino-2-deoxy-D-glucose, and galactose, to which glycerol is linked by a phosphodiester bridge; the latter also linked one of the primary hydroxy-groups of glycerol to a rhamnose residue. The repeating unit of both polysaccharides could be represented by one of the structures (59) or (59a). From serological studies it was shown that the *E. coli* examined did not contain a polysaccharide representing a K antigen.[507]

An acidic polysaccharide isolated from *E. coli* 08 : K87(B?) : H19 was shown to contain equimolar amounts of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-L-fucose, D-glucose, D-galactose, and D-glucuronic acid, as well as one *O*-acetyl group per five sugar residues. By means of periodate oxidation, hydrazinolysis, carboxy-group reduction, methylation, and partial acidic hydrolysis, the polysaccharide was shown to be built from about 270—300 pentasaccharide repeating units having the structure (60). From methylation studies of the polysaccharide before and after



(60)

carboxy-group reduction, it was found that the D-glucuronic acid residues in the polysaccharide are bound in the 4-position, suggesting that the repeating units in the polysaccharide are joined through D-galactosyl-(1 → 4)-D-glucuronyl bonds. Methylation studies also indicated that D-galactose is bound at C-6. From periodate-oxidation studies performed on the acidic polysaccharide before and after alkali treatment, it was concluded that either the D-galactose units within the polysaccharide chain or the non-reducing D-glucose units carry an *O*-acetyl group at C-2. With the aid of bacterial agglutination, passive haemagglutination, and immune precipitation, it was established that the acidic polysaccharide represents the K87 antigen of the *E. coli*. As determined by serological inhibition studies, non-reducing D-glucose units and *O*-acetyl groups on either D-glucose or D-galactose are essential parts of the determinant groups.[508] The O-specific polysaccharide from this strain of *E. coli* afforded D-mannose as the sole sugar constituent, whereas the (032)-specific polysaccharide from *E. coli* (032) : K87(B?) : H45 yielded D-glucose, D-galactose, D-glucuronic acid, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-L-fucose (1 : 1 : 1 : 1 : 1). The acidic polysaccharide from the 032-strain, as well as the (032)-specific polysaccharide moiety of

[507] B. Jann, K. Jann, G. Schmidt, I. Ørskov, and F. Ørksov, *European J. Biochem.*, 1970, **15**, 29.
[508] L. Tarcsay, B. Jann, and K. Jann, *European J. Biochem.*, 1971, **23**, 505.

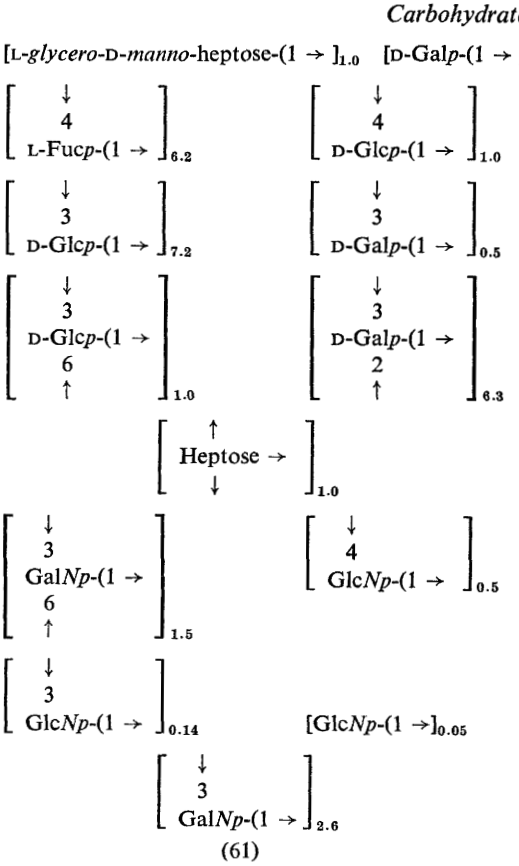
the lipopolysaccharide from that strain, have the same structure as the K87 antigen. Serological identity of the acidic polysaccharide and the (032)-specific polysaccharide moiety of the lipopolysaccharide from *E. coli* (032) : K87(B?) : H45 was shown. It was concluded that the acidic polysaccharide, which had been shown to be the K87-antigen,[508] is also responsible for O-specificity in the latter strain.[509] Extracts of *E. coli* (032) : K87(B?) : H45 gave identical immune electrophoretic patterns in both anti-O and anti-OK sera; in both systems, only one anodically migrating band was observed. It was also shown that many *E. coli* strains, including 032, contain negatively charged lipopolysaccharides.[510]

The 09-antigen from a strain of *E. coli* was shown to contain D-mannose and D-glucose (9 : 1). The time course of D-mannose incorporation from GDP-D-[^{14}C]mannose into acid-insoluble particles by an enzyme preparation from disrupted cells was biphasic, and the reaction was stimulated by UDP-D-glucose during the early part of the reaction. A product of high molecular weight was recovered, which yielded a single radioactive peak corresponding to D-mannose after total hydrolysis and chromatography. Since the polymer was precipitated with anti-09 serum in an antigen-binding assay, it is likely that the 09 antigen of this strain of *E. coli* is synthesized from GDP-D-mannose and UDP-D-glucose by reactions analogous to those of lipopolysaccharide biosynthesis in *Salmonella*.[511]

A homogeneous lipopolysaccharide was isolated from a strain of *E. coli*. The results of methylation and other analyses indicated that the molecule is highly branched with (1 → 3)-linked D-galactose forming the non-reducing end-groups and with other substituents attached at C-2. L-Fucose units are joined exclusively (1 → 4), whereas D-glucose units are linked β-(1 → 3) and β-(1 → 4), with some branching at C-6 in the (1 → 3)-linked units. The L-*glycero*-D-*manno*-heptose residues constitute a branched chain with (1 → 3)-linkages and with half of the heptose units as non-reducing terminal units. 2-Amino-2-deoxy-D-galactose was shown to be part of the polysaccharide structure, whereas 2-amino-2-deoxy-D-glucose is present in the backbone of the lipid A part of the lipopolysaccharide molecule. It was concluded that the lipopolysaccharide must contain the fourteen units shown in (61) in the relative proportions indicated.[512]

The chemical composition of *Klebsiella* lipopolysaccharides has shown that they contain small and comparable amounts of 3-deoxyoctulosonic acid (KDO), heptose, 2-acetamido-2-deoxy-D-glucose, D-glucose, and D-galactose residues as constituents of the common core. The sugars in the O-specific side-chains, which are the main sugar components present,

[509] B. Jann, K. Jann, G. Schmidt, F. Ørskov, and I. Ørskov, *European J. Biochem.*, 1971, **23**, 515.
[510] F. Ørskov, I. Ørskov, B. Jann, and K. Jann, *Acta Pathol. Microbiol. Scand.*, 1971, **79**, 142.
[511] D. K. Fitzgerald-Chandler and K. Jann, *European J. Biochem.*, 1971, **24**, 222.
[512] P. P. Singh and G. A. Adams, *Canad. J. Chem.*, 1970, **48**, 2500.



(61)

varied considerably.[513] Structural studies on *Klebsiella* 01 and 06 lipopolysaccharides have shown that they both consist of chains of α-(1 → 3)-linked D-galactopyranose residues. As no significant difference in structure was found between the two lipopolysaccharides, chemical evidence did not justify differentiation between groups 01 and 06.[514]

A survey of eighty bacterial species has demonstrated the presence of KDO and/or sialic acid. In most Gram-negative bacteria, KDO, but not sialic acid, was found in the wall material, whereas Gram-positive organisms contain neither compound.[515]

A number of unusual methylated, amino-, and deoxy-sugars have been found in lipopolysaccharides. Smooth and rough forms of lipopolysaccharides have been shown to contain 4-amino-4-deoxy-L-arabinose. Evidence was based on the fact that the reduced amino-sugar is identical

[513] W. Nimmich and G. Korten, *Pathol. Microbiol.*, 1970, **36**, 179.
[514] H. Björndal, B. Lindberg, and W. Nimmich, *Acta Chem. Scand.*, 1971, **25**, 750.
[515] D. C. Ellwood, *J. Gen. Microbiol.*, 1970, **60**, 373.

to 2-amino-2-deoxylyxitol and that, after degradation of the C-1-labelled, reduced amino-sugar, inactive L-serine was isolated.[516] A methylated sugar isolated from *Rhodopseudomonas capsulata* was characterized by chromatographic procedures and mass spectrometry as a 6-deoxy-3-*O*-methylhexose. Demethylation of the material released rhamnose, and rotational measurements identified the sugar as 3-*O*-methyl-L-rhamnose.[517] The same sugar was reported to be present in the lipopolysaccharide from a strain of *Klebsiella*.[518] The lipopolysaccharides of a *Klebsiella* and *E. coli* have been shown to contain 3-*O*-methylmannose,[519] whereas 3-*O*-methyl-D-xylose has been identified as a constituent of *Myxococcus fulvus* and 3-*O*-methyl-L-xylose is present in *Rhodopseudomonas viridis*.[520] Identification of 4-deoxy-D-*arabino*-hexose in four *Citrobacter* serotypes has been reported,[521] and 6-deoxy-L-altrose has been isolated from *Yersinia enterocolitica*.[522]

The serological properties of the lipopolysaccharide from strains of *Veillonella* have demonstrated that the determinant groups are carbohydrate in nature. Inhibition of haemagglutination by D-galactose and melibiose indicated that D-galactose occupies a terminal position in the structure that determines the serological type-specificity and is probably α-linked to C-6 of D-glucose.[523]

The major sugar components of the endotoxic lipopolysaccharide from *Sphaerophorus necrophorus* were identified as heptose, galactose, glucose, and 2-amino-2-deoxyglucose,[524] and the lipopolysaccharide isolated from *Bacteroides melaninogenicus* contained galactose, glucose, mannose, and rhamnose.[525] The major sugar components of *Bacteroides fragilis* were identified as glucose, galactose, fucose, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose.[526] Heptose and KDO were absent from the strains of *Bacteroides* and from two of the three strains of *Sphaerophorus*.

A lipopolysaccharide isolated from an avian strain of *Escherichia coli* was found to contain sugars qualitatively similar to those found in lipopolysaccharides of various other bacteria.[527]

The purified lipopolysaccharides of *Moraxella duplex* and *Micrococcus calco-aceticus* were shown to be essentially similar in overall compositions. Major differences occurred in the hexosamine contents of the lipopolysaccharide preparations from the two organisms: 2-amino-2-deoxy-D-

[516] W. A. Volk, C. G. Alanos, and O. Lüderitz, *F.E.B.S. Letters*, 1970, **8**, 161.
[517] J. Weckesser, H. Mayer, and G. Drews, *European J. Biochem.*, 1970, **16**, 158.
[518] H. Björndal, B. Lindberg, and W. Nimmich, *Acta Chem. Scand.*, 1970, **24**, 3414.
[519] W. Nimmich, *Biochim. Biophys. Acta*, 1970, **215**, 189.
[520] J. Weckesser, G. Rosenfelder, H. Mayer, and O. Lüderitz, *European J. Biochem.*, 1971, **24**, 112.
[521] J. Keleti, H. Mayer, I. Fromme, and O. Lüderitz, *European J. Biochem.*, 1970, **16**, 284.
[522] D. C. Ellwood and G. R. A. Kirk, *Biochem. J.*, 1971, **122**, 14P.
[523] T. Hofstad, T. Kristoffersen, and J. A. Maeland, *Acta Path. Microbiol. Scand. B*, 1971, **79**, 615.
[524] T. Hofstad and T. Kristoffersen, *Acta Path. Microbiol. Scand. B*, 1971, **79**, 385.
[525] T. Hofstad and T. Kristoffersen, *Acta Path. Microbiol. Scand. B*, 1971, **79**, 12.
[526] T. Hofstad and T. Kristoffersen, *J. Gen. Microbiol.*, 1970, **61**, 15.
[527] J. Lopes and W. E. Inniss, *Canad. J. Microbiol.*, 1970, **16**, 1117.

galactose is the main amino-sugar constituent of *M. duplex*, whereas 2-amino-2-deoxy-D-glucose is the major amino-sugar component of *M. calcoaceticus*.[528]

A soluble enzyme from *Mycobacterium phlei* catalysed the transfer of methyl groups from [^{14}C]methyl-*S*-adenosylmethionine to endogenous acceptors to produce a labelled lipopolysaccharide. D-Glucose oligosaccharides with α-(1 → 4)-linkages were good acceptors of the methyl transferase system, the most active oligosaccharides having 7—10 units; the product contained methylated sequences of at least six 6-*O*-methyl-D-glucose units. Since the lipopolysaccharides of the *Mycobacterium* species contain sequences of about ten α-(1 → 4)-linked 6-*O*-methyl-D-glucose units, it was concluded that the polysaccharide component of the lipopolysaccharide is probably methylated at the polymer level during its biosynthesis.[529]

The backbone of lipid A of the cell-wall lipopolysaccharide from *Neisseria sicca* is composed of (1 → 4)-linked 2-amino-2-deoxy-D-glucose units. 2-Amino-2-deoxy-D-galactose residues in the polysaccharide portion of the lipopolysaccharide did not survive methylation owing to the absence of an acyl-protecting residue on the amino-group. The distribution of D-glucose is 30% as non-reducing end-groups, 30% linked α-(1 → 2), and the remainder branched through C-3, C-4, and C-6.[530] Environmental conditions affected the content and the composition of the *N. sicca* lipopolysaccharide. High growth-rates produced greater amounts of lipopolysaccharide having a higher content of hexosamine and KDO and a higher ratio of 2-amino-2-deoxy-D-galactose to 2-amino-2-deoxy-D-glucose.[531]

Immunochemical studies on *Citrobacter* O-antigens showed that all the species studied contained glucose, galactose, heptose, KDO, and 2-amino-2-deoxyglucose. In addition to small amounts of D-xylose, other monosaccharides such as D-mannose, fucose, rhamnose, 6-deoxytalose, 4-deoxy-D-idose, abequose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-fucose, 3-amino-3,6-dideoxyglucose, and 3-amino-3,6-dideoxygalactose were detected.[532]

The presence of glyceromannoheptose has been reported in the lipopolysaccharides of *Veillonella*,[533] and 2-amino-2-deoxy-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-D-glucose (with ester- and amide-linked fatty acids) has been found in the lipid A of *Selenomonas ruminantium*.[534]

Two lipopolysaccharide fractions and a polysaccharide fraction isolated from *Brucella melitensis* contained different antigens.[535]

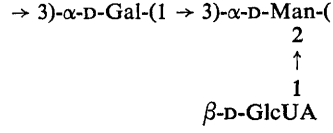
[528] G. A. Adams, C. Quadling, M. Yaguchi, and T. G. Tornabene, *Canad. J. Microbiol.*, 1970, **16**, 1.
[529] J. A. Ferguson and C. E. Ballou, *J. Biol. Chem.*, 1970, **245**, 4213.
[530] G. A. Adams, *Canad. J. Biochem.*, 1971, **49**, 243.
[531] I. J. McDonald and G. A. Adams, *J. Gen. Microbiol.*, 1971, **65**, 201.
[532] J. Keleti, O. Lüderitz, D. Mlynarčik, and J. Sedlak, *European J. Biochem.*, 1971, **20**, 237.
[533] M. J. Hewett, K. W. Knox, and D. G. Bishop, *European J. Biochem.*, 1971, **19**, 169.
[534] Y. Kamio, K.-C. Kim, and H. Takahashi, *J. Biochem.*, 1971, **70**, 187.
[535] A. Serre, J. Asselineau, C. Lacave, and S. Bascoul, *Ann. Inst. Pasteur*, 1971, **121**, 479.

Structural heterogeneity has been demonstrated in the lipopolysaccharides of *Aerobacter aerogenes*. After separation, one fraction contained lipid A and an oligosaccharide core which comprised KDO, heptose, glucose, galactose, and 2-amino-2-deoxyglucose. The second fraction was composed of lipid A together with three components containing galactan.[536]

Anti-polysaccharide antibodies have been reported to combine specifically with homologous antibody on lipopolysaccharides cross-linked with glutaraldehyde, but not with heterologous antibody.[537]

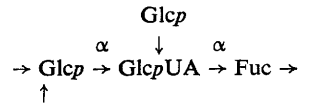
Complex polysaccharides have been reviewed with sections dealing with lipopolysaccharides, capsular polysaccharides, bacterial peptidoglycans, and yeast cell-wall polymers.[538]

A capsular polysaccharide produced by *Aerobacter aerogenes* was found to contain galactose, mannose, and glucuronic acid (2 : 1 : 1). Smith degradation indicated that all of the galactose residues are linked through C-3, all of the mannose residues are linked through both C-2 and C-3, and all of the glucuronic acid occurs at terminal, non-reducing positions. Digestion with a specific phage-induced polysaccharide depolymerase resulted in conversion into two limit oligosaccharides, termed *A* and *B*. Partial acidic hydrolysis, methylation, and Smith degradation indicated that *A* is the tetrasaccharide Gal-(1 → 3)-[GlcUA-(1 → 2)]-Man-(1 → 3)-Gal. Oligosaccharide *B* was characterized by Smith degradation and sequential enzymic degradation as an octasaccharide composed of two units of *A* connected by an α-(1 → 3)-linkage. The results indicated that the polysaccharide is composed of repeating units of the tetrasaccharide (62).[539]



(62)

Kinetic and sequence analyses showed that the capsular polysaccharide from *Klebsiella aerogenes* is made up of pentasaccharide repeating units. The repeating sequence most consistent with experimental data is (63).

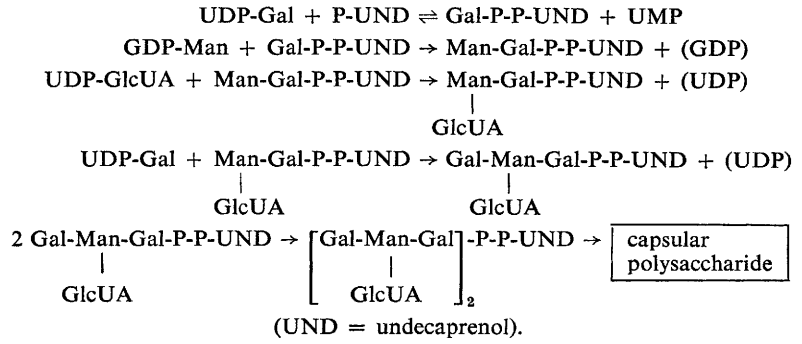


(63)

[536] D. E. Koeltzow and H. E. Conrad, *Biochemistry*,1971, **10**, 214.
[537] M. Eskenazy, *Nature*, 1970, **226**, 855.
[538] E. C. Heath, *Ann. Rev. Biochem.*, 1971, **40**, 29.
[539] E. C. Yurewicz, M. A. Ghalambor, and E. C. Heath, *J. Biol. Chem.*, 1971, **246**, 5596.

In addition, computer-assisted microanalytical procedures for polysaccharides and their derivatives were described. Two computer programs were presented for the processing of dual-label, liquid scintillation counting data and for the estimation of hydrolytic rate constants of individual glycosidic linkages.[540]

The mode of biosynthesis of the capsular polysaccharide from *A. aerogenes* was studied using a cell-envelope particulate fraction from a strain lacking UDP-D-galactose epimerase. Kinetic studies of D-[^{14}C]galactose transfer from UDP-D-[^{14}C]galactose to a lipid fraction and to the capsular polysaccharide suggested that a lipid-bound oligosaccharide is a precursor of the capsular polysaccharide. The lipid was identified as undecaprenyl phosphate. The particulate fraction catalysed the sequential transfer of mannose, glucuronic acid, and a second galactose unit to galactosyl-undecaprenol pyrophosphate to form di-, tri-, and tetrasaccharide derivatives, each of which was isolated and characterized, thus establishing the sequence illustrated in Scheme 4.[541] A correction to the postulated oligosaccharide sequence has been reported.[542]



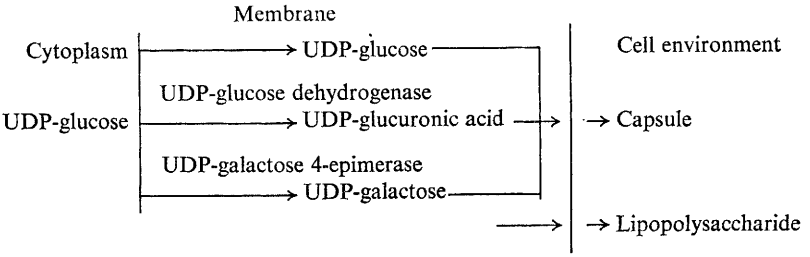
Scheme 4

Membrane preparations from *K. aerogenes* were shown to transfer D-glucose and D-galactose from their UDP-derivatives to a lipid and to an exopolysaccharide.[543] The ratio of sugars transferred was identical to the ratio of the sugars occurring in the native polysaccharide, with the transfer of D-galactose being dependent on prior glucosylation of the lipid. Significant amounts of D-glucuronic acid were incorporated *via* UDP-D-glucose but not by direct transfer from UDP-D-glucuronic acid. A model for the synthesis of the capsular polysaccharide was proposed (Scheme 5).

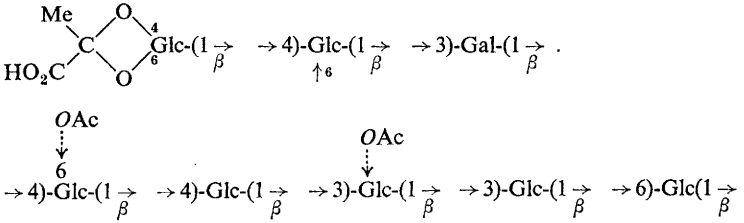
An extracellular, acidic polysaccharide produced by *Rhizobium meliloti* was found to consist of D-glucose, D-galactose, pyruvic acid, and *O*-acetyl

[540] M. P. Veniamin, *J. Chromatog. Sci.*, 1970, **8**, 173.
[541] F. A. Troy, F. E. Frerman, and E. C. Heath, *J. Biol. Chem.*, 1971, **246**, 118.
[542] F. A. Troy, F. E. Frerman, and E. C. Heath, *J. Biol. Chem.*, 1971, **246**, 1949.
[543] I. W. Sutherland and M. Norval, *Biochem. J.*, 1970, **120**, 567.

groups (7 : 1 : 1 : 1). Although it was not possible to establish the homogenity of the polysaccharide, methylation analysis of the native polysaccharide and of chemically modified materials revealed the structural features (64). No information on the mutual order of the sugar residues was obtained owing to the resistance of the molecule to enzymic attack.[544]



Scheme 5

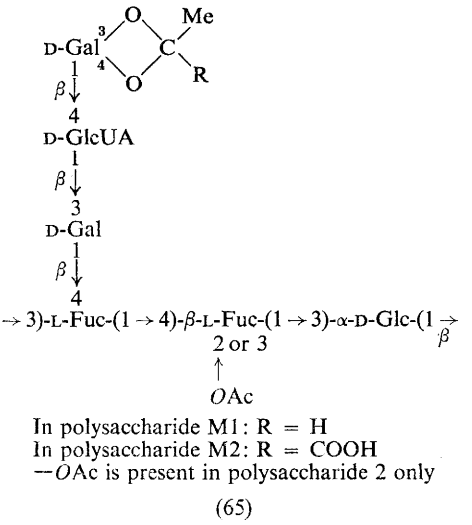


(64)

The structures of the extracellular polysaccharides (M-antigens) from two mucoid mutants (M1 and M2) of *Salmonella typhimurium* have been investigated by methylation of the original polysaccharides and of the polysaccharides obtained by mild acidic hydrolysis and by Smith degradation. It was concluded that the polysaccharides are composed of hexasaccharide repeating units. On the basis of these and previous studies, the complete structure (65) of the polysaccharides was proposed. The terminal β-D-galactopyranose residue in the repeating unit contains a 3,4-*O*-ethylidene group in the M1 polysaccharide and a 3,4-*O*-carboxyethylidene group in the M2 polysaccharide.[545] Structural studies have also been extended to an investigation of the M-antigens from three strains of *Escherichia coli* and *Aerobacter cloacae*. All the antigens seemed to have the same basic structure as the *Salmonella* M1- and M2-antigens, being composed of hexasaccharide repeating units. All but one (the *Salmonella* M1-antigen) were found to contain *O*-acetyl groups. Structural variation is provided

[544] H. Björndal, C. Erbing, B. Lindberg, G. Fåhraeus, and H. Ljunggren, *Acta Chem. Scand.*, 1971, **25**, 1281.

[545] P. J. Garegg, B. Lindberg, T. Onn, and T. Holme, *Acta Chem. Scand.*, 1971, **25**, 1185.



In polysaccharide M1: R = H
In polysaccharide M2: R = COOH
—*O*Ac is present in polysaccharide 2 only

(65)

by various alkylidene groups linked to the terminal D-galactopyranose residues.[546]

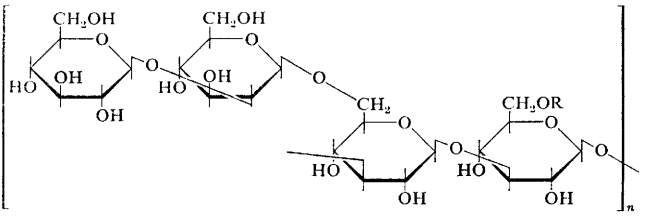
The isolation of oligosaccharides produced by the action of phage-induced enzymes on colanic acid has been reported. The products from colanic acid, produced by different strains of *Escherichia coli* and *Salmonella typhimurium*, were hexasaccharides differing in their acyl substituents. All contained fucose, glucose, galactose, and glucuronic acid, and appeared to have the same carbohydrate structure as that postulated for the repeating unit of colanic acid. All the hexasaccharides contained acetate and some pyruvate. The phage-induced enzymes only converted 30—35% of colanic acid into oligosaccharides, and the failure of the enzyme to degrade the residual molecule was attributed to the failure of a few fucose residues to become branch-points during biosynthesis of the polymer. These residues would not be distinguishable by chemical studies, but would alter the polymer sufficiently to arrest hydrolysis by an *exo*-enzyme, since it is necessary for a D-glucuronic acid residue to be present in the side-chain for the polysaccharide to be an effective substrate.[547]

Paper chromatographic separations of naturally acetylated, formylated, and pyruvylated oligosaccharides have been reported.[548]

Data presented on the composition of the capsular polysaccharide of *Pneumococcus* Type XXXVII indicate that it may be the simplest of all the pneumococcal polysaccharides examined. D-Glucose was the only sugar found on acidic hydrolysis of the molecule. Periodate oxidation indicated

[546] P. J. Garegg, B. Lindberg, T. Onn, and I. W. Sutherland, *Acta Chem. Scand.*, 1971, **25**, 2103.
[547] I. W. Sutherland, *European J. Biochem.*, 1971, **23**, 582.
[548] I. W. Sutherland, *J. Chromatog.*, 1971, **59**, 476.

that 31% of the molecule is resistant to attack owing to the presence of (1 → 3)-linked D-glucose units with branching at C-6. Identification of a disaccharide, obtained in low yield, as sophorose has led to the tentative suggestion of a structure (66) for the basic repeating unit.[549]



R = H or sophorose

(66)

The type-specific polysaccharide of *Pneumococcus* Type XIX has been isolated, purified, and shown to contain rhamnose, glucose, 2-acetamido-2-deoxyhexoses, and phosphate.[550] No evidence was found for the presence of galactose, pentose, anhydroribitol, or amino-acids (*cf.* J. Baddiley, J. G. Buchanan, and Z. A. Shavarova, *Biochim. Biophys. Acta*, 1962, **57**, 146). Chemical evidence suggested a minimum repeating unit for the polysaccharide containing L-rhamnose, D-galactose, and 2-acetamido-2-deoxy-D-mannose (2 : 1 : 1) linked by phosphate ester bonds to similar units.[551] From periodate oxidation and methylation studies, the repeating tetrasaccharide unit was depicted as either (67) or (68).[552]

β-L-Rha-(1 → 4)-α-D-Glc-(1 → 6)-β-D-Man*N*Ac-(1 → 2)-L-Rha

(67)

α-L-Rha-(1 → 6)-β-D-Man*N*Ac-(1 → 4)-β-D-Glc-(1 → 2)-L-Rha

(68)

Review articles have dealt with the immunological paralysis of mice with pneumococcal polysaccharide antigens[553] and with the immunity and tolerance to pneumococcal polysaccharides.[554]

Depolymerization of Type III pneumococcal polysaccharide occurred on heating aqueous solutions of the polymer, and this change was accompanied by a fall in immunogenicity, tolerogenicity, and antibody-neutralizing activity.[555]

The separation of cell-wall polysaccharides from capsular polysaccharides of the same and different pneumococcal strains has been reported. Both types

[549] J. C. Knecht, G. Schiffman, and R. Austrian, *J. Exp. Med.*, 1970, **132**, 475.
[550] T. Miyazaki, T. Yadomae, and J. K. N. Jones, *J. Biochem.*, 1970, **68**, 755.
[551] T. Miyazaki and T. Yadomae, *Carbohydrate Res.*, 1971, **16**, 153.
[552] T. Miyazaki and T. Yadomae, *Chem. and Pharm. Bull.* (*Japan*), 1970, **18**, 1249.
[553] W. J. Halliday, *Bacteriol. Rev.*, 1971, **35**, 267.
[554] G. W. Suskind, *Ann. New York Acad. Sci.*, 1971, **181**, 9.
[555] J. G. Howard, H. Zola, G. H. Christie, and B. M. Courtney, *Immunol.*, 1971, **21**, 535.

of polysaccharides were shown to be heterogeneous by immunological analyses, with the dissimilarities residing either in the mucopeptide portion of the molecule or in the region of its attachment to the teichoic acid.[556]

A dextran-like capsular polysaccharide has been isolated from the culture filtrates of *Streptococcus bovis*. Quantitative precipitin and inhibition studies suggested that isomaltose is a common feature of the selected strains. The immunological relationship existing among the various strains is dependent, in part, on the proportions of α-(1 → 6)- and α-(1 → 4)-linkages.[557]

Fatty-acid synthetase activity in *Mycobacterium phlei* has been reported to be regulated by polysaccharides. The multi-enzyme complex, which is able to catalyse the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA, required a heat-stable fraction for activity. The stimulating factor contains three polysaccharides, which exert their effect on fatty-acid synthetase by lowering the K_m for acetyl-CoA by about fifty-fold. One of the polysaccharides contains 3-*O*-methylmannose (95%) and mannose (5%), and the other two contain 6-*O*-methylglucose (55%) and glucose (45%).[558]

One of the principal immunochemical determinants of *Clostridium perfringens* was isolated under mild conditions from the intact highly encapsulated cells, and was shown to be an acidic polysaccharide composed of glucose, galactose, mannose, 2-amino-2-deoxyglucose, and glucuronic acid.[559]

An extracellular protease of a *Cytophaga* species was reported to be present as a complex with an acidic polysaccharide of the slime layer. The complex of basic protein (enzyme) and acidic polysaccharide indicated that the enzyme is not truly extracellular but is surface bound.[560]

The group A meningococcal polysaccharide was shown to be a homopolymer of 2-amino-2-deoxy-D-mannose phosphate, which is partially *N*- and *O*-acetylated. The principal glycosidic bond seemed to involve a (1 → 6)-phosphodiester.[561] Both groups B and C meningococcal polysaccharides have been shown to be polymers of sialic acid, but which differ both chemically and immunochemically. The C polysaccharide contains both *N*- and *O*-acetyl groups, but the B polysaccharide contains only *N*-acetyl groups.[562] Phagocytosis of Group A meningococci in the presence of certain Group A polysaccharide antisera was inhibited by 2-acetamido-2-deoxy-D-mannose, but not by 2-amino-2-deoxy-D-mannose, D-mannose,

[556] G. Schiffman, D. L. Bornstein, and R. Austrian, *J. Exp. Med.*, 1971, **134**, 600.
[557] J. Kane and W. W. Karakawa, *J. Immunol.*, 1971, **106**, 103.
[558] M. Ilton, A. W. Jevans, E. D. McCarthy, D. Vance, H. B. White, and K. Bloch, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1089.
[559] H. Baine and R. Cherniak, *Biochemistry*, 1971, **10**, 2948.
[560] J. Christison and S. M. Martin, *Canad. J. Microbiol.*, 1971, **17**, 1207.
[561] T.-Y. Liu, E. C. Gotschlich, E. K. Jonssen, and J. R. Wysocki, *J. Biol. Chem.*, 1971, **246**, 2849.
[562] T.-Y. Liu, E. C. Gotschlich, F. T. Dunne, and E. K. Jonnsen, *J. Biol. Chem.*, 1971, **246**, 4703.

2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, or neuraminic acid. Absorption studies showed that these polysaccharides are the major antiphagocytic determinants of Group A and Group C meningococci.[563]

E. coli K-235, an organism that synthesizes a polymer of *N*-acetylneuraminic acid called colominic acid, contains a particle-bound sialyltransferase that incorporates sialic acid into the polymer by transfer from cytidine-5-monophospho-*N*-acetylmuraminic acid. It was suggested that chain elongation proceeds at non-reducing termini of the polymer rather that at the reducing end, as in the case of bacterial lipopolysaccharides.[564]

Enzymes capable of degrading Vi antigen appeared to attack a Vi-protein complex rather than the free antigen. Immunoelectrophoresis demonstrated that de-*O*-acetylated Vi antigen did not form soluble complexes with albumin and did not protect albumin from precipitation with trichloroacetic acid, suggesting that the *O*-acetyl groups are required for formation of specific protein–Vi antigen complexes.[565] An enzyme preparation, containing pectate lyase and polygalacturonate lyase activities, made an eliminative attack on Vi antigen, releasing a series of uronic acid oligomers containing a double bond at C-4—C-5 of the non-reducing residue. The enzyme was also able to degrade de-*O*-acetylated Vi antigen, pectin, and polygalacturonic acid by an eliminative attack to give end-products analogous to those formed from Vi antigen.[566]

Partial acidic hydrolysis of the succinoglucan of *Alcaligenes faecalis* yielded cellobiose, gentiobiose, laminaribiose, 6-*O*-β-laminaribiosyl-D-glucose, 6-*O*-β-laminaritriosyl-D-glucose, and 3-*O*-β-cellobiosyl-D-galactose, thus indicating the presence of β-(1 → 3), β-(1 → 4)-, and β-(1 → 6)-linked D-glucose units, as well as β-(1 → 3)-linked D-galactose units.[567]

An acidic polysaccharide of *Clostridium welchii* was shown to resemble dermatan sulphate in containing equimolar proportions of sulphate, uronic acid, 2-amino-2-deoxyhexose, and *N*-acetyl groups with a β-(1 → 4)-linkage between 2-acetamido-2-deoxy-D-galactose and L-iduronic acid.[568]

The extracellular polysaccharides produced by two strains of *Arthrobacter simplex* grown on ethanediol contained glucose in one case and rhamnose, mannose, and glucose in the other.[569] A *Corynebacterium* species produced a homogeneous polysaccharide from propane-1,2-diol that is composed of rhamnose, mannose, glucose, and galactose.[570]

The specific substance from *Diplococcus pneumoniae* Type XXXI yielded L-rhamnose, D-galactose, and D-glucuronic acid. The polymer (*M* 120 000)

[563] R. B. Roberts, *J. Exp. Med.*, 1970, **131**, 499.
[564] F. D. Kundig, D. Aminoff, and S. Roseman, *J. Biol. Chem.*, 1971, **246**, 2543.
[565] L. A. McNicol and E. E. Baker, *Biochim. Biophys. Acta*, 1971, **229**, 233.
[566] L. A. McNicol and E. E. Baker, *Biochemistry*, 1971, **9**, 1017.
[567] H. Saito, A. Misaki, and T. Harada, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1683.
[568] G. K. Darby, A. S. Jones, J. F. Kennedy, and R. T. Walker, *J. Bacteriol.*, 1970, **103**, 159.
[569] S. Hagiwara and K. Yamada, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1283.
[570] S. Hagiwara and K. Yamada, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1402.

was very susceptible to acidic hydrolysis indicating that one or more components may occur in the furanose form.[571]

Hemophilus influenzae type b capsular polysaccharide was found to have $M > 200\,000$.[572]

A mannosyl transferase of *Cryptococcus laurentii* transferred D-mannosyl units from GDP-D-mannose to endogenous primer. Structural analysis of the product of enzymic action revealed that the D-mannosyl residues are newly linked to non-reducing ends of the enzyme-bound primer resulting in α-(1 → 2)- and α-(1 → 3)-mannosylmannose bonds.[573]

Kinetic studies on cellulose synthesis by *Acetobacter xylinum* indicated that a transfer reaction with D-glucose 1-phosphate and enzyme (E) produces a D-glucosyl-enzyme complex, which in turn reacts with the acceptor substrate (A), regenerating the enzyme in the following manner:[574]

$$\text{D-Glc-O-X(D-Glc-P)} + \text{E} \rightarrow \text{D-Glc-E} + \text{HOX}$$
$$\text{D-Glc-E} + \text{HO-A} \rightarrow \text{D-Glc-O-A} + \text{E}$$

Dextrans containing α-(1 → 3)-D-glucosidic linkages were more readily hydrolysed by animal dextran glucosidases than those with (1 → 2)-linkages or those containing more than 95% of (1 → 6)-linkages. Preliminary incubation of dextrans with concanavalin A inhibited subsequent hydrolysis by dextran glucosidase. Inhibition of hydrolysis by concanavalin A was greater with dextrans having a preponderance of (1 → 3)-bonds than with those having (1 → 2)- or (1 → 6)-bonds.[575]

The length of the side-chains of a dextran elaborated by *Leuconostoc mesenteroides* was studied by sequential degradation. Selective removal of terminal D-glucose residues was achieved by alkaline treatment of a methylated dextran in which hydroxymethyl groups had been replaced by *C-p*-tolylsulphonylmethyl groups, thus forming a new terminal unit in which the C-6 hydroxy-group is unsubstituted. The degraded material was subjected to a second degradation by using the same procedure. It was shown that 40% of all side-chains contain only one D-glucose unit, 45% are two units long, and the rest contain more than two units.[576]

A large proportion of gelatinous 'insoluble dextran' represents a complex between cell-bound dextransucrase and 'soluble dextran', which is released into the supernatant solution on completion of the synthesis.[577]

Results obtained from both periodate oxidation and methylation of a dextran produced by a cariogenic streptococcus, *Streptococcus mutans*, indicated that the molecule is highly branched and contains 69% of

[571] N. Roy, W. R. Carroll, and C. P. J. Glaudemans, *Carbohydrate Res.*, 1970, **12**, 89.
[572] L. P. Rodrigues, R. Schneerson, and J. B. Robbins, *J. Immunol.*, 1971, **107**, 1071.
[573] H. Ankel, E. Ankel, J. S. Schutzbach, and J. C. Garancis, *J. Biol. Chem.*, 1970, **245**, 3945.
[574] R. St.J. Manley, J. W. Jonker, D. Cooper, and T. C. Pound, *Nature New Biol.*, 1971, **229**, 88.
[575] M. E. Preobrazhenskaya and E. L. Rosenfeld, *Biokhimiya*, 1970, **35**, 735.
[576] O. Larm, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1971, **20**, 39.
[577] E. E. Smith, *F.E.B.S. Letters*, 1970, **12**, 33.

(1 → 6)-, 18% of (1 → 3)-, and 13% of (1 → 2)- or (1 → 4)-linkages.[578] The dextrans elaborated by two Streptococci appeared to contain a high proportion of D-glucosidic linkages other than α-(1 → 6)-linkages.[579]

In physicochemical studies on oligodextrans, the intrinsic viscosity–molecular weight relationship has been described.[580]

Polyaldehydes produced on periodate oxidation of carbohydrates have been coupled to the side-chain amino-groups of proteins. Oxidized dextran has been coupled to anti-red-cell antibody, and the method has been used for coating red cells with protein or polysaccharide.[581]

Glutaraldehyde-insolubilized concanavalin A showed the same specificity as concanavalin A in solution for binding certain dextrans, glycogen, or glycoproteins.[582]

Further studies on the structure of pullulan indicated that 7% of the polysaccharide occurs as maltotetraose units, the majority of which are linked through terminal D-glucose units by α-(1 → 6)-bonds.[583]

Conformational studies have shown that cycloamyloses containing fewer than six D-glucose residues could not be cyclized because of steric overlaps. Cyclohepta- and cyclo-octa-amyloses are stabilized by formation of intramolecular hydrogen bonds and, of the three possible cyclic compounds, cyclohexa-amylose has the lowest energy.[584] It was possible to predict the molecular rotation of cyclohexa-amylose in DMSO from *X*-ray and optical data.[585]

Systems have been developed for assessing the purity of individual cyclodextrins and for monitoring the appearance of these molecules during enzymolysis of starch.[586]

The stereospecific inclusion of the (−)-enantiomer of isopropylmethylphosphinate (I) into cyclohepta-amylose afforded (−)-I and (+)-I with optical purities of 66% and 17%, respectively.[587] Crystal structures of cyclodextrin complexes with several organic compounds were investigated by *X*-ray methods. Changes in the crystal structures caused by dehydration seemed to result from changes in packing arrangements of circular cylinders that were made by coaxial alignment of the dextrin molecules.[588] Different molecules enclosed within the void of the dextrins caused large changes in the diffraction patterns of the complexes. The crystal structure of the complexes could be accounted for by a closest packing of channel cylinders that resulted from coaxial alignments of the dextrin molecules.[589]

[578] W. J. Lewicki, L. W. Long, and J. R. Edwards, *Carbohydrate Res.*, 1971, **17**, 175.
[579] R. L. Sidebotham and H. Weigel, *Carbohydrate Res.*, 1971, **19**, 151.
[580] K. Gekko, *Makromol. Chem.*, 1971, **148**, 229.
[581] C. J. Sanderson and D. V. Wilson, *Immunochem.*, 1971, **8**, 163.
[582] E. H. Donnelly and I. J. Goldstein, *Biochem. J.*, 1970, **118**, 679.
[583] B. J. Catley and W. J. Whelan, *Arch. Biochem. Biophys.*, 1971, **143**, 138.
[584] P. R. Sundararajan and V. S. R. Rao, *Carbohydrate Res.*, 1970, **13**, 351.
[585] D. A. Rees, *J. Chem. Soc.* (*B*), 1970, 877.
[586] K. Takeo, Y. Kondo, and T. Kuge, *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 954.
[587] H. P. Benschop and G. R. Van den Berg, *Chem. Comm.*, 1970, 1431.
[588] K. Takeo and T. Kuge, *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 568.
[589] K. Takeo and T. Kuge, *Agric. and Biol. Chem.* (*Japan*), 1970, **34**, 1787.

Cyclodextrins reacted with 4(5)-chloromethyliminidazoles, which accelerated the hydrolysis of *p*-nitrophenylacetate by about 300-fold.[590]

Increasing temperatures led to an increasing partition coefficient in dextrans used in gel chromatography, reflecting a greater activity of solute in the gel phase when there are weaker gel–solvent interactions. Similarly, it was found that the better the solvent, the more is the partition coefficient changed in favour of the mobile solvent phase.[591] The separation of cellodextrins (up to cellohexaose) on polyacrylamide and dextran gels showed that the interaction between gel and solute increases with molecular weight, the effect being more pronounced with dextran gels.[592] The separation of substances of high molecular weight is improved with dextran gels that exhibit shrinking at elevated temperatures.[593] Results from the measurement of the partition coefficients of bovine serum albumin, dextran, and polyethylene glycol on dextran gels have been described by means of a simple thermodynamic treatment that makes use of virial coefficients of the gel and of the solute, and also of a coefficient that expresses their interaction. This coefficient is related to the exclusion volume of the gel for the solutes.[594]

The separation of bacterial polysaccharides and nucleic acids by column chromatography on hydroxyapatite has been shown to depend on the phosphate concentration of these substances, the degree of denaturation, and the presence or absence of phospholipids in the bacterial extracts.[595]

Fungal Polysaccharides

The distribution of the two known pathways for the biosynthesis of lysine could be correlated with allosteric controls of enzymes and the dichotomy of cellulose and chitin in fungi.[596]

The hyphal-wall compositions of six fungi of the genus *Leptosphaeria* were compared to assess whether gross changes had occurred in the composition of hyphal walls of closely related fungi that had become ecologically restricted to marine or terrestrial habitats. Qualitatively similar patterns were obtained with quantitative differences from species to species.[597]

Studies on walls synthesized by *Candida utilis* protoplasts showed that some modifications occur in the structural polysaccharides of the wall, which could be correlated with morphological changes in the reversion process. Spherical protoplasts, composed of chitin (15%), glucan (45%), and protein (20%), were converted into a tubular form, which later changed

[590] F. Cramer and G. Mackensen, *Chem. Ber.*, 1970, **103**, 2138.
[591] W. Brown, *J. Chromatog.*, 1971, **59**, 335.
[592] W. Brown, *J. Chromatog.*, 1970, **52**, 273.
[593] K. Lampert and H. Determann, *J. Chromatog.*, 1971, **56**, 140.
[594] A. G. Ogston and P. Silpanata, *Biochem. J.*, 1970, **116**, 171.
[595] G. Vidal, *J. Chromatog.*, 1971, **59**, 71.
[596] H. B. LéJohn, *Nature*, 1971, **231**, 164.
[597] P. J. Szaniszlo and R. Mitchell, *J. Bacteriol.*, 1971, **106**, 640.

to an ellipsoidal yeast composed of glucan (45%), mannan (25%), and protein (8%).[598]

Cell-wall synthesis in yeast protoplasts has been reviewed. The chemical analysis, kinetics of protoplast formation, electron microscopic, and physical properties were discussed; other sections dealt with cell-wall synthesis, cell-wall regeneration of species other than yeast genera, cellular morphogenesis, and cell-wall reproduction.[599]

The isolation, purification, and structure of polysaccharides from Eumycetes have been reviewed.[600]

Cell-wall compositions of various species of yeasts have been compared. In *Oospora suaveolens*, the mannan content is directly related to the age of the culture. *Torulopsis aeris* was shown to contain glucose and mannose, whereas *O. suaveolens* and *Gestrichum lactis* contain glucose, galactose, and mannose.[601]

The assessment of i.r. spectra as indicators of fungal cell-wall composition has been reported, allowing the method to be used for following the effect of chemical treatments designed to separate major wall components.[602] Chitin and cellulose were reported to be present in the cell walls of *Ceratocystis* on the basis of i.r. spectroscopy.[603]

The ultrastructural architecture of the walls of some hyphal fungi have been studied by electron microscopy. From the results of sequential treatment of the hyphae with proteolytic enzymes, cellulase, laminarinase, and chitinase, a coaxial distribution of certain wall polymers was inferred. Estimates of the thickness of each coaxial region were obtained from measurements of sections of walls that were subjected to identical enzymic treatments.[604] Treatment of the cell walls of *Schizophyllum commune* with chitinase and R-glucanase [a β-(1 $\rightarrow$ 6)-glucanase] resulted in dissolution of the hyphal septa, although the walls retained their structural integrity.[605]

Thermally induced changes in the cell-wall polysaccharides of *Blastomyces dermatitidis* produced a yeast-like form, composed of 95% of an α-glucan and 5% of a β-glucan, and a mycelial form, composed of 60% of an α-glucan and 40% of a β-glucan.[606]

The metabolic stability of carbohydrates in the walls of the hyphae of *Aspergillus clavatus* was investigated by using measurements of mycelial isotope distribution from D-[U-^{14}C]glucose and the ^{3}H to ^{14}C ratios of re-isolated monosaccharides when the organism was grown on labelled D-glucose as the sole carbon source. The results suggested that D-glucose

[598] M. Novaes-Ledieu and C. Garcia-Mendoza, *J. Gen. Microbiol.*, 1970, **61**, 335.
[599] C. Nečas, *Bacteriol. Rev.*, 1971, **35**, 149.
[600] T. Miyazaki, *Tampakushitsu Kakusan Koso*, 1970, **15**, 730.
[601] J. P. G. Ballesta and J. R. Villanueva, *Trans. Brit. Mycol. Soc.*, 1971, **56**, 403.
[602] A. J. Michell and G. Scurfield, *Austral. J. Biol. Sci.*, 1970, **23**, 345.
[603] A. J. Michell and G. Scurfield, *Trans. Brit. Mycol. Soc.*, 1970, **55**, 488.
[604] D. Hunsley and J. H. Burnett, *J. Gen. Microbiol.*, 1970, **62**, 203.
[605] F. A. Janzen and J. G. H. Wessels, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1970, **36**, 255.
[606] F. Kanetsuna and L. M. Carbonell, *J. Bacteriol.*, 1971, **106**, 946.

and 2-amino-2-deoxy-D-glucose become metabolically inert once they have been incorporated into wall polymers, and that the biosynthesis of 2-amino-2-deoxy-D-glucose and its incorporation into the cell wall are direct processes not subject to metabolic randomization at the precursor level.[607]

D-Glucose was the major neutral sugar found in the cell wall of *Cochliobolus miyabeanus*; 2-amino-2-deoxy-D-glucose (38%) and peptides were also present.[608] Methylation analysis and other evidence pointed to the existence of a β-(1 → 3)-glucan having branched units linked at C-6.[609]

Chitin and a β-linked glucan were identified as the major components of the cell walls of *Aspergillus nidulans*. Other monomeric residues to be identified were galactose, mannose, glucuronic acid, and 2-amino-2-deoxygalactose. The β-glucan contains (1 → 3)- and (1 → 6)-linkages. An α-glucan was also identified as a cell-wall component, but was distinguishable from nigeran by i.r. spectroscopy and by its low susceptibility to hydrolysis by an *endo*-α-(1 → 3) : α-(1 → 4)-glucan glucanohydrolase. Melanin was found to be distributed throughout the cell, but was associated particularly with the chitin fraction.[610] The presence of melanin is responsible for the resistance to lytic enzymes.[611]

The monosaccharide and chitin contents of the cell walls in both the yeast and mycelial phase cultures of *Histoplasma capsulatum* and *Blastomyces dermatitidis* have been recorded.[612]

The cell wall of *Piricularia oryzae* was shown to contain D-glucose, 2-amino-2-deoxy-D-glucose, and protein as well as small amounts of D-mannose and D-galactose. The three neutral sugars form part of a heteropolysaccharide complex, probably having α-glycosidic linkages. The isolation of 3-*O*-β-D-gentiobiosyl-D-glucose implied that the β-glucan is composed of β-(1 → 3)-linkages with β-(1 → 6)-branch points.[613]

The similarity in composition of the extracellular polysaccharide and cell-wall polysaccharide of some *Candida* species suggested that both are synthesized by the same enzyme system.[614]

The isolated cell walls of *Trichophyton mentagrophytes* contained glucose, mannose, and 2-amino-2-deoxy-D-glucose after density-gradient centrifugation and proteolytic digestion of disrupted cells. Ultrastructural investigations indicated a marked collapse of the lamellar structure of the walls.[615]

[607] D. L. Corina and K. A. Munday, *J. Gen. Microbiol.*, 1971, **65**, 253.
[608] H. Nanba and H. Kuroda, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 252.
[609] H. Nanba and H. Kuroda, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 448.
[610] A. T. Bull, *J. Gen. Microbiol.*, 1970, **63**, 75.
[611] A. T. Bull, *Arch. Biochem. Biophys.*, 1970, **137**, 345.
[612] J. E. Domer, *J. Bacteriol.*, 1971, **107**, 870.
[613] T. Nakajima, K. Tamari, K. Matsuda, H. Tanaka, and N. Ogasawara, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 553.
[614] L. Do Carmo-Sousa and C. Barroso-Lopes, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1970, **36**, 209.
[615] T. Noguchi, Y. Kitazima, Y. Nozawa, and Y. Ito, *Arch. Biochem. Biophys.*, 1971, **146**, 506.

Re-examination of the structure of pachyman, the cell-wall β-(1 → 3)-glucan of *Poria cocos*, has demonstrated the presence of β-(1 → 6)-linkages. Smith degradation furnished a linear glucan, which exhibited antitumour activity not present in the original polysaccharide.[616] Carboxymethylpachyman, labelled at the carboxymethyl residue with ^{14}C, has been prepared as a water-soluble polysaccharide with strong antitumour activity so that its distribution at cellular and subcellular levels could be investigated.[617] Pachyman accounted for about 90% of the cell wall of *P. cocos*; it contains about seven hundred (1 → 3)-linked β-D-glucose residues, with approximately three to six branch points (possibly at C-2), together with a few (1 → 6)-linked internal residues, which appear to be branched at C-4.[618]

An extracellular, stable, viscous glucan is normally produced *in vitro* by *Claviceps fusiformis*. A new strain of the fungus autolysed this glucan owing to the presence of constitutive β-(1 → 3)-glucanase and β-glucosidase systems, which could be detected as soon as the fungal hyphae differentiated to a sclerotial form.[619]

The cell walls of the yeast form of *Verticillium albo-atrum* were shown to contain an alkali-insoluble glucan having β-(1 → 3)- and β-(1 → 4)-linkages. Cellulose was considered to be absent since enzymic attack liberated β-(1 → 3)-linked oligomers as well as cellobiose. An alkali-soluble β-(1 → 6)-glucan was extracted from the walls, leaving an insoluble microfibrillar network of chitin.[620]

The gross chemical composition of material extracted from the cell walls of *Saccharomyces cerevisiae* was studied and attempts were made to locate these materials *in situ* by comparing electron micrographs of stained and sectioned walls with those of the residues of various extraction procedures. Two chemically distinct species of polymers were extracted with strong alkali: one was a mannan–protein complex and the other was a glucomannan–protein complex. The alkali-insoluble material also contained glucomannan and protein. It was shown that none of these polymers constitutes a physically distinct layer in the yeast cell wall. The results did not support the classical view that the yeast cell wall is made up of a network of fibres of a β-(1 → 3)- and β-(1 → 6)-linked glucan. Although the basic structural polymer is a glucan, it was suggested that 2-amino-2-deoxy-D-glucose is an integral part of the completed polysaccharide and could act as a handle for the attachment of protein or peptide moieties to both glucan and mannan components.[621]

[616] G. Chihara, J. Hamuro, Y. Maeda, Y. Arai, and F. Fukuoka, *Nature*, 1970, **225**, 943.
[617] J. Hamuro, Y. Yamashita, Y. Ohsaka, Y. Y. Maeda, and G. Chigara, *Nature*, 1971, **233**, 486.
[618] G. C. Hoffmann, B. W. Simson, and T. E. Timell, *Carbohydrate Res.*, 1971, **20**, 185.
[619] A. G. Dickerson, P. G. Mantle, and C. A. Szczyrbak, *J. Gen. Microbiol.*, 1970, **60**, 403.
[620] M. C. Wang and S. Bartnicki-Garcia, *J. Gen. Microbiol.*, 1970, **64**, 41.
[621] J. K. Bowded and B. Hodgson, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1970, **36**, 81.

A cytoplasmic glucan was found to act as reserve material for the growth of *Phytophthora cinnamomi*. Methylation, periodate oxidation, and enzymic studies indicated that the soluble glucan is composed of mainly β-(1 → 3)-linked residues with branches at C-3 and C-6.[622]

An extracellular glucan of *Pythium debaryanum* was reported to be a highly branched β-glucan containing both (1 → 3)- and (1 → 6)-glucosidic linkages.[623]

β-Glucans isolated from the Polyporaceae, *Gunoderma applanatum* and *Phellinus linteus*, showed marked antitumour activity against transplanted sarcoma 180 in mice.[624]

An examination has been made of an extracellular glucan produced by *Schizophyllum commune*. Enzymolysis with an *endo*-β-(1 → 3)-glucanase liberated 3-*O*-β-gentiobiosyl-D-glucose, whereas an *exo*-β-glucanase released two molar proportions of D-glucose and one of gentiobiose. It was concluded that the repeating unit of the polysaccharide is composed of three β-(1 → 3)-linked D-glucopyranose units to which is attached a single β-(1 → 6)-D-glucose unit.[625]

Two water-soluble polysaccharides were isolated from the fruit bodies of *Lentinus edodes*. One of the polysaccharides, obtained by extraction with trichloroacetic acid, was an α-glucan of the glycogen type with an average chain-length of about six D-glucose residues. The other polysaccharide, isolated by extraction with hot water, was a β-glucan. From the results of methylation analysis, periodate oxidation, and acetolysis, it was concluded that this polysaccharide consists of β-D-glucopyranose units with 66% having (1 → 3)-linkages and the remainder having (1 → 4)-linkages.[626]

Amylose-type polysaccharides were isolated from *Hericium ramosum* and *H. coralloides*. The results of iodine staining and β-amylolysis indicated that these polysaccharides differ from the amyloses of higher plants in consisting of short chains of 32—45 D-glucose units.[627]

A glucan having a high positive rotation was extracted, together with a galactomannan-peptide, from *Aspergillus fumigatus*. The polysaccharide appeared to be a highly branched α-(1 → 4)-glucan with branching at C-6, and did not show any immunological activity in Arthus and delayed skin reactions in sensitized rabbits and guinea-pigs, as did the galactomannan-peptide.[628]

[622] L. P. T. M. Zevenhuizen and S. Bartnicki-Garcia, *J. Gen. Microbiol.*, 1970, **61**, 183.
[623] T. Miyazaki and M. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 813.
[624] T. Sasaki, Y. Arai, T. Ikekawa, G. Chihara, and F. Fukuoka, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 821.
[625] S. Kikumoto, T. Miyajima, K. Kimura, S. Okubo, and N. Komatsu, *J. Agric. Chem. Soc. (Japan)*, 1971, **45**, 162.
[626] M. Shida, T. Mase, Y. Sasakawa, and K. Matsuda, *J. Agric. Chem. Soc. (Japan)*, 1971, **45**, 454.
[627] D. A. McCracken and J. L. Dodd, *Science*, 1971, **174**, 419.
[628] I. Azuma, H. Kimura, F. Hirao, E. T. Subura, Y. Yamamura, and A. Misaki, *Jap. J. Microbiol.*, 1971, **15**, 237.

