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VOLUME 9

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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 9

A Review of the Literature Published during 1975

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Preface

This Report, the ninth in the series, covers the literature available to us between mid-January 1975 and mid-January 1976. Once again every effort has been made to limit the size of the Report to roughly 500 pages, in the hope that this will keep its price in check: we have resisted the temptation to cover the carbohydrate literature selectively, feeling that the interests of our readers are best served by the comprehensive coverage achieved in past Reports.

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

This is the last Report for which we will have the benefit of Dr. N. A. Hughes's expert reporting: we are indebted to him for many notable contributions over the past three years. Dr. B. J. Catley has joined our team of Reporters for Part II.

We thank Dr. L. C. N. Tucker for reading and commenting on the whole of Part I, and Mrs. Alice Duncan, Miss Moira Endersby, and Miss Lynda Esplin for typing considerable proportions of this Report. The diagrams on pages 378 and 386 are reproduced with the kind permission of The American Chemical Society and that on page 430 with the kind permission of Elsevier Scientific Publishing Company.

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

August 1976 J. S. B.

Contents

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

1 Introduction 3

2 Free Sugars 5
Isolation and Synthesis 5
Physical Measurements 6
Reactions 8

3 Glycosides 10
O-Glycosides 10
Hydrolysis and Related Reactions 19
Other Reactions and Features of Glycosides 19
Natural Products 20
S-Glycosides 20
C-Glycosides 22

4 Ethers and Anhydro-sugars 28
Ethers 28
Methyl Ethers 28
Other Alkyl and Aryl Ethers 29
Silyl Ethers 30
Intramolecular Ethers (Anhydro-sugars) 31
Oxirans 31
Other Anhydrides 32

5 Acetals 35
Synthesis, Properties, and Reactions 35
Derivatives of Acyclic Sugars 38
Acetals Derived from Aldehydo-sugars 39

6 Esters 40
Carboxylic Esters 40
Acyloxonium Ions and Orthoesters 43
Phosphates 46
Sulphonates 50
Other Esters 51

7 Halogenated Sugars 54
Glycosyl Halides 54
Other Halogenated Derivatives 55

8 Amino-sugars 60
Natural Products 60
Synthesis 60
Reactions 65
Di- and Poly-amino-sugars 66

9 Hydrazones, Osazones, and Related Compounds 69

10 Miscellaneous Nitrogen-containing Compounds 71
Glycosylamines 71
Azides 72
Nitro-sugars 73
Heterocyclic Derivatives 74
Oximes 76

11 Thio- and Seleno-sugars 77

12 Derivatives with Nitrogen, Sulphur, or Phosphorus in the Sugar Ring 81
Nitrogen Derivatives 81
Sulphur Derivatives 81
Phosphorus Derivatives 85

13 Deoxy-sugars 86

14 Unsaturated Derivatives 91
Glycals 91
Other Unsaturated Derivatives 93

15 Branched-chain Sugars 99
Compounds with an $R^1—C—OR^2$ Branch 99
Compounds with an $R^1—C—R^2$ Branch 103
Compounds with an R—C—N Branch 106

16 Aldehydo-sugars, Aldosuloses, Dialdoses, and Diuloses 107

17 Sugar Acids and Lactones 111
Aldonic Acids 111
Aldaric Acids 114
Saccharinic Acids 115
Ulosonic Acids 115
Uronic Acids 117
Ascorbic Acid 118

18 Inorganic Derivatives 120
Carbon-bonded Compounds 120
Oxygen-bonded Compounds 122

19 Cyclitols 124

20 Antibiotics 131

21 Nucleosides 139
Synthesis 139
Double-headed Nucleosides and Homo- and Iso-nucleosides 146
Nucleosides with Branched-chain Components 148
C-Nucleosides 148
Unsaturated Nucleosides 150
Cyclonucleosides 152
Halogeno-sugar Nucleosides 156
Keto-nucleosides and Nucleoside Carboxylic Acids 157
Amino-sugar Nucleosides 159
Phosphate and Related Esters of Nucleosides 160
Other Derivatives 163
Reactions 165
Physical Measurements 166

22 Oxidation and Reduction 168
Oxidation 168
Reduction 170

23 N.M.R. Spectroscopy and Conformational Features of Carbohydrates 173
Acyclic Systems 174
Pyranoid Systems 174
Furanoid Systems 178
Di-, Oligo-, and Poly-saccharides 180
^{13}C N.M.R. Spectroscopy 180
Lanthanide Shift Reagents 183
Spin–Lattice Relaxation Times 183