An enzymic method for the large-scale preparation of nigerose and nigeran oligosaccharides has been reported. By using an α-(1 → 4)-glucanase (mycodextranase) in 40% DMSO, oligosaccharides were isolated that were shown to contain an even number of D-glucose units, to differ in DP by two units, and to have α-(1 → 3)-linkages at the reducing and non-reducing ends.[629]

A linear α-glucan has been isolated from *Tremella mesenterica* that resembles the neutral glucans obtained from *Pullularia* species. Maltose, isomaltose, panose, and isomaltotriose were identified in partial acidic hydrolysates. Together with results of periodate oxidation, it was concluded that the polysaccharide is linear and contains two hundred α-D-glucopyranose units, 66% of which are linked (1 → 6) and the remainder (1 → 4).[630]

Three species of lichens belonging to Umbilicaria contain partially *O*-acetylated pustulan in a water-soluble form. The purified polymers showed antitumour activity against implanted sarcoma 180 in mice.[631] The lichen *Parmelia caperata* was shown to contain a linear glucan with α-(1 → 3)- and α-(1 → 4)-linkages in equal proportions; blocks of adjacent (1 → 3)-D-glucose residues are probably absent.[632]

Aqueous extracts of the dried stroma from *Cyttaria harioti* contained D-glucose and D-*arabino*-hexulosonic acid. The results of methylation and partial acidic hydrolysis indicated the presence of both α-(1 → 3)- and α-(1 → 6)-linked D-glucopyranose units in a branched structure.[633]

Two similar glucuronoglucans were isolated from the fruit bodies of *Polyporus formentarius* and *P. igniarius*. It appeared that both polysaccharides contain a backbone of branched β-D-glucan in which D-glucose residues are connected by (1 → 3)- and (1 → 6)-linkages. Chains containing an average of four or five β-(1 → 4)-linked D-glucopyranosyluronic acid units are linked to the 3-position of some D-glucose residues in the backbone. Since the glucuronoglucan from either fungus could be separated into fractions having different uronic acid contents, a precise structure of the backbone could not be deduced.[634]

The cell wall of *Fusicoccum amygdali* yielded chitin and a β-glucan having both (1 → 3)- and (1 → 6)-D-glucopyranose linkages. Cell walls were stained by iodine, and were attacked by α-amylase with the liberation of D-glucose, maltose, and maltotriose, indicating the existence of chains of α-(1 → 4)-linked D-glucopyranose residues. About 30% of the cell wall was resistant to the action of hydrolytic enzymes and the resistant fraction was

[629] K. K. Tung and J. H. Nordin, *Analyt. Biochem.*, 1970, **38**, 164.
[630] C. G. Fraser and H. J. Jennings, *Canad. J. Chem.*, 1971, **49**, 1804.
[631] Y. Nishikawa, M. Tanaka, S. Shibata, and F. Fukuoka, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1431.
[632] T. Takeda, Y. Nishikawa, and S. Shibata, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1074.
[633] A. F. Cirelli and R. M. de Lederkremer. *Chem. Ind.*, 1971, 1139.
[634] H. Björndal and B. Lindberg, *Carbohydrate Res.*, 1970, **12**, 29.

shown to be predominantly a α-(1 → 3)-glucan.[635] A fragment isolated from the cell walls underwent attack by both α-amylase and *exo*-β-(1 → 3)-glucanase, providing evidence that it contains both α-(1 → 4)- and β-(1 → 3)-D-glucose linkages. Information from periodate oxidation, methylation, i.r. spectroscopy, and partial acidic hydrolysis showed the enzyme-resistant core to consist of linear chains of α-(1 → 3)-D-glucose units having blocks of one or two α-(1 → 4)-D-glucose units interspersed at intervals along the main chain. Enzymic digestion with *exo*- and *endo*-β-(1 → 3)-glucanases, periodate oxidation, and methylation analysis indicated that the β-glucan fraction is attached to a branched galactomannorhamnan core.[636]

A phosphomannanase has been purified to apparent homogeneity, but was shown to contain laminarinase activity, which was not active on yeast glucan. The enzyme brought about major changes in the yeast cell wall by releasing material from the outer surface of the wall and leaving a loose fibrillar structure that probably represented the remaining glucan. Electron microscopy demonstrated that the mannan is on the outer surface of the cell wall.[637]

Four purified mannans from *Candida albicans*, a mannan from *Saccharomyces cerevisiae*, and eight polysaccharide–protein complexes from *C. albicans* were tested for interferon-stimulating activity in explanted mouse peritoneal leukocytes. The purified mannans, as well as one of the polysaccharide–protein complexes from *C. albicans*, were able to stimulate release of interferon. The mannan from *S. cerevisiae* and glucan–protein complexes were inactive.[638] The immunodominant side-chain of *Saccharomyces cerevisiae* mannan is a tetrasaccharide Man*p*-(1 → 3)-Man*p*-(1 → 2)-Man*p*, since this oligosaccharide was the most effective inhibitor of the homologous precipitin reaction. Although *S. lactis* mannan contains this side-chain, it also contains a pentasaccharide of undetermined structure, which appears to be the immunodominant group in the mannan. Antiserum against *S. lactis* mannan contained at least two antibody specificities, one against the tetrasaccharide side-chain and the other against the pentasaccharide side-chain.[639] In order to provide further information on the chemical nature of the antigenic determinants of the *S. cerevisiae* mannan, the polymer was digested by an *Arthrobacter* α-mannosidase to produce a series of partially degraded mannans. Quantitative precipitin reactions indicated that the tetrasaccharide plays an important role as the antigenic determinant, whereas the core mannan is not responsible for antigenic activity, but functions merely as the 'carrier' of antigenic determinants.[640]

[635] K. W. Buck and M. A. Obaidah, *Biochem. J.*, 1971, **125**, 461.
[636] M. A. Obaidah and K. W. Buck, *Biochem. J.*, 1971, **125**, 473.
[637] W. L. McLellan, L. E. McDaniel, and J. O. Lampen, *J. Bacteriol.*, 1970, **102**, 261.
[638] V. Lackovic, L. Borecky, D. Sikl, L. Masler, and S. Bauer, *Proc. Soc. Exp. Biol. Med.*, 1970, **134**, 874.
[639] C. E. Ballou, *J. Biol. Chem.*, 1970, **245**, 1197.
[640] H. Sunayama and S. Suzuki, *Jap. J. Microbiol.*, 1970, **14**, 197.

Oligosaccharides, obtained by acetolysis of cell-wall mannans of a diploid strain of *Saccharomyces cerevisiae* and its two haploid mating types, have been separated by gel filtration and their ratios have been compared. Although the haploid forms gave less of the larger fragments than the diploid form, no dramatic differences were observed that might be related to a process of recognition between the two mating types.[641]

Determination of cell-wall polysaccharides during the course of synchronous proliferation of large cells of baker's yeast revealed that the mannan is synthesized linearly during cell-division cycles, whereas glucan synthesis proceeds intermittently.[642]

After addition of cycloheximide, the formation of acceptor polypeptides for the biosynthesis of yeast mannan was prevented, but glycosylation of acceptors present was completed and these molecules were incorporated into the cell wall. Glycosylation was then arrested for lack of acceptors and GDP-D-mannose accumulated, since its synthesis was not inhibited.[643]

The biosynthesis of polysaccharides by the cells of *Candida tropicalis* was studied by using labelled sodium acetate as the sole carbon source. Labelled, acetylated polysaccharide was obtained from the mannan in almost quantitative yield.[644]

A particle-bound enzyme preparation from *Cryptococcus laurentii* mediated the transfer of D-mannosyl units from GDP-D-mannose to endogenous primer. A polysaccharide fraction associated with the enzyme has been found to be similar to a polysaccharide isolated from intact cells and from cell-wall preparations. Both polysaccharides yielded mannose, galactose, xylose, arabinose, and glucose. The radioactive reaction product from GDP-D-[^{14}C]mannose remained particle bound and acidic hydrolysis indicated that D-mannose was the only ^{14}[C]-monomer present. Acetolysis of the enzymic product revealed that D-mannosyl residues were newly linked to non-reducing ends of the enzyme-bound primer, resulting in α-(1 $\rightarrow$ 2)- and α-(1 $\rightarrow$ 3)-mannosylmannose bonds. Comparison of electron micrographs of whole cell or cell-wall preparations, before and after extraction, suggested that a polymer similar to the enzymic product resides in the cell wall.[645] The transfer of D-mannosyl units from GDP-D-mannose to mono- and oligo-saccharide acceptors is catalysed by four different enzymes, which are required for the formation of four distinct D-mannosyl linkages, namely mannosyl-α-(1 $\rightarrow$ 2)-mannosyl, mannosyl-α-(1 $\rightarrow$ 6)-mannosyl, mannosyl-α-(1 $\rightarrow$ 3)-mannosyl, and mannosylxylosyl linkages. The occurrence of at least two, and possibly all four, of these linkages in the heteropolysaccharide of the cell wall of *C. laurentii* suggested the involvement of a number of enzymes in cell-wall biosynthesis.[646]

[641] T. R. Thieme and C. E. Ballou, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 621.
[642] M. Hayashibe, N. Sando, and M. Osumi, *J. Gen. Appl. Microbiol.*, 1970, **16**, 171.
[643] R. Sentandreu and J. O. Lampen, *F.E.B.S. Letters*, 1970, **11**, 95.
[644] N. P. Elinov, I. P. Sokolova, and G. E. Arkadyeva, *Mikrobiologiya*, 1970, **39**, 399.
[645] H. Ankel, E. Ankel, J. S. Schutzbach, and J. C. Garancis, *J. Biol. Chem.*, 1970, **245**, 3945.
[646] J. S. Schutzbach and H. Ankel, *J. Biol. Chem.*, 1971, **246**, 2187.

Particulate fractions were used as the enzyme source and GDP-D-mannose as the substrate source for the synthesis of the phosphomannan of *Hansenula capsulata*. Both D-mannosyl and phosphoryl residues appeared to be derived from GDP-D-mannose and the product was similar to the extracellular polysaccharide.[647]

Incorporation of 2-deoxy-D-*arabino*-hexose into the cell wall of *Saccharomyces cerevisiae* produced osmotically fragile cells with a lower mannan content.[648]

Changes in yeast cell-wall polysaccharides of biotin-deficient cells indicated that, in general, the total amounts of glucan and mannan show a parallelism with the thickness of the cell wall.[649] Growth of *S. cerevisiae* in a medium supplemented by methionine reduced the mannan content of the cell wall by 50%.[650]

Omission of inorganic phosphates from the culture media led to the production of altered phosphohexosans or neutral extracellular mannans by yeasts that otherwise elaborated phosphogalactans and phosphomannans. This demonstrated the potential for production of extracellular neutral hexosans by cultural manipulation of yeasts previously shown to elaborate phosphorylated polysaccharides.[651]

The phytohemagglutinin from the lentil *Lens culinaris* reacted with the phosphomannan from *Pichia pinus* in a precipitin reaction that was inhibited by such simple sugars as D-glucose, D-mannose, and their glycosides.[652] The ability of pea phytohemagglutinin to precipitate yeast mannan was abolished on removal of metal ions, but was stimulated in the presence of Mn^{2+} and Ca^{2+}.[653]

The sequence of linkages of the main-chain polysaccharide of the mannan from a *Candida* species was determined after successive periodate oxidation, borohydride reduction, and acetolysis. A 'block-type' of structure was indicated involving up to four consecutive α-(1 → 3)- and α-(1 → 6)-D-mannopyranose units.[654]

Further studies have used n.m.r. spectroscopy as an aid to the classification of yeast mannans of the genera *Candida*,[655] *Torulopsis*,[656] *Debaromyces*,

[647] R. M. Mayer, *Biochim. Biophys. Acta*, 1971, **252**, 39.
[648] P. Biely, Z. Krátký, J. Kovařík, and S. Bauer, *J. Bacteriol.*, 1971, **107**, 1211.
[649] T. Mizunaga, H. Kuraishi, K. Aida, and T. Uemura, *J. Gen. and Appl. Microbiol.*, 1971 **17**, 85.
[650] K. A. Killick, *J. Bacteriol.*, 1971, **106**, 931.
[651] M. Slodki, M. J. Safransky, D. E. Hensley, and G. E. Babcock, *Appl. Microbiol.*, 1970, **19**, 1019.
[652] N. M. Young, M. A. Leon, T. Takahashi, I. K. Howard, and H. J. Sage, *J. Biol. Chem.*, 1971, **246**, 1596.
[653] M. Paulová, G. Entlicher, M. Tichá, J. V. Koštíř, and J. Kocourek, *Biochim. Biophys. Acta*, 1971, **237**, 513.
[654] P. A. J. Gorin and J. F. T. Spencer, *Canad. J. Chem.*, 1970, **48**, 198.
[655] J. F. T. Spencer and P. A. J. Gorin, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1971, **37**, 75.
[656] J. F. T. Spencer and P. A. J. Gorin, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1970, **36**, 509.

and *Metschnkowia*.[657] The relationship of the n.m.r. spectra of mannans of some species of *Hansenula* to their phylogenetic significance has been reported.[658]

Cells of *Pichia bovis* and *Saccharomyces phaseolosporus* have been extracted with alkali and the solubilized polysaccharides purified by copper complexing. The polymers were shown to be monodisperse in aqueous solutions, giving single peaks on ultracentrifugation, and to contain predominantly D-mannose as well as 2-acetamido-2-deoxy-D-glucose and 6% protein. Methylation analysis indicated that the amino-sugar occurs as non-reducing end-groups on the polysaccharide chain. The location of the amino-sugar thus differs from that in the mannan–protein complex in which 2-acetamido-2-deoxy-D-glucose residues serve as a bridge between mannan and protein.[659] Methylation analysis of baker's yeast mannan showed the presence of a small proportion of 2-deoxy-3,6-di-*O*-methyl-2-methylamino-D-glucose. This sugar possibly arose from one or more amino-sugars in the form of 4-*O*-substituted 2-acetamido-(1-L-β-aspartamido)-1,2-dideoxy-β-D-glucose units, which probably form the bridge in one of the carbohydrate–peptide linkages in the cell-wall glycoprotein.[660]

A polysaccharide from *Sporothrix schenckii* was purified by precipitation of the borate complex with hexadecyltrimethylammonium bromide and by ion-exchange chromatography; it was found to contain D-mannose (50%) and L-rhamnose (33%). The polymer was almost entirely precipitated by concanavalin A without change in the D-mannose to L-rhamnose ratio, showing the sugars to be components of the same polymer. Methylation and partial acidic hydrolysis showed the basic structure to be a peptido-mannan to which numerous L-rhamnose residues are attached.[661]

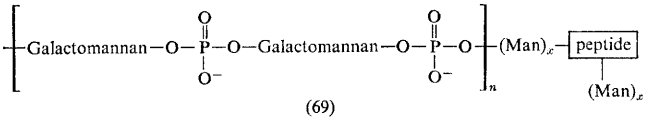
The results of investigations by methylation, n.m.r. spectroscopy, and g.l.c. indicated that the rhamnomannan from *Ceratocystis ulmi* is composed of a main chain of (1 → 6)-linked α-D-mannopyranose units substituted mainly with single α-L-rhamnopyranosyl units at C-3 and, probably, by a small proportion of *O*-L-rhamnopyranosyl-(1 → 4)-L-rhamnose side-chains. The corresponding polysaccharide isolated from *C. brunnea* was identified as a glucomannan having a main-chain of (1 → 6)-linked α-D-manno-pyranose units substituted at most of the 2-positions by α-D-glucose units and also by *O*-α-D-glucopyranosyl-(1 → 2)-α-D-mannopyranose and D-mannopyranose side-chains.[662]

A re-examination of the acetylphosphogalactan of *Sporobolomyces* has reported the presence of D-glucose (see M. Heidelberger and M. E. Slodki,

[657] J. F. T. Spencer and P. A. J. Gorin, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 1970, **36**, 135.
[658] J. F. T. Spencer, P. A. J. Gorin, and L. J. Wickerman, *Canad. J. Microbiol.*, 1970, **16**, 445.
[659] P. A. J. Gorin, J. F. T. Spencer, and A. J. Finlayson, *Carbohydrate Res.*, 1971, **16**, 161.
[660] P. A. J. Gorin, *Canad. J. Chem.*, 1971, **49**, 527.
[661] K. O. Lloyd and M. A. Bitoon, *J. Immunol.*, 1971, **107**, 663.
[662] P. A. J. Gorin and J. F. T. Spencer, *Carbohydrate Res.*, 1970, **13**, 339.

J. Exp. Med., 1968, **128**, 189), but the presence of D-glucose in no way affects the interpretations of the predicted cross-reactions.[663]

Aqueous extraction of the cells of *Cladosporium werneckii* and fractionation of the products yielded a galactomannan (14% of D-galactose and 78% of D-mannose) containing covalently bound phosphate and peptide. The polymer was shown to have a high molecular weight (150 000—200 000) and to contain both α- and β-linked sugar residues. Methylation analysis showed that D-mannose is mainly (1 → 2)-linked with smaller proportions of (1 → 6)- and (1 → 3)-substituted residues. All the D-galactose, which is present in both the pyranosyl and furanosyl forms, and some of the D-mannose residues are present at the non-reducing ends of chains. Ion-exchange chromatography of the original complex indicated that the peptidogalactomannan preparation consists of a family of polysaccharides having variable amounts of D-galactose and phosphate substituted on to a common mannan structure.[664] Fragments released by acidic hydrolysis, with concomitant scission of phosphate diester linkages, contained D-mannose, and most of the phosphate and much of the D-galactose of the original complex were identified as galactomannan phosphates of relatively low molecular weight (*ca.* 2500). The acid-stable fraction was rich in peptide and, on treatment with alkaline sodium borohydride, it released small, uncharged oligosaccharides containing D-mannose and D-mannitol. The structure (69) is in agreement with the results and is consistent with results obtained on reversing the sequence of the degradations in which alkaline treatment liberated low molecular weight oligosaccharides containing D-mannose as well as a high molecular weight nitrogen-free poly-(phosphogalactomannan).[665]



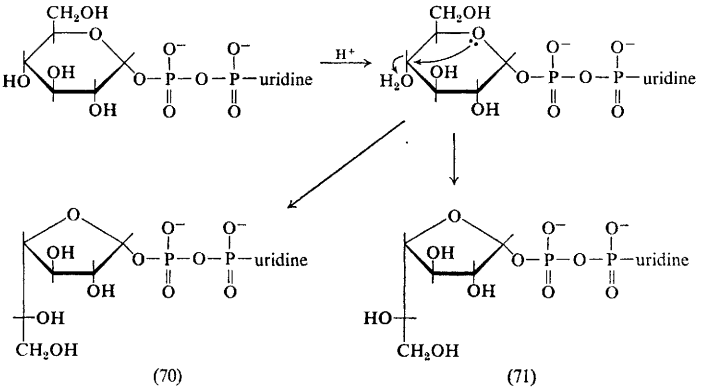
(69)

Pectin-polygalacturonase was reported to be associated with the galactan in the mycelia of *Aspergillus niger*.[666]

Investigations on the biosynthesis of galactocaralose, an extracellular β-D-(1 → 5)-linked polygalactofuranose, have led to the isolation and characterization of a new nucleotide, UDP-α-D-galactofuranose, which was shown to be an intermediate for the introduction of sugar units into the galactocaralose produced by *Penicillium charlesii*.[667] Cell-free extracts of *P. charlesii* utilized UDP-D-glucose and UDP-D-galactopyranose in the biosynthesis of galactocaralose, and it was shown from incorporation

[663] M. Heidelberger and M. E. Slodki, *J. Exp. Med.*, 1970, **132**, 1105.
[664] K. O. Lloyd, *Biochemistry*, 1970, **9**, 3446.
[665] K. O. Lloyd, *F.E.B.S. Letters*, 1970, **11**, 91.
[666] A. F. Abdel-Fattah, *J. Chem. U.A.R.*, 1969, **12**, 559.
[667] A. G. Trejo, G. J. F. Chittenden, J. G. Buchanan, and J. Baddiley, *Biochem. J.*, 1970, **117**, 637.

studies using [1-^{14}C]-, [6-^{14}C]-, and [U-^{14}C]-D-glucose that the D-galactofuranose residues in the polymer are formed from D-glucose without scission of the hexose carbon chain. When polymer synthesis was inhibited by F^- or Zn^{2+}, the major product was UDP-α-D-galactofuranose which, after isolation, was efficiently utilized for polymer synthesis. A possible mechanism for the direct transformation of either of the pyranose nucleotides into UDP-D-galactofuranose has been proposed. UDP-D-glucose could give the furanoside by the route outlined in Scheme 6. Protonation

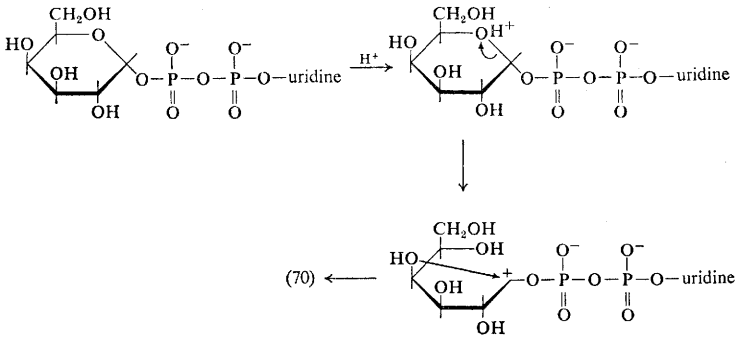


Scheme 6

of the hydroxy-group at C-4 is considered to be followed by rearward attack at C-4 by the lone pair of electrons on the ring-O atom. The fission of the C—O bond at position-5 would be accompanied by attack at that position by water. Although this attack could lead to either the D-galactose (70) or L-altrose (71) isomers in the final product, enzymic control could be assumed to specify the former. A mechanism for the direct transformation of UDP-D-galactopyranose into UDP-D-galactofuranose is given in Scheme 7. The mechanism proposes preferential protonation of the ring-O atom of the pyranose nucleotide followed by ring opening and rearrangement to the furanose form. The utilization of various substrates and the occurrence of a nucleotide possessing a galactofuranose residue indicated the overall route shown in Scheme 8 for the biosynthesis of galactocaralose from D-glucose in *P. charlesii*.[668]

A heterogalactan containing D-galactose, D-mannose, and L-fucose was elaborated by *Polyporus squamosus*. Methylation analysis and other evidence indicated that the polysaccharide is composed of chains of α-(1 → 6)-linked D-galactopyranose residues, 90% of which are substituted at C-2 with either α-L-fucopyranosyl, 3-*O*-α-D-mannopyranosyl-α-L-

[668] A. G. Trejo, J. W. Haddock, G. J. F. Chittenden, and J. Baddiley, *Biochem. J.*, 1971, **122**, 49.



Scheme 7

D-Glucose → D-Glucose 1-phosphate → UDP-D-glucose

↓

Galactocaralose ← UDP-D-galactofuranose ← UDP-D-galactopyranose

Scheme 8

fucopyranosyl, or short side-chains of α-(1 → 2)- and α-(1 → 3)-D-galactopyranosyl residues.[669]

Studies on the extracellular polysaccharide yeasts of the *Rhodotorula* genus have been reported. The main component of the polysaccharides produced from organisms of the subgenus *Rubrotorula* was shown to be mannose, whereas glucose, mannose, and xylose were detected in those polysaccharides isolated from the subgenus *Flavotorula*.[670] Polysaccharides isolated from *Rhodotorula* species using different conditions appeared to differ in monosaccharide composition.[671]

An extracellular polysaccharide from *Beijerinckia indica* was found to contain L-guluronic acid residues.[672]

Conditions of culture affected the composition of the cell walls of *Zygorhynchus vuilleminii* and the extent to which they were digested by mycolytic actinomycetes. The walls yielded 2-amino-2-deoxyglucose, galactose, fucose, and glucuronic acid, but no glucose. Wall fractions that were resistant to enzymic attack were found to be rich in uronic acid and fucose, whereas the readily degradable fractions contained 2-amino-2-deoxy-glucose.[673]

The relationship between taxonomy and chemical structure has been investigated by an examination of the acidic polysaccharides from the cell walls of *Absidia cylindrospora*, *Mucor mucedo*, and *Rhizopus nigricans*. The

[669] H. Björndal and B. Wågström, *Acta Chem. Scand.*, 1970, **23**, 3313.
[670] N. P. Elinov and G. A. Vitovskaya, *Biokhimiya*, 1970, **35**, 1187.
[671] N. P. Elinov and G. A. Vitovskaya, *Biokhimiya*, 1970, **35**, 622.
[672] A. Haug and B. Larsen, *Acta Chem. Scand.*, 1970, **24**, 1855.
[673] J.-P. G. Ballesta and M. Alexander, *J. Bacteriol.*, 1971, **106**, 938.

polysaccharides are all composed of L-fucose, D-galactose, and D-glucuronic acid, but in different molar ratios.[674]

The extracellular heteroglycans of *R. nigricans* were isolated and fractionated to give a main polysaccharide composed of L-fucose, D-mannose, D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose. The results of periodate oxidation indicated the presence of (1 → 2)- and (1 → 4)-hexopyranose residues as the main linkages. A minor polysaccharide isolated yielded L-fucose, D-galactose, and D-glucuronic acid.[675]

The slime mould *Physarum polycephalum* produced an acidic polysaccharide containing galactose and sulphate (2 : 1), together with traces of rhamnose. Electrophoretic studies suggested homogeneity, but ion-exchange chromatography indicated the presence of three major components. The slime appeared as an extracellular capsule adhering closely to the walls of the spherules.[676]

Rhizobial strains associated with the lotus species produced extracellular polysaccharides whose monosaccharide composition appears to be correlated with the division into acid- and non-acid-producing classes. The acid-producing strains gave polysaccharides containing glucose, galactose, and glucuronic acid, with little or no mannose. Non-acid-producing strains yielded polysaccharides containing glucose and mannose, with fucose and rhamnose occasionally present and with galactose and glucuronic acid nearly always absent.[677]

The culture filtrates of *Verticillium dahliae* contained a number of di- and tri-saccharides. Partial separation and chemical studies allowed the identification of *O*-β-D-fructofuranosyl-(1 → 6)-D-glucose, *O*-β-D-fructofuranosyl-(1 → 4)-D-glucose, *O*-β-D-fructofuranosyl-(1 → 2)-D-fructose, and *O*-β-D-fructofuranosyl-(1 → 1)-D-fructose.[678]

Yeast-phase cultures of *Histoplasma capsulatum* contained ten precipitinogens (four proteins and six polysaccharides), which were present in the broth filtrate. Gel filtration suggested that the molecular weights of the antigens range from < 50 000 for some of the carbohydrates to > 200 000 for some of the proteins.[679]

The cell wall of *Pityrosporum ovale* yielded higher amounts of lipid, protein, and 2-acetamido-2-deoxy-D-glucose than that of *Saccharomyces cerevisiae*.[680]

An extracellular, acidic polysaccharide from black yeast, containing 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucopyranosyluronic acid (2 : 1), was completely inert to periodate oxidation,

[674] T. Miyazaki and T. Irino, *Chem. and Pharm. Bull. (Japan)*, 1970, **18**, 1930.
[675] T. Miyazaki and T. Irino, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 1450.
[676] J. J. McCormick, J. C. Blomquist, and H. P. Rusch, *J. Bacteriol.*, 1970, **104**, 1110.
[677] R. W. Bailey, R. M. Greenwood, and A. Craig, *J. Gen. Microbiol.*, 1971, **65**, 315.
[678] Y. M. Choy and A. M. Unrau, *Canad. J. Chem.*, 1971, **49**, 894.
[679] G. H. Sweet, *Amer. Rev. Respirat. Diseases*, 1971, **104**, 401.
[680] E. Thompson and J. R. Colvin, *Canad. J. Microbiol.*, 1970, **16**, 263.

indicating that the linkages involved C-3 or C-4 or both. Optical-rotation values suggested the possibility of mixed α- and β-anomeric configurations.[681]

Autolysis of *Coprinus lagopus* was caused by degradation of the cell walls by the action of chitinases that are newly formed shortly before spore release begins. Chitinases and other hydrolytic enzymes are localized intracellularly in vacuoles and are released into the wall on cessation of metabolic activity in the senescing gills.[682]

A mutation of *Aspergillus nidulans* produced hyphal walls containing abnormally low amounts of amino-sugars. Temperature sensitivity was due to a single recessive mutation, and a block in the biosynthesis of 2-amino-2-deoxy-D-glucose presumably led to the formation of walls lacking chitin.[683]

Cell-free extracts of *Mucor rouxii* catalysed the synthesis of UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose from 2-amino-2-deoxy-D-[^{14}C]glucose. Incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose into chitin was demonstrated, but no evidence was found for the synthesis of chitosan, which is also a component of the wall.[684] Autoradiographic studies showed that chitin synthetase is localized preferentially in the apical region of the hyphal walls of *M. rouxii*; this is also the region of chitin deposition *in vivo*. The total content of chitin synthetase of a culture was shown to increase concomitantly with cell growth and with the hexosamine content of the cells.[685]

Electron-microscopic examination of extracted cell ghosts of *Saccharomyces carlsbergensis* revealed very thin cell envelopes with prominent bud scars in the shape of a hollow crater with a raised rim. The extracted cell ghosts had a high content of chitin, which could be removed by digestion with chitinase. This treatment did not destroy the integrity of the cell envelope, but largely eliminated the bud-scar rims. On the other hand, incubation with glucanase left only fragments in the shape of bud scars. It was concluded that chitin is localized in a ring around the bud scar sandwiched between two layers of glucan.[686] A particulate preparation from a spheroplast lysate of *S. carlsbergensis* was found to catalyse the transfer of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose to an endogenous acceptor. The reaction product, which remained bound to the particles containing the activity, was characterized as chitin by its insolubility in alkali, by the release of 2-amino-2-deoxy-D-glucose on acidic hydrolysis, and from the liberation of *NN'*-diacetylchitobiose and 2-acetamido-2-deoxy-D-glucose following enzymic hydrolysis. Polyoxin

[681] A. Jeanes, K. A. Burton, M. C. Cadmus, C. A. Knutson, G. L. Rowin, and P. A. Sandford, *Nature (New Biol.)*, 1971, **233**, 259.
[682] W. Iten and P. Matile, *J. Gen. Microbiol.*, 1970, **61**, 301.
[683] D. Katz and R. F. Rosenberger, *Biochim. Biophys. Acta*, 1970, **208**, 452.
[684] I. McMurrough and S. Bartnicki-Garcia, *Biochem. J.*, 1970, **119**, 11P.
[685] I. McMurrouch, A. Flores-Carreon, and S. Bartnicki-Garcia, *J. Biol. Chem.*, 1971, **246**, 3999.
[686] E. Cabib and B. Bowers, *J. Biol. Chem.*, 1971, **246**, 152.

A was a very potent competitive inhibitor of the chitin synthetase, but the antibiotic affected neither growth of intact cells nor the synthesis of chitin by naked spheroplasts, indicating that chitin synthetase is not located on the outside of the cytoplasmic membrane.[687] The soluble fraction of a spheroplast lysate from *S. carlsbergensis* and *S. cerevisiae* contained a heat-stable proteinaceous inhibitor of chitin synthetase. It was concluded from kinetic studies that the inhibitor and substrate interact with different parts of the enzyme system. The inhibitor was considered to be an allosteric effector that serves to regulate the enzyme *in vivo*.[688] The necessity of such regulation was indicated by the fact that chitin is deposited in a very restricted region of the cell wall and, probably, only during a specific phase of the budding process.[686]

Polyoxin D inhibited the incorporation of 2-amino-2-deoxy-D-[^{14}C]-glucose into the cell-wall chitin in *Neurospora crassa* at levels comparable with those required for the inhibition of fungal growth. At the same time, the antibiotic increased the accumulation of UDP-2-acetamido-2-deoxy-D-glucose, indicating inhibition of chitin synthesis. The chitin synthetase of *N. crassa* was strongly inhibited by polyoxin D as determined by the transfer of 2-acetamido-2-deoxy-D-[^{14}C]glucose from UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose to the particulate fraction. Inhibition was shown to be competitive and was specific for chitin synthetase. The formation of osmotically sensitive protoplast-like structures when *Cochliobolus miyabeanus* was grown in the presence of polyoxin D suggested that the primary site of action of the antibiotic is at the formation of cell-wall structures.[689] Polyoxin D did not inhibit the incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose into the cell wall of *C. miyabeanus*, but UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose was shown to accumulate in the mycelia.[690]

One of the main components of the cell wall of *C. miyabeanus* was demonstrated to contain 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose. The *X*-ray powder pattern suggested that chitin linked to some other unit is present. From the results of enzymic digestion, acidic hydrolysis, and methylation, the polysaccharide was shown to contain (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucose units with branch-points connected to 2-acetamido-2-deoxy-D-galactose units.[691]

The spores and spherules of *Physarum polycephalum* contain a polysaccharide composed of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose, which showed solubility characteristics similar to those of chitosan.[692]

[687] F. A. Keller and E. Cabib, *J. Biol. Chem.*, 1971, **246**, 160.
[688] E. Cabib and F. A. Keller, *J. Biol. Chem.*, 1971, **246**, 167.
[689] A. Endo, K. Kakiki, and T. Misato, *J. Bacteriol.*, 1970, **104**, 189.
[690] N. Ohta, K. Kakiki, and T. Misato, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1224.
[691] H. Nanba and H. Kuroda, *Chem. and Pharm. Bull. (Japan)*, 1971, **14**, 1402.
[692] J. J. McCormick, J. C. Blomquist, and H. P. Rusch, *J. Bacteriol.*, 1970, **104**, 1119.

5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

The application of affinity chromatography to the preparation of glycoproteins, including antigens and antibodies, has been the subject of a recent review.[693] A review on complex polysaccharides included sections on the structure and biosynthesis of glycoproteins and their role in virus structures.[694]

Details of the optimum conditions for hydrolysis, derivatization, and gas chromatography for the determination of carbohydrate constituents of glycoproteins have been reported.[695]

A gas-chromatographic method for the determination of neutral and amino-sugars (commonly found in glycoproteins of animal origin) as their alditol acetates has been described.[34] The monosaccharides could be determined in 1 mg of a glycoprotein containing at least 6% total carbohydrate.

Use of 3-methyl-2-benzothiazolone hydrazone for the estimation of *N*-sulphate and *N*-acetate groups in polysaccharides of animal origin has overcome the problems of interference experienced with the indole–acetic acid method.[13] The new method depends upon the prior reaction of *N*-sulphate and free amino-groups with nitrous acid, and on the stability of *N*-acetyl groups to nitrous acid. The method is also applicable to the estimation of total 2-amino-2-deoxyhexose after hydrolysis of the macromolecule.

A gas-chromatographic procedure for establishing the nature of the linkages of internal 2-acetamido-2-deoxy-D-glucose residues in oligosaccharide fragments of glycoproteins following methylation, methanolysis, and *O*-acetylation has been developed.[696]

In a review of the structures of glycoproteins and their enzymic degradation, covalent carbohydrate–protein compounds are classified according to their composition and the structure of the carbohydrate chains, but

[693] P. Cuatrecasas and C. B. Anfinsen, *Ann. Rev. Biochem.*, 1971, **40**, 259.
[694] E. C. Heath, *Ann. Rev. Biochem.*, 1971, **40**, 29.
[695] F. Mullinax, G. L. Mullinax, M. R. Cohen, C. L. Cromwell, and J. Deboe, *Immunochem.*, 1971, **8**, 551.
[696] B. Anderson, E. A. Kabat, S. Beychok, and F. Gruezo, *Arch. Biochem. Biophys.*, 1971, **145**, 490.

irrespective of source, as follows: (i) proteoglycans in which the carbohydrate moiety consists of small repeating units, usually a disaccharide, arranged in a linear structure of molecular weight 2.0—3.5 × 10^4, and (ii) glycoproteins in which the carbohydrate moiety contains 2-amino-2-deoxy-D-glucose and/or 2-amino-2-deoxy-D-galactose and one or more of the following monosaccharides: D-galactose, D-glucose, D-mannose, L-fucose, and sialic acid, the carbohydrate chain being nearly always branched and of low molecular weight.[697] So far the presence of a repeating unit in a glycoprotein has not been reported.

In summary, the glycopeptide linkages of glycoproteins involve (i) the relatively stable glycosylamine linkage involving the amido-group of L-asparagine, as observed in all plasma glycoproteins, (ii) the alkali-labile glycosidic linkage to the hydroxy-group of L-serine or L-threonine, as found in the proteoglycans, submaxillary mucins, and blood cell membrane glycoproteins, and (iii) the alkali-stable glycosidic linkage to the hydroxy-group of 5-hydroxy-L-lysine, as in collagen and basement membrane glycoproteins.[698] A homology was observed between the codon sequence and the linkage amino-acid, one of the codons for L-asparagine (AAU) giving rise to the codons for L-serine (AGU), L-threonine (ACU), and L-lysine (AAA) by single base substitutions.

A general method has been described for the radioactive labelling of glycoproteins containing terminal sialic acid residues, quantitative conversion of these residues into a radioactive 7-carbon analogue of sialic acid being achieved.[699]

The information presently available on the metabolism of sialic acids of animal glycoproteins has been reviewed.[700]

Microbial Glycoproteins

A glycopeptide, obtained from *Aspergillus fumigatus* by extraction with aqueous pyridine and purification by ion-exchange chromatography and gel filtration, contained galactose, mannose, and 2-amino-2-deoxyglucose.[628] The glycopeptide showed both an Arthus and delayed tuberculin-type skin reaction in sensitized animals, the former type being abolished by periodate oxidation and the latter by proteolysis.

Mannose-acceptor glycoproteins from subcellular fractions of *Dictyostelium discoideum* contain D-glucose, D-mannose, L-fucose, and 2-amino-2-deoxy-D-glucose.[701] Their lability to alkali indicated the presence of glycosidic linkages to β-hydroxyamino-acids as the main type of glycopeptide linkage. The mechanism of the enzymic transfer of D-mannose was investigated.

[697] A. Gottschalk, *Chimia* (*Switz.*), 1971, **25**, 77.
[698] M. Jett and G. A. Jamieson, *Carbohydrate Res.*, 1971, **18**, 466.
[699] L. Van Lenten and G. Ashwell, *J. Biol. Chem.*, 1971, **246**, 1889.
[700] T. Szymczyk and I. M. Jachimowicz, *Postepy Biochem.*, 1971, **17**, 417.
[701] R. Bauer, M. Rath, and H. J. Risse, *European J. Biochem.*, 1971, **21**, 179.

A lipopolysaccharide–protein complex from *Escherichia coli* contains heptose and 3-deoxyoctulosonate. The overall composition, together with the fact that the molecule is released from the cell by treatment with warm water, suggested that the complex is derived from the outermost layer of the cell wall.[500]

Mycoside C_2, a lipoglycopeptide isolated from *Mycobacterium avicum* and purified by column chromatography on silicic acid and silica gel G, yielded 3,4-di-*O*-methyl-L-rhamnose (1.0), 6-deoxy-L-talose (0.5), and 6-deoxy-3-*O*-methyl-L-talose (0.5 mole per mole).[702] Alkaline β-elimination studies indicated that the 6-deoxy-L-talose residue or its 3-*O*-methyl derivative is linked to an *allo*-threonine residue in the proposed structure (72).

Cultures of *Neurospora crassa* contained an extracellular glycoprotein which inhibited the growth of the organism and caused agglutination of its cells.[703] The glycoprotein released 2-amino-2-deoxygalactose (50%) and some neutral sugars. That the majority of the 2-amino-2-deoxygalactose residues are unacetylated was deduced from the observations that the molecule had a high ninhydrin value, which increased by a factor of only 2.4 on extensive hydrolysis, a high affinity for Dowex 50[H^+] ion-exchange resin, and an acetyl content of only 25% of that expected if all the 2-amino-2-deoxygalactose residues are *N*-acetylated. A molecular weight of 10^6 was estimated for the glycoprotein, complete acetylation of which abolished its biological activities.

Evidence for the release of a lipid–protein–carbohydrate complex from isolated envelopes and whole cells of *Pseudomonas* sp. type IV at low salt concentration has been reported.[704]

The cytoplasm of group A Streptococci contains a glycoprotein which cross-reacted immunologically with glycoproteins from mammalian cardiac valves.[705] A preparation of the glycoprotein, prepared by ethanol precipitation, was shown to contain hexoses (7.2%), 2-amino-2-deoxyhexose (0.5%), uronic acid (0.18%), muramic acid (0.12%), and protein (81%).

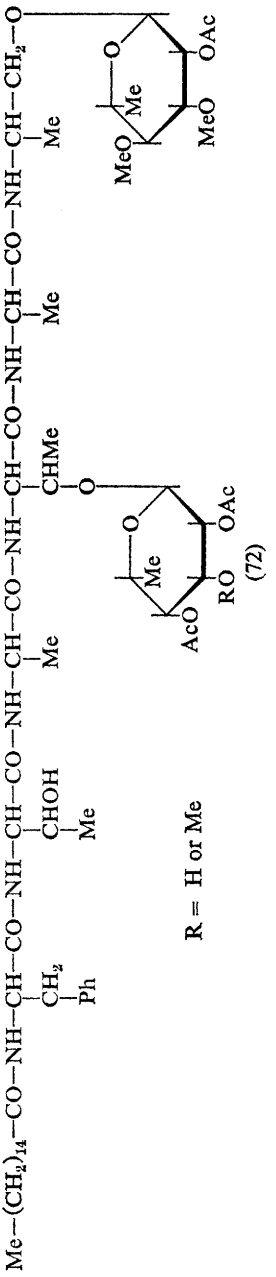
Isoelectric focusing of *Trypanosoma brucei* subgroup antigens in thin layers of polyacrylamide gel showed that a group of precipitating antigens are carbohydrate–protein complexes.[706]

Alkali-soluble material from the yeast form of *Verticillium albo-atrum* was found to be a heteropolysaccharide–protein complex containing galactose, mannose, glucose, 2-amino-2-deoxyglucose, and glucuronic acid.[620]

Plant Glycoproteins

Isoelectric focusing of concanavalin A from Jack-bean meal revealed a heterogeneity of this phytohaemagglutinin, the main components showing

[702] A. Voiland, M. Bruneteau, and G. Michel, *European J. Biochem.*, 1971, **21**, 285.
[703] J. L. Reissig and J. E. Glasgow, *J. Bacteriol.*, 1971, **106**, 882.
[704] F. L. A. Buckmire and R. A. MacLeod, *Canad. J. Microbiol.*, 1971, **17**, 713.
[705] B. Halpern, J. Parlebas, and I. Goldstein, *Compt. rend.*, 1971, **273**, *D*, 995.
[706] K. C. Humphryes, *J. Chromatog.*, 1971, **49**, 503.


Me–(CH2)14–CO–NH–CH–CO–NH–CH–CO–NH–CH–CO–NH–CH–CO–NH–CH–CO–NH–CH–CH2–O
CH2
Ph
CHOH
Me
Me
CHMe
O
Me
AcO
RO
OAc
Me
MeO
O
Me
MeO
OAc
R = H or Me
(72)

pI values in the pH range 4.5—5.5.[707] A simultaneous determination of pI by cellulose acetate electrophoresis gave a value of 5.1. That the tertiary structure of concanavalin A is essentially a mixture of β-form and random coil was indicated from o.r.d. and c.d. studies.[708] The effect on the spectra of the sequential addition of transition-metal ions, calcium ions, and methyl α-D-glucopyranoside was investigated. Molecular-weight studies under various conditions of pH and dissociating agents suggested that concanavalin A molecules are composed of four subunits of molecular weight 1.7×10^4,[709] whereas the results of cyanogen bromide analysis implied a composition of identical subunits of molecular weight 2.7×10^4.[710] Information has been presented which indicates that the binding of concanavalin A to simple neutral polyhydroxy-compounds represents a form of hydrogen bonding and that steric factors operate for compounds of higher molecular weight.[711] Some of the compounds previously thought to be non-inhibitory were found to inhibit, whereas others enhanced the binding of concanavalin A to neutral polysaccharides. Concanavalin A reacted with α-glucosylated but not with β-glucosylated teichoic acids; similarly it reacted with α-linked but not β-linked 2-acetamido-2-deoxyglucose-teichoic acids.[386] The usefulness of the interaction of concanavalin A with teichoic acids in studying the immunochemistry of these antigens was demonstrated. Bacterial cell walls were agglutinated by concanavalin A when they contained the appropriate glycosidic substituents on the teichoic acid constituents.[387] This finding has applications in the purification, structural analysis, and location of teichoic acids. It was proposed from the results of investigations on the mode of binding of aromatic residues to concanavalin A that apolar binding, involving hydrophobic interactions associated with the *o*- and *m*- but not the *p*-positions of the aromatic nucleus, is the predominant mode of binding the phenyl residue of phenyl β-D-glucopyranoside.[712] The binding of concanavalin A by normal and virus-transformed cells,[713] and the effects of the molecule on tumorigenesis [714] and inhibition of tumour-cell migration [715] have been studied. Concanavalin A insolubilized by cross-linking has been used as an adsorbent for the specific isolation of polysaccharides and glycoproteins.[582] A covalent concanavalin A–ferritin conjugate has been prepared.[716]

[707] G. Entlicher, J. V. Koštíř, and J. Kocourek, *Biochim. Biophys. Acta*, 1971, **236**, 795.
[708] W. D. McCubbin, K. Oikawa, and C. M. Kay, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 666.
[709] W. D. McCubbin and C. M. Kay, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 101.
[710] M. J. Waxdal, J. L. Wang, M. N. Pflumm, and G. M. Edelman, *Biochemistry*, 1971, **10**, 3343.
[711] E. P. Plow and H. Resnik, *Biochim. Biophys. Acta*, 1970, **221**, 657.
[712] R. D. Poretz and I. J. Goldstein, *Biochem. Pharmacol.*, 1971, **20**, 2727.
[713] M. J. Cline and D. C. Livingstone, *Nature New Biol.*, 1971, **232**, 155.
[714] D. Gericke, P. Chandra, and A. Wacker, *Naturwiss.*, 1971, **58**, 458.
[715] S. Friberg, A. J. Cochran, and S. H. Golub, *Nature New Biol.*, 1971, **232**, 121.
[716] G. L. Nicolson and S. J. Singer, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 942.

An anti A_1 phytohaemagglutinin from *Dolichos biflorus* seeds was found to be homogeneous by polyacrylamide gel electrophoresis and ultracentrifugation after gel filtration and CM-cellulose chromatography.[717] The molecule had an $S_{20,w}$ value of 6.3 *S* and a pI of 5.5, and liberated glucose (0.2%), mannose (1.5%), fucose (0.4%), arabinose (0.04%), xylose (0.1%), and 2-acetamido-2-deoxyhexose (1.25%). Periodate oxidation destroyed the erythroagglutinating activity of the molecule whereas proteolysis did not.

Phytohaemagglutinin from the common lentil (*Lens culinaris*) was purified by specific adsorption on Sephadex G-100 and subsequent displacement with D-glucose.[718] The preparation, which was homogeneous on ultracentrifugation and polyacrylamide gel electrophoresis, was shown to have a molecular weight of 6.3×10^4, and to contain glucose (6) and 2-amino-2-deoxyglucose (2 moles per mole). A phytohaemagglutinin from the same source was able to combine with D-mannose and methyl α-D-glucopyranoside but not with D-galactose.[719]

Fractionation of the phytohaemagglutinin of kidney bean (*Phaseolus vulgaris*) by polyacrylamide gel electrophoresis in sodium dodecyl sulphate yielded two major glycoprotein units with mobilities corresponding to molecular weights of 2.9×10^4 and 3.3×10^4.[720] The effects of heat and enzymes on phytohaemagglutinins from the same source have been studied.[721]

Demetalization and edta-treatment of phytohaemagglutinin from the pea (*Pisum sativum*) abolished its ability to precipitate mannan.[653]

A phytohaemagglutinin from *Robinia pseudoacacia* afforded mannose (23), fucose (6), xylose (6), and 2-amino-2-deoxyglucose (16 moles per mole).[722] Acid hydrolysis released the monosaccharides in the order fucose and xylose, mannose, and, finally, 2-amino-2-deoxyglucose. The neutral sugar residues were rapidly destroyed by oxidation with periodate, whereas the basic sugar residues were resistant.

Studies on the specificity of the lectin from *Soja hispida* revealed that the agglutinin could react with terminal α- and β-linked 2-acetamido-2-deoxy-D-galactose, but not with subterminal residues.[723]

Two mitogenic fractions (molecular weights 1.4×10^5) isolated from a commercially available phytohaemagglutinin by preparative gel electrophoresis contained hexose (2.90 and 4.30%) and 2-amino-2-deoxyhexose (1.15 and 2.00%), respectively.[724] Neither of the glycoproteins possessed haemagglutinating activity.

[717] J. Font, A. M. Leseney, and R. Bourrillon, *Biochim. Biophys. Acta*, 1971, **243**, 434.
[718] S. Toyoshima, T. Osawa, and A. Tonomura, *Biochim. Biophys. Acta*, 1970, **221**, 514.
[719] M. D. Stein, I. K. Howard, and H. J. Sage, *Arch. Biochem. Biophys.*, 1971, **146**, 353.
[720] D. Allan and M. J. Crumpton, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1143.
[721] H. G. Oswan and E. W. Jwanny, *J. Chem. U.A.R.*, 1969, **12**, 297.
[722] J. Font and R. Bourrillon, *Biochim. Biophys. Acta*, 1971, **243**, 111.
[723] W. Dahr and G. Uhlenbruck, *Blut*, 1971, **22**, 128.
[724] Y. H. Oh and R. A. Conrad, *Arch. Biochem. Biophys.*, 1971, **146**, 525.

Changes in the concentrations of various constituents of alfalfa herbage effected by drying at temperatures of 600—800 °C included increases of up to tenfold of the glycoprotein content.[309]

Glycoprotein I, isolated from *P. vulgaris* seeds, was shown to be homogeneous and free from proteases, and was found to undergo autodigestion.[725] The hydrodynamic and other properties of the molecule (molecular weight 6×10^4) indicated that it possesses a protein core bearing carbohydrate moieties. A partial characterization of a membrane-bound glycoprotein intermediate in the biosynthesis of *P. aureus* cell-wall polysaccharide from GDP-D-[^{14}C]mannose has been reported.[726] The glycoprotein (molecular weight 1.15×10^4) yielded mannose and cellotetraose on acid hydrolysis.

An improved method has been described for the isolation and purification of γ-globulin from rice embryo.[727] Although the preparation was apparently homogeneous on sedimentation analysis, gel electrophoresis fractionated it into two components which on subsequent DEAE–Sephadex chromatography yielded γ_1- and γ_2-, and γ_2- and γ_3-globulins, respectively. The presence of β-structure, random coil, and α-helix in γ_1-globulin were suggested by o.r.d., c.d., and i.r. measurements.[728] The blue glycoprotein isolated from rice bran contains hexose (5.49%) and pentose (4.01%), and copper (1.09 atoms per mole).[729] The physicochemical characteristics of the glycoprotein (molecular weight 1.83×10^4) have been studied by various techniques including o.r.d. spectroscopy.

A copper-containing glycoprotein, plastocyanin (molecular weight 1.5×10^4), purified from the leaves of wheat seedlings, contains glucose, arabinose, and 2-amino-2-deoxyhexose.[730] Wheat globulin has been quantitatively measured by disc electrophoresis.[731]

Blood-group Substances

Mild acidic hydrolysis of a human ovarian-cyst blood-group A substance followed by preparative chromatography resulted in the isolation and characterization of two disaccharides (73) and (74), a trisaccharide (75), and two tetrasaccharides (76) and (77).[696] The oligosaccharides appeared to be derived from the interior of the megalosaccharide chains of the glycoprotein. Methylation analyses were also presented for four oligosaccharides obtained from alkaline borodeuteride degradation of a precursor blood-group OG substance, which contains internal 4-substituted 2-acetamido-2-deoxy-D-glucose residues.

[725] A. Pusztai and I. Duncan, *Biochim. Biophys. Acta*, 1971, **229**, 785.
[726] C. L. Villemez, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 636.
[727] H. Sawai and Y. Morita, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 53.
[728] Y. Morita, H. Sawai, K. Hamaguchi, and K. Ikeda, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1231.
[729] Y. Morita, A. Wadano, and S. Ida, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 255.
[730] A. A. Mutuskin, K. V. Pshenova, S. K. Alekhina, and P. A. Kolesnikov, *Biokhimiya*, 1971, **36**, 239.
[731] V. Silano and F. Pocchiari, *Cereal Chem.*, 1971, **48**, 445.

A comparative study of the reaction of blood-group A_1 and A_2 glycoproteins from human ovarian-cyst fluid with human anti-A showed specificity differences between the two glycoproteins.[732] Structural bases for this observation were discussed.

β-D-Gal-(1 → 4)-D-Glc*N*Ac
(73)

β-D-Gal-(1 → 3)-D-Glc*N*Ac
(74)

β-D-Gal-(1 → 3)-β-D-Glc*N*Ac-(1 → 3)-D-Gal
(75)

β-D-Gal-(1 → 3)-β-D-Glc*N*Ac-(1 → 3)-β-D-Gal-(1 → 3)-D-Glc*N*Ac
(76)

β-D-Gal-(1 → 3)-β-D-Glc*N*Ac-(1 → 3)-β-D-Gal-(1 → 4)-D-Glc*N*Ac
(77)

The characteristics of human blood-group A and B substances degraded by the culture fluid from a *Streptomyces* sp. have been discussed in relationship to the glycosidases known to be present in the organism.[733]

Human saliva from secretors of blood-group A, B, O, and AB substances has been analysed by gel filtration and by testing of the fractions for blood-group A, B, and H antigens by haemagglutination inhibition. A major fraction of the saliva was excluded by filtration on Sephadex G-200 and was partially excluded from Sepharose 4B; a molecular weight of $2—50 \times 10^5$ was thereby estimated.[734] This fraction (*A*) was present in the saliva of all secretors of ABH substances and, in some cases, was associated with either or both of two group-specific fractions having molecular weights of $1.0—1.3 \times 10^4$ (*B*) and $\leqslant 2 \times 10^3$ (*C*).[735] The saliva from some non-secretors lacked fraction *A* but contained fraction *B* and/or *C*, whereas no active fraction was detected in saliva from other non-secretors (true non-secretors). In all, eight gel-filtration patterns for each of the A, B, and H antigens were identified, and a standardized technique for the analysis of salivary material was established. The ABH salivary gel-filtration pattern of a single individual was shown to be a stable characteristic. Gel filtration of solubilized human red-cell stroma yielded three group-specific fractions for each of the blood-group A, B, and H substances.[736] The three fractions had the same characteristics as the three salivary fractions. The identity of the third stromal fraction with the blood-group specific glycolipid isolated from erythrocytes was suggested.

[732] C. Moreno, A. Lundblad, and E. A. Kabat, *J. Exp. Med.*, 1971, **134**, 439.
[733] K. Oishi and K. Aida, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1101.
[734] A. Fiori, G. V. Giusti, G. Panari, and G. Porcelli, *J. Chromatog.*, 1971, **55**, 337.
[735] A. Fiori, G. V. Giusti, and G. Panari, *J. Chromatog.*, 1971, **55**, 351.
[736] A. Fiori, G. V. Giusti, and G. Panari, *J. Chromatog.*, 1971, **55**, 365.

Human gastric juice, saliva, and ovarian-cyst fluid were fractionated into glycoprotein components by equilibrium centrifugation in caesium chloride.[737] Glycoprotein fractions from the gastric juice of two blood-group O non-secretors, two group O secretors, and three group A secretors formed insoluble complexes with concanavalin A, those fractions showing the highest degrees of complexing having the highest activities. The sulphate content of the gastric glycoproteins was unrelated to their capacities to interact with concanavalin A. That the glycoproteins from the other sources were structurally different was indicated by their failure to interact with concanavalin A.

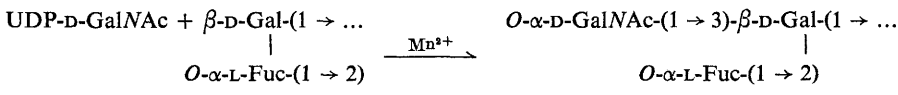
A partially purified blood-group-substance-like material obtained from human milk showed I activity on testing with anti-I sera.[738] Human ovarian-cyst substances considered to be precursors of blood-group A, B, H, Le^a, and Le^b substances showed comparable I activities. Furthermore, strong I activity could be produced by one-stage periodate oxidation and Smith degradation of human ovarian-cyst blood-group A + B substances and hog-mucin A + H substance, and by mild acidic hydrolysis of human saliva and ovarian-cyst blood-group B substance. The results indicated that I specificity appears at intermediate stages in the biosynthesis of A, B, H, Le^a, and Le^b substances.

M,N-Active human erythrocyte sialoglycopeptide readily formed a complex in aqueous solution with positively charged substances such as toluidine blue and bovine albumin.[739] The interaction was attributed to the formation of a reversible semipolar or ionic bond, and it was suggested that the M,N-active sialoglycopeptide possesses cation-exchange properties. The physicochemical properties, particularly the state of aggregation, of M,N substances prepared from the ghosts of human erythrocytes have been examined; the results of controlled, tryptic digestion of the glycoproteins indicated the presence of two trypsin-susceptible sites.[740] Evidence was presented for the dimerization of the fragments in aqueous solution.

An investigation of the effects of acylation, succinylation, and esterification on the M,N and Pr_1/Pr_2 activities of erythrocyte glycoproteins showed that the latter activities are unaffected by derivatization.[741] Only acylation and succinylation destroyed the M,N-activities, implying that the *N*-acetylneuraminic acid residues are not determinants of the activities.

An acetamidodeoxygalactosyltransferase occurring in human milk from donors of blood type A or AB, but not in that from donors of blood type B or O, catalysed a reaction (Scheme 9) which resulted in the formation of structural determinants of blood type A.[742]

[737] A. E. Clarke and M. A. Denborough, *Biochem. J.*, 1971, **121**, 811.
[738] T. Feizi, E. A. Kabat, G. Vicari, and B. Anderson, *J. Exp. Med.*, 1971, **133**, 39.
[739] S. Ohkuma and T. Furuhata, *Proc. Japan Acad.*, 1970, **46**, 185.
[740] A. Morawiecki and W. Wnuk, *Biochim. Biophys. Acta*, 1970, **222**, 680.
[741] W. Merz and D. Roelcke, *European J. Biochem.*, 1971, **23**, 30.
[742] A. Kobata and V. Ginsburg, *J. Biol. Chem.*, 1970, **245**, 1484.