24 Other Physical Methods 185
I.R. Spectroscopy 185
U.V. Spectroscopy 185
Mass Spectrometry 185
X-Ray Crystallography 187
Free Sugars and Simple Derivatives Thereof 187
Glycosides 187
Amino-sugar Derivatives 187
Acid Derivatives 188
Anhydro-sugar Derivatives 188
Nucleosides, Nucleotides, and their Derivatives 188
Antibiotics and Components Thereof 189
Other Derivatives 189
E.S.R. Spectroscopy 189

25 Polarimetry 190

26 Separatory and Analytical Methods 192
Chromatographic Methods 192
Gas–Liquid Chromatography 192

Column and Ion-exchange Chromatography 193
Paper Chromatography 193
Thin-layer Chromatography 194
High-pressure Liquid Chromatography 194
Other Analytical Methods 194

27 Alditols 197

28 The Synthesis of Optically Active Non-carbohydrate Compounds 200

Part II Macromolecules

1 Introduction 207

2 General Methods 208
By R. J. Sturgeon
Analysis 208
Structural Methods 215

3 Plant and Algal Polysaccharides 218
By R. J. Sturgeon
Starch 218
Cellulose 221
Gums and Mucilages 224
Pectins 226
Hemicelluloses 227
Algal Polysaccharides 233
Miscellaneous Polysaccharides 236

4 Microbial Polysaccharides 237
By R. J. Sturgeon
Teichoic Acids 237
Peptidoglycans 242
Lipopolysaccharides 246
Capsular Polysaccharides 253
Extracellular and Intracellular Polysaccharides 255
Fungal Polysaccharides 263
Glucans 263
Mannans 264
Chitin 265
Miscellaneous Polysaccharides 267

5 Glycoproteins, Glycopeptides, and Animal Polysaccharides 272
By B. J. Catley
Introduction 272
Microbial Glycoproteins 272

Plant Glycoproteins 276
Lectins 276
Blood-group Substances 281
Collagens 282
Glycogen 283
Glycosaminoglycans 289
Analytical Methods 289
Occurrence, Isolation, and Structure 290
Biosynthesis 294
Degradation 296
Functions 297
Pathology 298
Mammalian Cell and Tissue Glycoproteins 300
Cell-surface Glycoproteins 303
Hormonal Glycoproteins 309
Milk Glycoproteins 311
Serum Glycoproteins 311
Immunoglobulins 314
Erythrocyte Glycoproteins 316
Salivary and Mucous Glycoproteins 318
Urinary Glycoproteins 319
Miscellaneous Glycoproteins and Chitin 320
Analysis of Glycoproteins 322
Biosynthesis of Glycoproteins 323

6 Enzymes 327
By J. F. Kennedy
Introduction 327
Acetamidodeoxygalactosidases, Acetamidodeoxyglucosidases, and Acetamidodeoxyhexosidases 330
α-L-Arabinofuranosidases 336
β-D-Fructofuranosidases 336
α-L-, α-(1,2)-L-, and β-D-Fucosidases 337
D-Galactosidases, β-D-Galactosyl- and β-D-Galactosyl-D-glucosyl-ceramidases, and β-D-Galactosylsphingosidases 338
D-Glucosidases, β-D-Glucocerebrosidases, and β-1,6-D-Glucosidases 344
β-D-Glucuronidases 347
β-D-Glycylamidodeoxyglucosidases 350
α-D-Iduronidases 350
D-Mannosidases 350
Neuraminidases 351
β-D-Phosphogalactosidases 353
β-D-Phosphoglucosidases 353
Sucrose α-D-Glucohydrolases 354
β-D-Xylosidases 354
exo-β-D-Acetamidodeoxyglucanases and *exo*-β-D-Acetamido-deoxyglycanases 354

α-Amylases 355
β-Amylases 360
Arabinanases 361
Cellulases 362
Chitinases (*endo*-β-D-Acetamidodeoxyglucanases) 363
Chitosanases 364
Chondroitin Sulphate Lyases ABC 365
Chondroitin Sulphate Lyases AC 365
Chondroitin 4- and 6-Sulphate Lyases 365
Dextranases 365
endo-Galactanases 367
exo-β-D-Galactanases 367
endo-α-1,3-D-Glucanases 368
endo-β-1,3-D-Glucanases 368
endo-β-1,6-D-Glucanases 369
exo-α-1,6-D-Glucanases 369
exo-β-1,3-D-Glucanases 369
exo-β-1,4-D-Glucanases 370
D-Glucanases (Miscellaneous) 370
Glucoamylases 371
Glycanases 372
Hyaluronidases 373
Isoamylases 374
Keratan Sulphate Hydrolases 374
Laminarinases 374
Lichenases 374
Lysozymes 374
D-Mannanases (Miscellaneous) 381
Oligo-1,6-D-Glucosidases 381
Polygalacturonases 381
exo-Polygalacturonases 382
exo-Poly-α-D-Galacturonosidases 382
Pectate Hydrolases (Miscellaneous) 382
Pectate Lyases 383
Pectin Lyases 383
Pullulanases 383
αα-Trehalases 383
Xylanases (Miscellaneous) 384
Carbohydrate Isomerases 385
  D-Glucose Isomerases 385
  D-Xylose Isomerases 385
Carbohydrate Oxidases 385
  D-Galactose Oxidases 385
  D-Glucose Oxidases 385
Carbohydrate Transferases 386
  Cyclodextrin D-Glucanotransferase 386
  Dextransucrases 386

Glycopeptide Linkage Hydrolases 387
N-Acetylmuramyl-L-alanine Amidases 387
4-L-Aspartyl-β-D-glucosylamine Aminohydrolases 387
Miscellaneous 387
Proteinases 387
Bacterial Proteinases 387
Ribonucleases and Deoxyribonucleases 388
Sulphatases 388
2-Acetamido-2-deoxy-D-galactose 4-Sulphate Sulphatases 388
2-Acetamido-2-deoxy-D-glucose 6-Sulphate Sulphatases 388
Arylsulphatases 388
L-Ascorbic Acid 2-Sulphate Sulphatases 390
Cerebroside Sulphatases 390
Chondroitin 4-Sulphate Sulphatases 390
Chondroitin 6-Sulphate Sulphatases 390
Glycolipid Sulphate Sulphatases 391
Heparin Sulphatases 391
L-Iduronic Acid 2-Sulphate Sulphatases 391
Lactose Sulphate Sulphatases 391
Sulphatases (Miscellaneous) 392
Miscellaneous Enzymes 392
Glyco-α-lactalbumins 392
α-Lactalbumins 392
Lactose Synthases 392
Pectinesterases 392
Polysaccharide Deacetylases 392
Sulphamidases 392
D-Thioglucosidases 393
Index of Enzymes Referred to in Chapter 6 393

7 Glycolipids and Gangliosides 397
By R. J. Sturgeon
Introduction 397
Animal Glycolipids and Gangliosides 399
Plant and Algal Glycolipids 408
Microbial Glycolipids 409

8 Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids 412
By J. F. Kennedy
Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Enzymes, and Glycolipids 412
Polysaccharides 412
Oligosaccharides 412
Glycoproteins 413
Enzymes 414
Glycolipids 414

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides 414
Agaroses 414
Amylopectins 430
Amyloses 430
Celluloses 431
Chitosans 437
Chitins 437
Cycloamyloses 437
Dextran 438
Glycogen 440
Glycosaminoglycans 440
Miscellaneous Polysaccharides and Oligosaccharides 440
Starch 442
Xylans 444
Modification of Glycoproteins and Uses of Modified Glycoproteins 444
Albumins 444
Antibodies 444
Fetuins 444
Mucins 444
Phytohaemagglutinins 444
Orosomucoids 445
Ovalbumins 445
Immobile Glycoproteins 445
Modification of Enzymes and Uses of Modified Enzymes 449
β-D-Galactosidases 449
β-D-Glucosidases 449
α-Amylases 449
β-Amylases 450
Dextranases 450
D-Glucose Oxidase 450
Lysozymes 450
Immobilized Derivatives of Enzymes 451
Modification of Gangliosides and Glycolipids, and Uses of Modified Gangliosides and Glycolipids 461
Gangliosides 461
Glycolipids 462

Author Index 463

Abbreviations

The following abbreviations have been used;

ADP	adenosine diphosphate
ATP	adenosine triphosphate
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DBU	1,5-diazobicyclo[5,4,0]undec-5-ene
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dpm	dipivaloylmethanato
e.s.r.	electron spin resonance
fod	2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphortriamide
i.r.	infrared
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
RNA	ribonucleic acid
THF	tetrahydrofuran
Thp	tetrahydropyranyl
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