Scheme 9

Collagens

Papers presented at the 2nd European Symposium on Connective Tissue Research included contributions on the biosynthesis, enzymic degradation, and immunology of collagen, and its association with proteoglycans.[743]

Earlier reports that solubilization of collagen by α-amylase was a result of cleavage of carbohydrate chains have been refuted, the dispersion having been shown to result from the action of a protease contaminant of the α-amylase.[744]

Methods for the extraction of polymeric collagen from human skin have been assessed.[745] The solubility of skin collagen from normal subjects and patients with generalized scleroderma has been investigated.[746] Determination of the levels of glucosylgalactosylhydroxylysine and galactosylhydroxylysine in collagens from human skin and bone demonstrated that one-third of the total hydroxylysine in these tissues is glycosylated.[747] Glucosylgalactosylhydroxylysine predominated in skin, whereas galactosylhydroxylysine predominated in bone, the relative proportions being constant for a particular tissue.

All the insoluble, polymeric collagens from various simple soft human tissues (*e.g.* dermis, tendon, submucosa, sclera, and cornea) were extractable by the methods employing edta, whereas the more complex tissues (*e.g.* intercostal cartilage and intervertebral disc) yielded only small amounts of collagen.[748] The collagens released mannose, galactose, glucose, fucose, xylose, and 2-amino-2-deoxyhexose, the total hexose varying from 8.5—42.1 residues per 3000 amino-acid residues and the galactose : glucose ratio varying from 1 : 1.0 to 1 : 2.3. The presence of both galactosylhydroxylysine and glucosylgalactosylhydroxylysine sequences was recognized, corneal and tendon collagens containing 19.1 and 4.1 hydroxylysine-linked carbohydrate units per 3000 amino-acid residues, respectively. Variation in the disaccharide to monosaccharide unit ratio was also observed, being 1 : 1.2 and 1 : 4.1 for intercostal cartilage and submucosal collagens, respectively.

The collagen content of human trabecular bone and its extractability were assessed and related to the age and sex of the subject, and to the degree of porosity of the bone.[749] Human intervertebral protein–polysaccharide

[743] 2nd European Symposium on Connective Tissue Research, *Z. klin. Chem. klin. Biochem.*, 1971, **9**, 59.
[744] A. J. Bailey and D. J. Etherington, *Biochim. Biophys. Acta*, 1970, **214**, 238.
[745] M. J. O. Francis and D. C. Macmillan, *Biochem. J.*, 1971, **122**, 34P.
[746] J. Uitto, K. Øhlenschläger, and I. Lorenzen, *Clin. Chim. Acta*, 1971, **31**, 13.
[747] S. R. Pinnell, R. Fox, and S. M. Krane, *Biochim. Biophys. Acta*, 1971, **229**, 119.
[748] J. D. Schofield, I. L. Freeman, and D. S. Jackson, *Biochem. J.*, 1971, **124**, 467.
[749] J. Dequeker and W. Merlevede, *Biochim. Biophys. Acta*, 1971, **244**, 410.

light (PPL) fraction interacted with acetic acid-soluble calf skin and neutral, salt-soluble, rat skin tropocollagens in the isoelectric-focusing column to yield a single complex zone having a pI intermediate between those of the separate compounds.[750] It was shown that this association of the tropocollagen is largely brought about by the presence of the sulphate groups in the PPL fraction.

Purified collagen from human umbilical cord contains D-glucose (2.9) and D-galactose (2.3 residues per 1000 amino-acid residues).[751] The electrostatic interaction of chondroitin sulphate and chondroitin sulphate–proteoglycan with collagen was studied by column chromatography on a gel of cross-linked collagen. The binding observed between the macromolecules increased with decreasing pH and ionic strength, and was significant under physiological conditions. A study of the interaction between soluble collagen and chondroitin sulphate by a partition-equilibrium technique afforded similar results.

CM-Cellulose chromatography of collagens isolated from human glomerular basement membrane, sheep anterior lens capsules, and Descemet's membranes indicated that the molecules are composed of three identical α_1 chains.[752] The molecular weight of the chains from basement membranes is 1.1×10^5; this value is higher than that of interstitial collagens by an amount attributed to the excess of hexose. The basement membrane, lens capsule, and Descemet's membrane materials contained galactose (6.0, 6.3, and 5.2%) and glucose (5.5, 6.0, and 5.0%, respectively), and the first two materials also contained traces of mannose. The intermolecular cross-links of collagen in human and guinea-pig scar tissue have been examined.[753]

The results of n.m.r. and e.p.r. spectroscopy of hydrated collagen fibres in the presence of salts provided data on the mode of water binding of bovine collagen.[754] During the periodate-induced swelling of calf cornea, 90% of the hydroxylysine-linked galactose and glucosylgalactose units, and 70% of the heteropolysaccharides containing mannose were degraded.[755] This suggested that the carbohydrates are readily accessible in the macromolecular network of the stroma and that they have a role in the stabilization of the collagen fibres.

Insoluble elastins, prepared by several different methods from adult bovine and calf *ligamentum nuchae*, contained only trace amounts of neutral (0.13—0.35%) and basic (0.01—0.06%) carbohydrates.[756]

[750] V. Podrazky, F. S. Steven, D. S. Jackson, J. B. Weiss, and S. J. Leibovich, *Biochim. Biophys. Acta*, 1971, **229**, 690.
[751] B. Öbrink and Å. Wasteson, *Biochem. J.*, 1971, **121**, 227.
[752] N. A. Kefalides, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 226.
[753] L. Forrest and D. S. Jackson, *Biochim. Biophys. Acta*, 1971, **229**, 681.
[754] B. M. Fung and P. Trautmann, *Biopolymers*, 1971, **10**, 391.
[755] M. Moczar and E. Moczar, *Comp. Biochem. Physiol.*, 1971, **39B**, 173.
[756] M. E. Grant, F. S. Steven, D. S. Jackson, and L. B. Sandberg, *Biochem. J.*, 1971, **121**, 197.

The α_1- and α_2-chains of bovine and rat tendon collagen have molecular weights in the expected region of 1×10^5.[757] Heterogeneity of the α-components of collagens from lower species was also determined using polyacrylamide gel electrophoresis in sodium dodecyl sulphate, the α_1-components of cod and dog-fish skins having molecular weights intermediate between those of mammalian α_1 and α_2. The same technique has been used to analyse the α_1- and α_2-chains of acid-soluble collagen from calf and rat skin, carp swim bladder, and the soluble collagen from ray.[758]

In studies on chick embryo calvaria protocollagen, a precursor of proto-α_1 was identified.[759] The synthesis and extrusion of collagen by freshly isolated cells from chick embryo tendon has been investigated.[760]

The carbohydrate fraction released by proteolysis of body-wall connective tissue from the sea anemone (*Metridium dianthus*) was quantitatively recovered by gel filtration of the digest.[761] The carbohydrate components comprised galactose (9.5%), mannose (1.3%), glucose (12%), fucose (1.1%), ribose (0.1%), and 2-amino-2-deoxyhexose (1.5%). Since the mesenchymal tissue containing the collagen was free from glycosaminoglycans, it was concluded that the latter plays no part in collagen fibril formation or stabilization. Pepsin-solubilized collagen from *M. dianthus* contained, in its purified form, galactose (3.2%), glucose (3.8%), mannose (0.35%), fucose (0.3%), xylose (0.02%), 2-amino-2-deoxygalactose (0.1%), and 2-amino-2-deoxyglucose (0.4%).[762] Collagenase digestion and subsequent fractionations yielded a glycopeptide fraction, which contained 30% of the original carbohydrate consisting of mannose (3.5), fucose (0.7), xylose (0.1), 2-amino-2-deoxygalactose (0.5), and 2-amino-2-deoxyglucose (4.3 moles per mole). The principal units were released from the glycopeptide in the order fucose, mannose, and 2-amino-2-deoxyhexose on acid hydrolysis. Further studies indicated that the glycopeptide linkage involves 2-acetamido-2-deoxyglucose and asparagine residues.

The biosynthesis of collagen by the sex skin of oestrogen-treated monkeys [763] and the biosynthesis of the hydroxylysine-linked disaccharide unit of collagens from various species [764–766] have been studied.

Glycogens

Enzymic explorations of the structure of glycogen have been reviewed [767] and new proposals have been made for the arrangements of the unit chains.[199]

[757] B. C. Sykes and A. J. Bailey, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 340.
[758] H. Furthmayr and R. Timpl, *Analyt. Biochem.*, 1971, **41**, 510.
[759] P. K. Müller, E. McGoodwin, and G. R. Martin, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 110.
[760] P. Dehm and D. J. Prockop, *Biochim. Biophys. Acta*, 1971, **240**, 358.
[761] R. L. Katzman and R. W. Jeanloz, *Biochim. Biophys. Acta*, 1970, **220**, 516.
[762] R. L. Katzman and A. L. Oronsky, *J. Biol. Chem.*, 1971, **246**, 5107.
[763] J. P. Bentley, H. Nakagawa, and G. H. Davies, *Biochim. Biophys. Acta*, 1971, **244**, 35.
[764] R. G. Spiro and M. J. Spiro, *J. Biol. Chem.*, 1971, **246**, 4899.
[765] M. J. Spiro and R. G. Spiro, *J. Biol. Chem.*, 1971, **246**, 4910.
[766] R. G. Spiro and M. J. Spiro, *J. Biol. Chem.*, 1971, **246**, 4919.
[767] W. J. Whelan, *Biochem. J.*, 1971, **122**, 609.

A rapid enzymic method for the estimation of glycogen in small tissue samples depends on the combined action of α-glucosidase and α-amylase followed by enzymic determination of the D-glucose released using glucose oxidase and peroxidase.[768] In view of the high specificity of the enzyme system for glycogen, it is possible to make direct determinations.

An enzymic method for the determination of chain lengths of glycogen and other polysaccharides is based on oxidation with periodate and subsequent determination of the glycerol arising from terminal, non-reducing residues using glycerol kinase.[150] Application of the method to glycogens from rabbit and rat liver and *Ascaris lumbricoides* gave values of 14, 13, and 12, respectively, for the unit-chain lengths. An alternative method for determining the average chain lengths of glycogens utilizes the combined action of dextrin-1,6-glucosidase-transferase and β-amylase for debranching and liberation of terminal, non-reducing D-glucose residues, respectively, the latter being determined.[149] A third method involves the use of an isoamylase, which completely debranched glycogen but did not depolymerize the unit chains.[148]

Studies on the structure and metabolism of glycogen from liver and muscle tissues of human anencephalic babies showed that the glycogen contents of both tissues increase with gestation age, the muscle material having a slightly lower degree of branching than the corresponding liver glycogens.[769]

Isoamylase extensively cleaved the α-1,6-D-glucosidic branching linkages of rabbit-liver and oyster glycogen in a specific fashion to yield α-1,4-linked unit chains of $\overline{DP}$ 15 and 11, respectively.[770] The distribution of chain lengths of these products lay in the range 3—50. Pullulanase released malto-oligosaccharides of $\overline{DP}$ 7—8 from the glycogens to an extent of *ca.* 30%, and the branches in the pullulanase limit dextrins were cleaved completely by isoamylase to yield linear chains of $\overline{DP}$ 21 and 16 for the rabbit and oyster materials, respectively. The branching patterns in the glycogens were discussed on the basis of these findings.

The action of β-amylase on rabbit-liver and shellfish glycogens was shown to take place in two stages.[218] In the first, rapid reaction, the polysaccharide was degraded to a novel limit dextrin in which the unbranched chains were shortened to three or four residues in length. In the second, slower stage, the limit dextrin was degraded to the classical β-limit dextrin in which the unbranched side-chains were two or three residues in length.

Investigation of glycogenolysis and glycogen synthesis in a cell-free system from rat liver led to the recognition of a new glycogen polymer, which, in view of its partial resistance to β-amylase, appeared to be highly branched.[771]

[768] F. Huijing, *Clin. Chim. Acta*, 1970, **30**, 567.
[769] D. J. Manners, W. H. Schutt, J. R. Stark, and V. Thambyrajah, *Biochem. J.*, 1971, **124**, 461.
[770] H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 1971, **237**, 422.
[771] R. B. Scott and L. W. Cooper, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1071.

The variation in content of liver glycogen of chicks from day of hatch to adulthood has been determined.[772] The accompanying metabolic processes were studied. The glycogen in isolated mucosal and serosal fractions of turtle (*Pseudemys scripta*) bladder was measured and characterized; seasonal variations were observed for the parameters.[773] Investigation of the circadian variations in carbohydrate parameters in two teleosts (*Notopterus notopterus* and *Colisa fasciata*) revealed that the glycogen content of certain tissues shows cyclic variations over 24 h and that the variations are related to the habits of the species.[774]

Sections on the biosynthesis and degradation of glycogen are included in a review on metabolic regulation by chemical modification of enzymes.[775] A review[776] and the results of recent investigations[777, 778] of glycogen phosphorylase, an enzyme involved in the metabolic regulation of glycogen, have been published. Enzymes involved in the biosynthesis and degradation of rabbit skeletal-muscle glycogen were found to be bound to the polysaccharide.[779] A fraction containing a protein–glycogen complex was obtained from rabbit muscle by three different methods.[780] The 120 *S* component of this fraction consists of glycogen particles to which enzymes are attached.

The rapid action of glycogen phosphorylase on rabbit-liver and skeletal-muscle and shellfish glycogen was considered to arise from the high concentration of chain ends at the polysaccharide surfaces.[219] The effect on the rate of phosphorylation of debranching the polysaccharide chains with pullulanase was investigated.

The biosynthesis of glycogen in the hearts of normal and diabetic rats[781] and rat Novikoff ascites hepatoma cells[782] has been studied. Data on the biosynthesis of glycogen by rat and mouse ascites tumour cells revealed that they phosphorylate exogenous 2-amino-2-deoxy-D-glucose readily and transform a considerable proportion of the 2-amino-2-deoxy-D-glucose 6-phosphate produced into glycogen.[783]

[772] K. L. Raheja, J. G. Snedecor, and J. A. Freedland, *Comp. Biochem. Physiol.*, 1971, **39B**, 237.
[773] M. E. Le Fevre, L. J. Dox, J. F. Gennaro, and W. A. Brodsky, *Biochim. Biophys. Acta*, 1971, **241**, 628.
[774] P. V. Narasimhan and B. I. Sundararaj, *Comp. Biochem. Physiol.*, 1971, **39B**, 89.
[775] H. Holzer and W. Duntze, *Ann. Rev. Biochem.*, 1971, **40**, 345.
[776] E. H. Fischer, A. Pocker, and J. C. Saari, 'Essays in Biochemistry', ed. P. N. Campbell and F. Dickens, Academic Press, London, 1970, Vol. 6, p. 23.
[777] V. Kahn and J. J. Blum, *Arch. Biochem. Biophys.*, 1971, **143**, 80.
[778] V. Kahn and J. J. Blum, *Arch. Biochem. Biophys.*, 1971, **143**, 92.
[779] S. DiMauro, W. Trojaborg, P. Gambetti, and L. P. Rowland, *Arch. Biochem. Biophys.*, 1971, **144**, 413.
[780] F. Meyer, L. M. G. Heilmeyer, R. H. Haschke, and E. H. Fischer, *J. Biol. Chem.*, 1970, **245**, 6642.
[781] L. H. Opie, K. R. L. Mansford, and P. Owen, *Biochem. J.*, 1971, **124**, 475.
[782] S. Karasaki, *J. Ultrastruct. Res.*, 1971, **35**, 181.
[783] T. Sukeno, H. Kikuchi, H. Saeki, and S. Tsuiki, *Biochim. Biophys. Acta*, 1971, **244**, 19.

Shellfish glycogen was demonstrated to form a complex with glycogen phosphorylase, as judged from elution patterns on Sepharose 6B.[784] Aspects of glycogen metabolism in the rat pinworm (*Syphacia muris*) have been investigated.[785]

Glycosaminoglycuronans, Glycosaminoglycans, and their Protein and Peptide Derivatives

Papers presented at the 2nd European Symposium on Connective Tissue Research have been published.[743] Contributions included papers on the biosynthesis, occurrence, enzymic degradation, and immunology of glycosaminoglycans and their natural derivatives, and their association with collagen, *etc.*

Occurrence, Isolation, Measurement, and Structure.—Methods for determining the sulphur contents of glycosaminoglycans have been described. Sodium rhodizonate, which forms a red colour with barium ions, was employed in one procedure, the reduction of colour intensity of a standard amount of barium chloride indicating the quantity of sulphate present in a sample.[94] The quoted range of the method was 0—12 μg of sulphate. A procedure for the estimation of sulphate contents of less than 1 μg of glycosaminoglycan is based on the finding that the electrophoretic mobilities of the polymers in 0.1N hydrochloric acid are proportional to their sulphate contents.[95]

The c.d. traces obtained for aqueous solutions of the various glycosaminoglycans were all different.[786] Two bands in the region of amide and carboxyl transitions were observed. The first negative band was common to all polymers, whereas the characteristics of the second band were dependent upon polymer structure. 1,4-Linked 2-amino-2-deoxyhexose constituents gave rise to bands at wavelengths different from those arising from 1,3-linked 2-amino-2-deoxyhexose constituents.

The ^{1}H n.m.r. spectra of hyaluronic acid, chondroitin 4- and 6-sulphates, and dermatan sulphate were readily distinguishable.[787] The spectral data for a number of heparins, coupled with information obtained chemically for model compounds, indicated that they are composed principally of 1,4-linked α-L-idopyranosyluronic acid and 2-amino-2-deoxy-α-D-glucopyranosyl residues. The sulphate groups are located on position 2 of the former and positions 2 and 6 of the latter residues. D-Glucopyranosyluronic acid residues appeared to be only minor constituents of the heparins. The spectrum for heparan sulphate indicated that the structure is more complex than the structures of glycosaminoglycans possessing disaccharide

[784] V. Kahn and J. J. Blum, *Arch. Biochem. Biophys.*, 1971, **145**, 382.
[785] H. Van Den Bossche, J. Schaper, and M. Borgers, *Comp. Biochem. Physiol.*, 1971, **38B**, 43.
[786] A. L. Stone, *Biopolymers*, 1971, **10**, 739.
[787] A. S. Perlin, B. Casu, G. R. Sanderson, and L. F. Johnson, *Canad. J. Chem.*, 1970, **48**, 2260.

repeating units, whereas the spectrum obtained for keratan sulphate is somewhat inconsistent with the structure usually proposed.

It has been demonstrated that electrophoresis in polyacrylamide gels could be used to estimate the molecular weight of glycosaminoglycans on a micro scale.[788] The distance of migration of hyaluronic acid, chondroitin 4-sulphate, and chondroitin 6-sulphate was shown to vary linearly with the logarithm of the molecular size for each set of homologous polymers.

Assessment of the physicochemical properties of dilute solutions of hyaluronic acid by osmometry, light scattering, and diffusion sedimentation confirmed the flexible coiling nature of this polymer.[789] Using a model, a double logarithmic plot of the product of intrinsic viscosity and $\bar{M}_w$ against the degree of polymerization for the range $\bar{M}_w = 1—2 \times 10^4$ permitted a straight-line fit of the data available for hyaluronic acid, heparin, and the chondroitin sulphates.[158] The results suggested similarity of the short-chain hydrodynamic behaviour of these polymers.

Extensive autoxidative degradation of hyaluronic acid was achieved using an electrolysis cell and iron–edta as reductant at neutral pH.[324] The level of uronic acid fell at a rate which paralleled the formation of reducing properties. Terminal, reducing 2-acetamido-2-deoxy-D-glucose was produced at one-quarter of the apparent rate of generation of reducing sugar. Whereas reducing 2-acetamido-2-deoxy-D-glucose was evenly distributed between the dialysable and non-dialysable products, the remaining D-glucuronic acid was largely non-dialysable.

A method for the estimation of *N*-sulphate and *N*-acetate groups in glycosaminoglycans is based on nitrous acid treatment followed by reaction with 3-methyl-2-benzothiazolone hydrazone.[13] Amino- and sulphamido-groups could be determined directly using the intact polymer, whereas acetamido-groups could be determined after hydrolysis of the polymer to convert the acetamido-groups into amino-groups. The method was applied to chondroitin 4- and 6-sulphates and heparin.

Calculation of the total amount of anionic groups in sulphated glycosaminoglycans by summation of the data for individual determinations of sulphate and hexuronic acid residues can be inaccurate.[790] It was established that the complex cation hexa-amminecobalt(III) reacted with all anionic groups and could be used for the total determination of such groups in glycosaminoglycans. As determined by this method, the total anionic contents of chondroitin sulphate–keratan sulphate–protein hybrid and chondroitin 4-sulphate–peptide exceeded the summation values by 2—4 and 10—12%, respectively.

Chondroitin sulphates and related molecules underwent periodate oxidation in aqueous solution at a very slow rate.[791] However, the rate

[788] M. B. Mathews and L. Decker, *Biochim. Biophys. Acta*, 1971, **244**, 30.
[789] R. L. Cleland and J. L. Wang, *Biopolymers*, 1970, **9**, 799.
[790] S. M. Bychkov, W. N. Harlamova, and S. P. Kasakova, *Biokhimiya*, 1971, **36**, 1001.
[791] J. E. Scott and M. J. Tigwell, *Biochem. J.*, 1971, **123**, 46P.

increased dramatically when *p*-dioxan was used as the solvent. These observations were explained in terms of the degree of competition of the solvent for the oxidizable diol groups, and their application to the Schiff reaction was discussed.

Chondroitin 4-sulphate was degraded by a preparation from tadpoles, whereas dermatan sulphate, chondroitin 6-sulphate, and heparin were completely resistant.[792] This selectivity was proposed as a basis for the preparation of chondroitin 6-sulphate from mixtures of chondroitin 4- and 6-sulphates, and for the determination of the relative amounts of the two isomers in a mixture.

The thermodynamic parameters associated with the binding of acridine orange, acriflavine, azure A, crystal violet, methylene blue, and toluidine blue with chondroitin sulphate have been examined by pulse radiolysis.[793] The dependence of the extent of destruction of the complexes upon temperature was compared with data for their stability in the presence of ethanol and urea. The interaction of chondroitin sulphate with charged substances such as toluidine blue and bovine albumin was considered to be due to the reversible formation of a semipolar or ionic bond.[739] It was suggested that chondroitin sulphate possesses cation-exchange properties.

The ester sulphate and uronic acid contents, the anticoagulant properties, the gel-electrophoretic and gel-filtration patterns, and the i.r. spectrum of ([^{35}S]sulphamido)heparin, prepared by a route involving de-*N*-sulphation and re-*N*-sulphation, were consistent with the complete restoration of the sulphamido-groups.[794] The structure of heparin was discussed in the light of these and previous studies.

Component analysis and u.v. and ^{1}H n.m.r. spectral data obtained for a disaccharide representing 75% of the products of degradation of heparin by *Flavobacterium heparinum* enzymes indicated that it has the structure *O*-(4-deoxy-α-L-*threo*-hex-4-enopyranuronosyl 2-sulphate)-(1 → 4)-2-deoxy-2-sulphamido-D-glucose 6-sulphate.[795] The anomeric configuration of the unsaturated uronic acid residue of the disaccharide was deduced primarily from the fact that the olefinic 4-proton experiences long-range coupling with H-2 of the same residue. It was concluded that a [→ 4)-*O*-(α-L-idopyranuronosyl 2-sulphate)-(1 → 4)-*O*-(2-deoxy-2-sulphamido-α-D-glucopyranosyl 6-sulphate)-(1 →] repeating sequence occurs in heparin.

Oligosaccharides derived from keratan sulphate by degradation with an apparently specific enzyme from *Coccobacillus* sp. have been partially characterized.[796] Approximately 60% of the products were non-sulphated oligosaccharides and enzyme-resistant glycopeptides containing 80% of the original D-mannose units. The remaining oligosaccharides were sulphated

[792] J. E. Silbert and S. DeLuca, *J. Biol. Chem.*, 1970, **245**, 1506.
[793] D. M. Power, J. S. Moore, G. O. Phillips, and J. V. Davies, *Internat. J. Radiation Biol.*, 1971, **20**, 111.
[794] A. G. Lloyd, G. Embery, and L. J. Fowler, *Biochem. Pharmacol.*, 1971, **20**, 637.
[795] A. S. Perlin, D. M. Mackie, and C. P. Dietrich, *Carbohydrate Res.*, 1971, **18**, 185.
[796] S. Hirano and K. Meyer, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1371.

to various degrees and varied in their composition and chain length. One of the major fractions was characterized as a tetrasaccharide comprising D-galactose, 2-acetamido-2-deoxy-D-glucose, and sulphate (2 : 2 : 3).

A [^{3}H]-labelled iron–chondroitin sulphate colloid has been synthesized and the distribution of the label in mice on injection of the colloid was examined.[797] Water-insoluble covalent derivatives of chondroitin sulphate, dermatan sulphate, heparin, and heparan sulphate have been prepared.[798] The derivative of chondroitin sulphate was employed in an assay for hyaluronidase activity. An insoluble, covalent derivative of heparin has also been used as an affinity-chromatography matrix for the purification of enzymes.[799] The role of hyaluronic acid, chondroitin 4- and 6-sulphates, dermatan sulphate, heparin, and heparan sulphate as cofactors in the formation of platelet clumping substance has been investigated.[800]

Three methods have been used to prepare protein–polysaccharide light (PPL) complexes from human intervertebral discs; the compositions of the products from each method were identical.[801] Chromatography of the PPL preparations on DEAE–Sephadex separated them into five or six characteristic subfractions. Isoelectric focusing of the PPL preparations resulted in their dissociation into three major subunits, together with 2—4 minor components; isoelectric focusing of the subfractions from the ion-exchange chromatography gave the same patterns. The three major subunits in each case were sulphated and had low pI values. The PPL preparations interacted with tropocollagen in the isoelectric-focusing column, yielding a single complex zone having a pI value intermediate between those of the parent macromolecules.[750] The initial interaction was independent of the length of the glycosaminoglycan chains, and the complex zone was shown to contain both uronic acid and hydroxyproline. Chemical and enzymic modification of the PPL preparations demonstrated that the sulphate groups are largely responsible for the interaction with tropocollagen in the column.

Since it is established that chains of chondroitin sulphate and keratan sulphate are stable to strong alkali (2N sodium hydroxide at room temperature for at least 120 h), it has been possible to show the existence of two populations of glycopeptide linkage regions in chondroitin sulphate–protein and keratan sulphate–protein from the light and heavy fractions of the proteoglycan from human *nucleus pulposus*.[802] These linkage regions were classified as alkali-labile, which were split by 0.2N sodium hydroxide at room temperature, and alkali-stable, which were resistant to 0.2N

[797] Y. Nakanishi, M. Kishi, and Y. Minaki, *Yakugaku Zasshi*, 1970, **90**, 683.
[798] P. H. Iverius, *Biochem. J.*, 1971, **124**, 677.
[799] T. Olivecrona, T. Egelrud, P. Iverius, and U. Lindahl, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 524.
[800] H. Murase, H. Ijiri, T. Shimamoto, I. Kobayashi, T. Shimamoto, and H. Yamazaki, *Blood*, 1971, **37**, 684.
[801] V. Podrazky, F. S. Steven, M. E. Grant, and D. S. Jackson, *Biochim. Biophys. Acta*, 1971, **221**, 549.
[802] H. Lyons and J. A. Singer, *J. Biol. Chem.*, 1971, **246**, 277.

sodium hydroxide but were split by 2N sodium hydroxide at room temperature. Of the glycopeptide linkages of both chondroitin sulphate and keratan sulphate in the light fraction, 62% were of the former type, whereas the corresponding figure for the heavy fraction was 78%. The relative molecular sizes and the amounts of chondroitin sulphate and keratan sulphate bound by either linkage in both heavy and light fractions were investigated. It was observed that reducing-end group determination of the chondroitin sulphate by the copper-reduction method gave reliable values for the chain length of the more alkali-labile material when compared with values based upon determination of the linkage-region components D-xylose and D-galactose.

Dermatan sulphate and heparan sulphate were identified in amyloid preparations from various human organs, the total glycosaminoglycan contents lying in the range 0.2—1.8% dry weight of tissue.[803]

Cultures of ten established mammalian cell lines of different origin (lymphoid, epithelial, and fibroblastic) were found to produce and secrete into the medium a mixture of chondroitin 4- and 6-sulphates and dermatan sulphate.[804] The proportions of the individual mucopolysaccharides differed strikingly among the cell lines, and the use of chondroitin sulphate lyase ABC for their determination revealed the presence of some enzyme-resistant structures. Papain digestion of the acid-precipitated fractions from extracts of mammalian cell lines (CHO, Don C, L5178Y, BHK-C13, L-929, and HeLa) liberated acid-soluble heparan sulphate in each case.[805] It was concluded that heparan sulphate is a general cellular constituent rather than a differentiated cell product. Labelling experiments have revealed the production of chondroitin 4- and 6-sulphates and dermatan sulphate with additional sulphate contents or hybridized uronic acid components by cell lines of non-fibroblastic (human He-La-S_3) and fibroblastic (mouse L-929) origin.[806]

Small quantities of glycosaminoglycans have been detected in human cerebrospinal fluid.[807] Mean values of 8.1 and 24.8 mg/g creatinine were obtained for the hexuronic acid and 2-amino-2-deoxyhexose contents, respectively, of the non-dialysable constituents of human sera from subjects in the age range 18—75 years.[808] Mean values for lower age groups were greater. It was shown that the amount of bound hexuronic acid precipitated from human urine by quaternary ammonium salts could be as little as 25% of the value obtained by direct uronic acid analysis of the non-dialysable material. The levels of total glycosaminoglycan excretion in urine have been determined for children.[809] Experiments involving the

[803] M. Pras, Z. Nevo, M. Schubert, J. Rotman, and R. Matalon, *J. Histochem. Cytochem.*, 1971, **19**, 443.
[804] H. Saito and B. G. Uzman, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 723.
[805] P. Kraemer, *Biochemistry*, 1971, **10**, 1445.
[806] S. Suzuki, K. Kojima, and K. R. Utsumi, *Biochim. Biophys. Acta*, 1970, **222**, 240.
[807] G. Constantopoulos and A. S. Dekaban, *J. Neurochem.*, 1970, **17**, 117.
[808] L. Rosenfeld, *Clin. Chim. Acta*, 1971, **31**, 263.
[809] M. Mohanram and V. Reddy, *Clin. Chim. Acta*, 1971, **34**, 93.

precipitation of urinary glycosaminoglycans by quaternary ammonium salts revealed that the polysaccharides from juvenile urine comprise hyaluronic acid (0.8%), chondroitin 4-sulphate (60.6%), chondroitin 6-sulphate (29.1%), and heparan sulphate (1.7%).[810] Chondroitin 4- and 6-sulphates have also been recognized by others as the major glycosaminoglycans of juvenile urine.[811] Isolation of the non-ultrafilterable glycosaminoglycans from the urine of men, by a procedure which included digestion with pronase and sialidase, fractionation on ECTEOLA-cellulose, and preparative electrophoresis, showed that the polysaccharides comprise hyaluronic acid (4%), chondroitin (2%), chondroitin sulphate (60%), dermatan sulphate (1%), heparan sulphate (15%), and keratan sulphate (18%).[812] Chondroitin sulphate–dermatan sulphate hybrids occurred in trace amounts. Two distinct fractions of chondroitin sulphate were detected, having sulphate : 2-amino-2-deoxyhexose ratios of 0.5 : 1 and 1.0 : 1. The molar ratios of total sulphate to 2-amino-2-deoxyhexose for the heparan sulphate fractions varied from 0.5 : 1 to 0.9 : 1, but the molar ratio of *N*-sulphate to 2-amino-2 deoxyhexose was constant at 0.5 : 1. The molar ratio for sulphate and 2-amino-2-deoxyhexose in the case of keratan sulphate varied from 0.2 : 1 to 0.7 : 1. Human urinary chondroitin sulphates have been analysed by use of chondroitin sulphate lyases ABC and AC, and chondro-4- and 6-sulphatases.[813] Minor constituents of unsaturated, non-sulphated and unsaturated, disulphated disaccharides were demonstrated in the products from chondroitin sulphate lyase treatment in addition to the major components of 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranuronosyl)-D-galactose 4- and 6-sulphates, thus indicating the presence of under- and over-sulphated chondroitin sulphates. Component analysis of an oversulphated chondroitin sulphate isolated from human urine showed that the molecule contains hexuronic acid, 2-amino-2-deoxy-D-galactose, and sulphate (0.81 : 1.00 : 1.52), and that it is excreted as a glycopeptide having L-serine as the predominant amino-acid.[814] Digestion with chondroitin sulphate lyase ABC indicated the presence of two types of disaccharide unit in the original molecule, *viz.* 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranuronosyl)-D-galactose 4-sulphate and 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranuronosyl 2- or 3-sulphate)-D-galactose 4-sulphate. The heparan sulphate of human urine has been found to be heterogeneous and polydisperse with an average molecular weight of 1.8×10^4.[815]

Chest and sex skin from oestrogen-treated, female rhesus monkeys and skin from untreated monkeys have been examined for their glycosaminoglycan contents.[763] The major difference between the quantitative values

[810] R. Cherian, E. V. Chandrasekaran, and B. K. Bachhawat, *Indian J. Biochem.*, 1970, **7**, 174.
[811] J. Onisawa and T. Y. Lee, *Biochim. Biophys. Acta*, 1970, **208**, 144.
[812] E. Wessler, *Biochem. J.*, 1971, **122**, 373.
[813] K. Murata, T. Harada, T. Fujiwara, and T. Furuhashi, *Biochim. Biophys. Acta*, 1971, **230**, 583.
[814] D. P. Varadi and C. Griffiths, *Biochim. Biophys. Acta*, 1971, **230**, 248.
[815] N. Taniguchi, *Clin. Chim. Acta*, 1970, **30**, 801.

obtained was that the sex skin contained five times as much hyaluronic acid as skin from any other source.

The effect of sulphated proteoglycans from bovine intervertebral discs and hyaluronic acid proteoglycan from bovine synovial fluid on the swelling behaviour of gelatin gels immersed in osmotically active solutions of dextran was to affect markedly the internal osmotic contribution to the swelling pressure of the gel.[816] The internal osmotic pressures were considerably in excess of the sum of the osmotic activities of the individual components, and this was explained in terms of an entropic interaction between the gelatin and proteoglycan molecules. The role of the glycosaminoglycans in the physical behaviour of tissues was discussed in the light of these results.

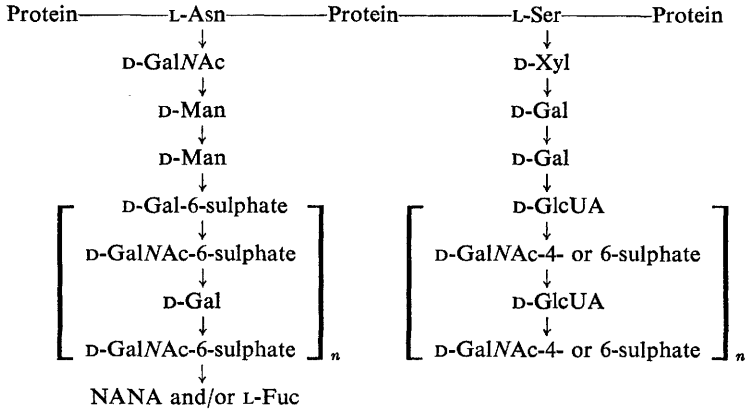
An investigation of the depolymerization of hyaluronic acid from bovine synovial fluid by ferrous ions and L-ascorbic acid showed that many organic buffers inhibit depolymerization.[817] Even variation of the order of mixing had a profound effect. A parallelism was observed between the degree of depolymerization of the hyaluronic acid and the extents of oxygen absorption and oxidation of L-ascorbic acid.

Bovine vitreous humour was separated into excluded and included fractions by gel filtration on Sepharose 4B.[818] There was no evidence for the reversible dissociation of the excluded material on treatment with L-ascorbic acid. The retarded material, however, showed evidence of dissociation when eluted with 1M sodium chloride and of association in 0.1M phosphate buffer of pH 7.2.

Proteoglycans were extracted from bovine cornea by high-speed homogenization with water.[819] Purification by chromatography on ECTEOLA-cellulose and gel filtration yielded six fractions having different contents of the chondroitin sulphate and keratan sulphate. Keratan sulphate predominated in five of the fractions, and some fractions had low sulphate contents. However, two proteoglycans isolated from the homogenate by chromatography on Dowex 1×2 contained predominantly chondroitin sulphate along with keratan sulphate, and also contained stoicheiometric amounts of sulphate. Hydrolysis of one of the keratan sulphate-rich proteoglycans yielded a small amount of a compound chromatographically indistinguishable from 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucosylamine. Proteolysis of the proteoglycan yielded a glycopeptide containing L-aspartic acid and D-mannose (1 : 2). On the basis of these results, a structure (78) was proposed for the corneal proteoglycan.

Periodate oxidation of calf corneal glycoproteins and proteoglycans *in situ* showed that the chondroitin sulphates and keratan sulphate are resistant.[755]

[816] F. A. Meyer, W. D. Comper, and B. N. Preston, *Biopolymers*, 1971, **10**, 1351.
[817] A. Herp, M. Harris, and T. Rickards, *Carbohydrate Res.*, 1971, **16**, 395.
[818] J. C. Caygill, *Biochim. Biophys. Acta*, 1971, **244**, 421.
[819] H. W. Stuhlsatz, R. Kisters, A. Wollmer, and H. Greiling, *Z. physiol. Chem.*, 1971, **352**, 289.



(78)

Glycosaminoglycuronans from bovine brain have been separated by ion-exchange chromatography and were identified as hyaluronic acid and chondroitin 4- and 6-sulphates.[820] Some uronic acid-containing components were resistant to chondroitin sulphate lyase AC, whereas β-elimination studies indicated the involvement of L-serine and L-threonine in the glycopeptide linkages of the chondroitin sulphates. The o.r.d. spectra of glycosaminoglycuronans as their methylene-blue complexes suggested that bovine brain chondroitin sulphates, as well as authentic chondroitin 4- and 6-sulphates, possess right-hand screw helices, whereas heparin possesses a left-hand screw helix. Neutral-sugar analysis of the total glycosaminoglycan and hyaluronic acid fractions, isolated from lipid-extracted and proteolysed bovine brain by precipitation with cetyltrimethylammonium bromide, revealed the presence of xylose and ribose, but the absence of arabinose.[821] The inability to detect arabinose is in contrast to previously published results (A. H. Wardi, W. S. Allen, D. L. Turner, and Z. Stary, *Arch. Biochem. Biophys.*, 1966, **117**, 44, and Vol. 1 of this Report, ref. 280).

Preparative agarose gel electrophoresis of heparan sulphate isolated from bovine lung tissue yielded four distinct fractions (*A*—*D*).[822] Fraction A alone contained *N*-acetyl groups, together with a small proportion of *O*-sulphate groups, whereas fractions *B*—*D* contained *N*- and *O*-sulphate groups in different amounts. Degradation of the heparan sulphates with heparinases from *Flavobacterium heparinum* showed that fraction *A* alone contained non-sulphated 2-acetamido-2-deoxy-D-glucose units, whereas the only forms of 2-amino-2-deoxy-D-glucose in fraction *B* were 2-deoxy-2-sulphamido-D-glucose and 2-deoxy-2-sulphamido-D-glucose 6-sulphate;

[820] K. Saigo and F. Egami, *J. Neurochem.*, 1970, **17**, 633.
[821] R. L. Katzman, *J. Neurochem.*, 1971, **18**, 1187.
[822] C. P. Dietrich, H. B. Nader, L. R. G. Britto, and M. E. Silva, *Biochim. Biophys. Acta*, 1971, **237**, 430.

the latter form also occurred in fractions *C* and *D*. All four fractions possessed very low anticoagulant activity, but were able to induce the formation of heparinases by *F. heparinum*.

A homogeneous proteoglycan containing dermatan sulphate (20%) and chondroitin sulphate (60%) was isolated from bovine arterial tissue.[823] A randomly coiled shape for the proteoglycan (molecular weight 2×10^6) was deduced from light-scattering measurements; the molecule consisted of a protein core carrying about eighty polysaccharide chains which are attached *O*-glycosidically to L-serine and L-threonine residues. Degradation of the proteoglycan with testicular hyaluronidase yielded dermatan sulphate and a series of oligosaccharides. A tetrasaccharide and higher molecular-weight fractions contained both L-iduronic and D-glucuronic acids, indicating a copolymeric structure. The molar ratio of L-iduronic acid to D-glucuronic acid for the oligosaccharides was found to increase with increase in chain length, whereas the molar proportions of sulphate remained constant.

The protein–polysaccharide complexes of bovine nasal cartilage have been prepared by a method involving column chromatography on Sepharose 4B.[824] Recoveries were as high as 90%, and the products had similar carbohydrate and sulphate contents to the complexes obtained by density-gradient centrifugation. The light fraction of the proteoglycan from bovine nasal cartilage was cleaved by treatment with 0.1M hydrochloric acid in acetone.[825] On gel filtration of the products on 4% agarose, two retarded fractions having Stokes' radii of 134 and 47 Å were detected. Both materials contained protein (20% and 4%, respectively) and possessed different molar ratios of 2-amino-2-deoxygalactose to 2-amino-2-deoxyglucose (4.8 : 1 and 23 : 1, respectively). Viscometric studies indicated that the former material undergoes reversible pH-dependent aggregation with a transition point at pH 4.9, and it was concluded that the fraction represents a subunit of the native proteoglycan.

A micro method developed for the determination of the molecular weights of chondroitin sulphate is based on Sephadex G-200 and Sepharose 6B chromatography, and the use of standard chondroitin sulphate fractions.[826] The molecular weight distributions of chondroitin sulphate from bovine nasal septum, puppy compact bone, and chick epiphyseal plate were determined using this method, and the effect of variations in the ionic strength of the eluent was also studied. Chondroitin sulphate was isolated from bovine nasal septum by precipitation with cetylpyridinium chloride after digestion of the tissue with papain.[827] Gel filtration of the material before and after treatment with hyaluronidase

[823] H. Kreese, H. Heidel, and E. Buddecke, *European J. Biochem.*, 1971, **22**, 557.
[824] M. Janado, *J. Biochem.* (*Japan*), 1971, **69**, 1123.
[825] A. Serafini-Fracassini, W. H. Stimson, and L. Floreani, *Biochem. J.*, 1971, **122**, 101.
[826] Å. Wasteson, *J. Chromatog.*, 1971, **59**, 87.
[827] Å. Wasteson, *Biochem. J.*, 1971, **122**, 477.

yielded eleven subfractions, component analyses of which revealed their similarity to chondroitin sulphate. The individual fractions were essentially monodisperse and possessed molecular weights in the range 2.4—36 × 10^3. The relationship between the intrinsic viscosities and molecular weights indicated that the chondroitin sulphate molecules assume a shape intermediate between that of a random coil and a stiff rod. The electrostatic interaction of bovine nasal chondroitin sulphate and chondroitin sulphate proteoglycan with collagen was studied by chromatography on collagen gel and by partition-equilibrium techniques.[751] The observed binding between the macromolecules increased with decreasing pH and ionic strength, being significant under physiological conditions.

The association of calcium and phosphate ions with bovine tracheal cartilage chondroitin 4-sulphate and nasal proteoglycans, porcine skin dermatan sulphate, a highly sulphated shark cartilage chondroitin sulphate, and puppy-rib cartilage sodium and calcium proteoglycans was studied by equilibrium dialysis.[828] A higher concentration of calcium ions or a lower concentration of phosphate ions was found on the polymer side of the semipermeable membrane of the dialysis cell. The formation complex for the bound calcium was affected principally by the ionic strength and the calcium : D-glucuronic acid ratio of the solutions. Two calcium-binding forms of the proteoglycans were apparent. The mode of preparation and characteristics of the puppy proteoglycans were described. The calcium-binding properties of bovine bone and shark chondroitin sulphates have been compared using the murexide method and by inhibition of calcium phosphate precipitation.[829]

Purified proteoglycans from porcine laryngeal cartilage were fractionated by equilibrium density-gradient centrifugation in the presence of guanidine hydrochloride.[830] A continuous distribution of both uronic acid and protein was observed in the gradient extending from a fraction containing most of the uronic acid to one containing little of the uronic acid. Gel chromatography on Sepharose 2B showed all the fractions to be polydisperse and heterogeneous in composition, but the chondroitin sulphate chains appeared to have the same average size in all fractions. The results suggest that there are several core proteins in chondroitin sulphate proteoglycan differing in length and also in the type, distribution, and number of attached carbohydrate chains.

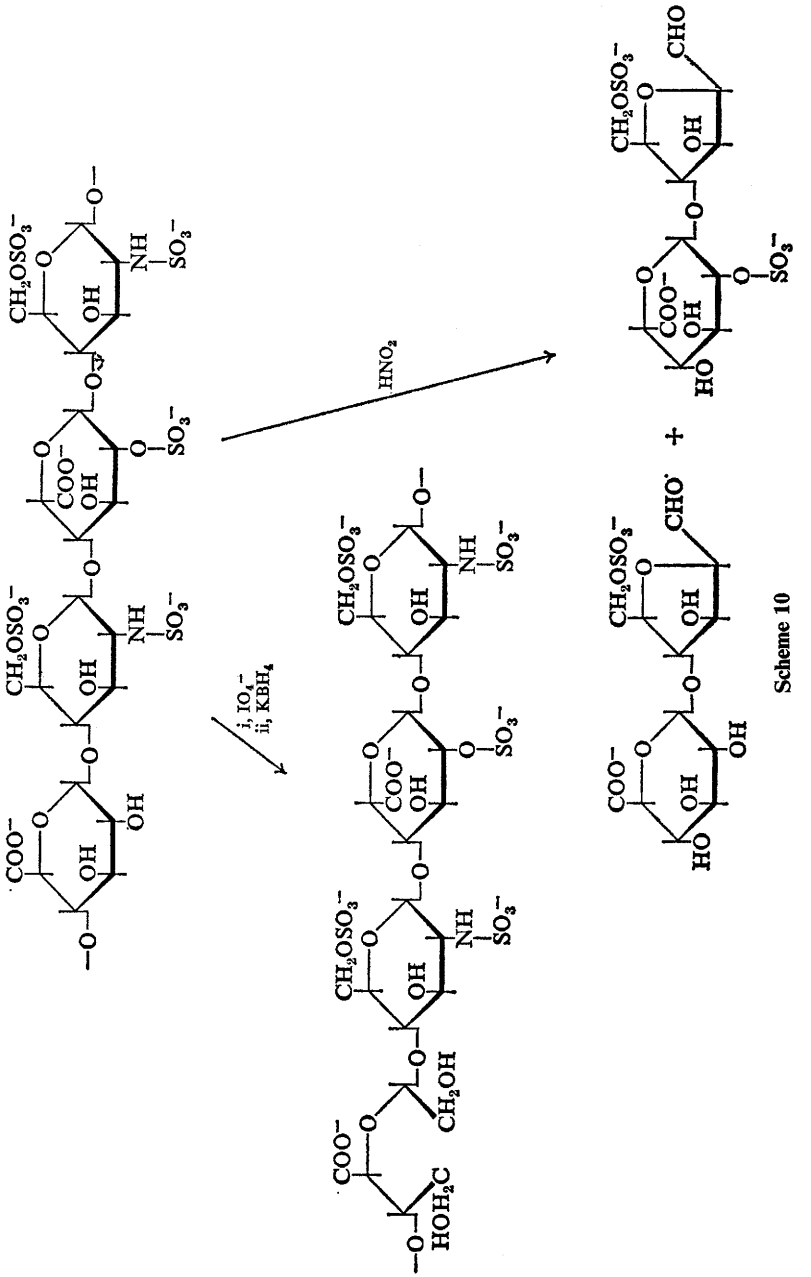
A series of extractions of porcine knee-joint cartilage with iso-osmotic sodium acetate brought one-third of the uronic acid content of the tissue into solution.[831] Purification of the extracted protein–polysaccharides by precipitation with 9-aminoacridine and gel filtration indicated that they are heterogeneous in size, the smallest molecules being most easily

[828] E. A. MacGregor and J. M. Bowness, *Canad. J. Biochem.*, 1971, **49**, 417.
[829] A. R. Chipperfield, *Biochem. J.*, 1970, **118**, 36P.
[830] C. P. Tsiganos, T. E. Hardingham, and H. Muir, *Biochim. Biophys. Acta*, 1971, **229**, 529.
[831] K. D. Brandt and H. Muir, *Biochem. J.*, 1971, **121**, 261.

extracted. There was a progressive increase in the protein content and in the molar ratio of 2-amino-2-deoxy-D-glucose to 2-amino-2-deoxy-D-galactose of successive extracts. In each preparation, this ratio was invariably largest for the smallest molecules. From the molar ratios of 2-amino-2-deoxy-D-galactose to pentose, it appeared that the chondroitin sulphate chains of each extract have an average length of 29 disaccharide units, although a somewhat higher proportion of shorter chondroitin sulphate chains occur in the larger molecules. In the final extract, where the largest molecules predominated, 50% of the molecules were reversibly dissociated by urea, whereas this reagent had no effect on the protein–polysaccharides of earlier extracts, even though they contained some large molecules. All except 2% of the remaining uronic acid content of the cartilage was solubilized after digestion with collagenase using urea and tris buffer.[832] The protein and keratan sulphate contents of the protein–polysaccharides of each extract were unrelated to their molecular sizes. High correlation between the molar ratios of 2-amino-2-deoxy-D-galactose to pentose and of 2-amino-2-deoxy-D-galactose to alkali-labile L-serine indicated that the chondroitin sulphate chains of the protein–polysaccharides are attached only to L-serine residues. Furthermore, it was estimated from these ratios that the chondroitin sulphate chains of each extract had the same average length, although this was shorter than that found for the chains of protein–polysaccharides extracted by sodium acetate. Alkali treatment also destroyed some of the L-threonine residues, indicating the attachment of keratan sulphate chains to them. It was concluded that the differences in size, composition, and physical state extended to all the cartilaginous protein–polysaccharides.

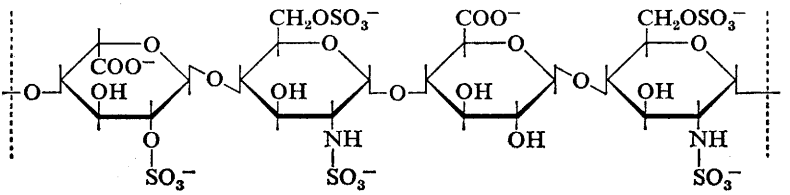
Degradation of purified heparin from porcine intestinal mucosae with nitrous acid at low temperature yielded four fractions which were characterized as non-sulphated uronic acid (*E*), non-sulphated uronosylanhydromannose (*F*), disulphated uronosylanhydromannose (*G*), and sulphated uronic acid (*H*).[833] Fractions *E* and *F* contained both D-glucuronic and L-iduronic acids, whereas fractions *G* and *H* contained only the L-*ido*-isomer. Thus, L-iduronic acid was recognized as the major sulphated uronic acid in heparin, and previous claims that nitrous acid treatment of heparin caused epimerization of D-glucuronic acid to L-iduronic acid residues were refuted. Periodate oxidation of the heparin destroyed 30% of the total uronic acid residues, including all the D-glucuronic acid residues, indicating that none of the latter are sulphated. The principal reactions occurring were assumed to be those shown in Scheme 10. Further studies of the reaction of nitrous acid with heparin yielded a series of glycosyl-serine compounds in which a uronic acid residue is located at the non-reducing terminals.[834] In addition, a tetrasaccharide with the proposed

[832] K. D. Brandt and H. Muir, *Biochem. J.*, 1971, **123**, 747.
[833] U. Lindahl and O. Axelsson, *J. Biol. Chem.*, 1971, **246**, 74.
[834] T. Helting and U. Lindahl, *J. Biol. Chem.*, 1971, **246**, 5442.



Scheme 10

structure uronosyl-(2-acetamido-2-deoxy-D-glucosyl)-uronosyl-anhydro-D-mannose was isolated. β-Glucuronidase liberated 33—100% of the terminal, non-reducing residues from these compounds as D-glucuronic acid, indicating that a large proportion of the glucuronidic linkages of heparin have the β-configuration. It was concluded that the structure (79) is involved in the polysaccharide chain.



(79)

Examination of the glycosaminoglycan contents of cat intervertebral discs by the cetylpyridinium chloride–cellulose column technique demonstrated that they consist of chondroitin sulphate and dermatan sulphate together with smaller amounts of hyaluronic acid and keratan sulphate.[835] The amounts of chondroitin sulphate and dermatan sulphate were directly related to the water content of the discs from which they were isolated.

Glycosaminoglycans isolated from rabbit bone-marrow cells comprise chondroitin 6-sulphate and small amounts of hyaluronic acid.[836] Gel filtration indicated that the chondroitin 6-sulphate exists both as a proteoglycan and as free polysaccharide chains. Recombination of the glycosaminoglycans and proteins obtained by equilibrium sedimentation yielded a precipitate, and it was suggested that the function of glycosaminoglycans in the leukocytes is to provide an acidic matrix to bind and neutralize the highly cationic proteins of the granules. Changes in the levels of aortic glycosaminoglycans of rabbits have been studied during pregnancy, enovid treatment, and hypercholesteraemia.[837] The major glycosaminoglycan isolated from rabbit compact bone was chondroitin 4-sulphate.[838] Component and other analyses of the bone glycosaminoglycans from normal and dexamethasone-treated animals were identical.

Guinea-pig costal cartilage contained up to 5.3% (dry weight) of 2-amino-2-deoxyhexose; 91% of this amino-sugar was derived from glycosaminoglycans.[839] Fractionation of the latter by the cetylpyridinium chloride–cellulose column technique showed that they comprise chondroitin 4- and 6-sulphates (88%), keratan sulphate (8%), and hyaluronic acid (3%). The

[835] W. F. Butler and C. M. Wells, *Biochem. J.*, 1971, **122**, 647.
[836] I. Olsson, *Exptl. Cell. Res.*, 1971, **67**, 416.
[837] K. J. Ho, J. E. Forestner, and P. Manalo-Estrella, *Proc. Soc. Exptl. Biol. Med.*, 1971, **137**, 10.
[838] L. Vejlens, *Z. physiol. Chem.*, 1971, **352**, 652.
[839] S. Lohmander, U. Friberg, and C. A. Antonopoulos, *Acta Chem. Scand.*, 1970, **24**, 3134.

concentrations of hyaluronic acid and chondroitin sulphate, and the molecular weight of the former, in the skin of normal guinea-pigs and those deprived of L-ascorbic acid have been measured.[840]

The glycosaminoglycan of three cellular fractions of Chinese hamster CHO-line cells was identified as (i) a cell-surface component removable with trypsin under conditions which prevented irreversible cell damage, (ii) a free and directly acid-soluble component of the 'cell sap', and (iii) a part of the residual acid precipitate that becomes soluble in acid following papain digestion.[841] The latter material could be divided into two further fractions. All fractions were identified as heparan sulphates, but the *N*- and *O*-sulphate contents of the glycosaminoglycuronan varied from fraction to fraction.