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

By

J. S. Brimacombe
R. J. Ferrier
N. A. Hughes
N. R. Williams

1
Introduction

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

Calcium and strontium salts have been used to alter the anomeric equilibria in the Fischer glycosidation of several aldoses, so allowing glycosides not normally obtained (*e.g.* methyl α-D-ribofuranoside, β-D-lyxo-furanoside and -pyranoside, α-D-talopyranoside, α- and β-D-talofuranosides) to be isolated (Chapter 3). Conditions necessary for successful halide-catalysed glycosidations have been defined by Lemieux and applied to the synthesis of several trisaccharides of biological importance (Chapter 3). A good deal of interest has also been shown in the synthesis of *C*-glycosides, much of it centred on the elaboration of *C*-glycosyl nucleosides of biological interest (see Chapters 3 and 21).

A point made again in Chapter 6 is that assessment of the relative reactivities of hydroxy-groups in sugars based on product distributions can lead to erroneous conclusions, since the reactivity of a hydroxy-group can change during the course of a multistage reaction.

The customary interest has been shown in unusual naturally occurring sugars, and syntheses of ristosamine (Chapter 8), axenose, and pillarose (Chapter 15) have been reported. Following the synthesis of derivatives of dihydrostreptomycin in 1974, Umezawa's group has now reported the total synthesis of streptomycin (Chapter 20). A combination of chemical and spectroscopic methods has been used to good effect in unravelling the highly intricate structures of such carbohydrate-containing antibiotics as everninomycin D (a truly monumental achievement!), the destomycins, hikizimycin, and several gentamycins and aldgamycins (Chapter 20). The bourgeoning activity in nucleoside chemistry is demonstrated by the fact that Chapter 21 contains roughly one-seventh of the total references appearing in Part I of this Report.

Although references to the use of carbohydrates in the total synthesis of optically active non-carbohydrate compounds have been included in previous Reports, they were usually scattered throughout several chapters. Because of the growing interest in using carbohydrates in this way, such references have been collected together in a new chapter at the end of Part I.

Some aspects of the chemistry of unsaturated sugars have been reviewed,[1] and several books [2, 3] of general interest have appeared over the past year.

[1] B. Fraser-Reid, *Accounts Chem. Res.*, 1975, **8**, 192.

The December issue [4] of *Carbohydrate Research* was dedicated to the memory of Dr. Hewitt G. Fletcher, jun. (1917—1973), and the March issue [5] was dedicated to Professor Michael Heidelberger to mark his distinguished contributions to carbohydrate chemistry.

[2] *Adv. Carbohydrate Chem. Biochem.*, Vol. 31, ed. R. S. Tipson and D. Horton, Academic Press, New York and London, 1975; *Synthetic Procedures in Nucleic Acid Chemistry*, Vol. 2, 'Physical and Physicochemical Aids in Characterization and in Determination of Structure', ed. W. W. Zorbach and R. S. Tipson, Wiley, New York, 1973.

[3] 'Industrial Gums, Polysaccharides, and their Derivatives', 2nd edn., ed. R. L. Whistler and J. N. BeMiller, Academic Press, New York and London, 1973; J. C. Colbert, 'Sugar Esters: Preparation and Applications', Noyes Data Corporation, Park Ridge (New Jersey) and London, 1974; Guthrie and Honeyman, 'Introduction to Carbohydrate Chemistry', 4th edn., Clarendon Press, Oxford, 1974.

[4] *Carbohydrate Res.*, 1975, vol. 45.

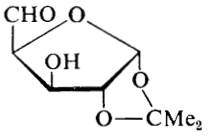
[5] *Carbohydrate Res.*, 1975, vol. 40.

2
Free Sugars

Browning reactions of sugars have been reviewed,[6] and the results of tests of Shallenberger's hypothesis relating the structure of sugars to their sweetness are reported in Chapter 4.