Data on the glycosaminoglycan contents of the skin of normal, athyroid, and L-tri-iodothyronine-treated athyroid rats have been published.[842] Normal skin was shown to contain hyaluronic acid (563), chondroitin 4-sulphate (69), chondroitin 6-sulphate (66), dermatan sulphate (220), heparin (71), and heparan sulphate (53 μg as μg uronic acid per g dry weight). The concentration of glycosaminoglycans in the skin and tracheal cartilage of normal and orchidectomized rats injected with testosterone has been measured.[843] The increase in concentration as a result of injection was attributed to an augmentation of the level of hyaluronic acid. Gel filtration and sedimentation analysis of heparin isolated from pronase-digested rat skin gave a molecular weight of 1.1×10^6.[844] The heparin proteoglycan was not depolymerized by dissociating agents such as urea, guanidine hydrochloride, and sodium chloride, but oxidative–reductive depolymerization with L-ascorbic acid gave rise to two fractions distinguishable by gel filtration and gel electrophoresis. A further study of these components led to the concept of a high molecular weight protein core, to which chains of heparin are bound by ascorbate-sensitive linkages. The xylosylserine linkages of the proteoglycan were found to be concentrated within the core; a core preparation, which represented only 8% of the weight of the undegraded macromolecule, contained 42% of the total L-serine. It was concluded that the heparin proteoglycan possesses a highly branched structure. Macromolecules containing glucuronic acid, which were degraded by hyaluronidase, have been identified in synaptosomes isolated from rat cerebral cortex.[845] The glycosaminoglycans of a transplantable chondrosarcoma of the rat, isolated after exhaustive proteolysis, comprised hyaluronic acid (1.2%) and chondroitin 4-sulphate (97.8%).[846] The latter was separated into three subfractions of different degrees of sulphation and

[840] P. J. Boumans and P. D. Mier, *Dermatologica*, 1970, **141**, 234.
[841] P. M. Kraemer, *Biochemistry*, 1971, **10**, 1437.
[842] J. A. Kofoed, *Experientia*, 1971, **27**, 702.
[843] J. A. Kofoed, C. E. Bozzini, and A. A. Tocci, *Acta Endocrinol.*, 1970, **63**, 193.
[844] A. A. Horner, *J. Biol. Chem.*, 1971, **246**, 231.
[845] B. W. Festoff, S. H. Appel, and E. Day, *J. Neurochem.*, 1971, **18**, 1871.
[846] H. U. Choi, K. Meyer, and R. Swarm, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 877.

chain length. Analysis of one subfraction (molecular weight 2.3×10^4) showed that it contained galactose and xylose. Direct extraction of the chondrosarcoma yielded two protein–polysaccharides having relatively similar carbohydrate compositions to those of the polysaccharides. Treatment of rat ascites hepatoma AH-130 cells with chondroitin sulphate lyase ABC demonstrated the presence of the chondroitin sulphates (particularly the 4-isomer) on the cell surface and indicated that these molecules contribute to the surface negative charge.[847]

Glycosaminoglycans from the cell layer and growth medium of mouse fibroblast line 3T6 were identified as chondroitin sulphate and heparan sulphate.[848] The cell layer contained the two glycosaminoglycans in equal amounts, whereas the former predominated in the growth medium. Different molecular-size distributions of the two glycosaminoglycuronans were indicated by gel filtration. Incorporation of radioactive precursors revealed that preparations of DNA from several mouse tissues contain glycosaminoglycans, particularly chondroitin sulphate,[849] and structures resembling protein–polysaccharide complexes have been detected in DNA preparations from mouse liver.[850]

The molecular weights of standard and commercial preparations of glycosaminoglycans have been determined by polyacrylamide gel electrophoresis, and the method was extended to the glycosaminoglycans of various avian tissues.[156] Identification of the glycosaminoglycans of chicken skin was aided by use of chondroitin sulphate lyases ABC and AC; hyaluronic acid, dermatan sulphate, and small amounts of chondroitin 4-sulphate, dermatan polysulphate, and heparan sulphate were identified. The age-dependence of the glycosaminoglycan levels was investigated.[851] Examination of the proteoglycan fractions from chicken medullary and cortical bone tentatively identified the heterosaccharide moieties as keratan sulphate and chondroitin sulphate, respectively.[852] The chondroitin sulphate chains of sternal cartilage from genetically micromelic chick embryos possessed molecular weights and sulphate contents similar to those of the chondroitin sulphate from non-mutant origin.[853] Cell lines derived from chick amnion synthesized sulphated and non-sulphated glycosaminoglycans *in vitro*.[854]

The existence of glycosaminoglycans attached to the Golgi membrane of chondrocytes of chick embryos was indicated by incorporation studies using [^{35}S]sulphate.[855] The bimodal sedimentation profile of a guanidine

[847] K. Kojima and T. Yamagata, *Exptl. Cell Res.*, 1971, **67**, 142.
[848] C. T. Bates and C. I. Levene, *Biochim. Biophys. Acta*, 1971, **237**, 214.
[849] D. G. Pritchard, R. M. Halpern, and R. A. Smith, *Biochim. Biophys. Acta*, 1971, **228**, 127.
[850] P. Wellauer and R. Weber, *Experientia*, 1971, **27**, 1171.
[851] K. Kondo, N. Seno, and K. Anno, *Biochim. Biophys. Acta*, 1971, **244**, 513.
[852] J. K. Candlish and F. J. Holt, *Comp. Biochem. Physiol.*, 1971, **40B**, 283.
[853] R. A. Fraser and P. F. Goetinck, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 494.
[854] R. Bischoff, *Exptl. Cell Res.*, 1971, **66**, 224.
[855] K. Kimata, M. Okayama, S. Suzuki, I. Suzuki, and M. Hoshino, *Biochim. Biophys. Acta*, 1971, **237**, 606.

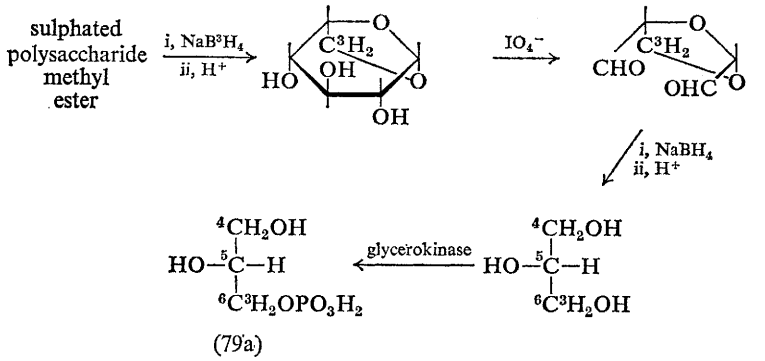
hydrochloride extract was compared with that for the protein–polysaccharides isolated from the extracellular matrix; 95% of the label of both components of the membrane material was released by the action of chondroitin sulphate lyases ABC and AC. A comparison of the glycosaminoglycan content of eggshell membranes with that of the isthmus region of hen oviduct showed that the membranes contain only hyaluronic acid (< 0.1% dry weight), whereas the isthmus region contains hyaluronic acid, chondroitin 4-sulphate, dermatan sulphate, and heparan sulphate.[856] The hyaluronic acid of the membrane was suggested to have a role in water-content maintenance and resistance of the egg to bacterial attack. Carbohydrate analysis of an ethanol-precipitable fraction of a papain digest of hen egg-white revealed the presence of 2-amino-2-deoxygalactose (4.6), 2-amino-2-deoxyglucose (7.0), neutral sugar (11.2), and uronic acid (1.5 mg/kg egg white); these components were considered to arise partly from glycosaminoglycans.[857]

A highly sulphated glycosaminoglycan isolated from the notochord of the hagfish (*Eptatretus burgeri*) released 2-amino-2-deoxyhexose, uronic acid, sulphate, and *N*-acetyl residues (1.00 : 1.09 : 1.89 : 1.01), together with small quantities of galactose, glucose, mannose, fucose, and xylose.[858] From the results of treatment with hyaluronidase and chondroitin sulphate lyases ABC and AC, it was concluded that the glycosaminoglycan is a dermatan polysulphate consisting mainly of [→ 4)-α-L-idopyranuronosyl-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl 4,6-disulphate)-(1 →] repeating units. Degradation with alkali indicated that the polysaccharide is linked to protein by *O*-glycosyl-serine linkages.

A glycosaminoglycan isolated from the mucus secreted by a snail (*Otella lactea*) appeared to be homogeneous after pronasic digestion and fractionation with cetylpyridinium chloride.[859] Physicochemical studies indicated a molecular weight in the range 7.5—9.0 × 10^5, and the dry weight of the material could be almost entirely accounted for by analyses for 2-amino-2-deoxy-D-glucose, L-iduronic acid, acetyl, and sulphate (1 : 1.5 : 1 : 1). The *ido*-configuration of the uronic acid was determined by identification of idosan as a product of hydrolysis of the methyl ester of the polysaccharide. The L-configuration of the uronic acid was determined using a new method (Scheme 11), which utilized the stereoselective phosphorylation of glycerol by glycerokinase followed by location of tritium in the product (79a) by periodate oxidation.

Clinical Conditions.—Individual determination of the glycosaminoglycans from urine of cases of Hunter syndrome further confirmed that dermatan sulphate and heparan sulphates constitute the major proportion.[811]

[856] C. I. Osuoji, *Biochim. Biophys. Acta*, 1971, **244**, 481.
[857] A. H. Johnson and J. R. Baker, *Biochem. J.*, 1971, **122**, 16P.
[858] K. Anno, N. Seno, M. B. Matthews, T. Yamagata, and S. Suzuki, *Biochim. Biophys. Acta*, 1971, **237**, 173.
[859] S. J. Pancake and M. L. Karnovsky, *J. Biol. Chem.*, 1971, **246**, 253.



(79a)

(carbon atoms numbered according to their original positions in the sugar ring)

Scheme 11

However, the data also revealed that chondroitin 4- and 6-sulphates, the major glycosaminoglycans of normal juvenile urine, also occur in considerably increased amounts. Dermatan sulphate (molecular weight 1.2×10^4) and heparan sulphate (molecular weight 5×10^3) fractions of the abnormal urine have been examined by thin-layer gel filtration; the heparan sulphate was shown to be monodisperse. These characteristics were compared with those of the corresponding polysaccharides isolated from normal urine.[815] Glycosaminoglycans were isolated from the urine of patients with Hunter, Hurler, and Morquio syndromes, and from the liver and spleen of the Hurler case, by a procedure which avoided further degradation.[860] The relative proportions of chondroitin sulphate, dermatan sulphate, and heparan sulphate in all samples from the Hurler case were similar; the molecules from the organs were of much lower molecular weight than normal and consisted of single chains of molecular weight of 5×10^3, together with multiples of up to four such chains attached to peptide moieties. The glycopeptide linkages of these chains involved L-serine. The output of urinary glycosaminoglycans by the Morquio case was less than that of the other two, and the material contained chondroitin sulphate (67%) and keratan sulphate (33%) as part of the same molecule. Degradation products of dermatan sulphate and heparan sulphate from the urine of patients with Hunter, Hurler, and Scheie syndromes showed considerable heterogeneity when compared with those isolated from normal urine.[861] Two distinct dermatan sulphate fractions were obtained for the two former syndromes, but not for the latter syndrome. The possibility that these heterogeneities may be responsible for the discrepancies reported between the clinical and chemical phenotypes in this group of metabolic

[860] M. F. Dean, H. Muir, and R. J. F. Ewins, *Biochem. J.*, 1971, **123**, 883.
[861] J. Onisawa and T. Lee, *Biochem. Med.*, 1970, **3**, 404.

disorders was discussed. An accumulation of dermatan sulphate and heparan sulphate to abnormally high levels was found in cerebrospinal fluid from cases of Hunter and Hurler syndromes.[807]

The literature on the chemical assessment of the Sanfilippo syndrome has been reviewed, and the changes involving glycosaminoglycan accumulation in ocular material have been investigated.[862] Fibroblasts cultured from the skin of Sanfilippo patients contained an excess of sulphated glycosaminoglycans, as judged by the incorporation of [^{35}S]sulphate.[863]

A highly sulphated chondroitin sulphate, detected in the urine of patients with hypothyroidism, exhibited the same characteristics as that isolated from normal urine.[814] The urinary excretion of glycosaminoglycans by juvenile cases of vitamin A deficiency and kwashiorkor was less than normal.[809] Restoration of the levels by vitamin A treatment was investigated. The glycosaminoglycans excreted by kwashiorkor children comprised chondroitin 4-sulphate (55.4%), chondroitin 6-sulphate (22.0%), partially sulphated chondroitin sulphate (17.4%), and heparan sulphate (1.2%).[810] The most striking differences between this pattern and that obtained for normals were the occurrence of the partially sulphated molecule and the absence of hyaluronic acid.

Evidence has been presented that the degradation of chondroitin sulphate–protein in synovial fluid of patients with inflammatory joint disease is effected by a protease.[864] The glycosaminoglycans of amyloid extracts of amyloid-laden organs from cases of rheumatoid arthritis, familial Mediterranean fever, Hodgkin's disease, and tuberculosis were identified as dermatan sulphate and heparan sulphate.[803] Comparisons with control extracts indicated that the differences are limited to the amounts of these glycosaminoglycans. Proteoglycans have been detected in bile from cases of functional disturbance of the liver and infections of the biliary tract.[865]

Biosynthesis and Metabolism.—The results of studies on the dissociation of bovine vitreous humour hyaluronic acid–protein are compatible with the hypothesis that the molecules are synthesized as smaller units, which undergo extracellular association to produce molecular aggregates in connective tissue.[818]

A microsomal preparation from horse leukocytes catalysed the incorporation of radioactivity from UDP-D-[^{14}C]glucuronic acid and UDP-2-acetamido-2-deoxy-D-[^{3}H]galactose into endogenous chondroitin sulphate.[866] The particulate enzyme also catalysed the incorporation of

[862] O. A. Jensen, *Acta Pathol. Microbiol. Scand., A*, 1971, **79**, 257.
[863] H. Kresse, U. Wiesmann, and M. Cantz, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 892.
[864] G. C. Wood, R. H. Pryce-Jones, D. D. White, and G. Nuki, *Ann. Rheum. Dis.*, 1971, **30**, 73.
[865] T. Matsushiro, T. Nemoto, M. Endo, and Z. Yosizawa, *Clin. Chim. Acta*, 1970, **30**, 645.
[866] I. Olsson and S. Gardell, *Biochim. Biophys. Acta*, 1971, **237**, 203.

radioactivity from UDP-2-acetamido-2-deoxy-D-[^{3}H]galactose into related exogenous hexa- and tetra-saccharides; analysis of the products indicated that a heptasaccharide and a pentasaccharide, respectively, had been formed. Maximal incorporation of radioactivity into both endogenous and exogenous receptors required the presence of both UDP-D-glucuronic acid and UDP-2-acetamido-2-deoxy-D-galactose.

Further investigation of the galactosyltransferase system of chick-cartilage microsomal particles revealed the presence of a third galactosyltransferase which transferred D-galactose from UDP-D-galactose to 2-acetamido-2-deoxy-D-glucose. It appeared that the catalytic centre involved in the reaction is distinct from those which catalyse the synthesis of the D-galactose moieties of chondroitin sulphate.[867]

Incubation *in vitro* of bovine arterial tissue with [^{35}S]sulphate resulted in labelling of the proteoglycan.[823] Degradation of the macromolecule with hyaluronidase yielded dermatan [^{35}S]sulphate and a series of labelled oligosaccharides. The specific activity of the sulphate groups of the former product was three times that of the sulphate groups of the latter products, providing evidence for a metabolic heterogeneity of the two constituent polysaccharides of a proteoglycan.

A quantitative study of the incorporation of a mixture of chondroitin 4- and 6-[^{35}S]sulphates into cultured Chinese hamster cells showed that the uptake is localized in the cytoplasm and that its magnitude is a function of polysaccharide concentration.[868] The process by which incorporated radioactivity was retained was temperature sensitive. Addition of sulphated glycosaminoglycans to the culture medium inhibited the incorporation of radioactivity, whereas non-sulphated glycosaminoglycans were without effect.[869]

One of the fragments arising from nitrous acid degradation of porcine intestinal mucosal heparin served, after digestion with β-glucuronidase, as an acceptor for D-glucuronic acid on incubation with UDP-D-[^{14}C]glucuronic acid and a particulate enzyme preparation from a heparin-producing mouse mastocytoma.[834] Pre-treatment of the fragment with β-glucuronidase was found to be mandatory for the exhibition of acceptor activity, and treatment of the product with the same enzyme released all the incorporated activity as D-[^{14}C]glucuronic acid. The biosynthesis of the neutral trisaccharide, *O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-xylose, of the heparin–protein linkage region has been investigated.[870] A microsomal fraction from the above mastocytoma catalysed the incorporation of D-[^{14}C]galactose from UDP-D-[^{14}C]galactose into endogenous substrates. Partial acidic hydrolysis of the D-[^{14}C]galactose-receptor yielded fragments with the characteristics of neutral oligosaccharides previously isolated from the linkage region of heparin–protein

[867] T. Helting, *Biochim. Biophys. Acta*, 1971, **227**, 42.
[868] H. Saito and B. G. Uzman, *Exptl. Cell Res.*, 1971, **66**, 90.
[869] H. Saito and B. G. Uzman, *Exptl. Cell Res.*, 1971, **66**, 97.
[870] T. Helting, *J. Biol. Chem.*, 1971, **246**, 815.

and of 2-acetamido-2-deoxy-4-*O*-β-D-galactosyl-D-glucose. The stepwise synthesis of the neutral trisaccharide was assessed using separate assays for the two galactosyltransferase reactions and exogenous receptors such as D-xylose (product: 4-*O*-β-D-galactosyl-D-xylose) and 4-*O*-β-D-galactosyl-D-xylose [product: *O*-β-D-galactosyl-(1 → 3)-*O*-β-D-galactosyl-(1 → 4)-D-xylose]. It was concluded that the two reactions are catalysed by different enzymes. A third galactosyltransferase, which catalysed the transfer of D-galactose to 2-acetamido-2-deoxy-D-glucose, was also detected. The metabolic fate of heparin in rats has been investigated using ([^{35}S]sulphamido)-heparin, [^{35}S]sulphamidochitosan, 2-deoxy-2-[^{35}S]sulphamido-D-glucose, and [^{35}S]sulphamino-L-serine.[871] Only in the case of labelled heparin was [^{35}S]sulphate excreted in the urine of animals to which the compounds had been administered. The relevant published data have been collated in a study of the kinetics of heparin in human, canine, and rabbit plasma.[872] The role of bovine lung heparan sulphates in the metabolism of heparin has been discussed.[822]

A sulphotransferase isolated from bovine lung catalysed the transfer of sulphate from adenosine 3′-phosphate-5′-[^{35}S]sulphatophosphate to de-*N*-sulphated heparan sulphate.[873] Degradation of the product with nitrous acid and *Flavobacterium heparinum* heparin lyase demonstrated that at least 75% of the radioactivity had been incorporated as *N*-sulphate.

Analysis of the *in vivo* incorporation of [^{35}S]sulphate into the chondroitin 6-sulphate of rabbit bone-marrow cells demonstrated that the largest molecules possessed the highest specific activity.[836] Sternal cartilage from genetically micromelic chick embryos incorporated labelled D-glucose, 2-amino-2-deoxy-D-glucose, and sulphate at reduced rates compared with normal cartilage.[853] The biosynthesis of the glycosaminoglycans of mouse 3T6 line fibroblasts,[848] rat cerebral cortex synaptosomes,[845] and the Golgi membrane of chick-embryo chondrocytes[855] has been studied. The rates of production and secretion of the sulphated glycosaminoglycans differed markedly among ten established mammalian cell lines.[804]

A differential effect of oestrogen upon the biosynthesis of the glycosaminoglycans of rhesus monkeys was observed in that greater amounts of D-[^{14}C]glucose were incorporated into dermatan sulphate and heparan sulphate by sex skin than by non-sex skin.[763] The rates of [^{35}S]sulphate uptake into the chondroitin sulphate of the skin of normal guinea-pigs and those deprived of L-ascorbic acid have been compared.[840] The effect of testosterone on the biosynthesis of the glycosaminoglycans of skin and tracheal cartilage from normal and orchidectomized rats was to increase the synthesis of hyaluronic acid only.[843] Glycosaminoglycan synthesis by cell lines derived from chick amnion was inhibited by 5-bromodeoxyuridine.[854]

[871] G. Embery, A. G. Lloyd, and L. J. Fowler, *Biochem. Pharmacol.*, 1971, **20**, 649.
[872] J. W. Estes, *Ann. New York Acad. Sci.*, 1971, **179**, 187.
[873] T. Foley and J. R. Baker, *Biochem. J.*, 1971, **124**, 25P.

Skin fibroblasts from cases of Sanfilippo syndrome showed excessive accumulation and prolonged turnover times of sulphated glycosaminoglycans, as determined by [^{35}S]sulphate incorporation.[863] The abnormalities could be corrected by a macromolecular factor found in the secretions of fibroblasts of differing genotypes and in normal human urine.

Miscellaneous Mammalian Bone, Cell, and Tissue Glycoproteins

In an investigation of the galactose, mannose, arabinose, and xylose contents of the brain and liver of cases of carbohydrate-storage disorders, it was found that greater than normal amounts of galactose, and galactose and mannose were liberated by acid hydrolysis of extracts of liver and brain, respectively, from cases of Hurler syndrome.[874] This was considered to be due to the storage of galactose-rich glycoproteins as a result of a deficiency in one of the β-galactosidase isoenzymes.

Amyloid extracted from amyloid-laden organs of cases of familial Mediterranean fever, Hodgkin's disease, rheumatoid arthritis, and tuberculosis contained 0.8% neutral sugars consisting of galactose (49%), glucose (10%), mannose (39%), and traces of ribose and xylose.[803]

A glycoprotein (molecular weight 7.0×10^4), isolated from bovine arterial tissue and purified by gel filtration and electrofocusing, contained 13% of carbohydrate.[875] Pronasic digestion of the glycoprotein yielded a glycopeptide (molecular weight 6.7×10^3) containing galactose (3), glucose (1), mannose (1), fucose (0.2), 2-amino-2-deoxyglucose (4), and *N*-acetylneuraminic acid (2 moles per mole). *In vitro* incubation of the tissue with uniformly labelled D-[^{14}C]glucose resulted in the specific labelling of the carbohydrate units of the glycoprotein indicating that its biosynthesis occurs within the arterial wall.

Studies on the metabolism and incorporation of L-[1-^{14}C]fucose into glycoproteins of bovine thyroid-gland tissue *in vitro* showed that the precursor is incorporated as L-fucose alone.[876] Intermediates in the biosynthetic pathway were identified as L-fucose 1-phosphate and GDP-L-fucose. The presence of puromycin in the culture medium inhibited the incorporation of L-[1-^{14}C]fucose into the glycoproteins, but not into the intermediates.

A polypeptide 2-acetamido-2-deoxyglucosyltransferase from bovine submaxillary glands effected the incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose into myelin protein (A1 protein) of bovine spinal cord.[877] Alkaline borohydride treatment of the resultant glycoprotein indicated that

[874] B. Hultberg, P. Öckerman, and A. Dahlqvist, *J. Clin. Invest.*, 1970, **49**, 216.
[875] V. Maier and E. Buddecke, *Z. physiol. Chem.*, 1971, **352**, 1338.
[876] J. L. Trujillo and J. C. Gan, *Biochim. Biophys. Acta*, 1971, **230**, 610.
[877] A. Hagopian, F. C. Westall, J. S. Whitehead, and E. H. Eylar, *J. Biol. Chem.*, 1971, **246**, 2519.

the incorporated units are linked *O*-glycosidically to the hydroxy-groups of L-threonine residues. The hydroxylation and *O*-acetylation of *N*-acetylneuraminic acid bound to glycoproteins of isolated subcellular membranes from bovine and porcine submaxillary glands have been investigated.[878]

During the periodate-induced swelling of bovine cornea, approximately 70% of the heteropolysaccharide moieties of the glycoproteins were destroyed.[755] Determination of the calcium-binding properties of bovine bone sialoprotein by inhibition of calcium phosphate precipitation and by the murexide method showed that the formation constant is considerably higher than that for chondroitin sulphates.[829]

A glycopeptide (molecular weight 8.1×10^4) characterized by the presence of hexose (23.1%), 2-amino-2-deoxyhexose (37.1%), and sialic acid (4.8%), and the absence of uronic acids and sulphate has been isolated in homogeneous form from porcine duodenal mucosae.[879] Pharmacological tests showed that the glycoprotein could inhibit certain ulcers and oedema, but that it had no anticoagulant properties. Pronase and trypsin prevented the formation of the glycoprotein cell-coat material on the surface of canine kidney cells.[880]

The existence of glycoproteins in guinea-pig rib cartilage has been noted.[839] The glycopeptides released proteolytically from the surface of control and Rous sarcoma virus-transformed hamster cells grown in the presence of radioactive L-fucose were fractionated by gel filtration.[881] It was found that the more rapidly migrating glycopeptides constitute a larger proportion of the glycopeptides from the surface of rapidly growing cells than from the surface of slowly growing or plateau cells.

Intracerebral injections of radioactive L-fucose into developing rats resulted in specific labelling of the brain glycoproteins in the L-fucose residues.[882] Considerable heterogeneity of the labelled glycoproteins was found on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. A procedure utilizing L-[^{3}H]fucose and L-[^{14}C]fucose, together with double-label counting techniques, for comparison of the electrophoretic patterns of newly synthesized glycoproteins from different tissue samples was reported; the incorporation pattern of 2-[^{3}H]acetamido-2-deoxy-D-mannose was also investigated. Further studies on the biosynthesis of glycoproteins in developing rat brain have led to the characterization of a UDP-D-galactose:glycoprotein galactosyltransferase.[883] Analysis of the macromolecular components of synaptosomes from rat cerebral cortex after incubation *in vitro* with 2-amino-2-deoxy-D-[1-^{14}C]glucose showed that radioactivity had been incorporated into the constituent

[878] R. Schauer and M. Wember, *Z. physiol. Chem.*, 1971, **352**, 1282.
[879] G. Bertellini, A. Butti, B. Piantanida, G. Prino, A. Riva, A. Rossi, and S. Rossi, *Arzneimittelforschung*, 1971, **21**, 244.
[880] G. Poste, *Exptl. Cell Res.*, 1971, **65**, 359.
[881] C. A. Buck, M. C. Glick, and L. Warren, *Biochemistry*, 1971, **10**, 2176.
[882] R. H. Quarles and R. O. Brady, *J. Neurochem.*, 1971, **18**, 1809.
[883] G. K. W. Ko and E. Raghupathy, *Biochim. Biophys. Acta*, 1971, **244**, 396.

2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, D-glucuronic acid, and *N*-acetylneuraminic acid units.[845]

Heterogeneous glycoproteins in rat liver mitochondrial preparations afforded small amounts of galactose, glucose, mannose, fucose, and arabinose.[884] The distribution of glycoproteins bound to the inner and outer mitochondrial membranes of rat liver has been determined by measurement of the 2-amino-2-deoxyhexose and sialic acid contents of these regions.[885] The biosynthesis of the glycoproteins of skin and tracheal cartilage of normal and orchidectomized rats was apparently unaffected by treatment *in vivo* with testosterone.[843]

Rat-intestinal surface-membrane glycoproteins could be labelled *in vivo* using 2-amino-2-deoxy-D-[1-^{14}C]glucose.[886] On Sepharose chromatography of the soluble product from brief proteolytic digestion of the labelled brush borders, most of the glycoprotein emerged as a single peak. This peak, which also contained maltase, sucrase, β-naphthylamidase, and alkaline phosphatase activities, was further resolved by electrophoresis. It was concluded that the surface glycoproteins are closely associated with enzyme activities. A study of the glycosyltransferases of microsomal subfractions of rat small intestinal mucosa suggested that the carbohydrate units of the glycoproteins are added by a stepwise procedure.[887] Glycoproteins containing galactose, glucose, mannose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid appeared to be components of membranes of rat ascites hepatoma.[888]

There was a lag of several hours between the incorporation of L-[^{3}H]-fucose into the glycoproteins of mouse whole brain fractions and into the soluble and particulate glycoproteins of the nerve end fractions.[889] However, no such lag was observed when 2-amino-2-deoxy-D-[1-^{14}C]-glucose was used as the precursor. It was concluded that the L-fucosyl glycoproteins are transported to the nerve endings after synthesis, whereas other glycoproteins are synthesized *in situ*.

The distribution of macromolecular 2-amino-2-deoxyhexose and sialic acid in organelles from mouse fibroblast L cells has been investigated.[890] Glycoproteins from mouse liver smooth microsomal and cell-membrane fractions were shown to contain neutral sugars and sialic acid.[891]

L-[^{3}H]Fucose- and 2-amino-2-deoxy-D-[^{3}H]glucose-labelled glycopeptides have been isolated from pronasic digests of H-2 transplantation allo-antigen glycoproteins purified from labelled spleen cells of inbred mice.[892]

[884] S. S. Martin and H. B. Bosmann, *Exptl. Cell Res.*, 1971, **66**, 59.
[885] B. de Bernard, M. C. Pugliarello, G. Sandri, G. L. Sottocasa, and F. Vittur, *F.E.B.S. Letters*, 1971, **12**, 125.
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Gel-filtration experiments indicated that the molecular weights of the glycopeptides (3.3×10^3) are identical with those of glycopeptides isolated from mouse-derived fibrosarcoma, (Meth-A)tumour, and lymphoma cell lines. The similarity between the glycopeptides from normal and tumour sources was further demonstrated by their identical behaviour on DEAE–Sephadex chromatography.

Glycolipoproteins obtained from mouse spleen cell surfaces by autolysis and papain digestion gave, on gel filtration and ion-exchange chromatography, a series of fractions each containing galactose, mannose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, glucuronic acid, and sialic acid.[893] The carbohydrate compositions were compared with those of corresponding glycoprotein preparations from two mouse genotypes.

Hormonal Glycoproteins

It has been agreed on an unofficial, but international, basis that the α- and β-nomenclature should be used for naming the subunits of hormonal glycoproteins. Thus, the 'common' subunit is now termed the α-subunit and the hormone specific subunit is termed the β-subunit.

Sedimentation analysis of human pituitary follicle-stimulating hormone gave a molecular weight of 3.26×10^4. DEAE–Sephadex chromatography of the urea-treated hormone yielded the α- and β-subunits, the biological activities of which were 2% and 6%, respectively, of the intact material.[894] Disc electrophoresis of the α- and β-subunits showed two basic and three acidic bands, respectively, indicating that the subunits were heterogeneous. Component analyses of the subunits showed that they are non-identical and it was suggested that they are linked non-covalently in the intact molecule. Attempted recombination by incubation regenerated 27% of the original activity.

Human and ovine follicle-stimulating hormones were modified by periodate oxidation followed by borohydride reduction to give molecules containing analogues of *N*-acetylneuraminic acid, in which the carbon chain had been shortened by one or two carbon atoms.[895] The treatment decreased the biological activity of the molecule but, even when all the neuraminic acid units had been modified, 50% of the original activity remained. Treatment of the modified follicle-stimulating hormone with neuraminidase resulted in the release of all the *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid) and 5-acetamido-3,5-dideoxy-D-*galacto*-octulosonic acid residues and part of the 5-acetamido-3,5-dideoxy-L-*arabino*-heptulosonic acid residues with total loss of biological activity. It was apparent that whereas part of the

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[894] B. B. Saxena and P. Rathnam, *J. Biol. Chem.*, 1971, **246**, 3549.
[895] M. Suttajit, L. E. Reichert, and R. J. Winzler, *J. Biol. Chem.*, 1971, **246**, 3405.

N-acetylneuraminic acid structure is essential for activity, an intact three-carbon, polyhydroxy side-chain is unnecessary. The ability of porcine and ovine follicle-stimulating hormone to inhibit the action of glyceraldehyde 3-phosphate dehydrogenase has been refuted.[896]

Luteinizing hormone isolated from bovine pituitaries possesses a molecular weight of 3×10^4.[897] A molecule isolated at the same time (molecular weight 1.5×10^4) had an activity of only 5 i.u. luteinizing hormone/mg and was considered to be a subunit of luteinizing hormone. Marked reductions of the *in vivo* activities of human luteinizing hormone and human chorionic gonadotrophin were observed when the molecules were desialylated.[898]

Studies of human luteinizing hormone by isoelectric focusing gave pI values of 7.52 and 9.10 according to the pH gradient used (6—8 and 3—10, respectively) and it appeared that in one of the systems the ampholine interacted with the hormone.[899] Use of a further pH gradient (7—10) resolved the hormone into several components of high biological and immunological activities. Digestion of the more acidic components converted them into one having a pI of 9.35, and it was concluded that even highly purified luteinizing hormone may contain a family of glycoproteins, which are similar in biological and immunological activities, but different in their sialic acid contents and in their charge properties. The pI's of bovine, porcine, ovine, rabbit, and rat luteinizing hormone fell within the range 8.6—10.7, whereas the equine hormone showed activity over the pH gradient 3.78—8.90. By comparison, the pI of human follicle-stimulating hormone had a range of 3.36—5.55, with ovine, bovine, and equine materials behaving similarly.

An apparently pure preparation of one of the subunits of bovine luteinizing hormone, LH-α, was found to contain two species, LH-α_A and LH-α_B, on examination by electrophoresis.[900] The carbohydrate contents of the two were determined. It was proposed that the carbohydrate chains in LH-α_A are attached to L-asparagine residues in the carboxy-terminal and inner portions of the subunit.

Three glycopeptides were isolated from a tryptic digest of bovine thyroid-stimulating hormone (thyrotropin) that had been *S*-carboxymethylated and reduced.[901] Investigation of reduced, *S*-carboxymethylated and maleylated, reduced, *S*-carboxymethylated α- and β-subunits of the hormone gave values of 1.36×10^4 and 1.47×10^4, respectively, for the molecular weights.[902] Amino-acid-sequence analyses of the subunits

[896] T. T. T. Yen, M. C. Wacholtz, and M. M. Greenberg, *Hormone Metabol. Res.*, 1970, **2**, 349.
[897] N. K. Assonova, F. J. Ryszka, and A. S. Khokhlov, *Biokhimiya*, 1971, **36**, 562.
[898] M. L. Dufau, K. J. Catt, and T. Tsuruhara, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1022.
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[900] G. C. Maghuin-Rogister and G. P. Hennen, *European J. Biochem.*, 1971, **21**, 489.
[901] B. Shome, T. Liao, S. M. Howard, and J. G. Pierce, *J. Biol. Chem.*, 1971, **246**, 833.
[902] T. Liao and J. G. Pierce, *J. Biol. Chem.*, 1971, **246**, 850.

demonstrated that TSH-α contained two carbohydrate moieties attached to L-asparagines 56 and 82, and that TSH-β contained only one carbohydrate moiety attached to L-asparagine 23. Comparison of the component and sequence analyses of TSH-α and the corresponding subunit of bovine luteinizing hormone (LH-α) showed that, whereas their amino-acid sequences are identical, the two chains differ in their carbohydrate contents, *viz.* galactose (0.13, 0.23), mannose (6.5, 5.6), fucose (0.55, 0.35), and 2-amino-2-deoxyglucose (5.6 and 6.4 residues per molecular weight of 1.4×10^4, respectively).[903]

The effect of thyroid-stimulating hormone releasing-hormone on the levels of thyroid-stimulating hormone in human serum has been studied.[904, 905] Properties of the releasing hormone have been investigated.[906]

A mixture of equal weights of the hormone-specific chain of bovine thyroid-stimulating hormone, TSH-β, and the α-subunit of human chorionic gonadotrophin, HCG-α, in solution yielded thyroid-stimulating activity similar to that observed by the recombination of TSH-α and TSH-β.[907] Recombination of HCG-α with bovine LH-β resulted in the generation of gonadotrophic activity.

Gel filtration of pregnancy urine on Sephadex G-100 yielded two species related to human chorionic gonadotrophin.[908] The slightly retarded fraction possessed biological, but no immunological activity, whereas the highly retarded fraction was immunologically active, but biologically inactive. The results of several experiments indicated that the highly retarded material is a component of urine, but not of serum, and that it is generated by the kidney. Gel filtration of purified preparations of human urinary chorionic gonadotrophin demonstrated that they were all inhomogeneous.[909] It was possible to separate chorionic gonadotrophin molecules devoid of detectable follicle-stimulating hormone activity from hormone molecules possessing such activity. Evidence was presented to indicate that at least some physiochemical manipulations utilized in the purification of human chorionic gonadotrophin from urine may introduce artefacts.

Subunits (HCG-α and HCG-β) have been isolated from human chorionic gonadotrophin by chromatography on DEAE–Sephadex and from reduced, carboxymethylated chorionic gonadotrophin by preparative polyacrylamide

[903] J. G. Pierce, T. Liao, R. B. Carlsen, and T. Reimo, *J. Biol. Chem.*, 1971, **246**, 866.
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[908] D. L. Matthies and E. Diczfalusy, *Acta Endocrinol.*, 1971, **67**, 434.
[909] D. L. Matthies, P. Petrusz, and E. Diczfalusy, *Acta Endocrinol.*, 1971, **67**, 445.

gel electrophoresis and gel filtration.[910] HCG-α was shown to possess a molecular weight of 1.8×10^4, a Stokes' radius of 23.3 Å, and less than 1% of the biological activity of the parent hormone; the corresponding values for HCG-β were 3×10^4, 30.2 Å, and 6%, respectively. Recombination of the subunits was effected by incubation at pH 7.0, more than 66% of the original activity being restored. Re-association was demonstrated by gel filtration and disc electrophoretic analysis of the product.

Thyroglobulin

Purified human thyroglobulin was divided into subfractions by chromatography on DEAE–cellulose.[911] The sialic acid content of each fraction increased according to the sequence in elution, demonstrating the heterogeneity of the thyroglobulin preparation and revealing differences in the number and arrangement of sialic acid residues at the surface of the molecule. However, it was evident from a comparison of the properties of normal and desialized thyroglobulin that the sialic acid content had only a minor effect upon the chromatographic elution pattern and that other properties contributed to the heterogeneity.

Glycopeptides isolated from a pronasic digest of porcine thyroglobulin were further separated by ion-exchange chromatography.[912] Two distinct types of heterosaccharide chain were recognized from the compositions and molecular weights of the glycopeptide fractions. One type (molecular weight $1.0—1.7 \times 10^3$) consisted of mannose (4—8) and 2-amino-2-deoxyglucose (2), whereas the other (molecular weight 3.3×10^3) contained galactose (3), mannose (3), fucose (1), 2-amino-2-deoxyglucose (5), and sialic acid (1—2.5 residues per mole). Partial acid hydrolysis of the glycopeptides liberated 2-acetamido-1-[(*N*-β-L-aspartyl)amino]-2-deoxy-β-D-glucopyranosylamine. One of the glycopeptides containing a complex mixture of monosaccharide units was reported to have the branched structure (80).[913]

The sedimentation rates of thyroglobulin from iodine-sufficient and iodine-deficient rats has been examined for a series of ionic media.[914] The results indicated that dissociation of thyroglobulin into subunits is preceded by unfolding of the molecule.

Milk Glycoproteins

It has been demonstrated that coagulation of bovine casein miscelles is brought about by lysozyme in a manner similar to the action of rennin; both enzyme-catalysed reactions were calcium-ion dependent and had

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[914] L. Valenta and S. Lissitzky, *Biochem. Biophys. Acta*, 1971, **236**, 376.

β-D-Galp
1
↓
4
β-D-GlcpNAc
1
↓
6 or 2
α-NeupNAc-(1 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2 or 6)-α-D-Manp
1
↓
?
α-NeupNAc-(1 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-α-D-Manp-(1 → ?)-D-GlcpNAc-(1 → ?)-D-Manp-(1 → ?)-D-GlcpNAc-Asn
3 or 6
↑
1
α-L-Fuc

(80)

similar pH optima.[915] It was concluded that the carbohydrate moiety of casein plays an important role in the stabilization of casein miscelles.

Aggregation phenomena and conformational properties of β-casein from bovine milk and the effect of carboxylation of the casein on the parameters involved have been studied.[916]

κ-Caseins of bovine milk, prepared by a number of methods, contain hexose and sialic acid (approximately 1.5 and 0.8%, respectively).[917] Ultracentrifugal analyses of the caseins gave single peaks (2.6—3.8 *S* and 14.4 *S*, respectively) in the presence and in the absence of urea. Isoelectric focusing indicated that all preparations have a pI of 6.0 and that they usually contain a second component having a pI of 3.6. DEAE–Cellulose chromatography allowed the recognition of two species in the caseins; compositional analyses indicated that both are components of the original κ-casein complexes.

Ion-exchange chromatography was employed in an attempt to determine the origin of the D-galactose, D-mannose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and *N*-acetylneuraminic acid constituents in preparations of bovine α-lactalbumin.[918] Two minor components were identified, one containing the majority of the carbohydrate of the preparations and the other containing principally D-galactose, 2-acetamido-2-deoxy-D-galactose, and 2-acetamido-2-deoxy-D-glucose.

A highly purified preparation of bovine milk α-lactalbumin contained a minor component, 'glyco-α-lactalbumin', with the same amino-acid composition as α-lactalbumin, but also containing galactose (1.4), mannose (4.0), fucose (1.0), 2-amino-2-deoxygalactose (1.1), 2-amino-2-deoxyglucose (3.1), and *N*-acetylneuraminic acid (0.64 mole per mole).[919]

A molecular weight of 7.5×10^4 was computed for human lactoferrin from the values for its partial specific volume and sedimentation and diffusion coefficients.[920] Molecular weights of 7.68×10^4 and 7.64×10^4 were estimated from agarose–polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and from component analyses, respectively. No decrease in molecular weight was observed when the glycoprotein in its reduced, alkylated or reduced, aminoethylated forms was treated with urea solution, indicating that it is composed of one chain only.

Lactoferrin was identified in milk from mare, cow, goat, pig, guinea-pig, and mouse on the basis of its electrophoretic mobility, the stability at acid pH of its iron complex, and its immunological properties.[921] A comparison

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[916] M. T. A. Evans, L. Irons, and M. Jones, *Biochim. Biophys. Acta*, 1971, **229**, 411.
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of the iron and lactoferrin concentrations of the milk failed to provide grounds for the assumption that lactoferrin plays a direct role in the excretion of iron into milk.

Serum, Plasma, and Blood Cellular Element Glycoproteins, and Immunoglobulins

Serum and Plasma Glycoproteins.—Alkaline hydrolysis followed by nitrous acid deamination of human plasma α_1-acid glycoprotein (orosomucoid) yielded a series of oligosaccharides containing trisaccharides, which consisted of D-galactose, 2,5-anhydro-D-mannose, and a sialic acid derivative presumed to be 3-deoxy-D-*glycero*-(D-*galacto*- or D-*gulo*-)-2-nonulosonic acid.[135] Similar treatment of the glycoprotein after initial desialylation yielded a disaccharide consisting of D-galactose and 2,5-anhydro-D-mannose. Periodate oxidation and other studies of these fragments indicated that the trisaccharide sequences (81), (82), and (83) are present

NANA-(2 → 3)-D-Gal-(1 → 4)-D-Glc*N*Ac
(81)

NANA-(2 → 2)-D-Gal-(1 → 4)-D-Glc*N*Ac
(82)

NANA-(2 → 6)-D-Gal-(1 → 4)-D-Glc*N*Ac
(83)

in the parent molecule. Evidence was also obtained for the presence of an *O*-sialylated 2-acetamido-2-deoxy-D-glucose terminal (1 mole per mole) in the α_1-acid glycoprotein.

Cyanogen bromide fragmentation of α_1-acid glycoprotein from human plasma showed that one of the two large glycopeptides derived from the carbohydrate-carrying portion of the molecule contains only one carbohydrate moiety, whereas the other contains four.[922] Both glycopeptides are derived from the *N*-terminal portion of the intact molecule, and all glycopeptide linkages involved aspartyl residues. Further studies on the amino-acid sequence of α_1-acid glycoprotein confirmed that the *C*-terminal portion is devoid of carbohydrate.[923]

Human plasma α_1-acid glycoprotein in which the terminal *N*-acetylneuraminic acid residues had been modified to 5-acetamido-3,5-dideoxy-L-*arabino*-[7-^{3}H]hydroxy-2-heptulosonic acid residues exhibited a normal half-life in rat plasma.[8] The modified residues could be removed with

[922] K. Schmid, M. Ishiguro, J. Emura, S. Isemura, H. Kaufmann, and T. Motoyama, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 280.
[923] T. Ikenaka, M. Ishiguro, S. Isemura, H. Kaufmann, W. Bauer, and K. Schmid, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 1142.

neuraminidase, and it appears that the 7-carbon analogue mimics *N*-acetylneuraminic acid. Further studies on the human glycoprotein showed that it inhibited the action of dipyridamol and became bound to this agent.[924]

Bovine plasma α_2-macroglobulin (molecular weight 8×10^5) has been purified until homogeneous and several of its physicochemical characteristics have been studied.[925] The glycoprotein released a total of 7.74% carbohydrate consisting of galactose (107), mannose (107), 2-amino-2-deoxyglucose (154), sialic acid (4 residues per mole), and traces of fucose. It was concluded that the molecule resembles α_2-macroglobulin isolated from human and porcine plasma.

During the growth period of field voles (*Micrototus agrestis*) significant changes in the levels of the serum α_2- and β-glycoprotein fractions were observed.[926] A 6.2 *S* β-glycoprotein isolated from human plasma by ion-exchange chromatography and gel filtration contained galactose (2.7%), mannose (2.7%), fucose (0.1%), 2-acetamido-2-deoxyhexose (4.2%), and *N*-acetylneuraminic acid (0.9%).[927] The glycoprotein is antigenically related to a γ-glycoprotein previously isolated from aged plasma and to an α_2-glycoprotein.

An ultramicrotechnique for estimating human serum albumin directly by using 5,5′-dibromo-*o*-cresolsulphonphthalein (bromocresol purple) has been described.[928] The method required a sample size of only 10 μl, was sensitive down to a level of 100 ng, and gave a high correlation with the results obtained by other methods. Measurement of human serum albumin using bromocresol green has also been described.[929]

The effect of complexing with aldehydes on the conformation of human serum albumin has been studied by o.r.d. spectroscopy.[930] The irreversible conformational transitions occurring in the albumin on heating at 60 °C and on treatment with formaldehyde resulted from despiralization of the α-helix.[931]

In vitro studies to determine the fate of human serum albumin [932] and the binding levels of urate ions by human serum albumin [933] have been reported. Human serum albumin levels have been monitored in cases of neonatal jaundice.[934] Human serum albumin has been mechanically trapped in the lattice of a highly cross-linked polyacrylamide gel and was employed in this form as an immunoadsorbent.[935]

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[933] P. C. Farrell, R. P. Popovich, and A. L. Babb, *Biochim. Biophys. Acta*, 1971, **243**, 49.
[934] P. J. N. Howorth, *Clin. Chim. Acta*, 1971, **32**, 271.
[935] S. Carrel and S. Barandun, *Immunochem.*, 1971, **8**, 39.

Chromatography of bovine plasma albumin on DEAE–cellulose gave a single asymmetric peak with considerable tailing; identical results were obtained for a cystinyl derivative of the albumin.[936] This behaviour was related to the presence of a series of albumins with different pI's, as revealed by isoelectric-focusing studies. Similar results were obtained for plasma albumins of human and mouse origin.

The intrinsic viscosity of bovine serum albumin in a 1 : 3 v/v dioxan–water mixture was below the range of values obtained for most globular species, and indicated that the albumin molecule approximates closely to an ideal Stokes–Einstein sphere in this solvent system.[937] The conformation[938] and monolayer properties[939] of bovine serum albumin have been studied. Equilibrium and kinetic isotope effects in bovine plasma albumin have been investigated for deuterium and tritium.[940]

Bovine albumin reacted readily in aqueous solution with chondroitin sulphate and blood-group M,N-active sialoglycopeptide to form a complex by either a reversible semipolar or an ionic bond.[739] The binding of sodium taurocholate[941] and the induction of spectral changes of various dyes[942] by bovine serum albumin have been investigated.

Rat serum albumin could be measured down to a level of 30 pg by a method involving ultramicrodisc electrophoresis and densitometry.[943] The pathway of secretion of rat serum albumin is apparently rough membranes, smooth membranes, Golgi bodies, and blood, in that order.[944] However, it has been reported that intracellular albumin is not a precursor of rat serum albumin.[945]

A comparative study of human, bovine, ovine, and porcine fibrinogens has been made by CM-cellulose chromatography of their sulphitolysis products.[946] Differences in the conformations of the plasmin degradation products of bovine fibrinogen, as revealed by hydrogen-exchange experiments and spectropolarimetry,[947] and the isoelectric-focusing properties of normal and cobalt-modified rabbit fibrinogens[948] have been reported.

Some of the glycoproteins isolated from the sera of Antarctic fishes (*Trematomus borchgrevinki* and *Dissosticus mawsoni*) possessed the unique property of depressing the freezing point of water more than expected on the basis of the number of particles present in solution. They were

[936] E. M. Spencer and T. P. King, *J. Biol. Chem.*, 1971, **246**, 201.
[937] S. F. Sun and N. O. del Rosario, *Chem. Comm.*, 1971, 669.
[938] W. G. M. Braam, B. J. M. Harmsen, and G. A. J. Van Os, *Biochim. Biophys. Acta*, 1971, **236**, 104.
[939] J. M. Trillo, S. G. Fernández, and P. S. Pedrero, *Anales de Quim.*, 1971, **67**, 115.
[940] B. E. Hallaway and E. S. Benson, *Biochim. Biophys. Acta*, 1971, **243**, 380.
[941] H. O. Green, J. Moritz, and L. Lack, *Biochim. Biophys. Acta*, 1971, **231**, 550.
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[943] D. E. Oken, *Microchem. J.*, 1970, **15**, 557.
[944] T. Peters, B. Fleischer, and S. Fleischer, *J. Biol. Chem.*, 1971, **246**, 240.
[945] J. D. Judah and M. R. Nicholls, *Biochem. J.*, 1971, **123**, 649.
[946] T. Cartwright and R. G. O. Kekwick, *Biochim. Biophys. Acta*, 1971, **236**, 550.
[947] A. Z. Budzynski, *Biochim. Biophys. Acta*, 1971, **229**, 663.
[948] S. Krantz, M. Lober, and H. Fiedler, *F.E.B.S. Letters*, 1970, **11**, 100.

shown to be principally composed of galactose (28%), 2-acetamido-2-deoxygalactose (29%), alanine (23%), and threonine (16%).[949] Other glycoproteins of the sera had similar compositions, containing proline in addition, but were inactive. The active glycoproteins could be separated into three distinct species which had identical compositions on a weight basis and molecular weights of 1.05×10^4, 1.70×10^4, and 2.15×10^4. The results of viscosity, c.d., and dialysis experiments indicated that the glycoproteins are expanded molecules, a property of importance to their function. Loss of activity occurred on acetylation of the carbohydrate hydroxy-groups, but activity was restored on deacetylation.[950] Sequential periodate oxidation demonstrated that galactose residues occupy external positions and β-elimination studies showed that 2-acetamido-2-deoxyglucose units are linked glycosidically to threonine residues. It was concluded that the glycoproteins have a repeating unit of alanylalanylthreonine, with the disaccharide 2-acetamido-2-deoxy-3(or 4)-*O*-D-galactosyl-D-glucopyranose attached to each threonine residue.[950, 951] Thermal hysteresis of the blood of Antarctic fish was considered to be due to the presence of these anti-freeze glycoproteins. Further investigation of the freezing and melting points of aqueous solutions of the glycoproteins suggested that hysteresis resulted from adsorption of the molecules on to the surface of ice crystals.[952] Complexing with borate removed the antifreeze properties presumably by complexing with terminal galactose residues of the repeating structure.

Hemopexin, a haeme-binding glycoprotein isolated from human and rabbit sera, contains 21.6% and 20.2% of carbohydrate, respectively, which consists of galactose (13.3 and 6.7), mannose (16.5 and 8.8), 2-amino-2-deoxyglucose (28.6 and 26.8), and *N*-acetylneuraminic acid (18.5 and 18.6 residues per mole, respectively). Native haemopexin and certain chemically modified forms did not show evidence of dissociation into subunits when subjected to a variety of conditions.[953]

Binding between Dextran Blue and prothrombin depends on the ionic strength of the medium, and the phenomenon has been applied to the separation of blood-coagulation factors.[954] The complexes were dissociable by treatment with highly ionic solutions or DEAE–cellulose. Isoelectric-focusing techniques have also proved useful in the separation of prothrombin from other blood-clotting factors.[955] Prothrombin has been isolated from the plasma of steers treated with dicumarol and was purified until apparently homogeneous; the physicochemical and immunological properties of the preparation were investigated.[956] Prothrombin has

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[950] S. K. Komatsu, A. L. DeVries, and R. E. Feeney, *J. Biol. Chem.*, 1970, **245**, 2909.
[951] A. L. DeVries, J. Vandenheede, and R. E. Feeney, *J. Biol. Chem.*, 1971, **246**, 305.
[952] A. L. DeVries, *Science*, 1971, **172**, 1152.
[953] Z. Hrkal and U. Muller-Eberhard, *Biochemistry*, 1971, **10**, 1746.
[954] A. C. W. Swart and H. C. Hemker, *Biochim. Biophys. Acta*, 1970, **222**, 692.
[955] L. Pechet and J. A. Smith, *Biochim. Biophys. Acta*, 1970, **200**, 475.
[956] O. P. Malhotra and J. R. Carter, *J. Biol. Chem.*, 1971, **246**, 2665.

been isolated in an undegraded form from bovine plasma and partial characterization revealed that it contains 2-amino-2-deoxyglucose.[957] The circulatory half-life of asialo bovine prothrombin in rats was 10% of that found for the intact glycoprotein, but removal of further carbohydrate units by other carbohydrases restored the half-life to a more normal value.[958]

A method involving ion-exchange chromatography has been described for the large-scale preparation of transferrin from human serum.[959] The homogeneity of the preparation was demonstrated by electrophoresis, immunoelectrophoresis, ultracentrifugation, and component analysis.

Treatment of human serum transferrin, which had been oxidized with performic acid, with chymotrypsin yielded a glycopeptide containing two carbohydrate moieties both of which were attached to L-asparagine residues in the polypeptide chain.[960] Two heterosaccharide moieties of human transferrin have also been isolated as two glycopeptides, one of which contained L-asparagine as the sole amino-acid, whereas the other contained L-asparagine and two other amino-acids.[961] Periodate oxidation of either glycopeptide yielded, among other products, (2-acetamido-2-deoxyglucosyl)mannose and (2-acetamido-2-deoxyglucosyl)-(2-acetamido-2-deoxyglucosyl)mannosyl-(2-acetamido-2-deoxyglucosyl)asparagine. Application in sequence of glycohydrolases, mild acid hydrolysis, and methylation also contributed to the structural identification of the glycopeptides. A structure (84) was proposed for each of the carbohydrate moieties of the parent molecule on the basis of the evidence obtained.

NANA-(2 → 6)-D-Gal-(1 → 3 or 4)-D-Glc*N*Ac-(1 → 3)-D-Man-(1 → 2 or 4)-D-Man-(1 → 2 or 4)-D-Man-(1 → 2, 4, or 6)-D-Man-(1 → 3 or 4)-D-Glc*N*Ac-Asn

NANA-(2 → 6)-D-Gal-(1 → 3 or 4)-D-Glc*N*Ac-(1 → 3 or 4)-D-Glc*N*Ac

(84)

Disc electrophoresis of each of the products of partial action of bacterial neuraminidases on human transferrin type CC gave five different bands; similar experiments with rabbit transferrin gave three bands.[962] Bovine transferrin type AA showed a reduced electrophoretic mobility after treatment with the enzymes. The effect of the amount of iron and copper bound by human transferrin on its chromatographic characteristics on DEAE–cellulose has been investigated.[963] The electrophoretic characteristics of transferrins from bighorn sheep (*Ovis canadensis*), doll sheep (*Ovis dalli*),

[957] A. C. Cox and D. J. Hanahan, *Biochim. Biophys. Acta*, 1970, **207**, 49.
[958] G. L. Nelsestuen and J. W. Suttie, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 198.
[959] N. Frénoy, Y. Goussault, and R. Bourrillon, *Clin. Chim. Acta*, 1971, **32**, 243.
[960] P. Charet and J. Montreuil, *Compt. rend.*, 1971, **273**, *D*, 533.
[961] G. A. Jamieson, M. Jett, and S. L. DeBernardo, *J. Biol. Chem.*, 1971, **246**, 3686.
[962] W. Scharmann, J. Brückler, and H. Blobel, *Biochim. Biophys. Acta*, 1971, **229**, 136.
[963] R. S. Lane, *Biochim. Biophys. Acta*, 1971, **243**, 193.

and mouflon (*Ovis musimon*) have been compared with those of reference transferrins from domestic sheep.[964] Polymorphism in the transferrins from rock dove (*Columba livia*), wood pigeon (*C. palumbus*), and barbary dove (*Streptopelia risoria*) was detected by gel electrophoresis, whereas the transferrin from collared dove (*S. decaoto*) was shown to be monomorphic.[965]

Vitellogenin, a glycoprotein induced in large amounts in the serum of the South African clawed toad (*Xenopus laevis*) by oestrogen treatment, was found to be precipitable in a pure form (molecular weight 6×10^5) by DMF.[966] The molecule yielded hexose (0.4%), 2-amino-2-deoxyhexose (0.77%), and sialic acid (0.18%). This and other compositional data led to the conclusion that it is a calcium-binding glycolipophosphoprotein. The biosynthesis of vitellogenin has also been investigated.[967]

Human factor VIII (antihaemophilic factor) was isolated from plasma by differential precipitation with polyethylene glycol and agarose gel filtration.[968] The homogeneous preparation was found to be a glycolipoprotein containing 10% hexose.

Valine, glutamic acid, and alanine 'apolipoproteins' from human plasma possess molecular weights of 7×10^3, 1×10^4, and 1×10^4, respectively.[969] The c.d. and o.r.d. spectra of the valine variant were consistent with a high content of α-helix, whereas those of the alanine variant suggested a random coil structure. The latter variant gave two components on DEAE-cellulose chromatography, both of which contained sialic acid (0.6 and 1.2 moles per mole). The concentrations of galactosylhydroxylysine and glucosylgalactosylhydroxylysine in human plasma were found to be very low compared with those for urine.[970]

Blood Cellular Element Glycoproteins.—The heterogeneity of human and animal red-cell glycoproteins, as revealed by isoelectric-focusing studies, was discussed in a review.[971] Several schemes for the isolation of the cell-surface glycoproteins were considered with respect to enzymic modification and the topographical arrangement of the glycoproteins. The review also included a list of established carbohydrate structures of red-cell glycoproteins, and the structures were compared with those of platelet and lymphocyte surface glycoproteins.

The membrane of human blood platelets has been characterized as a glycolipoprotein containing galactose, glucose, mannose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid (7% total).[972]

[964] C. F. Nadler, A. Woolf, and K. E. Harris, *Comp. Biochem. Physiol.*, 1971, **40B**, 567.
[965] A. Ferguson, *Comp. Biochem. Physiol.*, 1971, **38B**, 477.
[966] A. Q. Ansari, P. J. Dolphin, C. B. Lazier, K. A. Munday, and M. Akhtar, *Biochem. J.*, 1971, **122**, 107.
[967] P. J. Dolphin, A. Q. Ansari, C. B. Lazier, K. A. Munday, and M. Akhtar, *Biochem. J.*, 1971, **124**, 751.
[968] E. J. Hershgold, A. M. Davison, and M. E. Janzen, *J. Lab. Clin. Med.*, 1971, **77**, 185.
[969] W. V. Brown, R. I. Levy, and D. S. Fredrickson, *J. Biol. Chem.*, 1970, **245**, 6588.
[970] M. F. Lou and P. B. Hamilton, *Clin. Chem.*, 1971, **17**, 782.
[971] G. Uhlenbruck, *Chimia (Switz.)*, 1971, **25**, 10.
[972] A. J. Barber and G. A. Jamieson, *J. Biol. Chem.*, 1970, **245**, 6357.

Evidence has been obtained for the antigenic relationship between human red cell membrane and a urinary glycoprotein.[973]

Glycoproteins have been isolated from the surface of erythrocyte membranes by phenol–water extraction. The carbohydrate constituents released by hydrolysis were galactose, glucose, mannose, fucose, arabinose, xylose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and *N*-acetylneuraminic acid.[974] The anomalous behaviour of the major glycoprotein of human erythrocytes on electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulphate has been utilized in its purification.[975] Although the glycoprotein is found on the cell surface, it was concluded that the molecules extend through the membrane barrier to the interior surface of the cell membrane.