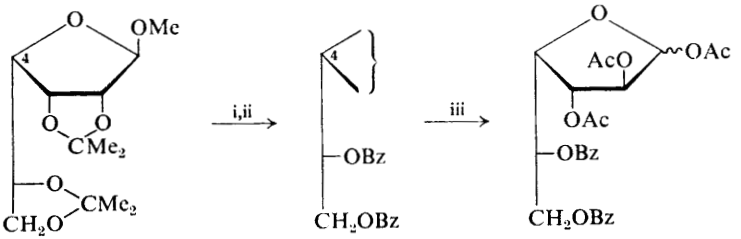
Isolation and Synthesis

D-[5-^{3}H]Xylose has been synthesized from the pentodialdose (1) by reduction with sodium borotritide, followed by removal of the protecting group.[7] D-Allose



(1)

and D-altrose were obtained in comparable amounts (45 : 55 parts) from D-ribose by the nitromethane route, the overall yield being 42%.[8] Molybdate-catalysed epimerization raised the proportion of D-allose to 60%, in contrast to other epimerizations where epimers with O-2 and O-3 *trans*-related are preferred. Epimerization of a D-*gulo*-compound at C-2 was used to prepare 1,2,3-tri-*O*-acetyl-5,6-di-*O*-benzoyl-D-idofuranose (2) (Scheme 1); the usefulness of this



(2)

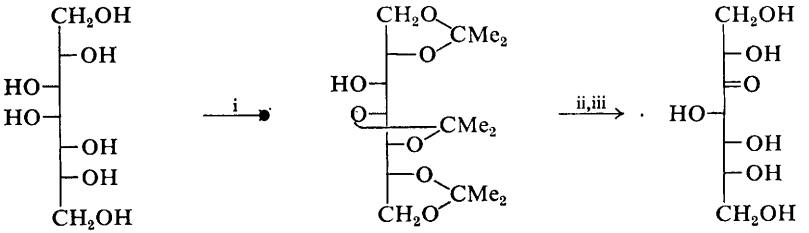
Reagents: i, H_3O^+; ii, BzCl–py; iii, Ac_2O–AcOH–H_2SO_4

Scheme 1

[6] H. G. Peer, *Misc. Pap.-Landbouwhogesch. Wageningen*, 1971, 105 (*Chem. Abs.*, 1975, **82**, 4483a).
[7] A. F. Bochkov and A. V. Rodionov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 667 (*Chem. Abs.*, 1975, **83**, 43 625p).
[8] V. Bilik, *Chem. Zvesti*, 1975, **29**, 114.

approach lies in the formation of a D-idofuranose derivative, rather than as a synthetic route to the free sugar.[9] L-Sorbose found in apple-cider vinegar appears to be formed by oxidation of D-glucitol, since it is not originally present in the cider.[10]

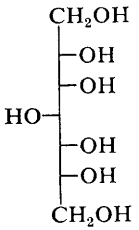
Application of the nitromethane route to L-mannose has furnished a mixture of L-*glycero*-L-*galacto*-heptose (43%) and L-*glycero*-L-*talo*-heptose (12%), which could be separated by ion-exchange chromatography.[11] A synthesis of D-*gluco*-hept-3-ulose (4) from perseitol (3) is shown in Scheme 2; the racemic form of (4)



(3) (4)

Reagents: i, Me_2CO–H^+; ii, P_2O_5–DMSO; iii, H_3O^+

Scheme 2



(5)

was similarly obtained from the *meso*-heptitol (5).[12] The following octuloses have been shown to accumulate when the leaves of red clover (*Trifolium pratense*) were allowed to imbibe solutions of the pentoses and hexoses indicated: L-*glycero*-L-*galacto*-octulose (L-arabinose or L-mannose),[13] D-*glycero*-L-*galacto*-octulose (D-xylose or D-gulose),[13] and D-*glycero*-D-*altro*-octulose (D-ribose or D-allose).[14]

Physical Measurements

Although aqueous solutions of D-xylose and D-glucose contain insignificant proportions of furanose forms, ^{1}H n.m.r. spectroscopy has indicated that ~15% of the β-furanoses are present in the tautomeric equilibria of the corresponding 3-*O*-methyl ethers; these equilibria are not governed by stereochemical factors

[9] L. M. Lerner, *J. Org. Chem.*, 1975, **40**, 2400.
[10] E. A. McComb, *Carbohydrate Res.*, 1975, **42**, 200.
[11] V. Bilik, D. Anderle, and J. Alfoldi, *Chem. Zvesti*, 1974, **28**, 668.
[12] T. Okuda, S. Saito, and Y. Shiobara, *Carbohydrate Res.*, 1975, **39**, 237.
[13] G. Haustveit, E. A. McComb, and V. V. Rendig, *Carbohydrate Res.*, 1975, **39**, 125.
[14] G. Haustveit, E. A. McComb, and V. V. Rendig, *Carbohydrate Res.*, 1975, **41**, 363.