One of the two major components located on the external surface of human erythrocytes by reaction with formylmethionyl sulphone methyl phosphate possessed a molecular weight of 9×10^4 and contained 50% and 70% of the total carbohydrate and sialic acid, respectively, of the cell surface.[976] A glycoprotein–glycolipid fraction isolated from human erythrocytes was shown to contain hexose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid.[977]

Immunoglobulins.—The structure, carbohydrate composition, subcellular distribution, biosynthesis, transport, and secretion of immunoglobulins have been reviewed.[978]

Immunological testing of a series of glycopeptides derived by enzymic digestion of human immunoglobulins IgA, IgG, and IgM showed that they make a small but definite contribution to the antigenic determinants on the heavy chains.[979] The cross-reactivity of different classes of immunoglobulins implied the existence of common structural features. The interaction of human secretory immunoglobulin IgA with free secretory piece has been studied.[980]

The different levels of immunoglobulin IgA in serum and saliva from normal humans, and lymphoid and myeloid leukaemias and paraproteinaemias have been measured.[981] In all situations, there was no correlation between the serum and saliva IgA levels. The serum levels of IgA, IgG, and IgM in normals have been compared with those in cases of hydatid disease.[982] An IgA deficiency has been recognized in the serum

[973] C. Bron and M. D. Poulik, *Immunochem.*, 1971, **8**, 447.
[974] J. Metz, W. Èbert, and H. Weicker, *Clin. Chim. Acta*, 1971, **34**, 31.
[975] M. S. Bretscher, *Nature New Biol.*, 1971, **231**, 229.
[976] M. S. Bretscher, *Biochem. J.*, 1971, **122**, 40P.
[977] B. Zvilichovsky, P. M. Gallop, and O. O. Blumenfeld, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1234.
[978] F. Melchers, *Histochem. J.*, 1971, **3**, 389.
[979] F. Miller, *Immunochem.*, 1971, **8**, 99.
[980] P. Brandtzaeg, *Acta Pathol. Microbiol. Scand., B*, 1971, **79**, 165.
[981] A. Patakfalvi, Z. Miszlay, and G. Böhm, *Blut*, 1971, **22**, 86.
[982] B. Seitanidis and B. Angelopoulos, *Clin. Chim. Acta*, 1971, **31**, 311.

of a case of desquamative interstitial pneumonia.[983] The serum concentrations of IgG, IgD, IgE, and IgM in patients with gastrointestinal dysfunction were shown to be normal, but it is questionable whether the apparent IgA deficiency is related to the disease.[984]

Quantitative and chromatographic studies have been made of immunoglobulins IgA, IgG, and IgM in bovine colostrum and milk and neonatal calf serum.[985] IgA, IgM, and IgG_1 are selectively secreted into the colostrum, with the latter being the predominant immunoglobulin of the secretions. The IgA of both colostrum and calf serum appeared in a range of molecular sizes, the predominant form being an 11.2 *S* component with secretory piece.

IgA was shown to be the major immunoglobulin of porcine milk, but IgG was found to be the main immunoglobulin of serum and colostrum.[986] The variation of the levels of IgA, IgG, and IgM in piglet serum with age was determined.

The characteristics of IgA isolated from canine serum and colostrum have been compared in terms of their molecular weights (3.57×10^5 and 2.80×10^5, respectively), antigenicities, and subunits.[987] It was proposed that both IgA molecules consist of two covalently linked subunits each composed of two α and two light chains.

Only one of the two forms of heavy chain in mouse myeloma IgA and IgM incorporated D-[^{3}H]glucose and 2-acetamido-2-deoxy-D-[^{3}H]glucose.[988] The rate of attachment of carbohydrate to the immunoglobulin subunits was investigated.

An improved method for the isolation of normal human IgE is based on use of an immunoadsorbent formed by the mechanical entrapment of anti-human myeloma IgE into a lattice of a highly cross-linked macroporous polyacrylamide gel.[989] Desorption of the IgE was achieved using sodium thiocyanate to give a product containing 20% of IgG.

Sheep anti-human myeloma IgE covalently coupled to cellulose *trans*-2,3-carbonate has been employed as an immunoadsorbent in the determination of myeloma IgE by radioimmunoassay.[990]

The carbohydrate compositions of immunoglobulins from the following classes of species have been reported: Mammalia, Reptilia, Avis, Chondrichtyes, and Osteichtyes.[991] All contain hexose, fucose, 2-amino-2-deoxyhexose, and sialic acid with totals lying in the range 0.8—12.1%, *e.g.*

[983] F. S. Tushan, Z. A. Zawadzki, C. L. Vassallo, and E. D. Robin, *Amer. Rev. Respiratory Diseases*, 1971, **103**, 264.
[984] A. Bjernulf, S. G. O. Johansson, and A. Parrow, *Acta Med. Scand.*, 1971, **190**, 71.
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[990] P. McLaughlan, D. R. Stanworth, J. F. Kennedy, and H. Cho Tun, *Nature New Biol.*, 1971, **232**, 245.
[991] D. Frommel, G. W. Litman, S. L. Chartrand, U. S. Seal, and R. A. Good, *Immunochem.*, 1971, **8**, 573.

human IgG (2.4%) and human IgM (12.1%). IgG from human, equine, bovine, ovine, porcine, canine, chicken, and rabbit sera released galactose, mannose, fucose, 2-amino-2-deoxyglucose, and sialic acid. Generally, most of the carbohydrate is located in the H-chains with only a small fraction present in the L-chains.[992] Chicken IgG is distinctive in that it contains 4.5% total carbohydrate compared with an approximate value of 2% for IgG's from all the other species. The presence of less than one residue of galactose, fucose, and sialic acid per H-chain indicated heterogeneity of the carbohydrate moieties of all the IgG's.

The existence and position of α-helical segments in human IgG[993] and the electrochemical reduction of the molecule[994] have been studied. Conformational transitions of fragment Fc(t) of human IgG incited by alkyl sulphates of various hydrophilic chain lengths have been described.[995] Human IgG and IgM mechanically entrapped in the lattices of highly cross-linked, macroporous polyacrylamide gels have found use as specific and stable immunoadsorbents.[935]

The characteristics of normal and myeloma forms of human IgG have been compared by isoelectric focusing.[996] Glycopeptides released from human normal and myeloma IgG's by proteolysis contained galactose (0.3—2.3), mannose (3), fucose (0.6—1.2), 2-acetamido-2-deoxyglucose (3.9—6.0), and sialic acid (0—2 residues per mole).[997] The variability in the number of galactose, fucose, and sialic acid residues was due to micro-heterogeneity of the oligosaccharide moieties, whereas the variability in the number of 2-acetamido-2-deoxyglucose residues reflected differences in the sequences of monosaccharides in the cores of the molecules. Two of the seven myeloma IgG's examined possessed an additional oligosaccharide chain. The structures (85)—(87) of the oligosaccharide moieties were determined using purified glycosidases, periodate oxidation, and methylation.

The monosaccharide compositions of six human myeloma IgG's, in which the carbohydrate was located on the Fab fragment, were similar consisting of hexoses (5—6), fucose (1), 2-amino-2-deoxyglucose (4—5), and sialic acid (1 mole per mole of light chain or Fd fragment).[998] The carbohydrate was located on the light chains in four cases, on the Fd fragment in one case, and on both the light chain and Fd fragment in another. Although investigation of the amino-acid sequences showed that the position of the glycopeptide linkage varies from one glycopeptide to another,

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[993] V. P. Zavyalov, *Biofizika*, 1971, **16**, 922.
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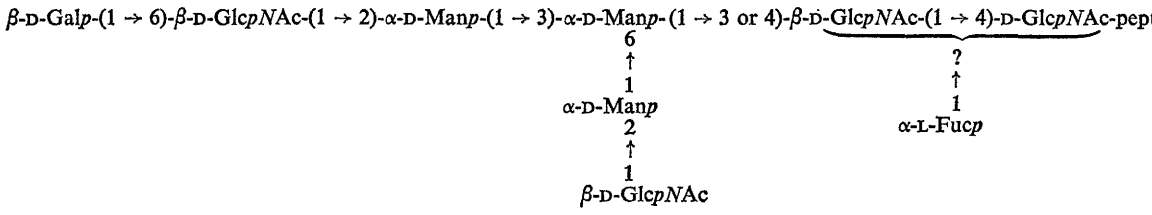
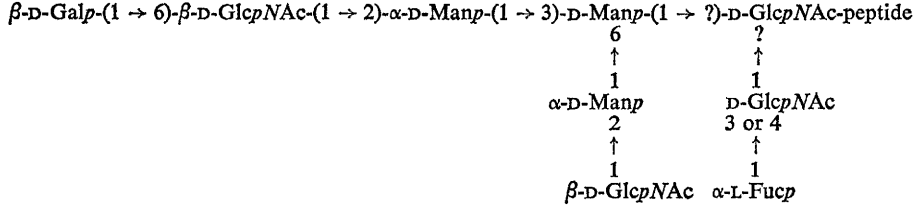
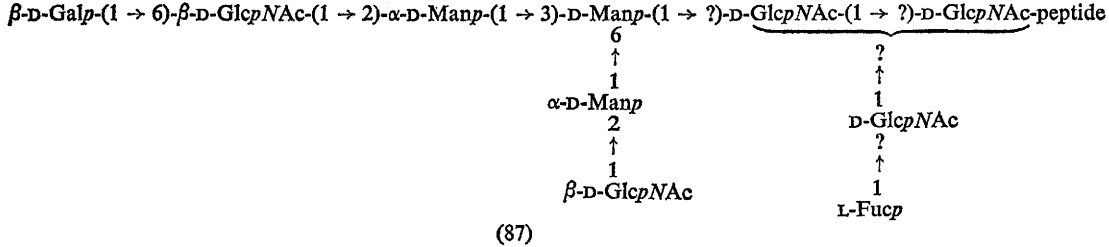

β-D-Galp-(1 → 6)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 3)-α-D-Manp-(1 → 3 or 4)-β-D-GlcpNAc-(1 → 4)-D-GlcpNAc-peptide
6
1
α-D-Manp
2
1
β-D-GlcpNAc
?
1
α-L-Fucp

(85)


β-D-Galp-(1 → 6)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 3)-D-Manp-(1 → ?)-D-GlcpNAc-peptide
6
1
α-D-Manp
2
1
β-D-GlcpNAc
?
1
D-GlcpNAc
3 or 4
1
α-L-Fucp

(86)


β-D-Galp-(1 → 6)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 3)-D-Manp-(1 → ?)-D-GlcpNAc-(1 → ?)-D-GlcpNAc-peptide
6
1
α-D-Manp
2
1
β-D-GlcpNAc
?
1
D-GlcpNAc
?
1
L-Fucp

(87)

it appears to be restricted to two regions within the variable portion of the light chains and Fd fragments—one near the *N*-terminus and the other near the *C*-terminus.

The κ-type light chains of myeloma immunoglobulins IgG obtained from two sera contained galactose, mannose, fucose, 2-amino-2-deoxyglucose, and sialic acid.[999] Glycopeptides isolated from tryptic digests of the κ-type light chains were derived from the variable region of the chain in both cases. The serum levels of IgG in normals and in a series of infantile conditions have been compared; the levels in cases of Down's syndrome (trisomy 21) were found to be low.[1000]

Details of the optimum conditions of hydrolysis, derivatization, and gas chromatography for determination of the carbohydrate contents of immunoglobulins have been reported from experiments using bovine IgG as a model.[695] The immunoglobulin released galactose (0.22%), mannose (0.50%), fucose (0.14%), and 2-amino-2-deoxyglucose (0.64%).

The products from papain and pepsin hydrolyses of guinea-pig IgG_1 and IgG_2 were isolated and characterized with respect to composition and antigenic specificity.[1001] The hexose was confined principally to the Fc fragments, the Fab and $(Fab')_2$ fragments containing not greater than 10% of hexose. The Fc′ fragments from both immunoglobulins were almost devoid of carbohydrate.

A simplified procedure for the preparation of Sephadex G-200 for the effective separation of rat serum immunoglobulins IgG and IgM involved boiling a mixture of the cross-linked dextran and isotonic, unbuffered sodium chloride for 2—3 min prior to packing of the gel bed.[111]

A new subclass (IgG3) of mouse serum immunoglobulin IgG exhibited a strong tendency to form non-covalent aggregates, which were dissociable with acid.[1002] IgG3 (molecular weight 1.3×10^5) contains a small proportion of 2-amino-2-deoxyglucose residues and the involvement of L-asparagine residues was also indicated.

The behaviour of human immunoglobulin IgM on discontinuous electrophoresis in polyacrylamide gel has been investigated.[1003] Structural investigation of the hinge region of the μ heavy chain of human IgM showed that it contains one of the five carbohydrate moieties of the chain, and that the glycopeptide linkage probably involves an asparagine residue.[1004] Cyanogen bromide fragmentation has been employed in a further attempt to localize the carbohydrate moieties of the heavy chain of human IgM; it was concluded that they are located in the Fc and Fd portions.[1005]

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[1004] C. Paul, A. Shimizu, H. Köhler, and F. W. Putnam, *Science*, 1971, **172**, 69.
[1005] J. M. Davie and C. K. Osterland, *Immunochem.*, 1971, **8**, 303.

The conformation of immunoglobulins IgM, from Waldenström macroglobulinaemia patients, in various solvents has been studied by i.r. spectroscopy.[1006] The results suggested that the molecules are non-helical in their natural state and contain the anti-parallel β-structure. The conformation of the IgM subunit was indistinguishable from that of the intact molecule. It was deduced from analysis of a series of five glycopeptides arising from cyanogen bromide treatment of each of two human Waldenström IgM's that the carbohydrate is attached at five sites in the constant sequence region of the μ heavy chain.[1007] The carbohydrate moieties are either of a simple type, containing mannose and 2-acetamido-2-deoxyglucose, or of a complex type, containing galactose, mannose, fucose, 2-amino-2-deoxyglucose, and *N*-acetylneuraminic acid; all are linked to aspartyl residues in the protein chain. A model showing the arrangement of the subunits in one IgM was presented.

Bovine IgM isolated from colostral whey has been characterized physicochemically and immunochemically.[1008] The molecular weight of the intact molecule was found to be 1.03×10^6, whereas those of the component μ and light chains were 7.60×10^4 and 2.25×10^4, respectively.

IgM from the murine plasmacytoma MOPC 104E of mice was prepared by an immunospecific procedure involving precipitation by dextran and dissociation of the complex by a hapten.[1009] The binding properties of the purified IgM were examined by equilibrium dialysis, and it was found that the molecule possesses ten binding sites for the hapten *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucitol. Investigation of the biosynthesis of the IgM suggested that light-chain synthesis is in excess of heavy-chain production.[1010]

A 14 *S* purified immune macroglobulin (molecular weight 6.1×10^5) from the longnose gar (*Lepisosteus osseus*) yielded a total of 4.97% of carbohydrate consisting of galactose (48), mannose (31), fucose (7), 2-amino-2-deoxyglucose (43), and sialic acid (20 residues per mole). Dissociation into heavy and light chains (molecular weights 7.0×10^4 and 2.3×10^4, respectively) was observed on treatment of the reduced, alkylated molecule with guanidine hydrochloride. Overall, the properties of the immunoglobulin resembled those of mammalian IgM's.[1011] Serum from the giant grouper (*Epinephelus itaira*) contained 16 *S* and 6.4 *S* immunoglobulins.[1012] The former (molecular weight 7.0×10^5) possesses a relatively high proportion of hexose and appears to be an IgM, whereas the latter appears to be a fragment of the 16 *S* species.

[1006] J. N. Miller, *Biochim. Biophys. Acta*, 1971, **236**, 655.
[1007] A. Shimizu, F. W. Putnam, C. Paul, J. R. Clamp, and I. Johnson, *Nature New Biol.*, 1971, **231**, 73.
[1008] T. K. S. Mukkur and A. Froese, *Immunochem.*, 1971, **8**, 257.
[1009] N. M. Young, I. B. Jocius, and M. A. Leon, *Biochemistry*, 1971, **10**, 3457.
[1010] R. M. E. Parkhouse, *Biochem. J.*, 1971, **123**, 635.
[1011] R. T. Acton, P. F. Weinheimer, H. K. Dupree, E. E. Evans, and J. C. Bennett, *Biochemistry*, 1971, **10**, 2028.
[1012] L. W. Clem, *J. Biol. Chem.*, 1971, **246**, 9.

The Fab fragment, heavy chains, and light chains of a 5.7 *S* immunoglobulin (molecular weight 1.18×10^5) from duck possess molecular weights of 4.8×10^4, 3.5×10^4, and 2.3×10^4, respectively.[1013] The 7.8 *S* immunoglobulin from the same source and its heavy chain have molecular weights of 1.78×10^5 and $6.2—6.6 \times 10^4$, respectively. The 5.7 *S* and 7.8 *S* immunoglobulins afforded 0.6% and 5% carbohydrate, respectively, consisting of hexose (1.9 and 31.0), fucose (1.0 and 4.4), 2-acetamido-2-deoxyglucose (1.4 and 12.5), and sialic acid (0.3 and 1.5 moles per mole, respectively). Both immunoglobulins are distinct from mammalian immunoglobulins.

Salivary, Mucous, and Other Mammalian Body-fluid Glycoproteins

Glycoproteins in human salivary secretion have been separated by electrophoresis on polyacrylamide gels in the presence of urea at acid pH.[1014]

A calcium-precipitable glycoprotein from human submaxillary saliva was found to contain covalently linked phosphate groups (5 per monomer, molecular weight 1.1×10^4).[1015] The glycoprotein existed as a dimer in aqueous solution, and aggregated in the presence of calcium ions owing to reduction of its electrostatic charges and changes in conformation.

The presence of a series of related basic glycoproteins in individual samples of human parotid saliva has been demonstrated by chromatographic, electrophoretic, and immunological studies.[1016] The glycoproteins contained differing amounts of hexose, fucose, and 2-amino-2-deoxyhexose, but no sialic acid; they also differed in their overall charge densities. One of the four proteinaceous macromolecules purified from human parotid saliva yielded neutral carbohydrate (4%).[1017]

Subfractions from the bile of normals and patients with functional disturbance of the liver or with infections in the biliary tract all contained glycoproteins, the carbohydrate components of which consisted of hexose (27 and 33%), fucose (6 and 6%), and 2-amino-2-deoxyhexose (28 and 34% maximum, normals and abnormals, respectively).[865]

The non-dialysable fractions from seminal plasma of stallion and boar contained galactose, mannose, and fucose (10 : 3 : 1) and galactose, glucose, mannose, and fucose (20 : 2 : 5 : 1), respectively.[1018] The corresponding fraction from bovine seminal plasma released galactose, 2-amino-2-deoxyhexose, and sialic acid.[1019]

The effect of gel porosity on the gel electrophoretic properties of bovine, ovine, porcine, and canine submaxillary mucus glycoproteins has been

[1013] B. Zimmerman, N. Shalatin, and H. M. Grey, *Biochemistry*, 1971, **10**, 482.
[1014] R. M. Stringham, *J. Chromatog.*, 1970, **50**, 345.
[1015] F. A. Bettelheim, *Biochim. Biophys. Acta*, 1971, **236**, 702.
[1016] R. D. Friedman, A. D. Merritt, and D. Bixler, *Biochim. Biophys. Acta*, 1971, **230**, 599.
[1017] A. Bennick and G. E. Connell, *Biochem. J.*, 1971, **123**, 455.
[1018] S. Baronos, *J. Reprod. Fert.*, 1971, **24**, 303.
[1019] S. Baronos, *J. Reprod. Fert.*, 1971, **25**, 219.

investigated.[1020] Some gels were suitable for characterization of the glycoproteins on a microgram scale and gave an indication of their relative molecular weights, degrees of polydispersity, and carbohydrate–protein compositions. The effects of physical deaggregation and disulphide-bond cleavage on the electrophoretic characteristics of submaxillary glycoproteins and canine tracheal mucus glycoproteins have also been studied.[1021]

Two distinct glycoproteins, which gave single bands on isoelectric focusing, were isolated from the gall-bladder secretions of pigs of blood group A and H.[1022] The carbohydrate components (70 and 72%, respectively) of the mucins comprised galactose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid (1.09 : 1.99 : 1.00 : 1.00 : 0.02 and 1.18 : 0.67 : 0.36 : 1.00 : 0.02, respectively). Degradation with alkali indicated that 2-acetamido-2-deoxygalactose, serine, and threonine units are involved in the glycopeptide linkages.

Sparingly soluble mucin obtained from anacid secretion of canine Heidenhain pouch was solubilized by the use of sodium sulphite and was then fractionated into a number of glycoproteins.[1023] The glycoproteins contained galactose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and *N*-acetylneuraminic acid, whereas serine, threonine, and proline together constituted more than half of the amino-acid residues. Alkaline borohydride studies indicated that *O*-glycosidic linkages exist between 2-acetamido-2-deoxyhexose and both serine and threonine residues.

Glycoprotein fractions of intestine and faeces of germ-free rats were found to contain galactose (19.5 and 18.4), mannose (3.7 and 3.8), fucose (9.7 and 8.3), arabinose (3.8 and 1.3), xylose (3.9 and 1.5), 2-acetamido-2-deoxygalactose (18.3 and 19.1), 2-acetamido-2-deoxyglucose (20.2 and 21.2), and sialic acid (6.0 and 6.3%, respectively).[1024]

On the basis of incorporation experiments with 2-amino-2-deoxy-D-1-[^{14}C]glucose, it was concluded that the mucosubstance secreted by rat Rous sarcoma cells contains 2-amino-2-deoxyglucose in a macromolecular form.[1025]

Urinary Glycoproteins

The carbohydrate compositions of Bence Jones glycoproteins isolated from urines of myeloma patients are similar to those of the corresponding myeloma IgG immunoglobulins.[998] They comprised hexose (5—6), fucose

[1020] K. G. Holden, N. C. F. Yim, L. J. Griggs, and J. A. Weisbach, *Biochemistry*, 1971, **10**, 3105.
[1021] K. G. Holden, N. C. F. Yim, L. J. Griggs, and J. A. Weisbach, *Biochemistry*, 1971, **10**, 3110.
[1022] D. H. Neiderhiser, J. J. Planter, and D. M. Carlson, *Arch. Biochem. Biophys.*, 1971, **145**, 155.
[1023] Y. S. Kim and M. I. Horowitz, *Biochim. Biophys. Acta*, 1971, **236**, 686.
[1024] J. K. Wold, R. Khan, and T. Midtvedt, *Acta Pathol. Microbiol. Scand., B*, 1971, **79**, 525.
[1025] D. M. Coe, J. T. Gallagher, J. S. Gibson, and J. C. Marsden, *Biochem. J.*, 1970, **120**, 21P.

(1), 2-amino-2-deoxyglucose (5—6), and sialic acid (3—4 moles per mole); aspartyl residues appear to be involved in the glycopeptide linkages. Human Bence Jones glycoprotein mechanically entrapped in a lattice of highly cross-linked macroporous polyacrylamide gel has been used as an immunoabsorbent.[935]

One of the thirty-six peptides separated from human urine appeared to be a glycopeptide containing galactose (2 moles per mole).[1026] Alkaline borohydride treatment indicated that serine is not involved in the glycopeptide linkage, and prolonged digestion with leucine aminopeptidase yielded a fragment composed of cysteine and galactose alone. As the possibility of a thiazolidine-type structure was excluded, it was concluded that the two galactose units are linked glycosidically to the sulphur atom of cysteine. The structure (88) was proposed for the glycopeptide.

Gal-Gal-Cys-Glu-His-Ser-His-Asp-Gly-Ala
(88)

Ion-exchange chromatography permitted the separation of glucosylgalactosylhydroxylysine and galactosylhydroxylysine from many of the other components of human urine. The two species were found to occur in a molar ratio of 1.0 : 1.1, respectively.[276] The constituents of dialysed human serum contained (on average) hexose (36.7), fucose (4.7), 2-amino-2-deoxyhexose (24.8), hexuronic acid (8.1), and sialic acid (21.9 mg/g creatinine) for the adult age group; values for children were higher.[135] A human urinary glycoprotein was demonstrated to be antigenically related to the red-cell membrane.[973]

Urinary tetra- and octa-saccharides from a case of G_{M_1}-gangliosidosis afforded different amounts of mannose and 2-acetamido-2-deoxyglucose.[1027] The oligosaccharides differed from those observed in the urine from cases of Tay–Sachs disease. The concentrations of maltose and isomaltose in the urine of severely injured humans were found to be greater than normal.[1028]

Rabbit Tamm–Horsfall urinary glycoprotein has been purified until homogeneous, and has been shown to contain hexose (14.4%), fucose (0.5%), 2-acetamido-2-deoxygalactose (0.2%), 2-acetamido-2-deoxyglucose (13.3%), and sialic acid (4.6%).[1029] Measurement of the molecular weight of the glycoprotein in the presence of sodium dodecyl sulphate gave a value of 8.4×10^4. The immunological properties of the molecule were studied.

Avian Egg Glycoproteins

An antigenic glycopeptide, chick allantoic antigen, which combines with antibody to influenza virus host material, contains D-galactose (26%), fucose

[1026] C. J. Lote and J. B. Weiss, *Biochem. J.*, 1971, **123**, 25P.
[1027] G. Strecker and J. Montreuil, *Clin. Chim. Acta*, 1971, **33**, 395.
[1028] V. Vítek, K. Vítek, and A. Cowley, *Clin. Chim. Acta*, 1971, **33**, 33.
[1029] A. M. S. Marr, A. Neuberger, and W. A. Ratcliffe, *Biochem. J.*, 1971, **122**, 623.

(4.3%), 2-amino-2-deoxygalactose (7.7%), 2-amino-2-deoxyglucose (17.9)%, and *N*-acetylneuraminic acid (1%).[1030] Mild acid hydrolysis of the glycopeptide liberated terminal, non-reducing residues of fucose and D-galactofuranose among other products.[1031] D-Galactofuranose residues were also shown to be present in the interior of the carbohydrate chains; cleavage at these residues with acid liberated 10% of the molecule as oligosaccharides. Periodate oxidation showed that most of the fucose residues and 50% of the D-galactose residues of the antigen are oxidizable, whereas the 2-amino-2-deoxyhexose residues are not.[1032] It was concluded that the fucose residues occupy predominantly terminal, non-reducing positions and that the oxidizable D-galactose residues are substituted at C-6 and/or C-2. Alkaline degradation showed that the glycopeptide contains glycosidic linkages between 2-acetamido-2-deoxygalactose and both serine and threonine residues.[1033] The 2-acetamido-2-deoxygalactose residues are also substituted glycosidically at C-3 and C-6. Structural features of the antigen were compared with those of an immunologically related glycopeptide from allantoic fluid. Oligosaccharides that combined with antibody to the chick allantoic sulphated glycopeptide were produced by dilute acid and alkali treatment of the glycopeptide.[1034] Chemical and immunological assays of the oligosaccharides, in conjunction with periodate oxidation and glycohydrolase studies, demonstrated that peptide, sialic acid, and 2-amino-2-deoxyhexose residues of the glycopeptide play no part in conferring activity, but that D-galactose and fucose residues are components of the active site.

Purified chicken egg-white avidin contains mannose (5) and 2-amino-2-deoxyglucose (4 residues per mole).[1035] One of the fragments released by the action of trypsin contained all the carbohydrate of the intact molecule.

The magnitude of the volume changes produced by the reaction of hen egg ovalbumin with acid and base in water and denaturing media has been determined by dilatometric analysis.[1036] Schematic immunochemical models have been constructed from the data obtained for the reactions of chicken-, duck-, and turkey-egg ovalbumin with goat IgG antibodies.[1037] A UDP-galactose:glycoprotein galactosyltransferase from rat serum was capable of catalysing the transfer of D-[^{14}C]galactose from UDP-D-[^{14}C]galactose to hen egg ovalbumin.[1038] Mild alkaline hydrolysis released less than 4% of the incorporated radioactivity, demonstrating that D-galactose is not attached to serine or threonine residues by *O*-glycosidic

[1030] M. J. How and J. D. Higginbotham, *Carbohydrate Res.*, 1970, **12**, 355.
[1031] M. J. How and J. D. Higginbotham, *Carbohydrate Res.*, 1970, **14**, 327.
[1032] M. J. How and J. D. Higginbotham, *Carbohydrate Res.*, 1971, **16**, 9.
[1033] M. J. How and J. D. Higginbotham, *Carbohydrate Res.*, 1970, **14**, 335.
[1034] J. D. Higginbotham, R. Schöyen, K. Mortensson-Egnund, M. J. How, and A. Harboe, *Acta Pathol. Microbiol. Scand., B*, 1971, **79**, 349.
[1035] T. Huang and R. J. DeLange, *J. Biol. Chem.*, 1971, **246**, 686.
[1036] S. Katz and J. E. Miller, *Biochemistry*, 1971, **10**, 3569.
[1037] M. S. Weintraub and M. Schlamowitz, *Comp. Biochem. Physiol.*, 1971, **38B**, 513.
[1038] R. R. Wagner and M. A. Cynkin, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 57.

linkages. The properties of ovalbumin from hen egg mechanically entrapped in a lattice of highly cross-linked macroporous polyacrylamide gel have been investigated.[935] The use of the preparation as an immunoadsorbent was tested.

Hen egg-white ovomucoid has been freed from the usual contaminants, lysozyme, ovoinhibitor, and ovalbumin, by successive batch treatments with anion and cation exchangers.[1039] Homogeneity of the product was demonstrated by subjecting it to a number of physicochemical techniques; it was shown to contain 2-amino-2-deoxyglucose (23.9 residues per mole) and to possess a molecular weight of 2.73×10^4.

The technique of flat-bed isoelectric focusing was used to examine the origin of charge heterogeneity in hen egg-white ovomucoid.[1040] Products from a variety of preparative techniques contained three major and two minor components. Desialylation with neuraminidase reduced the number of bands to two, indicating that only part of the heterogeneity is due to variation in the sialic acid contents of the species. Mild acid hydrolysis of the ovomucoid, under conditions required for removal of sialic acid, brought about extensive alterations in charge which were unrelated to the sialic acid content. However, it was concluded from the results of electrophoresis on cellulose acetate that the heterogeneity of preparations of ovomucoid is due to the presence of contaminants.[1041] Three major and two minor species of ovomucoid were also separated by chromatography on SE–Sephadex.[1042] The predominant sialic acid-free species was further resolved into three fractions by DEAE–cellulose chromatography. Wide variation was observed in the contents of galactose, 2-acetamido-2-deoxyglucose, and sialic acid, but not in the mannose contents of the ovomucoid species; charge heterogeneity was again related in part to variations in the content of sialic acid. The implications of variable carbohydrate composition on the structure and function of ovomucoid was discussed.

The mode of inhibitory action of chicken ovomucoid on proteinases has been investigated.[1043] Evidence indicating that turkey egg ovomucoid has independent binding sites for trypsin and chymotrypsin has been obtained.[1044]

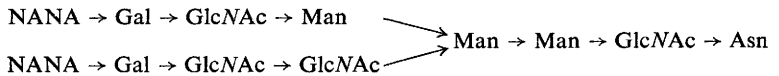
Hen egg ovomucin gel, solubilized by treatment with mercaptoethanol, was separated into two components by preparative electrophoresis in a density gradient. The fast- and slow-running components contained hexose (18.4 and 6.8), 2-amino-2-deoxyhexose (18.3 and 6.7), sialic acid (11.4 and 0.8), and sulphate (1.18 and 0.06%, respectively).[1045] Models for the structure of ovomucin gel and for the mechanism of egg-white thinning have been proposed.[1046]

[1039] J. G. Davis, C. J. Mapes, and J. W. Donovan, *Biochemistry*, 1971, **10**, 39.
[1040] J. G. Beeley, *Biochim. Biophys. Acta*, 1971, **230**, 595.
[1041] E. Jakubczak and J. Montreuil, *Compt. rend.*, 1971, **273**, *D*, 1430.
[1042] J. G. Beeley, *Biochem. J.*, 1971, **123**, 399.
[1043] J. Travis, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 793.
[1044] A. Gertler and G. Feinstein, *European J. Biochem.*, 1971, **20**, 547.
[1045] A. Kato and Y. Sato, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 439.
[1046] A. Kato, R. Nakamura, and Y. Sato, *Agric. and Biol. Chem. (Japan)* 1971, **35**, 351.

Hen egg-white ovotransferrin was shown to contain hexose (3.2) and 2-amino-2-deoxyglucose (4.2 moles per mole).[1047] The carbohydrate residues were stable to short-term exposure to periodate, which otherwise destroyed some of the amino-acids. The carbohydrate was similarly retained in the cases of iron and copper complexes of the ovotransferrin. Treatment of hen egg ovotransferrin with cyanogen bromide yielded three fragments in unit mole ratios.[1048] The fragments contained mannose (1, 1, and 0) and 2-amino-2-deoxyglucose (1, 2, and 0 residues per mole), with two moles of each fragment being recovered per mole of parent ovotransferrin cleaved. The molecular weights of the fragments were estimated as 21.0×10^3, 9.4×10^3, and 7.0×10^3, respectively, the sum of which is approximately 50% of the molecular weight of the intact molecule (7.66×10^4). It was concluded that ovotransferrin consists of several duplicate sections. Polymorphism in egg-white ovotransferrins of rock dove (*Columba livia*), wood pigeon (*C. palumbus*), and barbary dove (*Streptopelia risoria*) has been demonstrated by gel electrophoresis and was attributed to variations in the carbohydrate compositions.[965]

Analysis of the ethanol-precipitable fraction of a papain digest of hen egg-white revealed the presence of neutral sugar (11.2), 2-amino-2-deoxygalactose (4.6), 2-amino-2-deoxyglucose (7.0), and uronic acid (1.8 mg/kg egg white).[857]

Purified preparations of phosvitin, the phosphoglycoprotein of hen egg yolk, afforded 6.5% of carbohydrate consisting of galactose (3), mannose (3), 2-amino-2-deoxyglucose (5), and sialic acid (2 residues per molecular weight of 4.0×10^4).[1049] Pronasic digestion afforded a glycopeptide containing all the carbohydrate of the parent molecule attached to an aspartyl residue. A partial carbohydrate sequence (89) for the oligosaccharide moiety was proposed on the basis of sequential glycohydrolase hydrolysis and Smith degradation.[1050]



(89)

Miscellaneous Glycoproteins

Fractions obtained from chick-embryo fibroblast cell membranes contained sialic acid and neutral sugar.[1051]

Two glycopeptides obtained from proteolytic digests of fibroin from the silkworm (*Bombyx mori*) released mannose (3 and 5) and 2-amino-2-deoxyglucose (2 and 2 residues per mole).[1052] The glycopeptide linkage of

[1047] P. Azari and J. L. Phillips, *Arch. Biochem. Biophys.*, 1970, **138**, 32.
[1048] J. L. Phillips and P. Azari, *Biochemistry*, 1971, **10**, 1160.
[1049] R. Shainkin and G. E. Perlmann, *J. Biol. Chem.*, 1971, **246**, 2278.
[1050] R. Shainkin and G. E. Perlmann, *Arch. Biochem. Biophys.*, 1971, **145**, 693.
[1051] J. F. Perdue and J. Sneider, *Biochim. Biophys. Acta*, 1970, **196**, 125.
[1052] H. Sinohara, Y. Asano, and A. Fukui, *Biochim. Biophys. Acta*, 1971, **237**, 273.

both glycopeptides was shown to involve 2-amino-2-deoxyglucose linked glycosidically to asparagine residues. Structural studies on the oligosaccharides indicated that the partial structure (90) occurs in both glycopeptides.

$$\begin{array}{l} \text{Man} \rightarrow \text{Man} \rightarrow \text{Man} \rightarrow \text{Glc}N\text{Ac} \rightarrow \text{Glc}N\text{Ac} \rightarrow \text{Asn} \\ \qquad\qquad\quad \uparrow \\ \qquad\qquad\quad \text{Man} \end{array}$$

(90)

Calliphorin, a glycoprotein isolated from larvae and pupae of the blowfly (*Calliphora erythrocephala*), has been purified until homogeneous by a number of criteria; it was found to contain 0.4—0.5% of carbohydrate and to possess a molecular weight of 5.29×10^5.[1053]

The amount of uniformly labelled D-[^{14}C]glucose incorporated into the chitin of crayfish (*Orconectes obscurus*) served as an indication of the rate of chitin synthesis.[1054] Changes in the rate of chitin synthesis during the moulting cycle were observed.

Acidic hydrolysis of a glycoprotein extract from the jelly coat of sea-urchin (*Pseudocentrotus depressus*) eggs yielded *N*-glycolylneuraminic acid and another sialic acid identified as 5-acetoglycolamido-4-*C*-methyl-3,4,5-trideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid.[1055]

Periostracum, egg-capsule, and operculum glycoproteins, structural glycoproteins secreted extracellularly by the gastropod mollusc *Buccinum undatum*, all contain galactose, glucose, mannose, fucose, rhamnose, xylose, 2-amino-2-deoxygalactose, and 2-amino-2-deoxyglucose (3.30, 5.00, and 0.33% hexose; and 5.80, 0.57, and 0.21% 2-amino-2-deoxyhexose, respectively).[1056] The glycoproteins from the periostracum and egg capsule resemble α-fibroins, whereas the operculum glycoprotein is comparable to β-fibroins.

In a study of the biosynthesis of polysaccharides by encysting cells of *Acanthamoeba castellanii*, an enzyme system that catalysed the incorporation of D-[^{14}C]glucose from UDP-D-[^{14}C]glucose into alkali-soluble and alkali-insoluble products was identified.[1057] Both products were characterized as β-(1 → 4)-glucans on the basis of partial acid hydrolysis and acetolysis.

Proteolysis of body-wall connective tissue from the sea anemone, *Metridium dianthus*, followed by gel filtration yielded a fraction containing all of the soluble carbohydrate.[761] The latter comprised galactose (9.5%), glucose (12%), mannose (1.3%), fucose (1.1%), ribose (0.1%), and 2-amino-2-deoxyhexose (1.5% of the fraction).

[1053] E. A. Munn, A. Feinstein, and G. D. Greville, *Biochem. J.*, 1971, **124**, 367.
[1054] D. E. Hornung and J. R. Stevenson, *Comp. Biochem. Physiol.*, 1971, **40B**, 341.
[1055] K. Hotta, K. Kurokawa, and S. Isaka, *J. Biol. Chem.*, 1970, **245**, 6307.
[1056] S. Hunt, *Comp. Biochem. Physiol.*, 1971, **40B**, 37.
[1057] J. L. Potter and R. A. Weisman, *Biochim. Biophys. Acta*, 1971, **237**, 65.

A glycoprotein isolated and purified from experimental sponge granulomas in rat skin was shown to contain hexose (7.0%), comprising galactose, glucose, and mannose (15 : 73 : 12, respectively), and 2-amino-2-deoxyhexose (3.7%), comprising 2-amino-2-deoxygalactose and 2-amino-2-deoxyglucose (1 : 4).[1058] The glycoprotein possesses a pI of 4.1.

Glycoprotein has been found in the spikes of the membranes of Sindbis virus as evidenced by the incorporation of 2-amino-2-deoxy-D-[^{3}H]-glucose.[1059]

[1058] A. Rajamäki and E. Kulonen, *Biochim. Biophys. Acta*, 1971, **243**, 398.
[1059] R. W. Compans, *Nature New Biol.*, 1971, **229**, 114.

6
Enzymes

Various mechanistic hypotheses which have been suggested for the mode of action of glycosidases have been reviewed.[1060] The principles involved in the binding of enzymes to artificial matrices have been considered [1061] and methods of preparation of a number of insolubilized carbohydrases have been summarized.[1062] The uses of affinity chromatography in the preparation of enzymes [693] and the subject of metabolic regulation by modification of enzymes [775] have been reviewed.

Acetamidodeoxyhexosidases

The development of β-acetamidodeoxyglucosidase and β-acetamidodeoxygalactosidase activities in human brain has been followed during prenatal and postnatal periods.[1063] Subcellular fractions of human forebrain cortex also contain the two activities.[1064]

A number of methods have been examined for the differential assay of β-acetamidodeoxyhexosidases.[1065] Points of disagreement between the methods were highlighted and a procedure involving batch-wise separation on DEAE-cellulose was recommended as being the most reproducible.

β-Acetamidodeoxyhexosidases A and B were extracted from human liver and purified by column chromatography and isoelectric focusing.[1066] Both enzymes exhibited a molecular weight of 1.3×10^5 and possessed β-acetamidodeoxygalactosidase and β-acetamidodeoxyglucosidase activities. Their close relationship was further demonstrated in that isoenzyme A could be converted into a form which possessed the same pI as isoenzyme B. Either the A or both forms were missing in specimens from two common variants of Tay–Sachs disease. A microscale isoelectric focusing technique capable of separating the A and B enzymes has been advocated for the diagnosis of Tay–Sachs disease.[1067] Confirmation of the absence of

[1060] B. Capon, *Biochimie*, 1971, **53**, 145.
[1061] K. Mosbach, *Sci. Amer.*, 1971, **224**, No. 5, 26.
[1062] J. F. Kennedy, *Z. klin. Chem. klin. Biochem.*, 1971, **9**, 71.
[1063] R. Öhman and L. Svennerholm, *J. Neurochem.*, 1971, **18**, 79.
[1064] R. Öhman, *J. Neurochem.*, 1971, **18**, 89.
[1065] N. Dance, R. G. Price, and D. Robinson, *Biochim. Biophys. Acta*, 1970, **222**, 662.
[1066] K. Sandhoff and W. Wässle, *Z. physiol. Chem.*, 1971, **352**, 1119.
[1067] K. Harzer, *Z. analyt. Chem.*, 1970, **252**, 170.

β-acetamidodeoxyhexosidase activity in amniotic material from cases of Tay–Sachs disease provided the basis for prenatal diagnoses.[1068]

Human pregnancy serum contained β-acetamidodeoxyglucosidase isoenzymes A and 'P', the latter being a newly identified form.[1069] Isoenzymes A, B, and P were distinguishable by electrophoresis; A and P both had pH optima of 4.5 and were indistinguishable by inhibition studies. The thermal stability of isoenzyme P was intermediate between that of A and B. Traces of β-acetamidodeoxyglucosidase P were found with A and B forms in placenta and foetal membranes, whereas cord serum contained only isoenzyme A.

A 6.6 *S* β-acetamidodeoxyglucosidase has been isolated and purified from ovine thyroid gland.[1070] The activity of the enzyme against desialysed thyroglobulin and sialic acid-free glycopeptides from thyroglobulin was investigated. Application of electrodecantation to the initial purification of ovine testicular β-acetamidodeoxyglucosidase gave increases of up to ten-fold in the specific activity. Isoelectric focusing showed that the enzyme has a pI of 6.3.[1071] A procedure has been developed for the isolation of β-acetamidodeoxyglucosidase from porcine epididymus.[1072] The action of the enzyme on *m*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was inhibited by 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose; the enzyme was also active against disaccharides of the type 2-acetamido-2-deoxy-D-glucosyl-*X*, in which *X* represents either 1 → 3-, 1 → 4-, or 1 → 6-linked residues of D-galactose, D-glucose, or 2-acetamido-2-deoxy-D-glucose.

Polyomavirus - transformed BHK line cells derived from hamster kidney fibroblasts possessed greater β-acetamidodeoxyglucosidase and β-acetamidodeoxygalactosidase activities than the parent BHK line cells.[1073]

The distribution of β-acetamidodeoxyglucosidase in rat kidney glomeruli and tubular fragments has been assessed and multiple forms of the enzyme were separated by starch-gel electrophoresis.[1074] β-Acetamidodeoxyglucosidase has been solubilized from subcellular preparations of rat liver[1075] and glucagon had an effect on the same activity in the liver lysosomes.[1076] Characteristics of the β-acetamidodeoxyglucosidase of rat chorioallantoic placenta included an activity optimum at pH 4.5.[1077]

[1068] J. S. O'Brien, S. Okada, D. L. Fillerup, M. L. Veath, B. Adornato, P. H. Brenner, and J. G. Leroy, *Science*, 1971, **172**, 61.
[1069] J. L. Stirling, *Biochem. J.*, 1971, **123**, 11P.
[1070] O. Chabaud, S. Bouchilloux, and M. Ferrand, *Biochim. Biophys. Acta*, 1971, **227**, 154.
[1071] B. G. Winchester, M. Caffrey, and D. Robinson, *Biochem. J.*, 1971, **121**, 161.
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[1076] W. Guder, K. D. Hepp, and O. Wieland, *Biochim. Biophys. Acta*, 1970, **222**, 593.
[1077] R. L. Schultz and P. J. Jacques, *Arch. Biochem. Biophys.*, 1971, **144**, 292.

Liver extracts of the squid (*Ommastrephes sloani pacifus*),[1078] culture fluids from a *Streptomyces* sp.,[733] and washed suspensions of groups A, A L-form, C, and G Streptococci[1079] were shown to possess β-acetamidodeoxyglucosidase activities. The hydrolysis of various steroidal 2-acetamido-2-deoxy-D-glucosides by crystalline β-acetamidodeoxyglucosidase from Jack bean meal has been investigated.[1080]

α-Arabinofuranosidases

An improved method was used to obtain α-L-arabinofuranosidase in crystalline form from culture fluids of *Aspergillus niger*.[1081] The enzyme possesses a molecular weight of 5.3×10^4 and contains neutral carbohydrate. The production and properties of α-L-arabinofuranosidase from culture fluids of various strains of *Corticum rolfsii* have also been reported.[1082]

β-Fructofuranosidases

The question of the spatial relationship of brush border β-fructofuranosidase to the phlorizin-sensitive transport of D-glucose was examined in intact everted hamster intestinal sacs.[1083] β-Fructofuranosidase activity remained closely associated with rat intestinal brush border surface glycoproteins after subjection to a number of separative techniques, indicating that the enzyme may be a glycoprotein.[886] The enzyme has been obtained in a purified form from the same source and its rate of turnover has been measured.[1084]

The distribution of β-fructofuranosidase activity in the digestive organs of carp (*Cyprinus carpio*) has been studied.[1085]

Soluble β-fructofuranosidase (molecular weight 1×10^5) was purified from an extract of flies (*Drosophila melanogaster*); it showed maximum activity at pH 5.7, was apparently not dependent on ions, and was inhibited by 4-chloromercuribenzoate.[1086]

The activity of β-fructofuranosidase from *Saccharomyces cerevisiae* has been compared with that of its iodinated form and a mechanism (Scheme 12) involving a histidine residue and a carboxy-group of the enzyme has been proposed for its mode of action.[1087] Intracellular β-fructofuranosidase from *Neurospora crassa* contains mannose (11%) and 2-amino-2-deoxyglucose

[1078] Y. Kawai and K. Anno, *Biochim. Biophys. Acta*, 1971, **242**, 428.
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[1081] A. Kaji and K. Tawaga, *Biochim. Biophys. Acta*, 1970, **207**, 456.
[1082] A. Kaji and O. Yoshihara, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1249.
[1083] B. Sacktor and N. Wu, *Arch. Biochem. Biophys.*, 1971, **144**, 423.
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[1085] S. Kawai and S. Ikeda, *Bull. Jap. Soc. Sci. Fish.*, 1971, **37**, 333.
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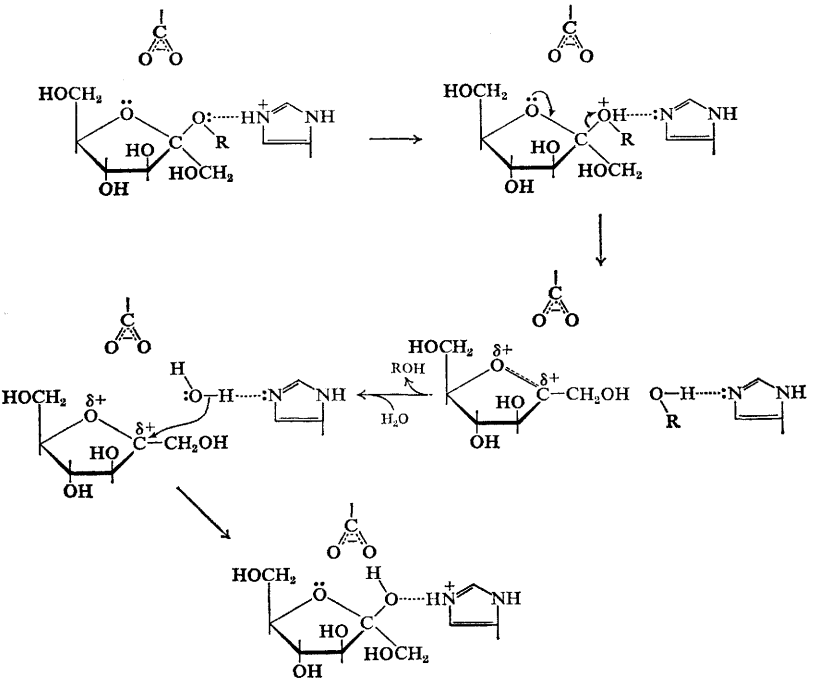
[1087] A. Waheed and S. Shall, *Biochim. Biophys. Acta*, 1971, **242**, 172.



Scheme 12

(3%) in its purified form.[1088] The observed molecular weight of 2.1×10^5 was reduced to 5.15×10^4 in the presence of guanidine hydrochloride, indicating that the enzyme structure is tetrameric. It seemed likely that at least some of the subunits are dissimilar, since an alkali-derived fragment of the molecule was active.

The cell wall of tomato pericarps showed considerable binding of endogenous β-fructofuranosidase.[1089] The degree of binding was affected by the presence of various ions and by the stage of ripening of the fruit. Changes in the pattern of β-fructofuranosidase development in gibberellin-treated dwarf-pea internodes,[1090] and the correlation between β-fructofuranosidase activity and mono-, oligo-, and poly-saccharides of resting and germinated seeds of red and white clover (*Trifolium pratense* and *T. repens*, respectively)[285] have been studied.

An active, insoluble derivative of β-fructofuranosidase has been prepared and its properties have been investigated.[1091]

[1088] Z. D. Meachum, H. J. Colvin, and H. D. Braymer, *Biochemistry*, 1971, **10**, 326.
[1089] H. Nakagawa, K. Sekiguchi, N. Ogura, and H. Takehana, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 301.
[1090] W. J. Broughton and A. J. McComb, *Annals Botany*, 1971, **35**, 213.
[1091] P. Monsan and G. Durand, *Compt. rend.*, 1971, **273**, *C*, 33.

Fucosidases

The α-fucosidase activities of human placenta and foetus liver have been followed through the gestation period.[1092]

The α-fucosidase of porcine kidney was separated into two forms by gel filtration on Sephadex G-200; the isoenzymes differed in their thermostability, stability on storage at various pH's, pH optima, and action on natural oligosaccharides containing L-fucose.[1093]

Polyoma virus transformed BHK line cells derived from hamster kidney fibroblasts contained greater α- and β-fucosidase activities than the parent BHK line cells.[1073] Gel filtration and ion-exchange chromatography of an extract from rat cerebral cortex yielded an α-fucosidase that appeared to be specific for (1 → 2)-glycosidic linkages.[1094]

A purified preparation of α-fucosidase obtained from the liver of *Charonia lampas* was free from other glycosidase activites judging from tests with aryl glycosides as substrates. The enzyme exhibited maximum activity at pH 3.3.[1095]

Galactosidases

The development of β-galactosidase activity in human brain has been followed during both the prenatal and postnatal periods.[1063] Subcellular fractions of human forebrain cortex also contained β-galactosidase activity.[1064]

Separative techniques and kinetic studies have demonstrated the presence of two β-galactosidases (A and B), which differ in their substrate specificities in normal human tissues. Tissues from cases of Hurler syndrome were deficient in isoenzyme A and this deficiency was considered to result in the accumulation of macromolecular D-galactose in the brain and liver.[874] Assays of human and Siamese cat brain tissue indicated that the β-galactosidase activities were defective in Tay–Sachs disease, human G_{M_1}-gangliosidosis, and the hereditary neurological disease of Siamese cats (cat G_{M_1}-gangliosidosis).[1096]

Kinetic, electrophoretic, and gel-filtration studies of β-galactosidase from human liver demonstrated the presence of a thermolabile component (optimum pH range 4.0—4.4) and a thermostable component (optimum pH range 5.0—6.5).[1097] The activity of the former was stimulated by chloride ions, whereas that of the latter was inhibited by these ions; these properties provided the basis for independent assays of the two enzymes. The latter enzyme also exhibited β-glucosidase activity. The complete deficiency of

[1092] G. Y. Wiederschain, E. L. Rosenfeld, A. I. Brusilovsky, and L. G. Kolibaba, *Biokhimiya*, 1971, **36**, 1026.
[1093] G. Y. Wiederschain and E. L. Rosenfeld, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 1008.
[1094] H. B. Bosmann and B. A. Hemsworth, *Biochim. Biophys. Acta*, 1971, **242**, 152.
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[1097] M. W. Ho and J. S. O'Brien, *Clin. Chim. Acta*, 1971, **32**, 443.

α-galactosidase activity in the liver of cases of Fabry's disease was considered to be the cause of accumulation of galactolipid.[1098]

Tests on 2-naphthyl β-D-galactopyranoside as a substrate for the β-galactosidases of the human small intestine showed that the glycoside was hydrolysed exclusively by the 'acid' β-galactosidase.[1099] The glycoside was therefore used in an assay specific for the latter enzyme with the liberated 2-naphthol being determined spectrofluorimetrically. Chromatographic analysis of the β-galactosidases from the same source on Sephadex G-200 revealed that the 'acid' galactosidase could occur in three forms, presumably representing monomer, dimer, and octamer.[1100] The characteristics of the forms, including their pH optima (4.0—4.5) and sensitivity to inhibition by *p*-chloromercuribenzoate and tris-chloride, were identical.

The α- and β-galactosidase activities of human placenta and foetus liver have been followed through the gestation period.[1092]

A 19 *S* β-galactosidase has been isolated and purified from ovine thyroid gland and the activity of the enzyme against desialysed thyroglobulin and a sialic acid-free glycopeptide derived from thyroglobulin was examined.[1070] Purified forms of α-galactosidases have been obtained from rabbit and rat small intestine, ficin, and coffee beans.[1101]

Polyoma virus transformed BHK line cells derived from hamster kidney fibroblasts contained greater activities of α- and β-galactosidases than the parent BHK cells.[1073] Purified β-galactosidase has been obtained from brush borders of rat intestine, and its rate of turnover has been measured.[1084] The specific activity of lysosomal β-galactosidase from rat intestine increased five-fold during starvation.[1102] β-Galactosidases have been found in the liver [1075] and chorioallantoic placenta [1077] of the rat; the enzyme from the latter source exhibited maximum activity at pH 4.5.

The distribution of α- and β-galactosidase activities in the digestive organs of carp (*Cyprinus carpio*) has been determined.[1085]

Growth of *Escherichia coli* on a medium containing melibiose induced the formation of an α-galactosidase, which was isolated as a soluble form.[1103] On account of its instability, the enzyme was not isolated in a pure form, but it appeared to possess a molecular weight of 2×10^5. Optimum activity was exhibited at pH 8.1 in the presence of manganous ions and 2-mercaptoethanol.

A study of β-galactosidase from *E. coli* at its pH optimum showed that the activities of the Mg^{2+}- and Mn^{2+}-free enzymes against various substrates are controlled by a protonated group, which ionizes in the alkaline

[1098] S. Handa, T. Ariga, T. Miyatake, and T. Yamakawa, *J. Biochem.* (*Japan*), 1971, **69**, 625.
[1099] N. Asp and A. Dahlqvist, *Analyt. Biochem.*, 1971, **42**, 275.
[1100] N. Asp, *Biochem. J.*, 1971, **121**, 299.
[1101] I. Bensaude, J. Callahan, and M. Philippart, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 913.
[1102] I. D. Desai, *Canad. J. Biochem.*, 1971, **49**, 170.
[1103] C. Burstein and A. Kepes, *Biochim. Biophys. Acta*, 1971, **230**, 52.

range, and by at least one unprotonated group, which ionizes in the acidic range.[1104] The β-galactosidase-catalysed reactions proceed *via* two intermediary complexes.

Immunological analysis of β-galactosidase from *E. coli* and a genetically defective β-galactosidase from another strain of the same organism did not reveal any differences between the two enzymes.[1105] However, the enzymic activity of the 'abnormal' enzyme was specifically increased several hundred-fold by the presence of antibodies directed against the normal version. The properties of the ribosome-bound β-galactosidase in *E. coli* cells were examined and compared with those of the soluble enzyme from the culture fluid.[1106] The effect of ATP and the catabolite repression of *E. coli* β-galactosidase has been assessed.[1107]

β-Galactosidase was extracted from *Lactobacillus bifidus* cells by ultrasonic oscillation and was purified by ion-exchange chromatography and gel filtration.[1108] The enzyme had a pH optimum of 7.0 and a temperature optimum of 50 °C, was activated by Mn^{2+} and Fe^{2+} ions, and was inactivated by Cu^{2+} and Hg^{2+} ions. Experiments in which oligosaccharides from human milk were used as substrates showed that the enzyme cleaved terminal β-(1 → 3)- but not β-(1 → 4)-D-galactosidic linkages.

A method has been devised for the isolation of α-galactosidase in crystalline form from the mycelia of *Mortierella vinacea*; the enzyme was shown to contain neutral sugar (10.8%) and 2-amino-2-deoxyglucose (2.7%).[1109] The effects of pH, substrate concentration, inhibitors, and temperature on the catalytic activity of the enzyme were described. Only α-D-galactopyranosyl-(1 → 4)- and -(1 → 6)-linkages were cleaved by the enzyme.

A β-galactosidase, with an activity optimum of pH 4, was found in the culture filtrates of *Neurospora crassa* when the mould was grown on certain carbohydrates.[1110] The enzyme is secreted extracellularly but is not a product of cell lysis; it possesses physicochemical and kinetic properties similar to those of the intracellular β-galactosidase of the same organism.

An assay for β-galactosidase, based on colour production by the enzyme in a semi-solid culture medium, has aided the detection of *Salmonella* and *Shigella* sp.[1111] The production of extracellular α- and β-galactosidases by a *Streptomyces* sp. was induced by the presence of D-galactose, lactose, raffinose, and melibiose in the culture medium.[733]

[1104] J. P. Tenu, O. M. Viratelle, and J. Garnier, *European J. Biochem.*, 1971, **20**, 363.
[1105] F. Celada, J. Ellis, K. Bodlund, and B. Rotman, *J. Exptl. Med.*, 1971, **134**, 751.
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[1107] M. Aboud and M. Burger, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 190.
[1108] T. Iwasaki, Y. Yoshioka, and T. Kanauchi, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 207.
[1109] H. Suzuki, S. Li, and Y. Li, *J. Biol. Chem.*, 1970, **245**, 781.
[1110] P. C. Comp and G. Lester, *J. Bacteriol.*, 1971, **107**, 162.
[1111] G. Giammanco and A. Falci, *Ann. Inst. Pasteur*, 1971, **120**, 525.

Multiple forms of α-galactosidase have been identified in dormant seeds from a number of higher plant species, including Aceraceae, Compositae, Leguminosae, Rosaceae, and Rubiaceae.[1112] The molecular weights of the enzymes were determined by gel filtration.

The use of glycosyl fluorides as substrates has facilitated polarimetric and g.l.c. determinations of the anomeric nature of the initial product of enzymic hydrolysis.[1113] Hydrolysis of α-D-galactopyranosyl fluoride by coffee-bean α-galactosidase was shown to proceed with retention of the anomeric configuration.

The α- and β-galactosidase contents of resting and germinating seeds of red and white clover (*Trifolium pratense* and *T. repens*, respectively) have been measured.[285] The increase in activity on storage at 4 °C of a lower molecular weight α-galactosidase of *Vicia faba* seeds was attributed to its conversion into a form of higher molecular weight. Further purification of the former enzyme by gel filtration prior to storage eliminated this effect. The purified, low molecular weight form of the enzyme was further resolved by CM-cellulose chromatography into two active fractions of similar molecular size.[1114] β-Galactosidase activity has been localized histochemically in the roots of *Zea mays*.[1115]

Active, immobilized forms of *E. coli* β-galactosidase have been prepared, and their properties have been investigated.[1116, 1117]

Glucosidases

The development of β-glucosidase activity in human brain has been followed for the prenatal and postnatal periods.[1063] Subcellular fractions of human forebrain cortex also contained β-glucosidase activity.[1064] One of the two β-galactosidases detected in normal human tissues [874] and the thermostable variant of the β-galactosidases of human liver [1097] also exhibited β-glucosidase activities.

Purified preparations of cattle liver lysosomal 'acid' α-glucosidase possessed the ability to hydrolyse glycogen completely.[1118] The α-glucosidase activity of the preparation was inhibited by D-glucose, but whereas the catalytic sites for α-glucosidase and oligo-1,6-glucosidase activities appeared to be identical, the relationship between the sites for α-glucosidase and glucoamylase activities appeared to be more complex.

Electrodecantation has been applied to the initial fractionation of porcine kidney β-glucosidase producing an increase in specific activity up

[1112] D. Barham, P. M. Dey, D. Griffiths, and J. B. Pridham, *Phytochemistry*, 1971, **10**, 1759.
[1113] J. E. G. Barnett, *Biochem. J.*, 1971, **123**, 607.
[1114] P. M. Dey, A. Khaleque, and J. B. Pridham, *Biochem. J.*, 1971, **124**, 27P.
[1115] A. E. Ashford, *Protoplasma*, 1971, **71**, 281.
[1116] P. J. Robinson, P. Dunill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1971, **242**, 659.
[1117] M. D. Lilly, *Biotechnol. Bioeng.*, 1971, **13**, 589.
[1118] V. Sica, A. Siani, C. B. Bruni, and F. Auricchio, *Biochim. Biophys. Acta*, 1971, **242**, 422.

to ten-fold.[1071] Isoelectric focusing of the enzyme showed that it has a pI of 4.9.

The kinetics, mode of action, and inhibition of rabbit muscle 'acid' β-glucosidase have been studied.[1119] Rabbit liver, kidney, and small intestine contained β-glucosidase activity.[1120] The purified enzyme exhibited a high affinity for oestrogen β-D-glucosides, which were appropriate substrates for assay of the enzyme.

The relationship of brush border α-glucosidase to the phlorizin-sensitive transport of D-glucose in intact everted hamster intestinal sacs has been examined.[1083] The α- and β-glucosidase activities of polyoma virus-transformed BHK cells derived from hamster kidney fibroblasts were greater than the corresponding activities of the parent BHK cells.[1073]

α-Glucosidase activity remained closely associated with rat intestinal brush border surface glycoproteins throughout a number of separation techniques, suggesting that the enzyme may be a glycoprotein.[886] The enzyme has been obtained in a purified form from the same source by sequences involving gel filtration and electrophoresis on polyacrylamide gel; the rate of turnover of the enzyme was investigated.[1084]

Polarimetric and g.l.c. determination of the anomeric configuration of the initial products released from D-glucosyl fluorides by rat intestinal and yeast α-glucosidases and almond emulsin β-glucosidase showed that the reactions proceeded with retention of configuration.[1113]

The distribution of α-glucosidase in the digestive organs of the carp (*Cyprinus carpio*), ayu (*Plecoglossus altivelis*), and Red Sea bream (*Pagrus major*) has been investigated; the distribution of β-glucosidase in carp has also been determined.[1085]

α-Glucosidase from *Aspergillus niger* has been used in conjunction with glucose oxidase for the coulometric determination of maltose.[85] The combined actions of *A. niger* α-glucosidase, α-amylase, glucose oxidase, and peroxidase are specific for the degradation of glycogen and could be used to determine the polysaccharide directly.[768] The influence of nitrogen and carbon sources and the pH of the nutrient on the synthesis and excretion of α-glucosidase by cultures of *A. oryzae* has been investigated.[1121]

A β-glucosidase of high molecular weight has been purified from culture filtrates of *Botryodiphodia theobromae* grown on cellobiose as the sole carbon source.[1122] The preparation was homogeneous by a number of criteria, possessed a molecular weight of 3.32×10^5, and was considered to be an aggregate of about thirty-two subunits.

The production of α-glucosidase by *Mycoplasma laidlawii* was induced by the presence of maltose.[1123] The locality of β-glucosidase in the cells of

[1119] T. N. Palmer, *Biochem. J.*, 1971, **124**, 713.
[1120] J. D. Mellor and D. S. Layne, *J. Biol. Chem.*, 1971, **246**, 4377.
[1121] J. Andrzejczuk-Hybel and J. Kaczkowski, *Bull. Acad. polon. Sci., Sér. Biol.*, 1971, **19**, 313.
[1122] G. M. Umezurike, *Biochim. Biophys. Acta*, 1971, **227**, 419.
[1123] M. L. Slater and C. E. Folsome, *Nature New Biol.*, 1971, **229**, 117.

Pseudomonas fluorescens has been determined.[1124] The synthesis of β-glucosidase in *Trichoderma viride* was induced by sophorose.[1125] The transglucosidase properties of brewer's yeast α-glucosidase have been employed for the synthesis of α-D-glucopyranosyl-D-fructoses.[1126]

Equal quantities of the two components (molecular weights 6.7×10^4 and 7.5×10^4) of sweet almond emulsin β-glucosidase participate in formation of the intact molecule.[1127] These components differed in their reaction to denaturants.

The α- and β-glucosidase activities of resting and germinating seeds of red and white clover (*Trifolium pratense* and *T. repens*) have been determined.[285] β-Glucosidase has been localized histochemically in the roots of *Zea mays*.[1115]

Active, insoluble derivatives of β-glucosidases,[1128] including mixed derivatives with other enzymes,[1129] and active, immobilized forms of the enzyme [1130–1132] have been prepared.

Glucuronidases

The occurrence, characteristics, and role of β-glucuronidase in mammalian tissues have been reviewed and the diagnostic usefulness of assaying the enzyme has been discussed.[1133]

An investigation of the levels of β-glucuronidase in rheumatoid and regenerated human synovium showed that the measurements could not be used as an index of inflammation.[1134]

A β-glucuronidase from rat liver lysosomes was homogeneous by a number of physicochemical criteria. The molecular weight (2.8×10^5) of the intact material was reduced to 7.5×10^4 by denaturing conditions and indicated a tetrameric structure for the enzyme.[1135] The specific activity of rat lysosomal β-glucuronidase was increased up to five times during starvation.[1102] Some of the characteristics of a β-glucuronidase (pH optimum 4.5) found in the lysosomes of rat chorioallantoic placenta have been reported.[1077] A homogeneous preparation of β-glucuronidase from the preputial glands of female rats exhibited a pI of 6.15 and showed optimum activity at pH 4.5.[1136] The properties and characteristics of the

[1124] K. Yamane, Y. Yoshikawa, H. Suzuki, and K. Nisizawa, *J. Biochem.* (*Japan*), 1971, **69**, 771.
[1125] T. Nisizawa, H. Suzuki, and K. Nisizawa, *J. Biochem.* (*Japan*), 1971, **70**, 387.
[1126] S. Chiba and T. Shimomura, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 1292.
[1127] T. Kleinschmidt and J. Horst, *Z. physiol. Chem.*, 1971, **352**, 603.
[1128] S. A. Barker, S. H. Doss, C. J. Gray, J. F. Kennedy, M. Stacey, and T. H. Yeo, *Carbohydrate Res.*, 1971, **20**, 1.
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[1132] R. A. Llenado and G. A. Rechnitz, *Analyt. Chem.*, 1971, **43**, 1457.
[1133] L. Konarska, *Postepy Biochemii*, 1971, **17**, 105.
[1134] J. L. Granda, C. S. Ranawat, and A. S. Posner, *Arthritis Rheum.*, 1971, **14**, 223
[1135] P. D. Stahl and O. Touster, *J. Biol. Chem.*, 1971, **246**, 5398.
[1136] K. Ohtsuka and M. Wakabayashi, *Enzymologia*, 1970, **39**, 109.

enzyme were compared with those of human and bovine liver β-glucuronidases.

A β-glucuronidase (molecular weight 3.1—4.5 × 10^5) isolated from mouse ascitic fluid mastocytoma cells by gel filtration, ion-exchange chromatography, and isoelectric focusing contained hexose and uronic acid.[1137]

Monitoring of the β-glucuronidase activity of the alimentary canal of tadpoles of *Raja tigrina* during prometamorphosis and metamorphosis indicated that the enzyme's pH optimum and response to activators and inhibitors varied significantly. The role of the enzyme was discussed.[1138] Liver extracts of the squid, *Ommastrephes sloani pacifus*, contained β-glucuronidase activity.[1078]

Mannosidases

The levels of α-mannosidase in placenta and liver of human embryos have been followed through the gestation period.[1092] The distribution, purification, and properties of the 10 *S* α-mannosidase of sheep thyroid gland have been reported, and the action pattern of the enzyme against desialysed thyroglobulin and sialic acid-free thyroglobulin glycopeptides has been examined.[1070]

A number of glycoproteins have been used as substrates in an investigation of the properties of an α-mannosidase from rat cerebral cortex.[1094] A comparison of the properties of the α-mannosidases of a lysosomal and a soluble cytoplasmic fraction showed that, although both enzymes are inhibited by D-mannono-(1,5-lactone, they possess different pH optima.[1139] The α-mannosidase activity of BHK line cells from hamster kidney fibroblasts was less than that of their polyoma virus-transformed variants.[1073]

The α- and β-mannosidase activities of resting and germinating seeds of red and white clover (*Trifolium pratense* and *T. repens*, respectively) have been determined.[285]

Sialidases

An investigation of the carbohydrases of human brain for both prenatal and postnatal periods showed that the development curve for neuraminidase differs from the curves for the other enzymes.[1063] Up to 77% of the sialidase of human forebrain cortex was located in the synaptosomal fraction.[1064] In both instances the enzymes appeared to be related to the ganglioside contents of the tissues. The endogenous substrates of human, rat, and chicken brain sialidases have been identified as gangliosides.[1140, 1141]

[1137] J. Preiss and H. Hilz, *Z. physiol. Chem.*, 1971, **352**, 947.
[1138] A. T. Varute and N. K. More, *Comp. Biochem. Physiol.*, 1971, **38B**, 225.
[1139] C. A. Marsh and G. C. Gourlay, *Biochim. Biophys. Acta*, 1971, **235**, 142.
[1140] A. Pretti, A. Lombardo, and G. Tettamanti, *Ital. J. Biochem.*, 1970, **19**, 371.
[1141] A. Lombardo, A. Pretti, and G. Tettamanti, *Ital. J. Biochem.*, 1970, **19**, 386.

A particulate enzyme, which catalyses the hydrolysis of *N*-acetylneuraminic acid only from (2-acetamido-2-deoxy-D-glucosyl)-(*N*-acetylneuraminosyl)-D-galactosyl-D-glucosyl-ceramide (Tay–Sachs GM_2-ganglioside), has been found in various tissues of the rat; it has a pH optimum of 5.0.[1142] The soluble and particle-bound neuraminidases present in the brain and liver of developing rats possess pH optima of 5.8 and 4.4, respectively.[1143]

Rat mammary glands contain a soluble neuraminidase (pH optimum 5.8) in the cytosol and a particulate neuraminidase (pH optimum 4.4) strongly bound to the lysosomes.[1144] The former enzyme only was inhibited by Cu^{2+}, Hg^{2+}, and Zn^{2+} ions, whereas the latter was inhibited by Li^+, Na^+, and K^+ ions. The dissimilar action patterns of the enzymes were determined using a number of glycoproteins and oligosaccharides as substrates. Sialidase activity has been found in all the cellular fractions from mouse fibroblast L cells.[890]

The neuraminidases from *Clostridium welchii* (*C. perfringens*), *Vibrio cholerae*, and influenza virus have been purified by affinity chromatography on an adsorbent containing oxamic acid groups.[1145] Columns containing this adsorbent completely removed neuraminidase activities from the extracts, whereafter quantitative elution of the enzymes was achieved using 0.2M sodium hydrogen carbonate buffer (pH 9.2).[1146] Differences in the physicochemical properties of the three neuraminidases were demonstrated by isoelectric focusing and these observations have been related to the different sensitivities of the enzymes to inhibitors.[1147] Examination of the specificities of *C. welchii* and *V. cholerae* neuraminidases using ceruloplasmin as substrate revealed that the enzymes possess preferential affinity for different sialic acid residues.[1148]

A molecular weight of 7.06×10^4 was estimated by gel filtration for each of four neuraminidase isoenzymes obtained from *Diplococcus pneumoniae*.[1149] The overall results of dissociation studies suggested that the isoenzymes are not composed of subunits and that the different forms of the enzyme arise from their different charges. The use of transferrins from various species as substrates demonstrated that the neuraminidases from *D. pneumoniae* and *V. cholerae* possess different action patterns from those of the corresponding enzymes from *Pasteurella hemolytica* and *P. multocida*.[962]

[1142] E. H. Kolodny, J. Kanfer, J. M. Quirk, and R. O. Brady, *J. Biol. Chem.*, 1971, **246**, 1426.
[1143] R. Carubelli and D. R. P. Tulsiani, *Biochim. Biophys. Acta*, 1971, **237**, 78.
[1144] D. R. P. Tulsiani and R. Carubelli, *Biochim. Biophys. Acta*, 1971, **227**, 139.
[1145] P. Cuatrecasas and G. Illiano, *J. Biol. Chem.*, 1971, **246**, 4938.
[1146] P. Cuatrecasas and G. Illiano, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 178.
[1147] A. R. Neurath, R. W. Hartzell, and B. A. Rubin, *Experientia*, 1970, **26**, 1210.
[1148] C. J. A. van den Hamer, A. G. Morell, I. H. Scheinberg, J. Hickman, and G. Ashwell, *J. Biol. Chem.*, 1970, **245**, 4397.
[1149] S. W. Tanenbaum and S. C. Sun, *Biochim. Biophys. Acta*, 1971, **229**, 824.

Xylosidases

The β-xylosidase activities of BHK line cells derived from hamster kidney fibroblasts and their polyoma virus transformed counterparts have been compared.[1073] The β-xylosidase of rat cerebral cortex was active against glycopeptide linkages of chondroitin sulphate proteoglycans.[1094]

A β-xylosidase was purified from commercially available *Aspergillus niger* 'hemicellulase preparations' by gel filtration and ion-exchange chromatography.[1150] Specificity studies revealed strict requirements at C-1, C-2, and C-3 of the D-xylopyranosyl unit of the substrate. Inversion of configuration at C-4 of the D-xylopyranosyl unit did not inhibit the activity of the enzyme but lowered its substrate affinity. However, substitution at C-4 prevented the substrate from acting as either a substrate or an inhibitor. Microheterogeneity of the β-xylosidase was apparent on isoelectric focusing across a narrow pH range.

α-Amylases

The mode of action of the major amylolytic enzymes, including α-amylase, has been summarized[1151] and industrial aspects of the degradation of starch by α-amylase have been discussed.[201] Earlier reports that solubilization of collagen by α-amylase was due to cleavage of carbohydrate chains have been refuted; the dispersion has been shown to arise, in part, from the action of a contaminating protease.[744]

Periodate-oxidized amylose proved to be a substrate for α-amylases, but not for β-amylases and glucoamylases; the substrate could therefore be used to detect α-amylase activity in preparations of the latter enzymes. In this way, an α-amylase impurity was detected in a supposedly pure preparation of glucoamylase from *Aspergillus niger*.[1152]

The α-amylase isoenzymes of saliva, serum, urine, and tissues from man, horse, pig, dog, cat, and rabbit have been separated by electrophoresis in agar and polyacrylamide gels.[1153] Marked variations of activities were observed between the species and the sources.

Electrophoresis of human salivary α-amylase in starch-containing gels indicated that the enzyme interacts with the starch so that starch-gel electrophoresis is inappropriate for examination of the enzyme.[1154] Human parotid salivary α-amylase, after purification by electrophoresis and gel filtration, was separated into two groups of isoenzymes by fractionation on DEAE–Sephadex.[1155] One group was resolved into two forms (pI's 5.80 and 6.65) and the other into three forms (pI's 5.32, 5.65, and 5.80) by isoelectric focusing. Electrophoretic examination of saliva from a man

[1150] M. Claeyssens, F. G. Loontiens, H. Kersters-Hilderson, and C. K. de Bruyne, *Enzymologia*, 1971, **40**, 177.
[1151] R. A. Knight and P. Wade (for D. J. Manners), *Chem. Ind.*, 1971, 568.
[1152] J. J. Marshall and W. J. Whelan, *Analyt. Biochem.*, 1971, **43**, 316.
[1153] R. Rajasingham, J. L. Bell, and D. N. Baron, *Enzyme*, 1971, **12**, 180.
[1154] B. Boettcher and F. A. de la Lande, *Analyt. Biochem.*, 1970, **34**, 1.
[1155] J. Andjic, A. Hayem, and M. Bonte, *Compt. rend.*, 1970, **270**, *D*, 407.

known to have little amylolytic activity showed an unusual α-amylase isoenzyme pattern; the usual isoenzymes were absent and the observed bands were considered to be inactive forms of the enzyme.[1156] The inhibitory action of wheat materials on human salivary α-amylase has been studied.[1157]

A method for the determination of serum amylase involved incubation with starch, treatment of the reducing carbohydrate liberated with Nelson's copper reagent, and coulometric titration with bromine of the Cu^+ ion formed; an analysis could be performed on 100 μl samples of serum.[1158] A method that employed a chromogenic substrate (Dy Amyl—a coloured derivative of amylopectin) for the determination of human serum and urinary α-amylase proved to be preferable to the older amyloclastic procedure.[1159] A deficiency of α-amylase was found in a number of tissues of a case of glycogen storage disease (Pompé's disease).[1160] An assay procedure for the α-amylase of human urine used blue starch incorporated into solidified agar as substrate.[1161]

Porcine pancreatic α-amylase has been used in conjunction with other enzymes for the specific and direct determination of glycogen.[768] Studies of the inhibition and action pattern of porcine α-amylase suggested that the enzyme possesses two types of substrate-binding locations: a random component, responsible for the rapid decrease in iodine stain of the substrate, and a non-random component, responsible for the production of small oligomers from the substrate.[1162] The effects of DMSO on pancreatic α-amylase [1163] and of urea on the u.v. absorption of the porcine enzyme [1164] have been studied. Partial amino-acid sequences have been obtained for the α-amylase isoenzymes.[1165]

A method has been described for the purification of detergent-solubilized rat liver amylase.[1166] The enzyme gave several anodic isoenzyme bands on electrophoresis on cellulose acetate and the amylases from serum and urine gave the same pattern. It was concluded that the liver is the major source of serum amylase in the rat.

The α-amylase activities of the digestive systems of various species of birds have been investigated.[1167] α-Amylase was found in a variety of tissues of the bullfrog (*Raja catesbiana*) and the leopard frog (*Raja pipiens*). Bullfrog α-amylase required the presence of chloride ions as activator and

[1156] B. Boettcher and F. A. de la Lande, *Enzymologia*, 1971, **40**, 234.
[1157] R. Shainkin and Y. Birk, *Biochim. Biophys. Acta*, 1970, **221**, 502.
[1158] J. R. Moody and W. C. Purdy, *Analyt. Chim. Acta*, 1971, **53**, 31.
[1159] K. Y. Chung, R. M. Sinha, and J. A. Trew, *Clin. Chem.*, 1971, **17**, 89.
[1160] D. Platt, *Z. physiol. Chem.*, 1970, **351**, 1320.
[1161] M. Ceska, *Clin. Chim. Acta*, 1971, **33**, 147.
[1162] W. Banks, C. T. Greenwood, and K. M. Khan, *Carbohydrate Res.*, 1971, **19**, 252.
[1163] D. R. Lineback and A. L. Sayeed, *Carbohydrate Res.*, 1971, **17**, 453.
[1164] P. Elödi and M. Krysteva, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1970, **5**, 449.
[1165] P. Cozzone, L. Pasero, B. Beaupoil, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1970, **207**, 490.
[1166] K. Hammerton and M. Messer, *Biochim. Biophys. Acta*, 1971, **244**, 441.
[1167] S. Bhattacharya and K. C. Ghose, *Comp. Biochem. Physiol.*, 1971, **40B**, 317.

possessed a pH optimum of 7.5; bullfrog tadpoles and other intermediate metamorphic stages also contained the enzyme.[1168] The distribution of amylase in the digestive organs of the carp (*Cyprinus carpio*), ayu (*Plecoglossus altivelis*), and Red Sea bream (*Pagrus major*) has been determined.[1085]

α-Amylase has been isolated from larvae of the beetle (*Callosobruchus chinensis*).[1169] The purified enzyme had a pH optimum of 5.2—5.4, but this range was elevated to 5.6—5.8 by the presence of Ca^{2+} ions or 2-mercaptoethanol, which acted as activators.[1170] Inhibition studies with iodoacetic acid and *N*-ethylmaleimide suggested that free thiol groups are essential for activity.

In a study of the effect of acidity of the culture medium on the production of amylolytic enzymes by *Aspergillus awamori*, it was found that maintenance of the pH within the range 4.5—5.0 prevented the biosynthesis of α-amylase. Lowering of the pH to 5 prior to culture gave the same result.[1171] Highly purified preparations of α-amylases obtained from *A. awamori*, *A. flavus*, and *A. oryzae* possessed the same amino-acid compositions. Modes of inactivation of the enzymes were studied.[1172]

The influence of nitrogen and carbon sources and the pH of the nutrient on the synthesis and the excretion of α-amylase by cultures of *A. oryzae* has been investigated.[1121] α-Amylase was purified from a culture of the organism grown on steamed rice by ion-exchange chromatography and crystallization with the aid of ammonium sulphate.[1173] The preparation was homogeneous on physicochemical analyses, while *X*-ray crystallographic studies showed that the enzyme forms single crystals of the monoclinic system.

The hydrolysis of linear maltodextrins ($\overline{DP}$ 2—117) catalysed by crystalline Taka-amylase A was studied to determine the rate parameters for each substrate. The K_m value decreased with increasing chain length, whereas the turnover number increased with chain length up to a length of seven units and then became constant. The results suggested that the region responsible for the specificity of the enzyme spans seven units of the substrate. Schematic models for the predominant productive enzyme–substrate complexes were presented.[1174] The inhibition of hydrolysis of phenyl α-maltoside and *p*-nitrophenyl α-maltoside by substrate analogues, carbohydrates, and alcohols was studied with a view to determining the inhibition constants and the type of inhibition.[1175] Most of the analogues exhibited competitive inhibition, but some possessed either non-competitive or mixed inhibitor properties. The enzymic activity of Taka-amylase A on

[1168] R. L. McGeachin and W. P. Welbourne, *Comp. Biochem. Physiol.*, 1971, **38A**, 457.
[1169] H. Podoler and S. W. Applebaum, *Biochem. J.*, 1971, **121**, 317.
[1170] H. Podoler and S. W. Applebaum, *Biochem. J.*, 1971, **121**, 321.
[1171] R. V. Feniksova and V. G. Ryzhakova, *Mikrobiologiya*, 1970, **39**, 974.
[1172] O. S. Tsyperovich, T. F. Kastrykina, and I. I. Perevozchenko, *Ukrain. biokhim. Zhur.*, 1971, **43**, 446.
[1173] Y. Morita and A. Wadano, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1128.
[1174] Y. Nitta, M. Mizushima, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1971, **69**, 567.
[1175] Y. Nitta, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1971, **69**, 577.

amylose, as measured by the blue-value method, increased to 180% of the original when one mole of mercaptosuccinyl group was introduced per mole of enzyme;[1176] similar results were observed when the activity was measured by the Somogyi–Nelson method. However, more extensive derivatization with the same group gave smaller increases of activity. The activity of the enzyme was lowered by succinylation and was inhibited by 4-chloromercuribenzoate and iodoacetamide. Structural investigation of a glycopeptide from Taka-amylase A showed the existence of three D-mannose residues, one of which is 1 → 2-linked, at the non-reducing terminal.[1177] The results of methylation and Smith degradation on a smaller glycopeptide composed of D-mannose (2), 2-acetamido-2-deoxy-D-glucose (2), and *N*-glycolylasparagine (1 moles per mole), together with those of previously reported enzymic studies, lead to the structure (91).

O-α-D-Man*p*-(1 → 6)-*O*-α-D-Man*p*-(1 → 4)-*O*-β-D-Glc*pN*Ac-
(1 → 4)-D-Glc*pN*Ac-AsnCCH$_2$OH
‖
O

(91)

Mutant strains of *A. sojae* exhibited co-ordinate increases of α-amylase and cellulase production when cultured in certain media. Analysis of this phenomenon suggested that mutation is due to a complex alteration rather than to a single gene mutation.[1178]

Water-insoluble, blue-starch particles incorporated into an agar medium were hydrolysed by α-amylase from *Bacillus subtilis* and the extent of hydrolysis under a number of conditions was determined.[1179]

A representational model based upon the concepts that the bond ruptured by a lytic enzyme hydrolysing a biopolymer reflects the positioning of the substrate on the specificity site and that, for a given substrate, selective positioning and bond rupture give rise to a discrete product distribution has been used to interpret the cleavage pattern of *B. subtilis* α-amylase on methyl α-maltodextrins (3—12 D-glucose residues).[1180] Data analyses led to the conclusions that the active site of the enzyme spans nine residues in the substrate and that the sub-sites exhibit a wide range of monomer affinities.

Difference spectra studies have been made of the interaction of maltose with *B. subtilis* α-amylase.[1181] The introduction of succinyl groups into the enzyme enhanced both its specific activity and its affinity for amylose.[1182]

[1176] S. Suzuki, Y. Hachimori, and R. Matoba, *Bull. Chem. Soc. Japan*, 1970, **43**, 3849.
[1177] H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Japan)*, 1970, **68**, 843.
[1178] S. Nasuno and T. Ohara, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 829.
[1179] M. Ceska, *European J. Biochem.*, 1971, **22**, 186.
[1180] J. A. Thoma, C. Brothers, and J. Spradlin, *Biochemistry*, 1970, **9**, 1768.
[1181] M. Ohnishi, *J. Biochem. (Japan)*, 1970, **68**, 933.
[1182] S. Suzuki, Y. Hachimori, R. Matoba, and K. Takasaki, *Bull. Chem. Soc. Japan*, 1971, **44**, 1147.

The increased affinity was considered to be due to an expansion of the enzyme molecule. The localization of amylase activity within *Pseudomonas fluorescens* cells has been investigated.[1124]

Culture filtrates of *Streptococcus equinus* contained α-amylase, which could be freed from transglucosylase activity by chromatography on DEAE-cellulose.[1183] The enzyme exhibited a pH optimum of 7.0 and required the presence of chloride ions for maximum activity. The α-amylase of *Trichoderma viride* has been fractionated and the biosynthesis of the enzyme was also studied.[1125]

Electrophoretic analysis of the sequential changes in the amylase isoenzymes during grain maturation in barley revealed a progressive change from α-amylase (molecular weight 4.2×10^4) to β-amylase (molecular weight 1.3×10^5).[1184] α-Amylase synthesis was induced in mustard (*Sinapis alba*) seedlings by the active form of phytochrome.[1185] Changes have been observed in the pattern of amylase development in dwarf pea internodes on treatment with gibberellic acid.[1090]

The products of the action of a purified α-amylase from malted rye flour on amylopectin were D-glucose, maltose, maltotriose, and a series of branched α-limit dextrins having DP $\geqslant 4$; panose was not a product.[1186] The identity of the α-limit dextrins was correlated with the ability of the enzyme to hydrolyse some, but not all, of the α-(1 → 4)-linkages in the vicinity of the α-(1 → 6)-interchain linkages of amylopectin. In an investigation of the effect of surfactants on the α-amylase activity of wheat flour, it was found that sodium dodecyl sulphate rapidly destroyed the enzymic activity of extracts from the flour. This denaturation was attributed to enzyme–detergent interaction.[1187]

A water-insoluble derivative of α-amylase, in which the enzyme was attached to a polyaldehyde support, has been reported.[1188]

β-Amylases

The actions of the major amylolytic enzymes, including β-amylase, have been summarized.[1151] Traces of α-amylases could be detected in preparations of β-amylases by using periodate-oxidized amylose as substrate, since only the former enzymes were active against the modified polysaccharide.[1152]

Analysis of the sequential changes in the amylase isoenzymes during barley grain maturation showed a progressive change from α- to β-amylase (molecular weights 4.2×10^4 and 1.3×10^5, respectively).[1184]

Sweet potato β-amylase has been crystallized, and crystallographic data have been obtained. A molecular weight of 2.06×10^5 was estimated for the enzmye using a method whereby molecular weights can be obtained for

[1183] E. W. Boyer and P. A. Hartmann, *J. Bacteriol.*, 1971, **106**, 561.
[1184] J. L. Stoddart, *Planta*, 1971, **97**, 70.
[1185] H. Drumm, J. Möller, and H. Mohr, *Naturwiss.*, 1971, **58**, 97.
[1186] D. J. Manners and J. J. Marshall, *Carbohydrate Res.*, 1971, **18**, 203.
[1187] E. E. McDermot and G. A. H. Elton, *J. Sci. Food Agric.*, 1971, **22**, 131.
[1188] R. Epton, J. V. McLaren, and T. H. Thomas, *Biochem. J.*, 1971, **123**, 21P.

crystals of macromolecules grown in concentrated salt solutions. The β-amylase structure was concluded to be tetrameric with molecular symmetry.[1189] The effect of DMSO on the activity of sweet potato β-amylase has been investigated.[1163]

Sweet potato β-amylase has been used in conjunction with dextrin-1,6-glucosidase transferase in determining the chain-lengths of glycogens and amylopectins.[149] The action of the β-amylase on glycogens has been shown to take place in two stages.[218] In the first rapid stage, the enzyme degraded the polysaccharide to a novel limit dextrin in which the unbranched chains were shortened to three or four units. In the second, slower stage, the enzyme degraded the limit dextrin to the classical β-limit dextrin in which the side-chains were two or three units in length. Similar results were obtained using amylopectin as substrate.

Glucoamylases

Industrial aspects of the enzymic degradation of starch using glucoamylase (γ-amylase) have been discussed.[201] Periodate-oxidized amylose served as a useful substrate for the detection of α-amylase contaminants of glucoamylase, since only the former degraded the modified polysaccharide.[1152] Whereas a pure preparation of *Rhizopus niveus* glucoamylase proved to be free from α-amylase activity, a supposedly pure preparation of *Aspergillus niger* glucoamylase was found to contain α-amylase.

Purified preparations of cattle liver lysosomal 'acid' α-glucosidase were able to debranch glycogen and to hydrolyse it completely to D-glucose by acting as an exo-glucoamylase.[1118] The α-glucosidase activity of the preparation was inhibited by D-glucose; whereas the catalytic sites for α-glucosidase and oligo-1,6-glucosidase activities appeared to be identical, the relationship between the sites for glucoamylase and α-glucosidase activities was more complex.

The kinetics, mode of action, and inhibition of rabbit muscle glucoamylase have been studied.[1119] The mechanism of the effect of adrenaline and other biogenic amines on the glucoamylase activity of rat liver and heart muscle has been investigated.[1190]

The production of glucoamylase by *A. awamori* was accelerated by use of a culture medium buffered at pH 4.5—5.0. Under these conditions, the biosynthesis of α-amylase was prevented. Lowering of the pH to 5 prior to culture gave the same result.[1171] From experiments using glucans of various degrees of polymerization and polydispersity as substrates, it was concluded that the mechanism of hydrolysis by *A. awamori* glucoamylase is determined by the average chain-length of the substrate.[1191]

[1189] P. M. Colman and B. W. Matthes, *J. Mol. Biol.*, 1971, **60**, 163.
[1190] V. S. Orlova, V. N. Sinyukhin, I. A. Popova, V. Z. Gorkin, and E. L. Rosenfeld, *Biokhimiya*, 1971, **36**, 555.
[1191] K. M. Bendetskii, V. L. Yarovenko, and L. N. Luk'yanova, *Biokhimiya*, 1971, **36**, 525.

Use of α-D-glucopyranosyl fluoride as substrate facilitated polarimetric and g.l.c. determinations of the anomeric configuration of the initial product of glucoamylase hydrolysis.[1113] In the case of the glucoamylase from *A. niger*, the reaction proceeded with retention of configuration.

Two separable glucoamylase isoenzymes (I and II) produced by *A. niger* possess molecular weights of 9.9×10^4 and 1.12×10^5, respectively.[1192] Both enzymes contain D-galactose (2 and 3), D-glucose (16 and 20), and D-mannose (69 and 128 residues per mole, respectively), and the glycopeptide linkages primarily involves L-serine and L-threonine residues. The results of incorporation studies using D-[1-^{14}C]glucose, D-[1-^{14}C]mannose, and D-[2-^{14}C]mannose suggested that the hexose residues are attached during biosynthesis mainly as intact residues, most likely by a nucleotide pathway. The glucoamylase from *R. delemar* released mannose (67) and 2-amino-2-deoxyglucose (20 residues per mole). The activities of *A. niger* glucoamylases I and II differed in their responses to treatment with DMSO.[1163]

The influence of pH and certain nitrogen and carbon sources on the synthesis and excretion of glucoamylase by cultures of *A. oryzae* was investigated.[1121]

Active, water-insoluble derivatives of glucoamylase have been prepared and their properties have been investigated.[1130, 1193, 1194]

Lysozymes

A sensitive immunoassay for human lysozyme (muramidase) is based on the inhibition of inactivation of the lysozyme–bacteriophage conjugate.[1195] The method is capable of determining both enzymically active and inactive forms.

Lysozyme from human saliva was separable from proteins and glycoproteins of the secretion by electrophoresis in polyacrylamide gel in the presence of urea at low pH.[1014] A method described for the isolation of lysozyme from human placenta involved chromatography on CM–Sephadex, CM–cellulose, and Sephadex.[1196] The preparation was homogeneous by physicochemical criteria, possessed a low molecular weight ($S_{20,w} = 1.8\ S$), and a pI of 11.7. Its specific activity was 3.2 times that of a purified lysozyme obtained from hen egg-white.

Human lysozyme, which had been acetylated, was 1.2 times as active against *Micrococcus lysodeikticus* cells as the unmodified enzyme.[1197] Nitration of one tyrosine residue of the enzyme resulted in loss of activity

[1192] J. H. Pazur, H. R. Knull, and A. Cepure, *Carbohydrate Res.*, 1971, **20**, 83.
[1193] H. Maeda and H. Suzuki, *J. Agric. Chem. Soc. Japan*, 1970, **44**, 547.
[1194] K. L. Smiley, *Biotechnol. Bioeng.*, 1971, **13**, 309.
[1195] E. Maron and B. Bonavida, *Biochim. Biophys. Acta*, 1971, **229**, 273.
[1196] K. Izaka, H. Shirakawa, M. Yamada, and T. Suyama, *Analyt. Biochem.*, 1971, **42**, 299.
[1197] E. L. Fawcett, T. J. Limbird, S. L. Oliver, and C. L. Borders, *Canad. J. Biochem.*, 1971, **49**, 816.

and polymerization. Neither nitration nor polymerization was prevented by the presence of 2-acetamido-2-deoxy-D-glucose, *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose (*NN'*-diacetylchitobiose), or *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose (*NN'N''*-triacetylchitotriose).

Rabbit anti-human lysozyme serum cross-reacted with hen, turkey, and Japanese quail lysozymes.[1198] *X*-Ray analysis of crystalline lysozyme, obtained from the urine of a leukaemic patient, showed that the enzyme has a tertiary structure similar to that of hen egg-white lysozyme.[1199] The primary structures of these lysozymes are distinct from that of goose egg-white lysozyme.[1200] Lysozyme (molecular weight 1.5×10^4) has been isolated from baboon milk.[1201]

Chinese goose egg lysozyme was strongly inhibited by oligosaccharides obtained from bacterial cell walls, but not by oligosaccharides derived from chitin.[1202] This was in contrast to the hen egg enzyme, which was more strongly inhibited by chitin oligosaccharides than by cell-wall materials. Extensive differences between the binding sites of the two enzymes were suggested. Lysozyme isolated from a pooled sample of Peking duck egg-white by ion-exchange chromatography and gel filtration contained three isoenzymes.[1203] The isoenzymes resembled hen egg-white lysozyme in some of their properties, but all four enzymes were distinguishable by electrophoretic and immunological criteria. Electrophoresis of the lysozyme from individual duck eggs on starch gel revealed that each egg contains either a combination of any two of the three isoenzymes or one of two of the isoenzymes alone.

Hen egg-white lysozyme was inactive in distilled water, being activated and inhibited by low and high salt concentrations, respectively.[1204] The effect of cations was found to vary with pH in such a way that the optimal cation concentration decreased with increasing pH. Thus, the activity of lysozyme could not be adequately represented by a pH–activity curve and the establishment of an activity–pH–cation profile was necessary.

Pure hen egg-white lysozyme released mucopeptide from the spore coats of *Bacillus megaterium* and this release was inhibited by bubbling n-butane into the solution. Similarly, the lysis of *M. lysodiekticus* cells by the enzyme was inhibited by n- and iso-butanes and propane. In contrast, none of the hydrocarbons inhibited the ability of lysozyme to hydrolyse glycolchitin,

[1198] A. Miller, B. Bonavida, J. A. Stratton, and E. Sercarz, *Biochim. Biophys. Acta*, 1971, **243**, 520.
[1199] C. C. F. Blake and I. D. A. Swann, *Nature New Biol.*, 1971, **232**, 12.
[1200] R. E. Canfield, S. Kamerman, J. H. Sobel, and F. J. Morgan, *Nature New Biol.*, 1971, **232**, 16.
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[1202] A. K. Allen and A. Neuberger, *Biochim. Biophys. Acta.*, 1971, **235**, 539.
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showing that they did not inhibit the chitinase activity of the enzyme.[1205] The kinetics of the lysozyme-catalysed hydrolysis of cell-wall oligosaccharides were assessed using a mathematical model. The various ways in which any substrate could associate with the enzyme were considered, and it was assumed that the association constant depends only on which sub-sites of the enzyme are filled. The rates of cleavage of bound substrate to form a glycosyl-enzyme intermediate, and the rates of hydrolysis or transfer from the intermediate to an acceptor, were assumed to be the same for any productively bound substrate. The model was solvable by computer and was fitted to experimental data.[432] The effect of human serum macromolecules on the lysis of *M. lysodeikticus* cells by hen egg-white lysozyme and on the assay of human serum lysozyme has been investigated.[1206]

Hen egg-white lysozyme brought about coagulation of casein micelles in a manner similar to the action of rennin; both reactions showed similar pH optima and calcium ion dependencies.[915]

The interaction of 2-acetamido-2-deoxy-α- and β-D-glucose with hen egg-white lysozyme has been studied by ^{1}H n.m.r. spectroscopy. The chemical shift of the *N*-acetyl protons of the bound anomers and the association constants were shown to be functions of pH, and this was related to the nature of the amino-acids in the active site of the enzyme. The effect of dimerization of the enzyme on substrate binding was also investigated.[1207] A crystallographic study of a complex between a spin-labelled inhibitor containing an acetamido-group and hen egg-white lysozyme enabled identification of the binding sites analogous to those involved in the binding of 2-acetamido-2-deoxy-D-glucose.[1208]

The action of the hen lysozyme was inhibited by 2-deoxy-2-trifluoroacetamido-αβ-D-glucose.[1209] The ^{19}F n.m.r. signal of the α-anomer, but not that of the β-anomer, showed an upfield shift in the presence of the enzyme, and a similar result was obtained for the ^{1}H n.m.r. signals of the anomeric protons of 2-acetamido-2-deoxy-αβ-D-glucose. Since the shifts for the two anomers are dissimilar, it was suggested that the anomers are bound differently. Use of the fluorinated analogue and ^{19}F n.m.r. techniques as a molecular probe of the structure of hen egg lysozyme has been reported by others; the halogenated analogues were found to be better inhibitors than 2-acetamido-2-deoxy-D-glucose.[161]

^{1}H N.m.r. spectroscopic studies of the action of hen egg-white lysozyme on *p*-nitrophenyl 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside suggested that any change in the dihedral angle between H-1 and H-2 of the D-glucose ring on substrate binding is small.[1210] It

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was concluded that the D-glucose ring is not distorted when the substrate becomes bound. However, the association constant for the binding by the enzyme of a lactone derived from *NN'N"N'"*-tetra-acetylchitotetraose, namely *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucono-1,5-lactone, was thirty-six times that for *NN'N"N'"*-tetra-acetylchitotetraose itself.[1211] This observation was taken as confirmation that the half-chair form of the substrate is most suitable for binding and that a strained ring form is involved in the lysozyme-catalysed reaction.

In an investigation of the reversibility of substrate-induced dissociation of hen egg-white lysozyme aggregates, it was found that the enzyme retained a low molecular weight form on reaction with *NN'N"N'"*-tetra-acetylchitotetraose and on subsequent removal of the substrate. It was suggested that the native and reacted enzyme possess different conformations.[1212] The interaction of alcohols with the enzyme has been assessed by c.d.[1213] and difference absorption spectral studies.[1214]

The ^{13}C n.m.r. analysis [1215] and the effects of urea and guanidine hydrochloride on the c.d. and u.v. spectra [1216] of hen egg lysozyme have been reported. The hydrogen-ion titration curve of the enzyme in guanidine hydrochloride was completely predictable from the p*K*'s of model compounds in the same solvent.[1217]

The reversible denaturation of hen egg-white lysozyme has been studied as a function of temperature, pH, and concentration of denaturant.[1218] The magnitude of the volume changes resulting from reaction of the enzyme with acid and alkali in water and denaturing media has been determined by dilatometric analysis.[1036] The enthalpy of denaturation of the hen lysozyme was determined by measurement of the heat capacity of an aqueous solution of the enzyme in the vicinity of the transition temperature.[1219] The results indicated that denaturation of the enzyme could be interpreted in terms of a two-state model; successive measurements showed that the transition was 80% reversible under the conditions used. The effect of dansylation on the renaturation of the hen egg enzyme has been investigated.[1220]

Changes have been observed in the conformation of hen egg-white lysozyme on modification of the tyrosyl and tryptophanyl residues [1221] and

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on treatment with cyanogen bromide.[1222] In the latter case, a collapse of the α-helical conformation occurred. Changes in other properties of the enzyme on modification of the tryptophan residues have also been reported.[1223] Only minor changes occurred on γ-irradiation of lysozyme in the dry state, but on subsequent solution fundamental changes in structure took place.[1224]

Chemical, immunological,[1225] and ^{1}H n.m.r.[1226] studies of hen egg-white lysozyme and α-lactalbumin led to the conclusion that conformational and other specific differences exist between the two molecules. However, similarities between their open-chain structures have been claimed,[1227] and all of the observed differences in the small-angle *X*-ray scattering of their solutions could be accounted for by dimerization of the α-lactalbumin.[1228]

Lysozyme has been identified in a *Limax* amoeba, and it was purified by ion-exchange chromatography.[412] The enzyme was active against a number of cells and was able to degrade cell-wall fragments that were resistant to hen egg-white lysozyme. Lysozyme from a *Chalaropsis* sp. possesses a molecular weight of 2.34×10^4; compositional and structural data showed that the enzyme differs from the lysozymes of hen egg-white, papaya, and T_4 phage.[1229]

Other Polysaccharidases and Oligosaccharidases

Agarases.—Examination of the activity of an extract from supernatants of cultures of *Agarbacterium pastinator* against galactans and other polysaccharides showed that the enzyme present has a high specificity for polysaccharides containing 3,6-anhydro-L-galactose residues.[1230] The action of the enzyme on agarose gave a series of homologous neoagarosaccharides, the smallest product being neoagarobiose and the largest an octadecasaccharide, suggesting that agarase cleaved the β-(1 → 4)-linkages of agar.

Cellulases.—Extracts of lesions caused by *Ascochyta pisi* and *Mycosphaerella pinodes* on detached pea leaflets contained cellulase.[1231] Mutant strains of *Aspergillus sojae* exhibited co-ordinate increases in cellulase and α-amylase production when cultured in certain media; analysis of the phenomenon yielded information on the type of mutation involved.[1178] Cellulase activity has been observed in rumen bacteria, *Butyrivibrio fibrisolvens*, *Cillobacterium cellulosolvens*, *Ruminococcus albus*, and *R.*

[1222] Y. Ohta, Y. Hibino, K. Asaba, K. Sugiura, and T. Samejima, *Biochim. Biophys. Acta*, 1971, **236**, 802.
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flavefaciens,[1232] and in culture filtrates of *Cercosporella herpotrichoides*.[1233]

Two extracellular cellulases (*A* and *B*) and one cell-bound cellulase (*C*) from *Pseudomonas fluorescens* were purified until each showed a single peak on ultracentrifugation and electrophoresis and was free from other enzyme activities.[1234] All three enzymes contain galactose, glucose, mannose, and fucose; in addition *A* and *B* contain 2-amino-2-deoxyglucose and *C* contains 2-amino-2-deoxygalactose. Cellulases *A* and *B* degraded cellopentaose and higher cello-oligosaccharides to cellobiose and cellotriose, whereas *C* hydrolysed cellotriose and higher cello-oligosaccharides to cellobiose. Determination of the amounts of the enzymes within the cell showed that only *C* is present in significant quantities and that it is localized in the periplasmic fraction.[1124]

Cellulase activity was found in the filtrates from cultures of virulent, but not avirulent, strains of *Sclerotium bataticola*.[1235] Inocculated sunflower plants were also tested for the enzyme.

The presence of lactose in the culture medium induced the synthesis of cellulase by *Trichoderma lignorum*.[1236] Even greater amounts of the enzyme were produced under laboratory and plant conditions when whey was used as the source of lactose.

Testing of a wide range of di- and oligo-saccharides and their derivatives showed that only sophorose and gentiobiose enhanced the cellulase production of *Trichoderma viride*.[1237] Sophorose induced cellulase activity much more effectively than gentiobiose. Inhibition studies were carried out with actinomycin and puromycin,[1237] and the biosynthesis of the enzyme and its behaviour on fractionation were also studied.[1125] Cellulase production by cultures of *Trichurus cylindricus* was shown to be due to the presence of a contaminant, *Micrococcus varians*.[1238] Cellulase has been demonstrated as an extracellular product of *Xanthomonas malvacearum*, the incitant of cotton bacterial blight.[1239]

Changes in the pattern of cellulase development in gibberellin-treated dwarf pea plant internodes have been reported.[1090]

Chitinases.—Immunodiffusion experiments have indicated that the chitinases of lysostaphin and *Staphylococcus aureus* are unrelated structurally; evidence was obtained for the existence of isoenzymes of the *S. aureus* chitinase.[1240] A procedure for the preparation and purification of *Streptococcus* sp. chitinase has been developed and an electrophoretically and

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centrifugally homogeneous form of the enzyme (molecular weight 2.9×10^4) was crystallized.[1241] Calcium was essential for the manifestation of enzymic activity, four atoms being associated with each enzyme molecule.

Chondroitin Sulphate Hydrolases and Chondroitin Sulphate Lyases.—The active principle of human serum capable of degrading hyaluronic acid, chondroitin 4- and 6-sulphates, and dermatan sulphate behaved as a chondroitin sulphate lyase ABC on electrophoresis in polyacrylamide gel.[1242]

Chondroitin 4-sulphate was degraded by a preparation from tadpole tail fin, whereas chondroitin 6-sulphate, dermatan sulphate, and heparin were completely resistant to its action.[792] The selectivity was proposed as a basis for distinguishing between the chondroitin sulphates and for the preparation of chondroitin 6-sulphate.

Dextranases.—A spectrophotometric method for the determination of dextranase activity has been described. Blue Dextran was used as the substrate and the increase in intensity of the blue colour as cleavage proceeded was determined.[1243] *Penicillium luteum* produced large quantities of extracellular dextranase when grown aerobically in a medium containing dextran.[1244] An electrophoretically homogeneous preparation of the enzyme exhibited a pH optimum of 5.0. The dextranase hydrolysed a series of isomaltodextrins and dextran and its derivatives, but was inert against amylopectin, pullulan, panose, and isomaltosylmaltose. A dextranase has also been isolated from oral strains of *Streptococcus mitis*.[1245] The action of the enzyme is obstructed by branch points in the oligosaccharide chain of the substrate.

Chymotrypsin A attached covalently to water-insoluble, cross-linked dextran could be resolubilized by the action of a dextranase from *Cytophaga* sp. on the conjugate.[1246] Insolubilization of dextranase to water, with retention of enzymic activity, has been reported.[1188]

Dextrin-1,6-glucosidases.—A study of the distribution of dextrin-1,6-glucosidase (amylo-1,6-glucosidase) in subcellular fractions of human and rabbit skeletal muscle showed that the enzyme, which is involved in the biosynthesis and metabolism of glycogen, is bound to the polysaccharide rather than to the sarcoplasmic reticulum.[779]

Glucanases.—Barley β-glucan stained with reactive dyes was employed as substrate in an assay of β-glucanase activity.[1247]

The extent of degradation of dextrans by oligo-1,3-glucosidases from bovine, porcine, and rabbit spleen and rabbit liver increased with increasing

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proportions of (1 → 3)-linkages in the substrate.[575] Isolation and characterization of the products from partial degradation of the glucans by the enzymes showed a preferential loss of (1 → 3)-linkages compared with (1 → 2)- and (1 → 6)-bonds. Pretreatment of the substrates with concanavalin A inhibited the degradation of (1 → 3)-linkages by the enzyme to greater extents than the degradation of other linkages.

Investigation of possible pullulanase production by *Aspergillus niger* showed that the organism produced a pullulan-degrading enzyme, which is distinctly different from glucoamylase and pullulanase.[1248] The enzyme hydrolysed the α-(1 → 4)-linkages in pullulan liberating isopanose, *O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucose.

An extracellular enzyme from cultures of *Pseudomonas stutzeri* produced large quantities of maltotetraose from linear and branched substrates such as amylopectin and glycogen.[1249] The fourth glycosidic bonds from the non-reducing, terminal units of maltohexaose and maltoheptaose were hydrolysed specifically. The enzyme has a broad pH–activity curve, which shows a maximum at pH 8, and possesses a molecular weight of 1.25×10^4; it exhibited concentration-dependent, quaternary association to give active dimers, tetramers, hexamers, octamers, and decamers.

An amylolytic enzyme with physicochemical properties differing from those of α- and β-amylase was purified from barley. The enzyme had a unique action pattern, the products of hydrolysis always containing maltotriose.[1250]

A glucanase from germinated barley hydrolysed the β-(1 → 4)-linkages of barley β-D-glucan, but neither cellulose nor laminarin was affected.[1251] The enzyme was stimulated by the presence of sodium chloride, but was inhibited by cellobiose and D-glucono-1,5-lactone.

Hyaluronidases and Hyaluronate Lyases.—Hyaluronidase activity could be measured using a water-insoluble form of chondroitin sulphate as substrate and determining the amount of uronic acid released into solution by the enzyme.[798] Hyaluronidase deficiency in several tissues of a case of Pompé's disease (glycogen storage disease) has been reported.[1160] The hyaluronidase activities of human semen and sperm suspensions subjected to temperature shock and freezing,[1252] and the hormonal regulation of the enzyme in rats during spermatogenesis[1253] have been studied. The specific activity of hyaluronidase in subcellular fractions of certain rat tissues was increased by pre-treatment of the animals with hydrocortisone.[1254] The hyaluronidase in liver extracts of the squid, *Ommastrephas sloani pacifus*, exhibited

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maximum activity in the pH range 4.5—5.0 on partial purification, and was completely inhibited by Cu^{2+} and Fe^{3+} ions.[1078] The products from the action of the enzyme on hyaluronic acid were shown to be identical with those from the action of testicular hyaluronidase; the enzyme was also active against chondroitin and certain sulphated glycosaminoglycuronans.

A reductometric method, which measures the increase of reducing groups resulting from enzymic action, was used in an assay of the hyaluronate lyase produced by *Streptomyces hyalurolyticus*.[1255] The purified enzyme was highly resistant to heat treatment. Other differences in its properties from those of testicular hyaluronidase included its resistance to inhibition by high molecular weight sulphate esters such as chondroitin 4- and 6-sulphates, dermatan sulphate, keratan sulphate, heparin, and potassium polyvinyl sulphate.

Isoamylases.—A partially purified isoamylase (glycogen 6-glucanohydrolase; molecular weight 1.2×10^5) from *Cytophaga* sp. showed the ability to hydrolyse the branch linkages of amylopectin and glycogen with the complete dismemberment of the macromolecules.[1256] The unit chains were not depolymerized and the enzyme was used to determine the average chain-length of glycogen and related polysaccharides.[148]

Isoamylase (molecular weight 9.5×10^4) was also purified from the culture fluid of a *Pseudomonas* sp. and the enzyme hydrolysed all α-(1 → 6)-linkages in glycogen, amylopectin, and their phosphorylase limit dextrins.[1257] The branch points of β-amylase limit dextrins, however, were incompletely hydrolysed. The enzyme preferentially removed α-maltotriosyl side-chains, rather than α-maltosyl units, from various substrates. The enzyme has been used to investigate the structures of certain glycogens.[770]

Keratan Sulphate Hydrolases.—A partially purified enzyme from extracts of *Coccobacillus* sp. specifically degraded keratan sulphate.[796] The products of its action were oligosaccharides of varying chain-length and glycopeptides containing 80% of the original D-mannose units. One of the major oligosaccharide products was identified as a tetrasaccharide composed of D-galactose (2), 2-acetamido-2-deoxy-D-glucose (2), and sulphate (3 equivalents per mole). Since the reducing, terminal unit is D-galactose, it was concluded that the enzyme cleaves D-galactosyl bonds in keratan sulphate.

Laminarinases.—Laminarinase has been purified from the culture medium of *Bacillus circulans* and some of its properties have been defined.[637]

Mannanases.—An assay for 'phosphomannanase' was based on the ability of the enzyme to release soluble mannan from the cell walls of

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Saccharomyces sp.[637] The enzyme was extensively purified by gel filtration and isoelectric focusing from the culture medium of *Bacillus circulans*, but the copending laminarinase activity could not be removed. The 'phosphomannanase' cleaved phosphomannan from yeast cell walls by glycosyl cleavage adjacent to the phosphodiester-linked D-mannose unit.

Oligo-1,3-glucosidases.—The oligo-1,3-glucosidase in *Basidiomycete* sp. quantitatively inverted the anomeric configuration in its action on laminarin, yielding α-D-glucose as the product.[1258] It was assumed that the enzyme catalysed a single displacement mechanism in which the rate-limiting step involved a back-side nucleophilic attack by water. An enzyme (molecular weight 1.7×10^4) from the same source, which acted on yeast cells, was also identified as an oligo-1,3-glucosidase.[1259] The enzyme was stable to heat and to acid, possessed a pH optimum of 4.0, and randomly hydrolysed β-(1 → 3)-glucosidic linkages; β-(1 → 4)- and β-(1 → 6)-glucans were resistant to the action of the enzyme.[1260] Barley aleurone cells were found to accumulate oligo-1,3-glucosidase activity, and release of the enzyme was a gibberellic acid-dependent process.[1261]

Oligo-1,6-glucosidases.—An oligo-1,6-glucosidase obtained from a cattle liver lysosomal fraction debranched glycogen and then further depolymerized it by acting as a glucoamylase.[1118] The enzyme also possessed α-glucosidase activity and the relationship between the catalytic centres was studied by kinetic experiments.