alone, and solvation effects may be important.[15] The rate constants for the forward and reverse reactions in the mutarotation of α-D-glucopyranose in 70% *p*-dioxan have been determined.[16] The rate of crystallization of lactose has been related to the rate of cooling and to the rate constants for mutarotation.[17] The complex mutarotation shown by some monosaccharides has been analysed mathematically based on a scheme of three components; detailed analysis of D-galactose showed that the slow and fast processes of the complex mutarotation correspond approximately to pyranose–pyranose and pyranose–furanose interconversions, respectively.[18] The ionization and mutarotation of hexoses in aqueous alkaline solution have been examined using ^{13}C n.m.r. spectroscopy; the rate-determining step in mutarotation is the rotation about the C-1—C-2 bond of the acyclic intermediate.[19] The β-anomer is the more acidic D-glucopyranose, whereas the reverse holds for the D-mannopyranoses, indicating that an *eq.–ax.* sequence of the anomeric and adjacent hydroxy-groups is less favourably disposed towards ionization than either *ax.–ax.* or *eq.–eq.* sequences. Related studies have used i.r. spectroscopy to follow the reaction of α-D-glucopyranose with hydroxide ions.[20]

Two groups have used physical measurements (*e.g.* density, viscosity, refractive index, and optical rotation) to determine the compositions of solutions of water–sucrose–D-glucose (or invert sugar) at various temperatures.[21, 22] The diffusion coefficient for self-diffusion of D-fructose in an aqueous system has been measured over a range of concentrations and temperatures.[23] The yields of trapped electrons and radicals have been determined following γ-irradiation of frozen aqueous solutions of sugars.[24]

The alkali-catalysed reactions of trioses (glyceraldehyde and dihydroxyacetone) have again been investigated, but this time using a pH-stat to maintain a constant concentration of the catalyst and employing a polarographic method to determine the concentration of triose.[25] The preferred course of aldolization, giving such products as fructose, sorbose, and dendroketose, depends on the reaction conditions, especially the concentration of triose. The kinetics and mechanism of aldolization were partly elucidated.

Oxidation of seven aldoses by vanadium(V) in sulphuric acid or perchloric acid has been examined by kinetic methods [26] [for studies with cerium(IV) see *J. Org. Chem.*, 1974, **39**, 1788]. It was concluded that the rates correlate with the proportions of *aldehydo*-forms present, although this may really mean the relative

[15] P. J. Garegg, B. Lindberg, and C.-G. Swahn, *Acta Chem. Scand.*, 1975, **B29**, 631.
[16] R. Mitzner, E. Behrenwald, K. F. Luessow, and H. Rieche, *Z. Chem.*, 1975, **15**, 230 (*Chem. Abs.*, 1975, **83**, 193 597v).
[17] M. V. Milyukin, K. K. Polyanskii, and A. G. Shestov, *Izvest. Vyssh. Uchebn. Zaved., Pishch. Tekhnol.*, 1975, 38 (*Chem. Abs.*, 1975, **83**, 193 601s).
[18] H. Kijima and N. Go, *Carbohydrate Res.*, 1975, **40**, 235.
[19] G. De Wit, A. P. G. Kieboom, and H. van Bekkum, *Tetrahedron Letters*, 1975, 3943.
[20] H. Schmid, *Z. phys. Chem. (Frankfurt)*, 1975, **94**, 83.
[21] A. R. Mynott, S. J. Higgs, and R. Jowitt, *J. Sci. Food Agric.*, 1975, **26**, 135.
[22] A. V. Zubchenko, A. Ya. Oleinikova, and V. I. Buravleva, *Khlebopek. Konditer. Prom.*, 1975, 22 (*Chem. Abs.*, 1975, **83**, 43 636t).
[23] M. N. Dadenkova, L. P. Zhmyrya, V. M. Danileiko, and R. S. Burdykova, *Izvest. Vyssh. Uchebn. Zaved., Pishch. Tekhnol.*, 1974, 156 (*Chem. Abs.*, 1975, **83**, 43 624n).
[24] J. Bardsley, P. J. Baugh, and G. O. Phillips, *J.C.S. Perkin II*, 1975, 614.
[25] J. Königstein and M. Fedoroňko, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1183.
[26] A. Kumar and R. N. Mehrotra, *J. Org. Chem.*, 1975, **40**, 1248.