Polygalacturonases and Polygalacturonate Lyases.—The characteristics and action patterns of polygalacturonate lyases have been reviewed.[1262] Polygalacturonase activity has been detected in the hepatopancreas of Japan Sea invertebrates and a surface snail (*Succinea putris*),[1263] certain strains of *Arthrobacter* and *Brevibacterium* sp.,[1264] and culture filtrates from *Cercosporella herpotrichoides*.[1233]

The polygalacturonase of a flax anaerob (*Clostridium felsineum*) degraded flax pectin but not orange pectic acid; the action of the enzyme was inhibited by calcium and magnesium chlorides.[1265] The polygalacturonases secreted by the fungal plant pathogens, *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, and *Sclerotium roflsii*, were inhibited by proteins extracted from

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the cell walls of certain plants, apparently by a mechanism involving complex formation.[1266]

U.v.-irradiation or the addition of nalidiscic acid, mitomycin C, or bleomycin to the culture medium stimulated the production of polygalacturonase by *Erwinia aroideae*.[1267] Bivalent metal ions inhibited *Ganoderma lucidum* polygalacturonase, which had been purified until it contained no pectinesterase activity.[1268] The enzyme has a pH optimum of 5.4 and high temperature optima. The *in vivo* and *in vitro* production of polygalacturonase by *Gilbertella persicaria*, a phytopathogenic fungus, has been studied and the enzyme has been partially purified by chromatography on cellulose.[1269]

A polygalacturonase of unusual thermostability has been identified in *Rhizopus nigricans*; the enzyme possessed a half-life of three minutes at 100 °C and its survival in canned foods was considered to be the cause of post-canning softening and disintegration of fruits.[1270] Since the presence of lactose in the culture medium induced the synthesis of polygalacturonase by *Sclerotina sclerotium*, it was found that whey is an equally suitable inducer for production of the enzyme under plant conditions.[1236]

Polygalacturonase was purified from the culture fluids of *Verticillium albo-atrum* by ion-exchange chromatography and gel filtration; the final preparation (molecular weight 3.0×10^4) was homogeneous by a number of physicochemical criteria and contained carbohydrate (1.2%).[1271] The enzyme, which catalyses the hydrolysis of sodium pectinate to short-chain oligouronides, was inhibited by heavy metal ions, but not by classical sulphydryl or serine antagonists. Polygalacturonase was shown to be an extracellular product of cultures of *Xanthomonas malvacearum*, the incitant of cotton bacterial blight.[1239]

Extracts of lesions of detached pea leaflets caused by *Ascochyta pisa* and *Mycosphaerella pinodes* contained polygalacturonate lyase.[1231] The polygalacturonate lyase from the culture fluid of *Aspergillus sojae* is able to macerate various types of plant tissue.[1272] An extracellular polygalacturonate lyase from *Cytophaga johnsonii* exhibited a pH optimum of 9.0 and required the presence of calcium ions for maximum activity.[1273] Studies of its action pattern showed that the enzyme cleaves polygalacturonic acid randomly and that it is able to cleave the glycosidic linkages adjacent to non-reducing, terminal units. Pectinaceous materials and an acetone extract of potatoes induced the production of polygalacturonate lyase by a strain of *Pseudomonas fluorescens*, an organism responsible for

[1266] P. Albersheim and A. J. Anderson, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1815.
[1267] H. Tomizawa and H. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 191.
[1268] H. L. Kumari and M. Sirsi, *J. Gen. Microbiol.*, 1971, **65**, 285.
[1269] M. D. Mehrotra, P. Kaiser, and C. Reynaud, *Ann. Inst. Pasteur*, 1971, **120**, 81.
[1270] K. A. Harper, *Chem. Ind.*, 1971, 462.
[1271] M. C. Wang and N. T. Keen, *Arch. Biochem. Biophys.*, 1970, **141**, 749.
[1272] S. Ishii and T. Yokotsuka, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1157.
[1273] A. Sundarraj and J. V. Bhat, *Arch. Microbiol.*, 1971, **77**, 155.

soft rot in potatoes.[1274] Culture filtrates of virulent extracts of *Sclerotium bataticola* contained polygalacturonate lyase activity and the presence of the enzyme in sunflower plants inoculated with the organism has been investigated.[1235]

Pullulanases.—The differences between the action patterns of bacterial pullulanase and other debranching glycohydrolases have been summarized.[1151] An enzyme showing pullulanase activity has been purified from sweet corn and separated on hydroxyapatite into what appeared to be two isoenzyme fractions of identical specificities.[1275] Pullulan, α-limit dextrins, and amylopectin β-limit dextrin were rapidly hydrolysed by the enzyme, whereas amylopectin was slowly debranched and glycogen was not significantly degraded.

Trehalases.—The relationship of the trehalase of hamster intestinal sacs to the transport of D-glucose has been studied.[1083] Soluble trehalase from flies (*Drasophila melanogaster*) has been purified to an extent that only one band was detectable by disc gel electrophoresis; the enzyme possesses a molecular weight of 1.0×10^5 and a pH optimum of 5.7.[1086] Trehalase has also been localized in the flight muscle of the blow fly (*Phormia regina*) and its properties were investigated.[1276] Trehalase has been isolated from *Pullularia pullulans* cells by a series of steps including DEAE-cellulose chromatography.[1277] The enzyme is specific for trehalose and exhibits maximum activity at pH 4.0.

Xylanases.—A β-xylosidase obtained from *Aspergillus niger* appeared to have xylanase activity.[1150] The production of xylanase by *Trichoderma viride* was stimulated by the presence of sophorose.[1125] Purification of an extract of the organism by DEAE-Sephadex chromatography yielded two xylanase-active fractions.[1278] One of these fractions was crystallized after chromatography on CM-Sephadex and possessed a pH optimum of 3.5.

Carbohydrate Oxidases

Glucose Oxidases.—Glucose oxidase from *Aspergillus niger* has been used in a coulometric determination of maltose [85] and in combination with other enzymes in a specific assay for glycogen.[768] The absolute specficity of glucose oxidase (from *Penicillium amagasakiense*) for β-D-glucose was the basis for a polarographic microdetermination of the D-glucose anomers, the β-anomer being determined before and after attainment of mutarotational equilibrium.[46]

[1274] M. Zucker and L. Hankin, *Canad. J. Microbiol.*, 1971, **71**, 1313.
[1275] E. Y. C. Lee, J. J. Marshall, and W. J. Whelan, *Arch. Biochem. Biophys.*, 1971, **143**, 365.
[1276] W. D. Reed and B. Sacktor, *Arch. Biochem. Biophys.*, 1971, **145**, 392.
[1277] E. Merdinger, C. F. Lange, and B. F. Booker, *J. Bacteriol.*, 1971, **106**, 1034.
[1278] S. Hashimoto, T. Muramatsu, and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 501.

Sedimentation analyses of glucose oxidase from *P. amagasakiense* gave a molecular weight of 1.6×10^5 for the native enzyme.[1279] Analysis in the presence of guanidine hydrochloride reduced this value to 8.1×10^4, and this value was reduced to 4.5×10^4 by addition of mercaptoethanol. It was concluded that the glucose oxidase molecule consists of four chains of equal size, two chains being covalently bound to form a dimer and two such dimers being non-covalently bound to give the intact enzyme. Glucose oxidase, purified from *Penicillium vitale* by gel filtration until homogeneous, was shown to contain galactose, mannose, and 2-amino-2-deoxyglucose.[1280]

Water-insoluble derivatives of glucose oxidase have been prepared and their properties compared with those of the soluble enzyme.[1281, 1282]

Gulonolactone Oxidases.—The distribution of gulonolactone oxidase in the *locus coerulus* of rabbits has been assessed.[1283]

Peptidases

Bromelain.—The possible non-involvement of the carbohydrate moiety of pineapple-stem bromelain in the catalytic mechanism of the enzyme was examined by periodate oxidation of the sulphydryl-derivatized enzyme.[1284] The unoxidized material afforded 4.76% of carbohydrate consisting of mannose (2.44), fucose (1.34), xylose (0.98), and 2-amino-2-deoxyglucose (1.82 moles per mole). When oxidation had proceeded to the extents that only 20, 5, or 2% of the neutral sugar residues remained, enzyme activity was reduced to 83, 72, and 64%, respectively. According to c.d. and o.r.d. measurements, no conformational changes are brought about by oxidation.

Thrombins.—A method has been described for the preparation of human thrombin from Cohn Fraction III.[1285] The recovered material migrated as a single component (molecular weight 3.1×10^4) on electrophoresis, but autolysis occurred on storage. The chromatographic purification of bovine thrombin from commercially available starting material has been described and the product was shown to contain 2-amino-2-deoxy-D-glucose (2—4 residues per mole).[1286] Analysis of a further purified preparation of bovine thrombin showed that the molecule contains D-galactose (0.7%), D-mannose (0.6%), L-fucose (0.3%), 2-acetamido-2-deoxy-D-glucose (0.5%), and sialic acid (0.5%).[1287] Treatment with appropriate glycosidases released

[1279] T. Yoshimura and T. Isemura, *J. Biochem.* (*Japan*), 1971, **69**, 839.
[1280] T. A. Abalikhina, A. D. Morozkin, V. P. Bogdanov, and E. D. Keversneva, *Biokhimiya*, 1971, **36**, 191.
[1281] M. K. Weibel and H. J. Bright, *Biochem. J.*, 1971, **124**, 801.
[1282] H. H. Weetall, *Biochim. Biophys. Acta*, 1970, **212**, 1.
[1283] K. Iijima, *Histochemie*, 1971, **25**, 107.
[1284] Y. Yasuda, N. Takahashi, and T. Murachi, *Biochemistry*, 1971, **10**, 2624.
[1285] J. W. Fenton, W. P. Campbell, J. C. Harrington, and K. D. Miller, *Biochim. Biophys. Acta*, 1971, **229**, 26.
[1286] G. Glover and E. Shaw, *J. Biol. Chem.*, 1971, **246**, 4594.
[1287] K. Skaug and T. B. Christensen, *Biochim. Biophys. Acta*, 1971, **230**, 627.

D-galactose (34%), 2-acetamido-2-deoxy-D-glucose (26%), and sialic acid (90%) without affecting the clotting activity of the molecule. However, the glycoprotein nature of thrombin has been disputed and the carbohydrate content of the bovine molecule was considered to be an artefact of the purification procedure.[1288]

Miscellaneous Enzymes

Carbohydrate Isomerases.—An enzyme preparation from cultures of *Azotobacter vinelandii* epimerized the D-mannuronic acid residues of alginic acid into L-guluronic acid residues in the presence of calcium ions.[1289] The properties of the mannuronate isomerase were defined and an assay was devised for measuring its activity based on the different extinction coefficients of mannuronic and guluronic acids in the carbazole reaction.

Ceruloplasmin.—Human ceruloplasmin in which the terminal *N*-acetylneuraminic acid residues had been modified to 5-acetamido-3,5-dideoxy-L-*arabino*-7-[^{3}H]hydroxy-2-heptulosonic acid residues exhibited a normal half-life in rat plasma.[699] The modified sialic acid residues could be removed by the action of neuraminidase, indicating that the seven-carbon analogue simulates *N*-acetylneuraminic acid.

Removal of less than 20% of the terminal sialic acid residues from labelled human and rat ceruloplasmins, with consequent exposure of penultimate D-galactosyl residues, resulted in the rapid disappearance of the molecules from circulation when administered to rats.[1148] It was estimated that exposure of no more than two D-galactosyl residues is sufficient to manifest this property, and, by use of the different specificities of two bacterial neuraminidases, it was shown that removal of any two sialic acid residues from ceruloplasmin is incompatible with the continued survival of the molecule in the circulation. The catabolism of desialysed human and rat ceruloplasmin labelled with ^{64}Cu and with ^{3}H in the D-galactose residues was investigated[1290] and *N*-acetyl[1-^{14}C]neuraminic acid-labelled ceruloplasmin has also been used to study the fate of the glycoprotein.[1291]

Porcine ceruloplasmin yielded galactose (8), mannose (12), fucose (4), 2-amino-2-deoxyglucose (12), and *N*-acetylneuraminic acid (8 residues per mole).[1292] Tryptic digestion yielded four principal glycopeptides (0.6—1.0 moles each per mole) each containing the five monosaccharides listed (2 : 3 : 1 : 4 : 2, respectively). Compositional and ultracentrifugal analysis

[1288] C. W. Batt, T. W. Mikulka, K. G. Mann, C. L. Guarracino, R. J. Altiere, R. G. Graham, J. P. Quigley, J. W. Wolf, and C. W. Zafonte, *J. Biol. Chem.*, 1970, **245**, 4857.

[1289] A. Haug and B. Larsen, *Carbohydrate Res.*, 1971, **17**, 297.

[1290] G. Gregoriadis, A. G. Morell, I. Sternlieb, and I. H. Scheinberg, *J. Biol. Chem.*, 1970, **245**, 5833.

[1291] J. Hickman, G. Ashwell, A. G. Morell, C. J. A. van den Hamer, and I. H. Scheinberg, *J. Biol. Chem.*, 1970, **245**, 759.

[1292] A. Matsunaga and Y. Nosoh, *Biochim. Biophys. Acta*, 1970, **215**, 280.

gave molecular weights in the range 3.3—4.8 × 10^3 and it was concluded that the parent molecule contains four heterosaccharide moieties.

Cytochromes.—Cytochrome P-450_{cam}, isolated from *Pseudomonas putida*, consisted of a single polypeptide chain bearing a small carbohydrate moiety, one of the components of which is 2-amino-2-deoxyglucose.[1293]

Formyltetrahydrofolate Synthetase.—Formyltetrahydrofolate synthetase from *Clostridium cylindrosporum* was shown to contain neutral carbohydrate (4 moles per mole maximum).[1294] The molecular weight of 2.4 × 10^5 was reduced to 6.0 × 10^4 on treatment with guanidine hydrochloride, indicating a tetrameric structure for the intact molecule.

α-Lactalbumin.—A chromatographic procedure has been reported for the isolation of α-lactalbumin from human milk whey.[1295] The molecule resembled that from bovine and guinea-pig sources in a number of its parameters, but differed in its immunological properties. Minor structural differences between human, sheep, goat, and porcine milk α-lactalbumins have been reported.[1296] The various carbohydrates identified in preparations of bovine α-lactalbumin have been attributed to the presence of minor glycoprotein components.[918, 919]

It has been reported that bovine α-lactalbumin and lysozyme have similar conformations in solution,[1228] whereas the results of chemical, immunological,[1225] and n.m.r.[1226] studies lead to the conclusion that specific differences exist between them. Immunological cross-reaction between bovine α-lactalbumin and lysozyme was observed only after conversion into the reduced, carboxymethyl derivatives.[1227]

β-Naphthylamidase.—In spite of extensive purification, a glycoprotein fraction from rat intestinal brush borders retained β-naphthylamidase activity, and the possibility exists that the enzyme is a glycoprotein.[886]

Pectinesterases.—A spectrophotometric technique for the determination of methanol, as formaldehyde, has been employed in the estimation of pectinesterase activity.[100] Equations that predict the activity of pectinesterases as a function of cation and enzyme concentrations and pH have been presented.[1297] The empirical relationships are statistically significant and quantitatively represent the kinetic differences between pectinesterases of fungal and plant origin. Extracellular pectinesterase activity has been detected in cultures of *Cercosporella herpotrichoides*,[1233] *Clostridium felsineum* (a flax anaerobe),[1265] and *Xanthomonas malvacearum* (the incitant of cotton bacterial blight).[1239] In the last case, the presence of pectin was

[1293] K. Dus, M. Katagiri, C. Y. D. L. Erbes, and I. C. Gunsalus, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 1423.
[1294] W. H. Welch, D. H. Buttlaire, R. T. Hersh, and R. H. Himes, *Biochim. Biophys. Acta*, 1971, **236**, 599.
[1295] N. I. Phillips and R. Jenness, *Biochim. Biophys. Acta*, 1971, **229**, 407.
[1296] D. V. Schmidt and K. E. Ebner, *Biochim. Biophys. Acta*, 1971, **243**, 273.
[1297] H. Mayorga and C. Rolz, *J. Agric. Food Chem.*, 1971, **19**, 179.

found to be necessary for the production of the enzyme. The properties of a pectinesterase produced by a phytopathogenic fungus (*Gilbertella persicana*) have been studied.[1269]

Changes in the pattern of pectinesterase development in gibberellic acid-treated dwarf pea plant internodes have been reported.[1090] Tomato pectinesterase was found to possess a molecular weight of 2.4—2.7 × 10^4 and a pI of 8.4.[1298] Extensive binding was demonstrated between the cell wall of tomato pericarps and the endogenous pectinesterase.[1089]

Phosphatases.—An orthophosphate-repressible acid phosphatase (molecular weight 8.5 × 10^4), obtained in physicochemically pure form from *Neurospora crassa*, afforded 9.5% of carbohydrate consisting of mannose (34.3) and 2-amino-2-deoxyglucose (15.6 moles per mole).[1299] The molecule dissociated into two indistinguishable subunits on treatment with guanidine hydrochloride in mercaptoethanol.

Rat intestinal, surface-membrane glycoprotein could not be dissociated from alkaline phosphatase activity even after extensive purification.[886] The intestinal alkaline phosphatase of a mollusc (*Semperula maculata*) appeared to be a sialoglycoprotein.[1300]

Sulphatases.—Excessive excretion of sulphatides by patients with sulphatidosis (metachromatic leukodystrophy) was taken as confirmation of a deficiency in arylsulphatase.[1301] Liver extracts of the squid (*Ommastrephes sloani pacifus*) contained chondrosulphatase.[1078] A fucosulphatase from a mollusc (*Patella vulgata*) was found to hydrolyse L-fucose 2-, 3-, and 4-sulphates at similar rates.[1302]

Index of Enzymes Referred to in Chapter 6*

Trivial Name	*Systematic Name*	*EC No.*	*Page*
β-Acetamidodeoxy-galactosidase	β-2-acetamido-2-deoxy-D-galactoside acetamidodeoxygalactohydrolase		345
β-Acetamidodeoxy-glucosidase	β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	345
β-Acetamidodeoxy-hexosidase	β-2-acetamido-2-deoxy-D-hexoside acetamidodeoxyhexohydrolase		345
Acid phosphatase	orthophosphoric monoester phosphohydrolase	3.1.3.2	378
Agarase			367
Alkaline phosphatase	orthophosphoric monoester phosphohydrolase	3.1.3.1	378
α-Amylase	α-1,4-glucan 4-glucanohydrolase	3.2.1.1	357
β-Amylase	α-1,4-glucan maltohydrolase	3.2.1.2	361
α-Arabinofuranosidase			347

[1298] H. Declincée and B. J. Radola, *Biochim. Biophys. Acta*, 1970, **214**, 178.
[1299] M. M. Jacobs, J. F. Nyc, and D. M. Brown, *J. Biol. Chem.*, 1971, **246**, 1419.
[1300] A. T. Varute and V. A. Patil, *Histochemie*, 1971, **25**, 77.
[1301] M. Philippart, L. Sarlieve, C. Meurant, and L. Mechler, *J. Lipid Res.*, 1971, **12**, 434.
[1302] P. F. Lloyd and P. F. Forrester, *Biochem. J.*, 1971, **124**, 21P.

* See Introduction (Part II, Chapter 1).

Trivial Name	*Systematic Name*	*EC No.*	*Page*
Polygalacturonase	poly-α-1,4-galacturonide glycanohydrolase	3.2.1.15	372
Polygalacturonate lyase	poly-α-1,4-D-galacturonide lyase	4.2.99.3	372
Pullulanase			374
Sialidase†			355
Sulphatase			378
Thrombin		3.4.4.13	375
Trehalase	trehalose 1-glucohydrolase		374
Uronic acid isomerase			376
Xylanase	β-1,4-xylan xylanohydrolase	3.2.1.8	374
β-Xylosidase	β-D-xyloside xylohydrolase	3.2.1.37	357

† Sialidase is used generally to include neuraminidase and unspecified neuraminate glycohydrolases.

7
Glycolipids and Gangliosides

A number of reviews have appeared covering various aspects of glycolipids and gangliosides as follows: analysis, structure, synthesis, biosynthesis, metabolism, and biological function of glycosphingolipids;[1303] the chemistry and biochemistry of glycosphingolipids, including carbohydrate structures, and biosynthesis in normal and clinical cases;[1304] the classification of glycolipids and the biosynthesis of glycolipids and gangliosides;[694] the biosynthetic and degradative pathways of glycosphingolipids and glycosylglycerides in mammalian tissues;[1305] and isolation, purification, comprehensive structural details, biosynthesis, cellular location, and function of bacterial glycolipids.[1306]

The quantitative isolation of total glycosphingolipids on a microgram scale from crude lipid extracts without contamination by lipids of other classes has been reported.[1307] The method consisted of acetylation of the total lipids with acetic anhydride and pyridine, followed by separation of the acetylated glycolipids from non-glycolipids on a magnesia–silica gel column, and deacetylation with sodium methoxide. Gel filtration on Sephadex G-100 was shown to be a simple and rapid technique for the purification of gangliosides, since it overcame the problems of contamination by amino-acids, peptides, phospholipids, and carbohydrates.[1308] Sephadex columns have also been exploited for the separation of gangliosides from corticosteroids and water-soluble non-lipids of lipid extracts.[1309]

A method advocated for the determination of cerebral cerebrosides and sulphatides employed t.l.c., but did not require column chromatography.[1310] A quantitative procedure reported for the spectrophotometric determination of gangliosides is based on the sulphophospho-vanillin reaction and does not require prior hydrolysis of the sample.[11] In conjunction with the resorcinol procedure for measuring *N*-acetylneuraminic acid, the method is capable of determining whether a purified ganglioside is of the

[1303] W. Stoffel, *Ann. Rev. Biochem.*, 1971, **40**, 57.
[1304] W. Gielen, *Chimia (Switz.)*, 1971, **25**, 81.
[1305] B. Czartoryska, *Postepy Biochemii*, 1971, **17**, 3.
[1306] N. Shaw, *Bacteriol. Rev.*, 1970, **34**, 365.
[1307] T. Saito and S. Hakomori, *J. Lipid Res.*, 1971, **12**, 257.
[1308] I. F. de Raveglia and N. E. Ghittoni, *J. Chromatog.*, 1971, **58**, 288.
[1309] C. Terner, E. I. Szabo, and N. L. Smith, *J. Chromatog.*, 1970, **47**, 15.
[1310] E. Mesdjian, *J. Chromatog.*, 1971, **57**, 448.

mono-, di-, or tri-sialo type. All the components of glycosphingolipids, *i.e.* hexose, 2-amino-2-deoxyhexose, neuraminic acid, and sphingosine base, were determinable by a single run on g.l.c. after methanolysis and trifluoroacetylation.[36] D-Galactose, derived from galactolipids by enzymic hydrolysis, could be determined *in situ* without interference by uncleaved galactosyl residues using a galactose dehydrogenase–nicotine adenine dinucleotide (NAD) system, the reduced NAD being determined.[1311]

Mass spectrometric analysis of microgram quantities of a series of glycosphingolipids of known structure after trimethylsilylation provided a technique for structural identification.[1312] Reproducible ratios were obtained for the intensities of certain carbohydrate fragment ions to the total intensity of ions characteristic of the sphingolipid bases, and these were used to determine the number of monosaccharides in the glycosyl moiety and how many units were unsubstituted at C-3. 2-Acetamido-2-deoxyhexose residues were readily detected, and further characteristic fragments appeared if they were present as terminal residues of the oligosaccharide chain. It was also possible to distinguish between the *N*-glycolyl and *N*-acetyl derivatives of neuraminic acid and to determine the number of these residues present.

Monolayer characteristics of, and calcium-adsorption on to, cerebroside and cerebroside sulphate orientated at an air–water interface have been investigated.[1313]

A study of the behaviour of glycolipids in autolysing human brain white matter showed that the cerebroside levels remain unchanged for at least 24 days, thus supporting the hypothesis that they cannot be hydrolysed extracellularly but only by certain intravital reactions.[1314] The neuraminidase content of human brain appeared to be related to the ganglioside content,[1063, 1064] and the gangliosides were identified as endogenous substrates of neuraminidase in human, rat, and hen brains.[1140, 1141] Development profiles for the gangliosides of human and rat brain have been compared.[1315]

Glycosphingolipids isolated from brain white matter of a patient with globoid cell leukodystrophy (Krabbe's disease) have been identified as gluco- and galacto-cerebrosides, lactosylceramide, digalactosylglucosylceramide, tetrahexosylceramides, and a sulphatide containing galactose as the sole monosaccharide.[1316] The literature on the biochemical assessment of the Sanfilippo syndrome has been reviewed, and the accumulation of gangliosides in cerebral material has been investigated.[862] The ganglioside content of brain from cases of Tay–Sachs disease (G_{M_1}-gangliosidosis) was shown to be

[1311] S. Gatt, *Biochim. Biophys. Acta*, 1970, **218**, 173.
[1312] G. Dawson and C. C. Sweeley, *J. Lipid Res.*, 1971, **12**, 56.
[1313] P. J. Quinn and W. R. Sherman, *Biochim. Biophys. Acta*, 1971, **233**, 734.
[1314] C. Leube and F. Lindlar, *Z. physiol. Chem.*, 1971, **352**, 1100.
[1315] M. T. Vanier, M. Holm, R. Öhman, and L. Svennerholm, *J. Neurochem.*, 1971, **18**, 581.
[1316] Y. Eto and K. Suzuki, *J. Neurochem.*, 1971, **18**, 503.

higher than normal.[1096] An accumulation of the same ganglioside was found in the brains of Siamese cats suffering from a hereditary neurological disease. The release of *N*-acetylneuraminic acid from 2-acetamido-2-deoxy-galactosyl[*N*-acetylneuraminosyl]galactosylglucosylceramide (Tay–Sachs ganglioside G_{M_2}) was effected by an enzyme from rat tissues.[1142]

By use of purified α- and β-galactosidases, ceramide trihexosides [galactosyl-(1 → 4)-galactosyl-(1 → 4)-glucosyl-(1 → 1)-ceramide] isolated from normal human kidney, kidney from a case of Fabry's disease, human erythrocytes, hamster fibroblasts, and Nakahara–Fukuoka sarcoma were found to contain terminal α-D-galactosyl and penultimate β-D-galactosyl residues.[1317] Enzymic studies of ^3H- or ^{14}C-labelled trihexosylceramide from cases of Fabry's disease corroborated the α-configuration of the terminal D-galactose unit and the existence of an α-galactosidase deficiency.[1101] Other independent work has also demonstrated a deficiency of this enzyme; the anomeric linkages of the galactose residues were established by n.m.r. spectroscopy and confirmed by mass spectrometry of a trifluoroacetylated, reduced oligosaccharide obtained from the trihexoside by ozonolysis and fragmentation with alkali.[1098]

The galactocerebroside and glucocerebroside contents of kidney tissues from patients with globoid cell leucodystrophy (Krabbe's disease) were greater than those found in controls by 25 and 30%, respectively.[1318] Thus, despite the genetic defect of the degradative enzyme (β-galactosidase), there is no specific accumulation of galactocerebroside.

In a study of the gangliosides of skin fibroblasts derived from normal children and patients with several hereditary diseases, only mono- and di-sialohematosides were found.[1319] The content of lipid-bound *N*-acetyl-neuraminic acid was found to be greater than normal in cases of Hurler syndrome and globoid cell leucodystrophy.

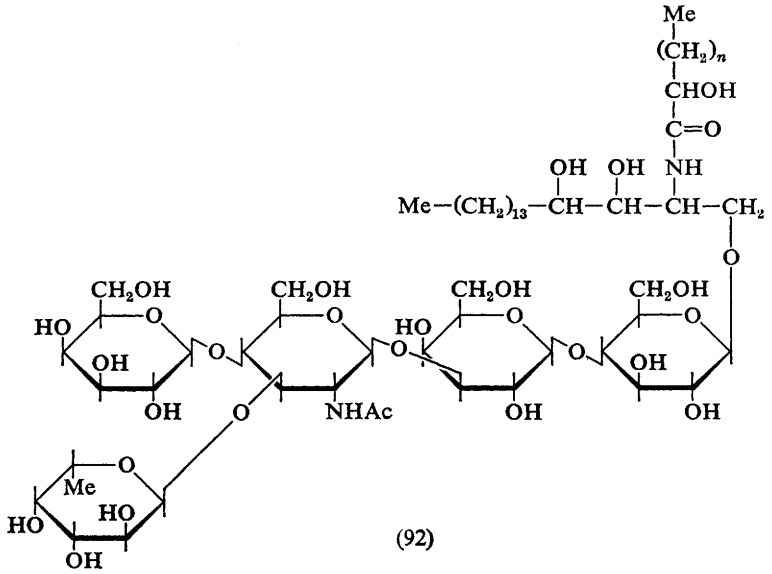
A fraction obtained from human blood red-cell stroma is probably identical with the blood-group specific glycolipid isolated from erythrocytes.[736] A glycolipid–glycoprotein fraction isolated from human erythrocyte membranes yielded hexose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid.[977] Glucosylceramide and lactosylceramide have been found in human leukocytes and in leukaemic leukocytes from patients with acute, chronic myelogeneous and chronic lymphocytic leukaemia.[1320] The amounts of each, however, varied widely amongst the conditions. Glucosylceramide was the only glycolipid found in normal and leukaemic lymphocytes, whereas lactosylceramide was found in platelets. Lactosylceramide was the major glycolipid in preparations consisting mainly of polymorphonuclear, myeloid, and blastic cells.

The urinary, neutral glycosylceramides and sulphatides from humans have been fractionated on DEAE-cellulose and silicic acid columns;

[1317] Y. Li and S. Li, *J. Biol. Chem.*, 1971, **246**, 3769.
[1318] K. Suzuki, *Lipids*, 1971, **6**, 433.
[1319] L. Hof, R. Matalon, and A. Dorfman, *Z. physiol. Chem.*, 1971, **352**, 1329.
[1320] J. Hildebrand, P. Stryckmans, and P. Stoffyn, *J. Lipid Res.*, 1971, **12**, 361.

dihexosylceramides were shown to be excreted in larger amounts by women than by men, and sulphatides were detected in all specimens.[1301] In cases of sulphatidosis (metachromatic leukodystrophy), excessive sulphatide excretion was apparent and this was taken to confirm a deficiency in arylsulphatase A. The urinary ceramide trihexosides from cases of Fabry's disease are similar to those that accumulate in the liver and kidney.[1321] The ceramide dihexosides contain sulphur, but this is attached to the ceramide rather than the hexose residues.

The carbohydrate structure of a novel glycosphingolipid isolated from human adenocarcinomas was elucidated from methylation, Smith degradation, recognition of the cross-reactivity with type XIV Pneumococcal polysaccharide, and the reactivity with wheat-germ agglutinin.[1322] The ceramide moiety of the overall structure (92) was represented as *N*-(α-hydroxy fatty acyl)-4-hydroxysphinganine, since this structure represents the major component.



(92)

The ganglioside content in brains of representatives of six vertebrate classes (monkey, rabbit, rat, tortoise, triton, frog, hen, pigeon, carp, sheatfish, lamprey, and ray) has been determined and, in most cases, was found to correlate with the level of nervous organization.[1323] The ganglioside compositions of the brains of higher vertebrates (mammals, birds, and reptiles) are similar; four principal gangliosides containing up to three *N*-acetylneuraminic acid residues were found to constitute up to 90% of the

[1321] P. E. Gregoire, G. Jonniaux, and W. Voet, *Clin. Chim. Acta*, 1971, **33**, 387.
[1322] H. Yang and S. Hakomori, *J. Biol. Chem.*, 1971, **246**, 1192.
[1323] N. F. Avrova, *J. Neurochem.*, 1971, **18**, 667.

total ganglioside *N*-acetylneuraminic acid. The gangliosides of lower vertebrates (fish and amphibia) contain a higher proportion of polysialogangliosides having four or five *N*-acetylneuraminic acid residues.

A method of separating gangliosides by DEAE-cellulose column chromatography followed by preparative t.l.c. has been used in obtaining appreciable quantities of the seven normal, bovine brain gangliosides.[1324] Studies of the c.d. spectra of bovine gangliosides indicated the possibility of making structural assignments by using this technique.[786] Isolation and purification of neuraminic acid-free glycosphingolipids from bovine spleen yielded duplicate pairs of galactosyl-, glucosyl-, diglycosyl-, and triglycosylceramides with each pair differing in the chain length of the fatty acid residue.[1325] Permethylation and hydrolysis, and partial hydrolysis showed that galactosyl-(1 → 4)-glucosyl and galactosyl-(1 → 4)-galactosyl-(1 → 4)-glucosyl sequences are present in the di- and tri-glycosylceramides, respectively.

The major glycolipids in porcine erythrocytes are of the globoside (tetraglycosylceramide) type and occur along with smaller amounts of other glycosyl ceramides and traces of gangliosides.[1326] Two major globosides isolated were demonstrated to contain galactose, glucose, and 2-amino-2-deoxygalactose (1.00 : 2.15 : 1.18 and 1.00 : 2.20 : 1.15, respectively); limited methylation studies revealed that they possess the same basic carbohydrate structure (93), but differ in the fatty acid structures.

$$\text{Glc}N\text{Ac} \rightarrow \text{Gal} \rightarrow \text{Gal} \rightarrow \text{Glc} \rightarrow \text{ceramide}$$
(93)

Separations of the cerebral gangliosides of rabbit and rat showed qualitative similarities but quantitative differences.[1327] In cases of intoxication with organophosphoric inhibitor of cholinesterase, the relative amounts of the gangliosides remained unaltered, but the absolute content was normal in the rat and reduced in the case of the rabbit. Investigation of glycolipid biosynthesis in normal and transformed hamster NIL 2 cells, using [1-^{14}C]palmitate as precursor, showed that mono- and di-hexosylceramide are present in comparable amounts in both cell types, but that three larger glycolipids are absent from the transformed line.[1328]

Non-specific effects of behavioural stimulation of rats undergoing swimming trials included decreased net incorporation of injected 2-amino-2-deoxy-D-[1-^{14}C]glucose into monosialogangliosides, cerebrosides, and sulphatides, and also changes in the content of certain gangliosides of the brain.[1329] Specific effects included decreased uptakes of radioactivity into

[1324] C. C. Winterbourn, *J. Neurochem.*, 1971, **18**, 1153.
[1325] G. Tschöpe, *Z. physiol. Chem.*, 1971, **352**, 71.
[1326] D. J. Hanahan, J. E. Ekholm, and B. Benson, *Biochim. Biophys. Acta*, 1971, **231**, 343.
[1327] N. P. Taranova and V. I. Rozengart, *Biul. Eksp. Biol. i Med.*, 1971, **77**, 39.
[1328] P. W. Robbins and I. A. MacPherson, *Proc. Roy. Soc.*, 1971, **B177**, 49.
[1329] L. N. Irwin and F. E. Samson, *J. Neurochem.*, 1971, **18**, 203.

disialogangliosides. The contents of glycolipid and ganglioside have been determined during myelination in cases of neuronal perikarya and astrologia of rat brain.[1330] Incorporation of 2-amino-2-deoxy-D-[1-^{14}C]glucose into synaptosomes isolated from rat-cerebral cortex [845] and of 2-[^{3}H]acetamido-2-deoxy-D-mannose into developing rat brain [882] has been assessed. Glycolipids containing galactose, glucose, fucose, 2-amino-2-deoxy-galactose, and sialic acid have been identified in membranes of rat ascites hepatoma.[888]

Glycolipids comprised 20.1 and 15.7% of normal and quaking mouse brain myelin, respectively.[1331] Glucosylceramides containing 2-hydroxy-acids have been isolated from mouse brains,[1332] and the biosynthesis of cerebrosides by mouse brain microsomes has been studied using UDP-D-[^{14}C]galactose and UDP-D-[^{14}C]glucose.[1333]

The gangliosides of mouse L-929 cells and secondary cultures of mouse embryo cells were identified by their chromatographic behaviour and their response to mild treatment with acid.[1334] Monosialogangliosides were predominant in the L-cells, whereas the secondary cultures contained more complex gangliosides, but the D-[1-^{14}C]glucose incorporated by both cultures was principally distributed in 2-amino-2-deoxy-D-galactose, *N*-acetylneuraminic acid, and *N*-glycosylneuraminic acid moieties. Mono-, di-, and tri-glycosylceramides were detected in L-cells, whereas secondary cultures contained mono-, di-, and tetra-glycosylceramides. The sialic acid content of the glycolipid fraction from mouse fibroblast L cells has been reported.[890]

Glycosphingolipids containing neuraminic acid have been isolated from two lines of mouse neoplastic mast cells; compositional and separative analyses indicated the presence of *N*-acylneuraminosyl-monoglycosyl-ceramide, *N*-acylneuraminosyl-mono-(2-amino-2-deoxyhexosyl)triglycosyl-ceramide, and di-(*N*-acylneuraminosyl)-mono-(2-amino-2-deoxyhexosyl)-diglycosylceramide.[1335] The cerebroside sulphate isolated yielded hexose (1), sulphate (1), and sphingosine (1 mole per mole). The intracellular distribution of all these components was investigated.

The diglycosylceramide, which is present in trace amounts in normal, female mouse kidney and in substantial amounts in the kidneys of mice treated with testosterone, has been identified as digalactosyl-ceramide.[1336]

[1330] W. T. Norton and S. E. Poduslo, *J. Lipid Res.*, 1971, **12**, 84.
[1331] N. Singh, N. Spritz, and B. Geyer, *J. Lipid Res.*, 1971, **12**, 473.
[1332] S. Hammarström, *European J. Biochem.*, 1971, **21**, 388.
[1333] P. Morell, E. Constantino-Ceccarini, and N. S. Radin, *Arch. Biochem. Biophys.*, 1970, **141**, 738.
[1334] G. Yogeeswaran, J. R. Wherrett, S. Chaterjee, and R. K. Murray, *J. Biol. Chem.*, 1970, **245**, 6718.
[1335] K. Masek, K. Bensch, and H. W. Felsenfeld, *Biochem. Pharmacol.*, 1971, **20**, 2309.
[1336] G. M. Gray, *Biochim. Biophys. Acta*, 1971, **239**, 494.

Certain aspects of the biosynthesis of galactocerebroside by embryonic chicken brain [1337] and of cerebroside by chicken liver [1338] have been reported.

Deposits within the swim bladders of two deep-ocean fish, *Coryphaenoides acroleposis* and *Antimora rostrata*, have been found to contain glycolipids (4.3—8.9%).[1339] A shallow-water fish, the kelp bass (*Paralabrax clathratus*), has similar amounts of glycolipids in the swim-bladder lining. A glycosphingophospholipid has been isolated from a marine shell-fish, *Turbo cornutus*, and was purified by silicic acid column chromatography and mild alkaline hydrolysis; the molecule yielded galactose (1 mole per mole).[1340]

The mono- and di-galactosyldiacylglycerols from several plant species of different orders have been studied.[1341] The galactolipid contents of bundle sheath chloroplasts were shown to be higher than those of mesophyll chloroplasts in both maize (*Zea mays*) and sorghum (*Sorghum bicolor*) plants.[1342] The relative proportion of monogalactosyldiacylglycerol to digalactosyldiacylglycerol lay in the range 1.6—2.5 and the ratio reflected the degree of grana formation in the chloroplast. The enzymic conversion of 2-acyl-3-monogalactosylglycerols to 1,2-diacyl-3-monogalactosylglycerols by spinach-leaf homogenates has been studied.[1343] Column chromatography of a solvent extract of a red marine alga, *Porphyra tenera*, yielded three glycolipid fractions, which were identified as monogalactosyl-lipid, digalactosyl-lipid, and lyso-type digalactosyl-lipid.[1344]

The glycolipids from *Arthrobacter crystallopoietes*, *A. pascens*, and *A. globiformis* comprised mono- and di-galactosyldiglyceride, dimannosyldiglyceride, and traces of tri- and tetra-glycosyldiglycerides.[1345] Quantitative differences were observed in the glycolipids of the mesosomal and plasma membranes from *Bacillus megaterium* and *Micrococcus lysodeikticus*.[1346] Changes in the galactolipid composition of cells of *Bifidobacterium bifidum* have been observed as a result of inhibition of the cells.[1347] The decrease in galactose resulted both from a lowering of all galactolipids and from a shift from oligogalactosyl- to monogalactosyl-lipids.

A cerebroside isolated from the yeast *Hansenula ciferri* by solvent extraction contained only D-glucose.[1348] Periodate oxidation indicated that the sugar is linked to C-1 of the sphingosine unit. Fractionation of a

[1337] S. Basu, A. M. Schultz, M. Basu, and S. Roseman, *J. Biol. Chem.*, 1971, **246**, 4272.
[1338] S. Ito, T. Negishi, M. Nakano, and Y. Fujino, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 143.
[1339] T. Patton and A. J. Thomas, *J. Lipid Res.*, 1971, **12**, 331.
[1340] A. Hayashi and F. Matsuura, *Biochim. Biophys. Acta*, 1971, **248**, 133.
[1341] G. Auling, E. Heinz, and A. P. Tulloch, *Z. physiol. Chem.*, 1971, **352**, 905.
[1342] D. G. Bishop, K. S. Andersen, and R. M. Smittie, *Biochim. Biophys. Acta*, 1971, **231**, 412.
[1343] R. Safford, R. S. Appleby, and B. W. Nichols, *Biochim. Biophys. Acta*, 1971, **239**, 509.
[1344] S. Sato, *Bull. Japanese Soc. Sci. Fish.*, 1971, **37**, 326.
[1345] N. Shaw and D. Stead, *J. Bacteriol.*, 1971, **107**, 130.
[1346] D. J. Ellar, T. D. Thomas, and J. A. Posgate, *Biochem. J.*, 1971, **122**, 44P.
[1347] F. A. Exterkate and J. H. Veerkamp, *Biochim. Biophys. Acta*, 1971, **231**, 545.
[1348] B. Kaufman, S. Basu, and S. Roseman, *J. Biol. Chem.*, 1971, **246**, 4266.

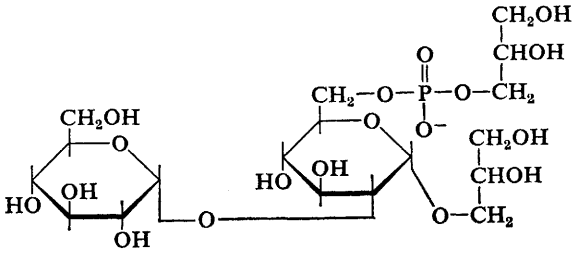
(94) R = H or Me

lipid extract from a *Micromonospora* sp. yielded a phosphatidylinositomonomannoside and a minor fraction identified as a glycolipid containing αα-trehalose acylated by two fatty acid residues.[1349]

A glycosulpholipid from *Mycobacterium tuberculosis* was identified as a 2,3,6,6′-tetra-*O*-acyltrehalose 2′-sulphate derivative.[1350] Mycoside C_2, a lipoglycopeptide isolated from *Mycobacterium avicum*, was shown to contain 3,4-di-*O*-methyl-L-rhamnose (1), 6-deoxy-L-talose (0.5), and 6-deoxy-3-*O*-methyl-L-talose (0.5 moles per mole).[702] Alkaline β-elimination indicated that the 6-deoxy-L-talose residues are linked to an *allo*-threonine residue in the proposed structure (94).

Evidence has been presented for the release, at low salt concentrations, of a carbohydrate–lipid–protein complex from isolated envelopes and whole cells of a marine pseudomonad (*Pseudomonas* type IV).[704] The cell-wall glycolipid from a mutant strain of *Salmonella minnesota* has yielded 2-amino-2-deoxyglucose (12.6%).[1351]

Characterization of a phosphoglycolipid from membranes of *Streptococcus faecalis* was facilitated by the development of methods which specifically labelled constituent units of the molecule with radioisotopes. The compound was identified as a tetra-acylated derivative of α-D-glucopyranosyl-(1 → 2)-*O*-(6-*O*-glycerophosphoryl-α-D-glucopyranosyl)glycerol (95).[1352]



(95)

[1349] H. Tabaud, H. Tishovska, and E. Vilkas, *Biochimie*, 1971, **53**, 55.
[1350] M. B. Goren, O. Brokl, B. C. Das, and E. Lederer, *Biochemistry*, 1971, **10**, 72.
[1351] E. T. Rietschel, C. Galanos, A. Tanaka, E. Ruschmann, O. Lüderitz, and O. Westphal, *European J. Biochem.*, 1971, **22**, 218.
[1352] R. T. Ambron and R. A. Pieringer, *J. Biol. Chem.*, 1971, **246**, 4216.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Glycolipids, and Gangliosides

Polysaccharides.—An α-(1 → 6)-linked polysaccharide has been prepared by the phosphorus pentachloride catalysed polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranose.[1353] Higher reaction temperatures and monomer concentrations were required compared to those employed for the polymerization of the corresponding D-glucose and D-mannose derivatives. Debenzylation of the resultant galactopolymer gave a polysaccharide that apparently was soluble only in aqueous lithium hydroxide–borate mixtures and in DMF–nitrogen tetroxide solutions. Periodate oxidation demonstrated that the polymer was as stereoregular as the previously synthesized glucan and mannan and, therefore, of essentially pure α-configuration.

A D-fructose-rich polymer, which interacted with concanavalin A, has been synthesized enzymically using a water-insoluble preparation of dextransucrase.[1354]

Oligosaccharides.—Homopolymers and copolymers with styrene have been formed from methyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*p*-vinylbenzoyl)-α-D-glucopyranoside, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-β-D-glucopyranose, 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-β-D-glucopyranosyl chloride, and 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-vinylbenzoyl)-α-D-glucopyranosyl bromide.[1355] These polymers have been prepared as supports for the synthesis of oligosaccharides.

The synthesis of gentiobiose [*O*-β-D-glucopyranosyl-(1 → 6)-D-glucose] and 6^2-β-D-glucosylcellobiose [*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 4)-D-glucose] was effected by the β-D-glucosyltransferase activity of extracts of *Tetrahymena pyriformis* using D-glucose and cellobiose, respectively, as acceptors and cellobiose as donor.[1356] Similar

[1353] T. Uryu, H. Libert, J. Zachoval, and C. Schuerch, *Macromolecules*, 1970, **3**, 345.
[1354] S. Ogino, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1268.
[1355] R. D. Guthrie, A. D. Jenkins, and J. Stehlicek, *J. Chem. Soc.* (*C*), 1971, 2690.
[1356] D. J. Manners, J. R. Stark, and D. C. Taylor, *Carbohydrate Res.*, 1971, **16**, 123.

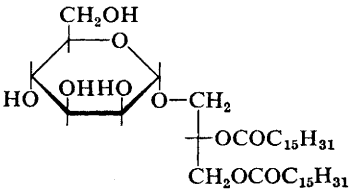
experiments with an extract from *Ochromonas malhamensis* gave rise to cellotriose and 6^2-β-D-glucosylcellobiose. The same extracts could also use gentiobiose as the glucosyl donor giving higher gentiosaccharides as products.

The transglucosidase properties of brewer's yeast α-glucosidase have been applied to the synthesis of such α-D-glucopyranosyl-D-fructoses as maltulose (1 → 4) and turanose (1 → 3).[1126]

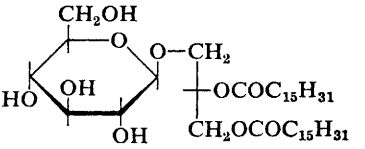
The preparation of straight-chain D-xylose oligosaccharides (DP 2—7) from Esparto grass xylan by acid hydrolysis at low temperatures has been reported.[108]

Glycoproteins.—A glycoprotein has been formed enzymically from myelin protein (A1 protein) with the aid of a polypeptide-2-acetamido-2-deoxy-galactosyl transferase.[877] Treatment with alkaline borohydride showed that the incorporated 2-acetamido-2-deoxy-D-galactose units are linked *O*-glycosidically to the hydroxy-group of threonine residues.

Glycolipids and Gangliosides.—A section in a review on glycosphingolipids was devoted to their chemical synthesis.[1303] Routes for the synthesis of



(96)



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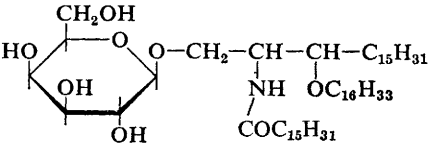


(98)

the α-D-mannopyranosyl diglyceride (96), the β-D-glucopyranosyl diglyceride (97),[1357] and β-D-galactopyranosyl-*N*-palmityl-3-*O*-hexadecyldehydrosphingosine (98)[1358] have been reported. Methyl 6-*O*-mycolyl-α-D-glucopyranoside and 6,6′-di-*O*-mycolylsucrose have been prepared by routes involving methyl 6-*O*-tosyl-α-D-glucopyranoside and 6,6′-di-*O*-tosylsucrose, respectively.[1359]

[1357] A. I. Bashkatova, G. V. Smirnova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1971, **7**, 1644.

[1358] I. G. Zhukova, G. P. Smirnova, N. V. Chekareva, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 411.

[1359] M. Kato and J. Asselineau, *European J. Biochem.*, 1971, **22**, 364.

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

Uses of modified polysaccharides for the preparation and purification of enzymes, antibodies, antigens, and glycoproteins,[693] and peptides and nucleic acids [1360] by affinity chromatography have been reviewed. Uses of certain polysaccharides for the preparation of insolubilized enzymes have been summarized.[1062]

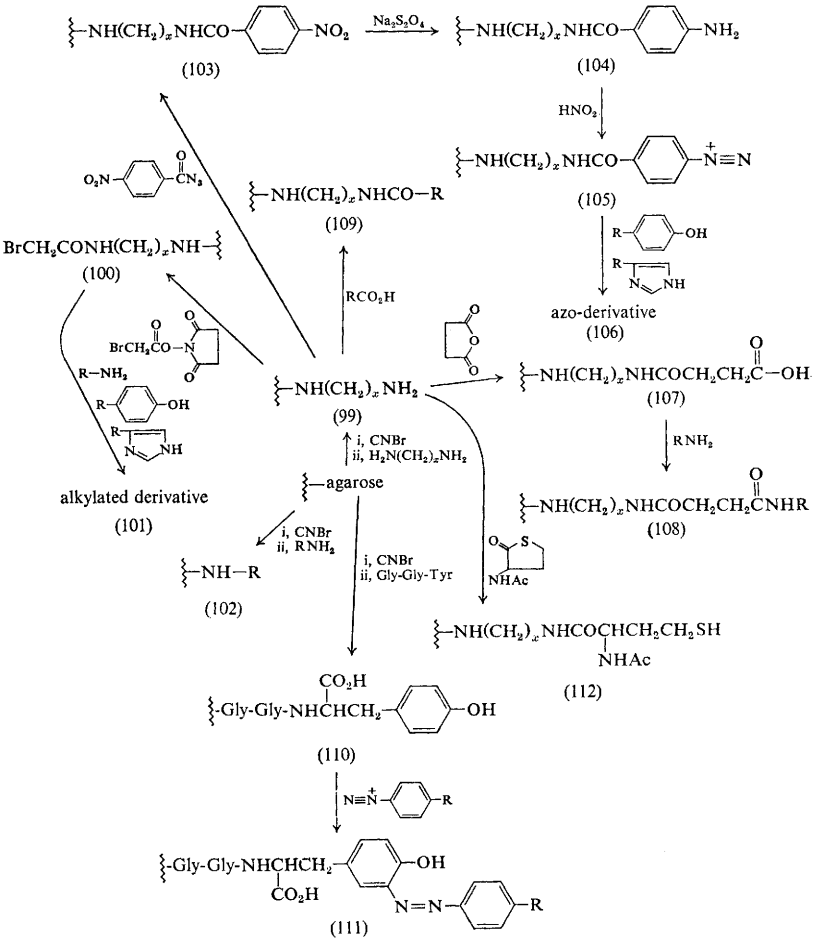
Agar, Agaropectin, and Agarose.—Addition of an acidified aqueous solution of chitosan to a solution of agar in formamide caused coprecipitation of the sulphated entities and the procedure was employed for the removal of agaropectin from agar to give an agarose-rich fraction containing only 0.15% of sulphate.[1361] The complex could also be formed using chitin or chitosan in a heterogeneous phase reaction.

Sepharose (a commercially available macroporous agarose) pretreated with 2-amino-4,6-dichloro-*sym*-triazine has been used as a support in the preparation of a water-insoluble derivative of chymotrypsin A.[1362]

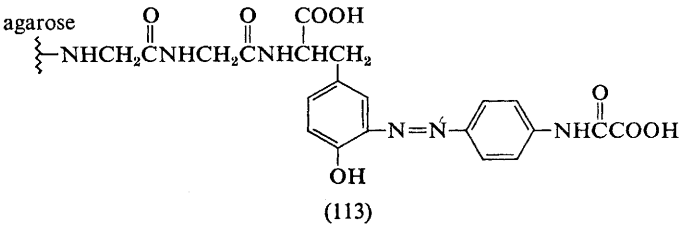
Sepharose 4B modified to contain cyclic iminocarbonate groups (CIC-agarose) has been used extensively as an intermediate in the formation of more complex derivatives of agarose, the initial activation being achieved by treatment of Sepharose with cyanogen bromide. Routes (Scheme 13) for the preparation of agarose derivatives containing such groups as ω-aminoalkyl (99), bromoacetyl (100), alkyl (101) and (102), nitrophenyl (103), aminophenyl (104), diazo (105), azo (106), carboxylic acid (107), substituted amide (108) and (109), and also peptide (110) and modified peptide (111) side-chains have been reported.[1363] Sulphydryl derivatives of agarose (112) could be prepared by treating ω-aminoalkylagarose (99) with *N*-acetylhomocysteine thiolactone; ligands containing free carboxy-groups could then be coupled to such derivatives by reaction in the presence of water-soluble carbodi-imides. Reaction of the CIC-agarose tripeptide derivative (110) with diazotized *N*-(*p*-aminophenyl)oxamic acid provided a product (113) that was used for the purification of neuraminidases by affinity chromatography.[1145, 1146]

Treatment of CIC-agarose with 3,3′-diaminodipropylamine yielded an aminoagarose, which on reaction with cortisol hemisuccinate and DCC gave a cortisol-agarose.[1364] This material was used for the chromatographic purification of corticosteroid-binding globulin from plasma. CIC-Agarose treated with 4-aminophenylmercuric acetate has been applied to the separation of active and inactive forms of papain.[1365] Further derivatives

[1360] G. Feinstein, *Naturwiss.*, 1971, **58**, 389.
[1361] G. G. Allan, P. G. Johnson, Y. Lai, and K. V. Sarkanen, *Carbohydrate Res.*, 1971, **17**, 234.
[1362] G. Kay and M. D. Lilly, *Biochim. Biophys. Acta*, 1970, **198**, 276.
[1363] P. Cuatrecasas, *Nature*, 1970, **228**, 1327.
[1364] W. Rosner and H. L. Bradlow, *J. Clin. Endocrinol. Metab.*, 1971, **33**, 193.
[1365] L. A. Æ. Sluyterman and J. Wijdenes, *Biochim. Biophys. Acta*, 1970, **200**, 593.


(103)
(104)
(105)
azo-derivative
(106)
(107)
(108)
(109)
(100)
(99)
alkylated derivative
(101)
(102)
(112)
(110)
(111)

Scheme 13


agarose
(113)

of CIC-agarose have been produced by treatment with pyridoxamine 5′-phosphate, *N*′-(ω-aminohexyl)pyridoxamine 5′-phosphate, and *N*′-(ω-aminododecyl)pyridoxamine 5′-phosphate.[1366] The latter two derivatives selectively bound apoglutamic-oxaloacetic transaminase and this property was exploited in affinity chromatography.

CIC-Agarose reacted with 4-[4-(*p*-aminophenyl)butanamido]phenyl β-D-fucopyranoside to give a product suitable for the purification, by affinity chromatography, of a regulatory protein required for expression of the L-arabinose operon in *Escherichia coli*.[1367]

The reactive groups of CIC-agarose could be inactivated by treatment with ethanolamine, and covalent attachment of heparin to the modified agarose has been described.[799] CIC-Agarose has also been employed for the insolubilization of such glycosaminoglycuronans as chondroitin sulphate, dermatan sulphate, heparin, and heparan sulphate.[798]

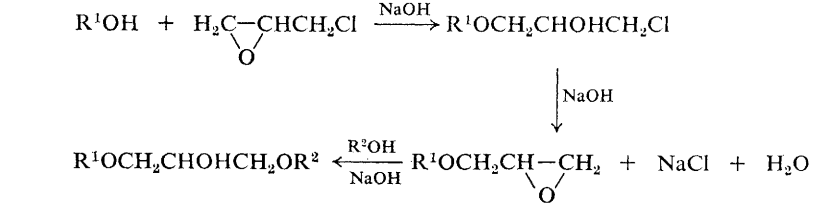
Bovine serum albumin [1368] and modified forms of lysozyme and α-lactalbumin [1227] reacted with CIC-agarose to give products that were useful immunoabsorbents. Conversely, antibodies to modified enzymes have been reacted with CIC-agarose and the products employed in the isolation of fragments from the enzymes.[1369] Activation of Sepharose 4B with a limited amount of cyanogen bromide minimized the subsequent attachment of aldolase to CIC-agarose by more than one group per enzyme molecule.[1370]

Polyinosinic acid (poly rI, a single-strand ribonucleic acid) was covalently bound through its terminal 5′-phosphate group to CIC-agarose, and annealing of the product with polycytidilic acid (poly rC) yielded a matrix-bound poly(rI:rC).[1371] Reaction of the cyclic iminocarbonate function with poly rI was considered to proceed by addition of the 5′-phosphate group to a cyanic ester to yield an activated 5′-phosphate group which, in turn, reacted with an adjacent hydroxy-group of the agarose to give a phospho-diester. Ribonucleic acids, deoxyribonucleic acids, and deoxyribonucleic acid polymerase have been attached similarly to CIC-agarose; in certain cases, the single-stranded nucleic acids coupled with greater ease than the double-stranded molecules.[1372]

Prealbumin coupled to CIC-agarose has been used in affinity chromatography of human retinol binding protein.[1373] Prolactin and insulin derivatives of CIC-agarose, formed from Sepharose 2B, have been employed in the stimulation of ribonucleic acid synthesis.[1374]

[1366] R. Collier and G. Kohlhaw, *Analyt. Biochem.*, 1971, **42**, 48.
[1367] G. Wilcox, K. J. Clemetson, D. V. Santi, and E. Englesberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2145.
[1368] H. Inouye, S. Fuchs, M. Sela, and U. Z. Littauer, *Biochim. Biophys. Acta*, 1971, **240**, 594.
[1369] M. Wilcher, V. Bocchini, M. Becker, and D. Givol, *Biochemistry*, 1971, **10**, 2828.
[1370] W. W.-C. Chan, *Biochim. Biophys. Res. Comm.*, 1970, **41**, 1198.
[1371] A. F. Wagner, R. L. Bugianesi, and T. Y. Shen, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 184.
[1372] M. S. Poonian, A. J. Schlabach, and A. Weissbach, *Biochemistry*, 1971, **10**, 424.
[1373] A. Vahlquist, S. F. Nilsson, and P. A. Peterson, *European J. Biochem.*, 1971, **20**, 160.
[1374] R. W. Turkington, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1362.

Alginic Acid.—Alginic acid has been cross-linked with epichlorohydrin (Scheme 14) to give an alkali-resistant ion-exchanger.[1375] The properties of alginic acid were not substantially modified by the reaction, but the ion-exchange efficiency of the product was greater since it could be used at more suitable pH's.



R^1 and R^2 represent different molecules of alginic acid

Scheme 14

The carboxy-groups of alginic acid have been converted into substituted amides by activation with 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDC, a water-soluble carbodi-imide) followed by nucleophilic attack of [1-^{14}C]glycine methyl ester on the activated carboxy-group (38.4% conversion) (*cf.* Scheme 22).[1376]

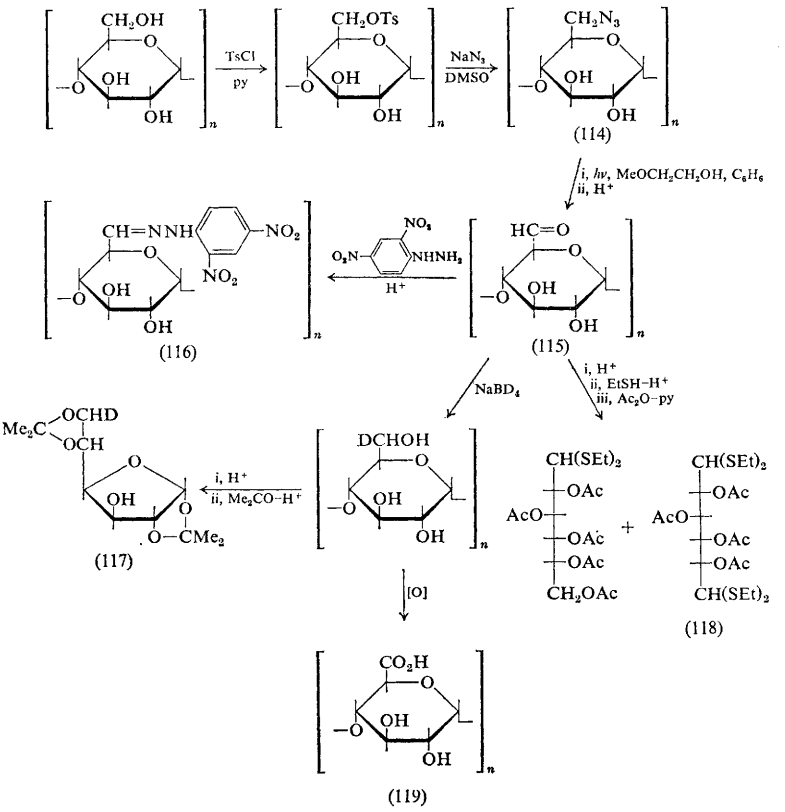
Methods have been described for the preparation of degraded sodium alginate by oxidative–reductive depolymerization and by γ-irradiation; the rate of degradation by the former process is governed by more than one rate constant. The products prepared by either method were shown to have gross chemical and biological properties similar to those of a derivative of sodium alginate prepared by hydrolytic degradation.[1377]

The D-mannuronic acid residues of alginic acid could be converted into L-guluronic acid residues by using an epimerase from *Azotobacter vinelandii*.[1289]

Amylopectin.—A coloured derivative of amylopectin, reactone Red-2B amylopectin, has been employed for the measurement of α-amylase activity.[1159]

Amylose.—The 6-aldehydo-derivative of amylose (115) has been prepared (Scheme 15) by photolysis of the corresponding 6-azido-6-deoxy-derivative (114).[1378] The 6-aldehydo-derivative was characterized by formation of the 2,4-dinitrophenylhydrazone (116) and also by reduction with sodium borodeuteride followed by hydrolysis, acetonation, and

[1375] M. R. C. Mazza and F. Ferrero, *Ann. Chim.* (*Italy*), 1971, **61**, 348.
[1376] I. Danishefsky and E. Siskovic, *Carbohydrate Res.*, 1971, **16**, 199.
[1377] E. R. Humphreys and G. R. Howells, *Carbohydrate Res.*, 1971, **16**, 65.
[1378] D. M. Clode and D. Horton, *Carbohydrate Res.*, 1971, **17**, 365.



Scheme 15

examination of the resulting 1,2:5,6-di-*O*-isopropylidene-α-D-[6-^{2}H]gluco-furanose (117) by mass spectrometry. 6-Aldehydoamylose was also characterized by hydrolysis and identification of the liberated D-*gluco*-hexodialdose by conversion into the crystalline 2,3,4,5-tetra-*O*-acetyl-D-*gluco*-hexodialdose tetraethyl bis(dithioacetal) (118). The formation of the aldehyde made available a route from the glucan to the corresponding glucuronan (119).

It was concluded from measurements of intrinsic viscosity and specific optical rotation and from potentiometric titrations that carboxymethyl-amylose exists as a random coil in aqueous solution.[1379] Methodology for the permethylation of amylose using methyl iodide, DMSO, and the methyl-sulphinyl carbanion has been reported.[132] The permethylated derivatives of polysaccharides of various degrees of polymerization could be prepared in up to 90% yield with only 10—20% degradation. Methylated amylose

[1379] J. R. Patel and R. D. Patel, *Biopolymers*, 1971, **10**, 839.

containing 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3,6-tri-*O*-methyl-D-glucose, in a 1 : 421 molar ratio, has been prepared and used in assessing the anomeric configuration of polysaccharides by ^{1}H n.m.r. spectroscopy.[362]

Hydrodynamic measurements on amylose tricarbanilate fractions in a pyridine–water theta-mixture have been used to provide a test of theoretical treatments of limiting viscosity number and frictional coefficient, which had been developed on the basis of a partial, free-draining model.[1380]

Monte-Carlo computer simulation techniques applied to investigations of nearest-neighbour auto-inhibitory effects in the periodate oxidation of amylose provided evidence for the rapid establishment of equilibria between the aldehydo-groups of oxidized units and the hemiacetals formed with the hydroxy-groups on neighbouring, unoxidized units.[229] Periodate-oxidized amylose, which was resistant to the action of β-amylase and glucoamylase, proved to be a useful substrate for the detection of α-amylase in the presence of the other amylolytic enzymes.[1152]

A kinetic study has been made of the reaction between amylose and iodine.[224] O.r.d. and c.d. spectra have been obtained for amylose–iodine complexes formed from a series of amyloses of different degrees of polymerization.[225]

The unit-cell dimensions for wet and dry states of complexes of amylose with straight-chain aliphatic mono- and poly-ketones have been calculated from *X*-ray diffraction analyses.[214] It was found that the helix-packing diameter of the complex is dependent upon the linear chain length of the complexed ketone. Chemical and physicochemical analyses of a crystalline complex isolated from solutions of amylose in ethylenediamine revealed a complexing ratio of two D-glucose units to one ethylenediamine molecule.[1381] The complex was studied by *X*-ray diffraction and its structure was shown to be almost identical with that of an amylose–DMSO complex.

Cellulose.—The induction of graft polymerization to cellulose by pre-irradiation and simultaneous irradiation methods has been reviewed.[1382] The nature of the resultant graft copolymers and techniques for the proof of grafting were described. Using styrene, vinyl acetate, or 2- or 4-vinylpyridines as monomers, it was shown that the extent of graft polymerization to pre-irradiated cellulose per unit radiation dose was significantly lower than that achieved by simultaneous irradiation.[1383]

Modification of cellulose by heat treatment, to form ultimately 1,6-anhydro-β-D-glucopyranose, was found to occur by two competitive reactions for which mechanisms have been postulated.[236]

Cellulose has been modified to give cross-linked products by treatment with divinyl sulphone in alkali.[1384] Identification of the substituted and

[1380] W. Banks, C. T. Greenwood, and J. Sloss, *Makromol. Chem.*, 1970, **140**, 119.
[1381] T. D. Simpson, *Biopolymers*, 1970, **9**, 1039.
[1382] P. W. Moore, *Rev. Pure Appl. Chem.*, 1970, **20**, 139.
[1383] S. Dilli and J. L. Garnett, *Austral. J. Chem.*, 1971, **24**, 981.
[1384] V. O. Cirino, A. L. Bullock, and S. P. Rowland, *Carbohydrate Res.*, 1971, **17**, 67.

cross-linked D-glucoses obtained after hydrolysis showed that the mole fractions of reagent residues in the form of single substituents, simple cross-links, and complex structures were 0.20, 0.72, and 0.08, respectively. The substituent linkages and cross-linkages to the D-glucose residues were found to involve principally O-6.

The generation of carbonyl groups at C-2 and/or C-3 or C-6 in the D-glucose residues of celluloses and hemicelluloses on oxidation with chlorine or hypochlorite rendered the adjacent β-D-glucosidic bonds more labile to alkali.[1385] This process can be related to the shortening of carbohydrate chains occurring during bleaching and removal of lignin.

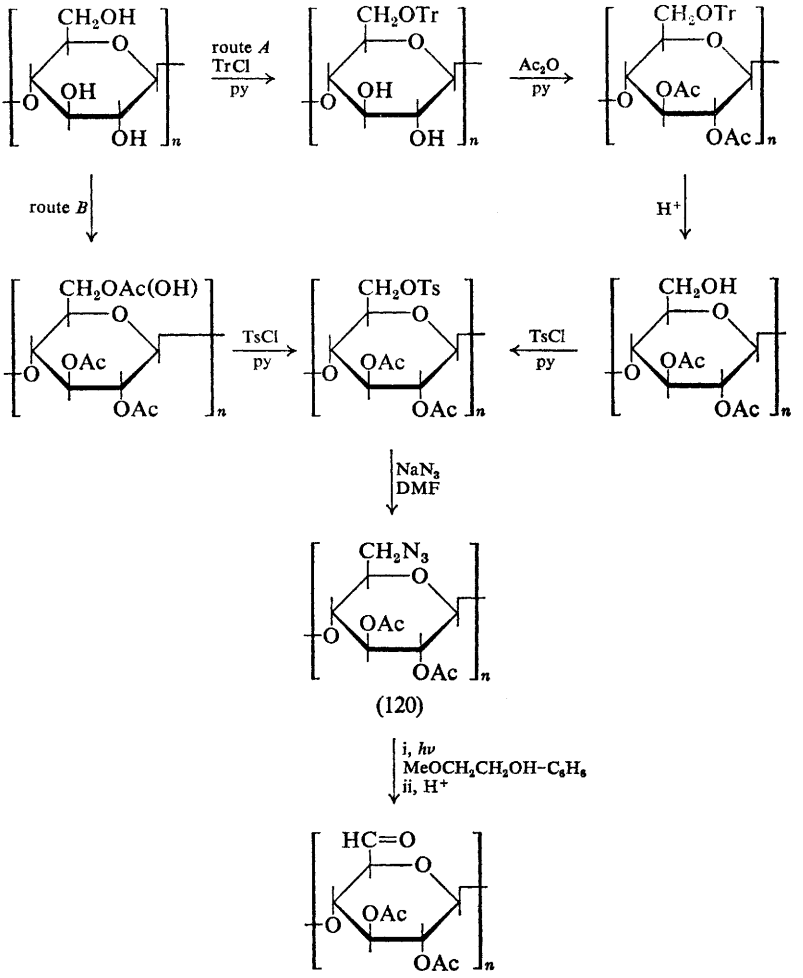
Chitosan-impregnated cellulose has been employed for the ion-exchange chromatography of nucleic acid fragments.[1386] Ion-exchangers have been prepared by binding protamine and methylated albumin to cellulose,[1387] and the products were particularly useful for the separation of nucleic acids. The kinetics of the conversion of cellulose into protein by *Myrothecium verrucaria* have been investigated as a mathematical model for diffusion-controlled fermentation processes.[1388]

D-Glucosylation of cellulose acetate (DS 2.2) with 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(t-butyl orthoacetate), followed by saponification and reduction with sodium borohydride, gave a water-soluble glucan.[1389, 1390] Periodate oxidation and partial acetolysis revealed that the polysaccharide contains a main chain of the cellulose type with branching (at approximately every second residue) by β-D-glucopyranosyl residues mainly through the secondary hydroxy-groups of the main chain. The predominant D-glucosylation of the secondary hydroxy-groups was explained by inter-monomeric acetyl migrations during the reaction.

Two specific routes have been described for the introduction of 6-aldehydo-groups into cellulose (Scheme 16); both procedures involved photolysis of 6-azido-6-deoxycellulose (120).[1391] 6-Aldehydocellulose (121) was characterized by formation of its 2,4-dinitrophenylhydrazone and by mass spectrometry after conversion into 1,2:5,6-di-*O*-isopropylidene-α-D-[6-^{2}H]glucofuranose (see Scheme 15). The latter procedure provided a means of determining the degree of substitution of 6-aldehydocellulose and it was found that route *A* gave rise to a higher DS than route *B*.

Aminoethylcellulose has been used for the preparation of an insoluble derivative of trypsin, the enzyme being coupled with the aid of glutaraldehyde.[1392]

[1385] T. Krause, *Angew. Chem.*, 1971, **83**, 548; *Angew. Chem. Internat. Edn.*, 1971, **10**, 522.
[1386] K. Nagasawa, H. Watanabe, and A. Ogama, *J. Chromatog.*, 1971, **56**, 378.
[1387] S. O. Kudinov and E. M. Makohonenko, *Ukrain. biochem. Zhur.*, 1971, **43**, 389.
[1388] L. W. Ross and D. M. Updegraff, *Biotechnol. and Bioeng.*, 1971, **13**, 99.
[1389] N. K. Kochetkov, A. F. Bochov, and T. A. Sokolovskaya, *Carbohydrate Res.*, 1971, **19**, 1.
[1390] A. F. Bochkov, T. A. Sokolovskaya, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1971, **41**, 2761.
[1391] D. M. Clode and D. Horton, *Carbohydrate Res.*, 1971, **19**, 329.
[1392] C. K. Glassmeyer and J. D. Ogle, *Biochemistry*, 1971, **10**, 786.



Scheme 16

The optimum conditions for the formation of *trans*-2,3-cyclic carbonate derivatives of cellulose have been established.[1393] Use of ethyl chloroformate yielded a product bearing cyclic groups on 54% of the D-glucose residues as well as acyclic carbonate (ethoxycarbonyl) groups; use of other chloroformates gave poorer conversions. Cellulose *trans*-2,3-carbonate has

[1393] S. A. Barker, H. Cho Tun, S. H. Doss, C. J. Gray, and J. F. Kennedy, *Carbohydrate Res.*, 1971, **17**, 471.

been used in the preparation of insoluble derivatives of β-glucosidase [1128] and antibodies to human myeloma IgE.[990]

Free-rotation dimensions have been obtained for sodium carboxymethylcellulose.[158] Comparison of the values obtained with those for other ionic polysaccharides suggested similarities of their short-chain hydrodynamic behaviour. The azido-derivative of carboxymethylcellulose has been used for the preparation of covalent, water-insoluble derivatives of chymotrypsin A [1394, 1395] and glucoamylase.[1193] Carboxymethylcellulose hydrazide has proved useful for the production of apoenzymes corresponding to a number of pyridoxal phosphate-dependent enzymes.[1396] Carboxymethylcellulose and DEAE-cellulose, which had been treated with 2-amino-4,6-dichloro-*sym*-triazine, formed the bases of insoluble derivatives of chymotrypsin A.[1362]

β-Galactosidase [1117] and glucoamylase [1194] derivatives of DEAE-cellulose have been prepared by adsorption of the enzymes on to the polymer. Benzoylated [1397] and benzoylated-naphthoylated [1398] derivatives of DEAE-cellulose have been applied successfully to the separation of deoxyribonucleic acids and related compounds.

Procion brilliant-orange DEAE-cellulose has been prepared by treating DEAE-cellulose with the dye in alkaline solution; the derivative was used as a matrix support for cholinesterase.[1399] DEAE-Cellulose, after treatment with 2-amino-4,6-dichloro-*sym*-triazine, has also been used as a support for chymotrypsin.[1400]

A method reported for the determination of the ethoxyl contents of samples of *O*-ethyl- and *O*-ethyl-hydroxyethyl-cellulose is based on conversion of the groups into acetic acid, which is then measured by g.l.c.[102] Fluorescence depolarization and viscometric studies have been carried out on concentrated solutions of *O*-(2-hydroxyethyl)cellulose and on the same polysaccharide to which fluorescein had been attached.[1401] *O*-(2-Hydroxypropyl)cellulose has been prepared by reaction of cellulose with propylene oxide and epichlorohydrin, whereas treatment of the polysaccharide with epichlorohydrin and boron trifluoride yielded *O*-(chlorohydroxypropyl)cellulose.[1402] Reaction of the latter derivative with ammonia and various primary, secondary, or tertiary amines gave rise to a wide range of lipophilic anion-exchangers suitable for chromatography in organic solvents.

[1394] V. I. Surovtsev, L. V. Kozlov, and V. K. Antonov, *Doklady Akad. Nauk S.S.S.R.*, 1970, **195**, 1463.
[1395] V. I. Surovtsev, L. V. Kozlov, and V. K. Antonov, *Biokhimiya*, 1971, **36**, 199.
[1396] I. Mezzasoma, C. Borri-Voltattorni, A. Giartosio, A. Orlacchio, and C. Turano, *Boll. Soc. ital. Biol. sper.*, 1970, **46**, 913.
[1397] R. Stern, *Biochemistry*, 1971, **10**, 2963.
[1398] V. N. Iyer and W. D. Rupp, *Biochim. Biophys. Acta*, 1971, **228**, 117.
[1399] R. O. Stasiw, H. D. Brown, and F. X. Hasselberger, *Canad. J. Biochem.*, 1970, **48**, 1314.
[1400] S. P. O'Neill, J. R. Wykes, P. Dunill, and M. D. Lilly, *Biotechnol. and Bioeng.*, 1971, **13**, 319.
[1401] D. Biddle and S. Pardhan, *Arkiv. Kemi*, 1971, **32**, 43.
[1402] B. Almé and E. Nyström, *J. Chromatog.*, 1971, **59**, 45.

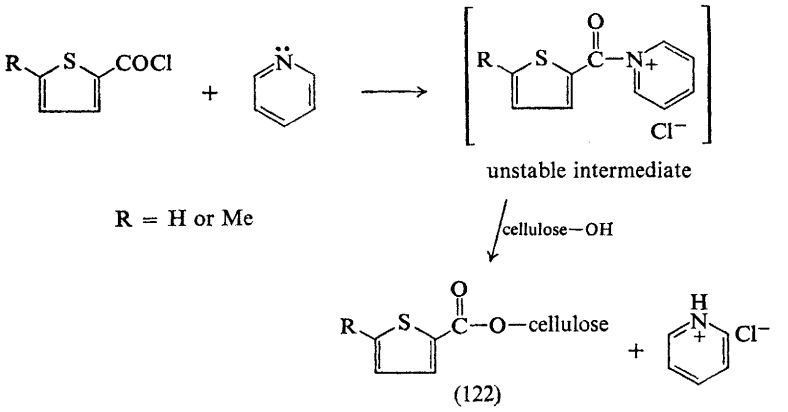
Treatment of cellulose with cyanogen bromide yielded the cyclic iminocarbonate derivative, which has been used to form insoluble derivatives of intrinsic factor[1403] and of antibodies to human chorionic gonadotrophin.[1404] Nicotine adenine dinucleotide (NAD), ε-aminocaproate, glycylglycylglycine, and 2-aminophenol derivatives of cellulose have also been prepared *via* the cyclic iminocarbonate.[1405] The three latter derivatives were further modified by reaction with NAD and 1-cyclohexyl-3-(2-morpholinoethyl)-carbodi-imide–*p*-tolylsulphonylmethane. Diazo-NAD-cellulose was prepared by treating 2-hydroxyanilinocellulose with diazotized benzidine followed by NAD. All the foregoing NAD derivatives of cellulose were used as insoluble co-factors in the separation of NAD-dependent and non-dependent enzymes.

Per-*O*-methylcellulose was obtained in good yield by a two-step process using dimethyl sulphate.[132] Permethylated and partially methylated celluloses have been obtained by using the dimethylsulphinyl carbanion procedure.[246] The corresponding deuteriated derivatives of per-*O*-methylcellulose, in which the D-glucopyranose units were of the 2,3-di-*O*-methyl-6-*O*-[$^2H_3$-methyl], 3,6-di-*O*-methyl-2-*O*-[$^2H_3$-methyl], or 2,3,6-tri-*O*-[$^2H_3$-methyl] type, were prepared by a variety of routes. ^1H N.m.r. studies of these cellulose derivatives permitted an estimation of the degree of substitution at each position. ^1H N.m.r. studies have also been carried out on a per-*O*-methylcellulose containing 2,3,4,6-tetra-*O*-methyl- and 2,3,6-tri-*O*-methyl-D-glucose residues in a 1 : 110 ratio.[362]

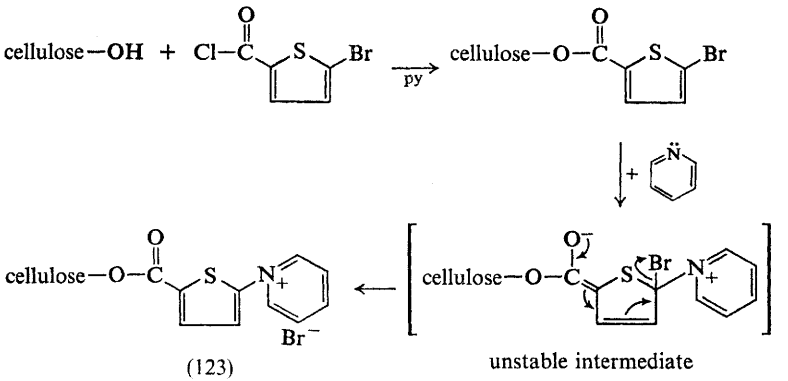
Oxidative decomposition of cellulose nitrate was effected using water at high temperatures and pressures.[1406] The product was completely soluble in water and could be used for the safe estimation of sulphate present in the original cellulose nitrate.

The 2-thenoate and 5-methyl-2-thenoate of cellulose (122) have been prepared by reaction of the polysaccharide with 2-thenoyl chloride and its 5-methyl derivative, respectively (Scheme 17).[1407] Analogous reactions have been achieved using 5-bromo-2-thenoyl chloride, 2-thiophenacryloyl chloride, and 5-bromo-2-thiophenacryloyl chloride; the bromo-substituents underwent nucleophilic displacements to give quaternary salts (123) corresponding to the bases used (*e.g.* pyridine, Scheme 18). Cross-linked heterocyclic esters of cellulose (124) have been obtained by extensions of these reactions in which 5-bromo-2-furoyl chloride and 5-bromo-2-thenoyl chloride reacted with cellulose in the presence of 1,3-bis-(4-pyridyl)-propane (Scheme 19).[1408] Use of 5-bromo-2-thiophenacryloyl chloride gave a similar product (125). The degrees of substitution and tensile properties of the foregoing derivatives were determined.

[1403] M. Ceska and U. Lundkvist, *Clin. Chim. Acta*, 1971, **32**, 339.
[1404] J. Arends, *Acta Endocrinol.*, 1971, **68**, 425.
[1405] C. R. Lowe and P. D. G. Dean, *F.E.B.S. Letters*, 1971, **14**, 313.
[1406] A. S. Dawoud and A. A. Gadalla, *Analyst*, 1970, **95**, 823.
[1407] S. Singh and J. C. Arthur, *Carbohydrate Res.*, 1971, **17**, 353.
[1408] S. Singh, J. C. Arthur, and R. H. Wade, *Carbohydrate Res.*, 1971, **18**, 449.



Scheme 17

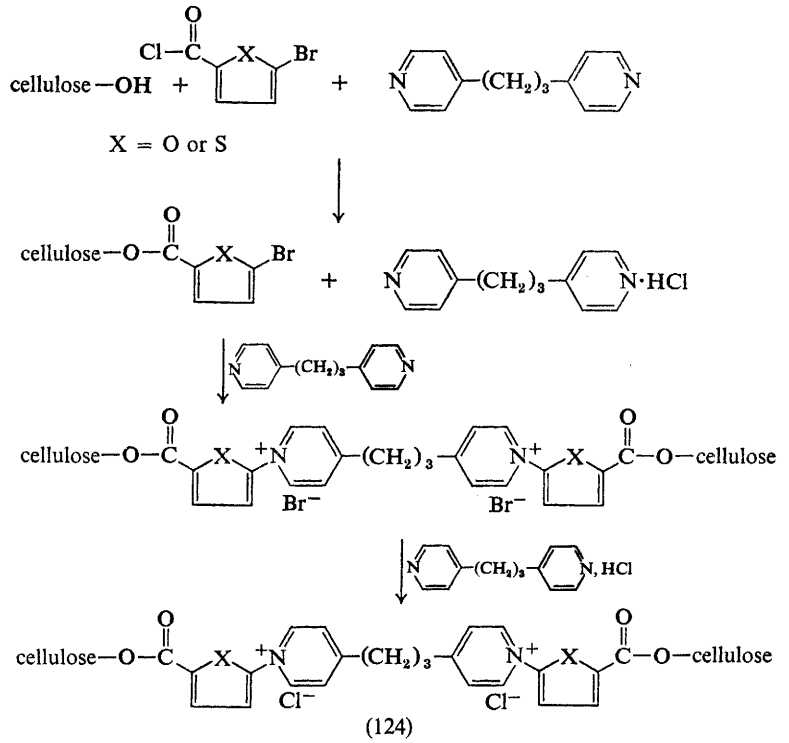


Scheme 18

The dissolution and stability of cellulose triacetate in acetone have been examined; samples with DS 2.80—2.90 could be dissolved by first cooling the mixture to 190 K before warming to 293 K.[1409] It was concluded that acetone is able to dissolve the smaller crystallites and amorphous regions in the fully acetylated material, but could not dissolve larger, well-ordered regions unless the sample contains a significant number of hydroxy-groups.

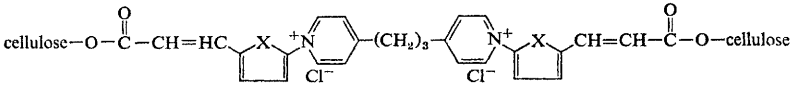
Cellulose pre-treated with alkali-metal hydroxides reacted with trichloroacetonitrile to give cellulose 2,2,2-trichloroacetimidate (126).[1410] Trichloroacetylated units also formed, but were readily hydrolysed under the mild alkaline conditions (Scheme 20). The optimal conditions for achieving high degrees of substitution were established and hydrolysis of the

[1409] J. M. G. Cowie and R. J. Ranson, *Makromol. Chem.*, 1971, **143**, 105.
[1410] T. L. Vigo and C. M. Welch, *Carbohydrate Res.*, 1971, **17**, 145.



(124)

Scheme 19



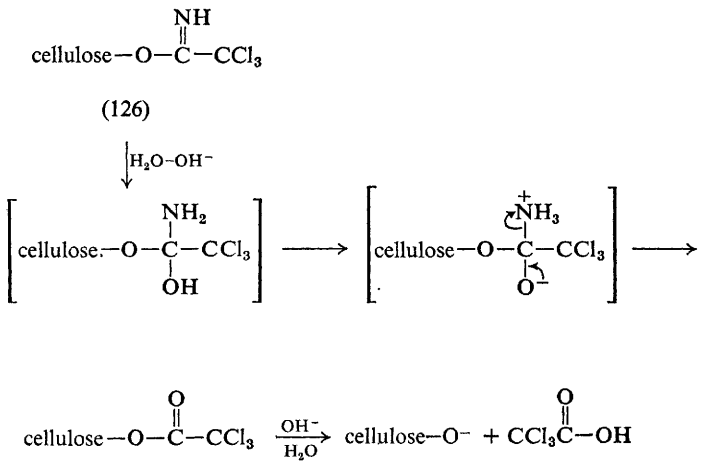
(125)

2,2,2-trichloroacetimidate groups was largely avoided. The tensile properties of the modified cellulose were described.

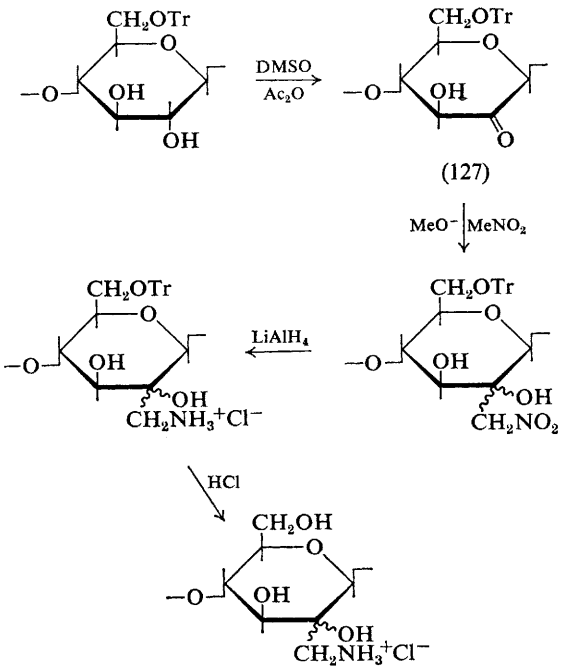
6-*O*-Tritylcellulose has been oxidized by DMSO–acetic anhydride mixtures to give a ketocellulose (127) (Scheme 21).[1411] Condensation of the 2-ketocellulose with nitromethane, followed by reduction and detritylation, gave the *C*-(aminomethyl)cellulose (128). A publication on xanthate chemistry included summaries of some of the literature on xanthates of cellulose.[1412]

[1411] Z. I. Kuznetsova, V. S. Ivanova, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, **879**.

[1412] S. R. Rao, 'Xanthate Chemistry', Dekker, New York, 1971.



Scheme 20

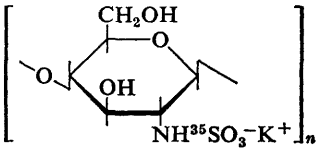


Scheme 21

Chitin.—A method has been reported for the determination of the degree of de-*N*-acetylation in chitin.[13]

A complex formed by chitin or chitosan with agaropectin has been used for the separation of agar into agarose and agaropectin.[1361]

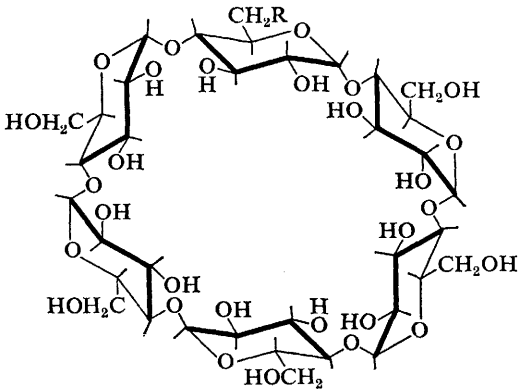
Chitosan-impregnated cellulose layers have been employed for the separation of nucleic acid components.[1386] [^{35}S]Sulphoaminochitosan (129)



(129)

has been prepared by reaction of chitosan with trimethylamine and [^{35}S]-sulphur trioxide.[794] The chemical and physicochemical properties of the product were consistent with complete derivatization of the free amino-groups. [2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)]$_3$-2-acetamido-2-deoxy-D-glucono-1,5-lactone has been prepared from tetra-*N*-acetyl-chitotetraose by oxidation with alkaline hypoiodite.[1211]

Cycloamyloses.—Crystalline derivatives of cyclohexa-amylose (α-cyclo-dextrin) (130) specifically substituted at one of the α-D-glucopyranosyl residues have been prepared.[1413] After purification, the 6-*O*-*p*-tolyl-sulphonyl derivative (131) was converted into 6-azido-6-deoxy (132), 6-bromo-6-deoxy (133), 6-chloro-6-deoxy (134), and 6-deoxy-6-iodo (135) derivatives by appropriate nucleophilic displacement of the sulphonate group. Reduction of the 6-azido-6-deoxy (132) and 6-deoxy-6-iodo (135) derivatives gave 6-amino-6-deoxy (136) and 6-deoxy (137) cyclohexa-amyloses, respectively. 6-*O*-Tritylcyclohexa-amylose (138) was also prepared.



(130) R = H
(131) R = OTs
(132) R = N_3
(133) R = Br
(134) R = Cl
(135) R = I
(136) R = NH_2
(137) R = H
(138) R = OTr

[1413] L. D. Melton and K. N. Slessor, *Carbohydrate Res.*, 1971, **18**, 29.

X-Ray diffraction analyses of the structures of a number of complexes of cyclohexa-amylose with organic molecules have been reported.[589] The stereospecific inclusion properties of cyclohexa-amylose and cyclohepta-amylose have been investigated, and these molecules have been employed for the partial resolution of isopropyl methylphosphinate and related compounds.[1414] Stereospecific reactions of isopropyl 4-nitrophenyl methylphosphonate with cyclohexa-amylose in alkaline media have been observed [1415] and chiral sulphoxides have been partially resolved by stereospecific inclusion into cyclohepta-amylose.[1416]

Dextran.—Comparison of the elution volumes of HOD and $H_2{}^{18}O$ from Sephadex G-10 (a commercially available, macroporous cross-linked dextran) permitted a physicochemical assessment of tritium exchange by the polysaccharide.[110] Ion-exchangers have been prepared by binding protamine and methylated albumin to Sephadex.[1387] The latter material proved to be suitable for the separation of nucleic acids.

Sephadex G-25 has been etherified with 1-allyloxy-2,3-epoxypropane, and the mercuriated dextran, obtained by treating the product with mercuric acetate, provided a matrix suitable for the fractionation of mononucleotides.[1417]

The binding of Blue Dextran to Sephadex gels has been examined and it was found that Blue Dextran could, in turn, bind proteinaceous materials *in situ*.[106] This property has been exploited in the separation of prothrombin and other blood coagulation factors,[954] and in the affinity chromatography of phosphofructokinase.[1418] In the latter case, Blue Dextran was used in a form immobilized by fixation in cross-linked polyacrylamide gel. The utility of Blue Dextran as a substrate in the determination of dextranase activity has been demonstrated.[1243]

Treatment of Sephadex LH-20 with boron trifluoride and epichlorohydrin gave chlorohydroxypropyl-Sephadex LH-20.[1402] Reaction of this derivative with ammonia, or primary or secondary or tertiary amines, yielded a series of lipophilic anion-exchangers; dibutylaminohydroxypropyl-Sephadex LH-20 was particularly suitable for the separation of phospholipid mixtures.

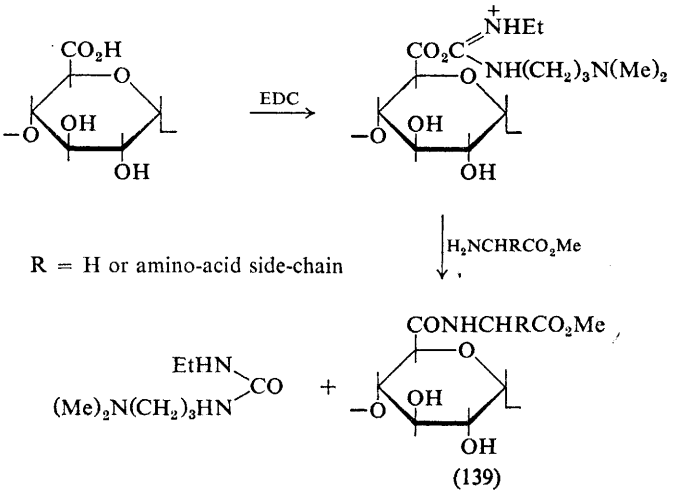
Aminoacylase [1419] and dextranase [1354] have been complexed with DEAE-Sephadex to provide water-insoluble derivatives of the enzymes. Sephadex bearing cyclic iminocarbonate groups, produced by treatment of the cross-linked polysaccharide with cyanogen bromide, has been used as a support in the production of insoluble, covalent derivatives of chymotrypsin A.[1246] In cases where the generation of cyclic groups was limited, the enzyme could

[1414] H. P. Benschop and G. R. Van den Berg, *Chem. Comm.*, 1970, 1431.
[1415] C. Van Hooidonk and C. C. Groos, *J. Roy. Netherlands Chem. Soc.*, 1970, **89**, 845.
[1416] M. Mikolajczyk, J. Drabowicz, and F. Cramer, *Chem. Comm.*, 1971, 317.
[1417] D. W. Gruenwedel and J. C. C. Fu, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2002.
[1418] G. Kopperschälger, W. Diezel, R. Freyer, S. Liebe, and E. Hofmann, *European J. Biochem.*, 1971, **22**, 40.
[1419] T. Tosa, T. Mori, and I. Chibata, *Enzymologia*, 1971, **40**, 49.

be liberated by the action of dextranase. Cyanogen bromide-activated Sephadex G-50C has been employed in the preparation of a multi-enzyme conjugate in which β-glucosidase was one of the enzymes.[1129] Intrinsic factor has also been coupled to cyanogen bromide-activated Sephadex and the product was used in the radiosorbent assay of vitamin B_{12}.[1403]

An iron–dextran complex has proved useful in the therapy of synovitis.[1420] Permethylation of dextran by using the methylsulphinyl carbanion in a one-step process has been reported.[132] Dextran and Sephadex, activated by treatment with 2-amino-4,6-dichloro-*sym*-triazine, have been shown to react with chromotrypsin to provide active, soluble and insoluble derivatives, respectively, of the enzyme.[1400] A similarly derivatized Sephadex was employed in the preparation of an active, insoluble derivative of chymotrypsin A.[1362]

Glycosaminoglycuronans.—The carboxy-groups of glycosaminoglycuronans have been converted into substituted amides of amino-acids (139) by activation with 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDC) followed by reaction of an amino-acid methyl ester with the activated carboxy-group (Scheme 22).[1376] The reaction proceeded rapidly under



(139)

Scheme 22

mild conditions and no other modification of the polysaccharide occurred. Amide derivatives of heparin were obtained using glycine methyl ester (96%), phenylalanine methyl ester (74%), and glycylphenylalaninamide (81% yield), and the reaction was extended by using [1-^{14}C]glycine methyl ester with hyaluronic acid (39%), chondroitin 4-sulphate (33%), chondroitin

[1420] A. G. Mowat, T. F. Disney, and J. H. Vaughan, *Ann. Rheumatic Diseases*, 1971, **30**, 187.

6-sulphate (36%), dermatan sulphate (28%), heparin (87%), and heparan sulphate (86% yield).

Alkaline hydrolysis of chondroitin sulphate-peptide removed the repeating disaccharide units and subsequent treatment of the residue with β-galactosidase yielded the β-xylosylpeptide.[1094]

Chondroitin sulphate reacted readily in aqueous solution with positively charged substances such as toluidine blue and bovine albumin.[739] Pulse radiolysis has been used to examine the thermodynamic parameters associated with the binding by chondroitin 4-sulphate of the cationic dyes Acridine Orange, Azure A, acriflavine, Crystal Violet, Methylene Blue, and Toluidine Blue.[793] The extent of destruction of the complexes on raising the temperature was compared with data for their stability in the presence of ethanol and urea. O.r.d. studies of glycosaminoglycuronan–methylene blue complexes suggested that chondroitin 4- and 6-sulphates possess right-handed screw helices, whereas heparin has a left-handed screw helix.[820]

Sodium chondroitin sulphate labelled with tritium has been converted into a tritiated iron–chondroitin sulphate colloid whose fate *in vivo* was examined.[797]

Chondroitin sulphate, dermatan sulphate, heparin, and heparan sulphate have been shown to react with Sepharose 4B containing cyclic imino-carbonate groups to form covalent complexes.[798] The bond is probably mediated by the amino-group of a serine or peptide residue at the reducing end of the mucopolysaccharide chains. Direct analysis of the washed and freeze-dried products revealed that only one-third of the uptake was attached firmly to the gels. The use of the gels was exemplified by application of one of them to an assay of hyaluronidase. Heparin similarly bound to Sepharose 4B was applied to the affinity chromatography of lipoprotein lipase.[799]

([^{35}S]Sulphoamino)heparin has been prepared by de-*N*-sulphation of heparin with Amberlite CG-120 resin (H^+ form) followed by treatment with trimethylamine and [^{35}S]sulphur trioxide.[794] The physical and anti-coagulant properties of the product accorded with complete restoration of the sulphamino-groups.

Starch.—Comments on derivatives of starch and their industrial uses have been reported[1421] and summaries of some of the literature on the xanthation of starch were incorporated in a review.[1412] Deoxy and deoxy-carbonyl fragments were formed as a result of γ-irradiation of starch and mechanisms for the formation of these fragments have been proposed.[1422]

The 6-aldehydo-derivative of starch has been prepared by photolysis of the 6-azido-6-deoxy-derivative (see Scheme 15).[1378] Blue starch polymers included in agar gels have proved useful as immobilized substrates for an assay of α-amylase.[1161, 1179]

The potential of *O*-(2-hydroxyethyl)starch as a plasma expander has been

[1421] G. A. Barber, *Biochem. J.*, 1971, **123**, 3P.

[1422] N. K. Kochetkov, L. I. Kudryashov, S. M. Jarovaya, and S. V. Voznesenskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1970, 201.

examined and, for a constant degree of substitution, it was found that higher molecular weight materials persisted longer in the blood stream.[1423] *O*-(2-Hydroxyethyl)starch (molecular weight 2.16×10^5, DS 0.54) has been hydrolysed with various concentrations of acid and the physicochemical and biological properties of the products tested. ^{1}H N.m.r. spectroscopy has been evaluated as a rapid means of determining the hydroxypropyl content of modified starch.[101] The terminal methyl group of the hydroxypropyl side-chain appeared as a distinct doublet, which was utilized as a basis for the quantitation of the substituent in commercially modified starches.

Miscellaneous Polysaccharides.—Although the sodium salt of a sulphated galactan from *Laingia pacifica* was unaffected by heating with DMSO, heating of the pyridinium salt with the same solvent or with DMF gave a desulphated polymer (75% yield).[1424] Gel filtration showed that the chain-length of the polysaccharide had decreased during the reaction. However, improved yields (up to 90%) and decreased degradation were achieved when 2% pyridine was included in the reaction mixture; the product then contained only 1.4% sulphate. Corresponding values for the yield and sulphate content of the product obtained by desulphation with methanolic hydrochloric acid according to the standard procedure were 40% and 1.2%, respectively.

Barley β-glucan stained with reactive dyestuffs was used as a substrate for measuring β-glucanase activity.[1247] Permethylated mannan and pullulan have been obtained, in high yield, from the parent polysaccharides by a one-step process.[132]

Modification of Glycoproteins and Uses of Modified Glycoproteins

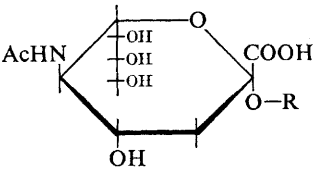
The uses of modified glycoproteins for the isolation and purification of macromolecules by affinity chromatography have been reviewed.[693] A general method for the radioactive labelling of glycoproteins containing terminal sialic acid residues has been described.[699] Quantitative conversion of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (*N*-acetylneuraminic acid) units (140) into labelled 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid units (141) was achieved by short-term periodate oxidation followed by reduction with sodium borotritide (Scheme 23). The identity of the labelled units was confirmed, and these units were found to be the sole site of tritium incorporation into carbohydrate chains of the macromolecule. Orosomucoid and other glycoproteins were modified by this method.

The fully acetylated version of a glycoprotein elaborated by *Neurospora*

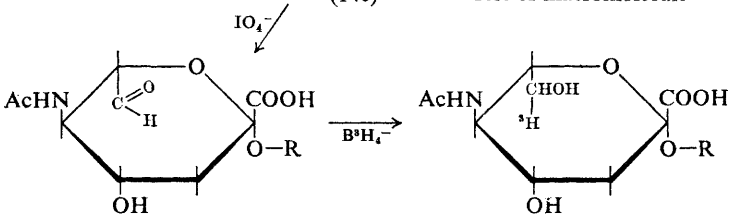
[1423] T. Tamada, K. Okada, R. Ishida, K. Kamishita, and T. Irikura, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 286.

[1424] A. I. Usov, K. S. Adamyants, L. I. Miroshnikova, A. A. Shaposhnikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **18**, 336.

crassa, which contains unacetylated 2-amino-2-deoxygalactose units in its native state, has been prepared.[703] Concanavalin A has been derivatized by maleylation [709] and by coupling to ferritin with glutaraldehyde.[716]



(140) R = rest of macromolecule



(141)

Scheme 23

Human-erythrocyte, blood-group MN-active sialoglycopeptide formed a complex with positively charged substances such as bovine albumin and Toluidine Blue.[739] Acyl, succinyl, and other esterified derivatives of blood-group MN and Pr_1/Pr_2-active erythrocyte glycoproteins have been prepared.[741] Modification of blood-group substances by γ-irradiation has been investigated and mechanisms were proposed for the formation of the products.[1422]

Modification of the structure of collagen by chromium-formalin tanning provided a matrix suitable for chromatography; the retardation of certain proteins during chromatography was studied.[1425] Collagen has also been modified by cross-linking with glutaraldehyde,[751] and tropocollagen has been derivatized by acetoacetylation and guanidination.[750]

Terminal *N*-acetylneuraminic acid units of follicle-stimulating hormone have been modified to their eight- and seven-carbon analogues by sequential periodate oxidation and borohydride reduction.[895] The 5-acetamido-3,5-dideoxy-D-*galacto*-2-octulosonic acid and 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid units so formed were susceptible to hydrolysis by neuraminidase. Reduced, carboxymethylated derivatives of thyroid-stimulating hormone [901] and human chorionic gonadotrophin [910] have been prepared. Desialized thyroglobulin has been produced by enzymolysis of the native molecule.[911]

Carboxyacyl derivatives of β-casein [916] and reduced, alkylated, and reduced aminoethylated derivatives of lactoferrin [920] have been prepared.

[1425] C. Dennison, *J. Chromatog.*, 1970, **53**, 381.

Human serum albumin, immunoglobulins IgG and IgM, Bence-Jones glycoprotein, and ovalbumin mechanically trapped in the lattices of highly cross-linked, macroporous polyacrylamide gels provided a series of specific and stable immunoadsorbents.[935] Kieselguhr treated with methylated serum albumin has been used for the fractionation of nucleic acids,[1426] and methylated albumin bound to cellulose or Sephadex has been used as an ion-exchanger for a similar purpose.[1387] The formation of sulphenyl iodide and sulphenyl periodide derivatives of bovine serum albumin has been studied.[1427] Antigenic substances have been formed by the coupling of periodate-oxidized inosine, adenosine, and guanosine to bovine serum albumin; the albumin itself coupled to Sepharose was used as an immunoadsorbent.[1368] Bovine serum albumin has also been modified by coupling with gastrin and related peptides using 2,4-dichloro-6-methoxy-1,3,5-triazine as a bridge.[1428] A half-cystinyl derivative of bovine plasma albumin has been prepared and the compound was further modified by mono-[^{14}C]acetylation.[936] Amino-acid derivatives of bovine serum albumin have been produced enzymically.[1429] The induction of spectral changes in bovine serum albumin by various dyes was attributed to their binding in hydrophobic environments of low polarity on the albumin molecule.[942] The binding of ^{82}Br- and ^{14}C-labelled cytotoxic dibromohexitols, *viz.* 1,6-dibromo-1,6-dideoxy-D-mannitol and 1,6-dibromo-1,6-dideoxygalactitol, by bovine serum albumin is due to complex formation, and the necessity of the CH_2Br group for this reaction was demonstrated.[1430]

Modified fetuins have been obtained by treatment of the glycoprotein with neuraminidase, galactosidase, and β-acetamidodeoxyglucosidase;[1431] the desialysed, degalactosylated molecule was obtained by mild acidic hydrolysis followed by periodate oxidation.[883] Other derivatives of fetuin have been formed either by mild acidic hydrolysis (to remove residues of *N*-acetylneuraminic acid) followed by treatment with β-galactosidase, oxidation with periodate, and hydrolysis, or by treatment with β-acetamidoglucosidase.[1094] *N*-Acetylneuraminic acid-free porcine submaxillary mucin glycoprotein and ovalbumin free of 2-acetamido-2-deoxy-D-glucose have been formed by using the appropriate glycosidases.

Reduced and reduced-carboxymethylated derivatives of hemopexin [953] and asialo prothrombin [958] have been prepared. Prothrombin was found to form a complex with Dextran Blue.[954]

Alkylated and reduced-alkylated derivatives of human secretory immunoglobulin IgA and free secretory piece,[980] and a supposed immunoglobulin IgM from the long-nose gar (*Lepisosteus osseus*),[1011] have been formed.

[1426] J. P. Garel, G. Nullans, and P. Mandel, *J. Chromatog.*, 1971, **56**, 154.
[1427] L. Jirousek and E. T. Pritchard, *Biochim. Biophys. Acta*, 1971, **229**, 618.
[1428] K. L. Agarwal, S. Grudzinski, G. W. Kenner, N. H. Rogers, R. C. Sheppard, and J. E. McGuigan, *Experientia*, 1971, **27**, 514.
[1429] M. J. Leibowitz and R. L. Soffer, *J. Biol. Chem.*, 1971, **246**, 4431.
[1430] E. Institoris and L. Holczinger, *Biochem. Pharmacol.*, 1971, **20**, 1183.
[1431] A. P. Baker and J. R. Munro, *J. Biol. Chem.*, 1971, **246**, 4358.

Water-insoluble derivatives of antibodies to human myeloma immunoglobulin IgE and to human chorionic gonadotrophin, suitable for radioimmunoassay, have been produced by coupling the antibodies to cellulose *trans*-2,3-cyclic carbonate [990] and cyanogen bromide-activated cellulose,[1404] respectively. Antibodies to human chorionic gonadotrophin have also been cross-linked with ethyl chloroformate and glutaraldehyde for the same purpose.[1432] Insolubilized antibodies have also been used in the isolation of fragments from specifically modified proteins.[1369]

Tamm–Horsfall urinary glycoprotein has been alkylated and reduced.[1029] Ovalbumin has been modified enzymically by galactosylation; it was shown that the D-galactose residues introduced are attached neither to serine nor to threonine residues by *O*-glycosidic linkages.[1038]

Modification of Enzymes and Uses of Modified Enzymes

The principles of binding enzymes to artificial matrices to give active, insoluble derivatives have been reviewed [1061] and descriptions of certain insolubilized enzymes, including carbohydrases, have been given.[1062]

The reaction of β-fructofuranosidase with iodine has been investigated [1087] and the enzyme has been insolubilized by fixation to mineral supports, such as bentonite and glass, which had been activated with sulphuryl or thionyl chloride.[1091] The activity of the insoluble bentonite derivative was stable and was maintained in the presence of substrate, in contrast to the activity of the free enzyme.

Stability tests over three years on β-galactosidase attached to porous sheets of DEAE-cellulose showed that 81% of the initial activity had been lost at the end of this period.[1117] β-Galactosidase and chymotrypsin A have been immobilized by attachment to porous aminoalkylsilyl glass using glutaraldehyde as a bridge; 50% and 36%, respectively, of the specific activities of the free enzymes were retained after coupling.[1116]

An active, insoluble derivative of β-glucosidase has been formed by covalent attachment of the enzyme to cellulose *trans*-2,3-carbonate.[1128] The reaction was considered to involve nucleophilic attack of ε-amino-groups in the enzyme on the five-membered carbonate rings; the optimum pH for the reaction was pH 7.8. The pH–activity profiles of the bound and free enzymes were similar, but the bound form was more stable to heat. β-Glucosidase and glucoamylase were similarly coupled to a poly(allyl carbonate) containing eight-membered carbonate rings.[1130] The efficiency of the coupled reactions catalysed by β-glucosidase, hexokinase, and glucose 6-phosphate dehydrogenase simultaneously bound to cyanogen bromide-activated Sephadex was higher prior to reaching a steady state than that of the reaction catalysed by the corresponding soluble system.[1129] β-Glucosidase, immobilized by inclusion in polyacrylamide gel, has been used as the coating in an enzyme electrode for amygdalin.[1131, 1132]

[1432] S. Isojima, O. Naka, K. Koyama, and H. Adachi, *J. Clin. Endocrinol. Metab.*, 1970, **31**, 693.

Reaction of neuraminidase with potassium cyanate afforded the carbamyl derivative.[1149]

The potential of the polyaldehyde arising from cross-linked polyacryloylaminoacetaldehyde dimethylacetal for the preparation of insolubilized enzymes has been demonstrated by the formation of such derivatives from α-amylase, dextranase, papain, and trypsin.[1188] The introduction of succinyl groups into *Bacillus subtilis* α-amylase enhanced the specific activity of the enzyme.[1182] The increased affinity of the enzyme for amylose was ascribed to expansion of the molecular structure of the enzyme on derivatization. The activity of Taka amylase A (an α-amylase from *Aspergillus oryzae*) was increased to 180% when one mole of mercaptosuccinyl group was introduced per mole of enzyme.[1176] More extensive derivatization gave lower increases in activity, whereas succinylation of the free enzyme reduced its activity.

Water-insoluble glucoamylase, prepared by binding the enzyme to carboxymethylcellulose azide, retained 90% of the specific activity of the free enzyme.[1193] However, the stability of the enzyme derivative to heat and to a pH of 2.0 was less than that of the native enzyme, and the derivative was not effective for the hydrolysis of substrates of molecular weight greater than 8×10^3. Partially purified glucoamylase, immobilized by absorption on to DEAE-cellulose, was used for the continuous conversion of starch-type substrates into D-glucose.[1194] Transglycosylation products were not present to any greater degree than in soluble digests of starch.

Lysozyme acetylated with *N*-acetylimidazole was 1.2 times as active as the unmodified enzyme in tests with *Micrococcus lysodiekticus* as substrate.[1197] Nitration caused polymerization of the enzyme and reduced its activity by 75%. Lysozyme has also been modified by treatment with *N*-bromosuccinimide,[1223] by dansylation,[1220] and by the sequence nitration, reduction, and treatment with 2-nitrophenylsulphenyl chloride.[1221] Lysozyme and α-lactalbumin have also been modified by reaction with tetranitromethane and trinitrobenzenesulphonic acid,[1225] and by carboxymethylation and reduction.[1227] In the latter case, the products were subsequently attached to Sepharose 4B for use as immunoadsorbents.

Glucose oxidase was insolubilized by attachment to either alumina or hydroxyapatite or porous glass, whereas trypsin and papain were insolubilized by attachment to silica *via* sulphonamide linkages.[1282] Trypsin, ficin, and papain were also attached to porous glass by means of azo and sulphonamide linkages; the properties of all the enzyme derivatives, as well as those of commercially available derivatives, were studied. Enzymes coupled covalently to inorganic carriers were found to have greater stabilities than those attached to organic carriers when stored for several weeks at 4 or 23 °C in the dry or wet state. Enzymes coupled covalently to the inorganic carriers by sulphonamide linkages were not as stable during storage as enzymes coupled by azo-linkages.

The tetrathionite-blocked derivative of bromelain has been prepared.[1284] Active, water-insoluble derivatives of chymotrypsin A have been prepared and their properties compared with those of the natural enzyme.[1246, 1362, 1394, 1395] A soluble 'immobilized' form of chymotrypsin was afforded by covalent attachment of the enzyme to a dextran.[1400]

Pepsin was immobilized by attachment to porous glass.[1433] Pronase has been coupled to an arylamino-derivative of glass [1434] and to a diazotized copolymer of leucine and 4-aminophenylalanine.[1435] The preparation of alkyl derivatives of thrombin has been reported.[1286] Insoluble derivatives of trypsin have been formed by attachment to nylon tubes [1436] and to aminoethylcellulose with the aid of glutaraldehyde.[1392]

Bovine pancreatic ribonuclease was reacted with diethylpyrocarbonate to prepare polymeric markers for polyacrylamide gel electrophoresis.[1437] Water-insoluble derivatives of aminoacylase [1419] have been produced.

Ceruloplasmin labelled with *N*-acetyl-[1-^{14}C]neuraminic acid has been prepared enzymically from desialysed ceruloplasmin and has been used in a study of the fate of the enzyme *in vivo*.[1291] Terminal *N*-acetylneuraminic acid units of ceruloplasmin have also been labelled by periodate oxidation, to give the seven-carbon derivative, followed by reduction with sodium borotritide.[699] ^{64}Cu-Labelled, desialylated ceruloplasmin has been prepared.[1290]

Water-insoluble derivatives of cholinesterase [1399] and dextransucrase [1354] have been prepared, and yeast phosphofructokinase has been derivatized by binding to Blue Dextran.[1418]

Modification of Glycolipids and Uses of Modified Glycolipids

Succinyl and dinitrophenyl derivatives of the methyl ester of a cell-wall glycolipid (somatic antigen) from *Salmonella minnesota* have been prepared and used in an investigation of the endotoxicity of the glycolipid.[1351]

Mono- and di-galactolipids isolated from *Sinapis alba* and *Spinacia oleracea* plants have been modified to give a series of compounds in which identical or different acyl groups were attached to C-1 and C-2 of the glycerol residues.[1438]

[1433] W. F. Line, A. Kwong, and H. H. Weetall, *Biochim. Biophys. Acta*, 1971, **242**, 194.
[1434] G. P. Royer and G. M. Green, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 426.
[1435] P. Cresswell and A. R. Sanderson, *Biochem. J.*, 1970, **119**, 447.
[1436] W. E. Hornby and H. Filippusson, *Biochim. Biophys. Acta*, 1970, **220**, 343.
[1437] B. Wolf, P. M. Pausarot, J. A. Lesnaw, and M. E. Reichmann, *Biochim. Biophys. Acta*, 1970, **200**, 180.
[1438] E. Heinz, *Biochim. Biophys. Acta*, 1971, **231**, 537.

Author Index