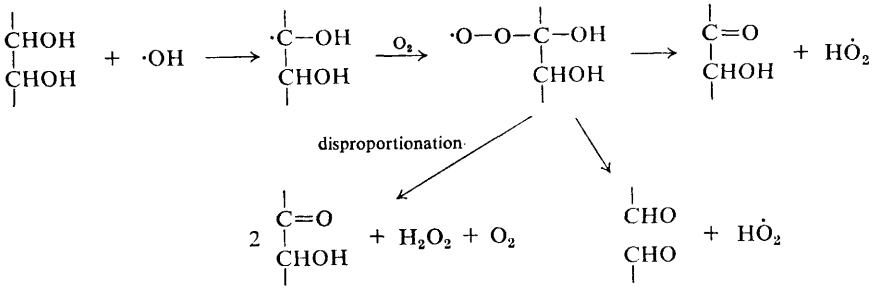
rates of attainment of the *aldehydo*-forms; the observed order of rates, arabinose = xylose > galactose > mannose > glucose, is consistent with this view. Other workers have studied the kinetics and mechanism of oxidation of a number of free sugars by silver(I) in the presence of ammonium hydroxide; the intermediacy of an ene-diolate anion was suggested, since the rates of oxidation were effectively the rates of enolization of the sugars.[27] Related studies on the oxidation of D-sorbose with chloramine-T in highly alkaline solution have been reported.[28] Only two species, D-glucose.$B_4O_7^{2-}$ and free D-glucose, have been found to be involved in a polarimetric titration of D-glucose by tetraborate ($B_4O_7^{2-}$), but no bis(bidentate) species [(D-glucose)$_2$.$B_4O_7^{2-}$] was detected.[29] LCAO calculations have been used to provide information on the electronic configurations of D-glucopyranose, D-glucofuranose, and *aldehydo*-D-glucose and its hydrated form; dipole moments for α- and β-D-glucopyranose were calculated.[30]

Reactions

Labelling studies have established that intramolecular hydride-transfer occurs from C-2 to C-1 during the acid-catalysed isomerization of hexoses; thus, D-[2-^{3}H]glucose afforded D-[1-^{3}H]fructose containing equal proportions of the *R*- and *S*-forms.[31] The reaction is similar to the enzyme-catalysed, but not to the base-catalysed, isomerization.

Deuterium-labelled derivatives of D-glucose have been used in a ^{1}H n.m.r. spectroscopic study of the action of alkali on carbohydrates; it appears that degradation of 3-*O*-methanesulphonyl-D-glucose does not involve H-1.[32] Cyclopentane-1,2-dione has been detected among the products of alkaline degradation of a number of carbohydrates.[33] In addition to minor amounts of pentose, tetrose, and triose derivatives, γ-radiolysis of D-glucose in aerated aqueous solution yielded D-glucono-1,5-lactone, D-*arabino*-hexosulose, D-*ribo*-hexos-3-ulose, D-*xylo*-hexos-4-ulose, and D-*xylo*-hexos-5-ulose by the overall reaction shown in Scheme 3.[34] Among the products identified on γ-radiolysis of D-glucose in deoxygenated aqueous solutions saturated with nitrous oxide were D-gluconic acid, hexuloses, dialdoses, and lower sugars.[35] The oxidation of D-xylose in acidic solutions containing transition-metal ions has been studied; eight carboxylic (oxalic, glycolic, glyceric, maleic, fumaric, tartaric, pyromucic, and formic) acids and carbonic acid were formed in the presence of cupric sulphate, whereas ferric sulphate, mercuric nitrate, and ceric sulphate gave

[27] M. P. Singh, H. S. Singh, S. C. Tiwari, K. C. Gupta, A. K. Singh, V. P. Singh, and R. K. Singh, *Indian J. Chem.*, 1975, **13**, 819.
[28] R. Sanehi, K. C. Gupta, R. N. Mehrotra, and S. P. Mushran, *Bull. Chem. Soc. Japan*, 1975, **48**, 330.
[29] S. Aronoff, T. C. Chen, and M. Cheveldayoff, *Carbohydrate Res.*, 1975, **40**, 299.
[30] N. P. Borisova, M. A. Ivanov, S. N. Ionina, and V. N. Khodyreva, *Trudy Vses. Nauchno-Issled. Inst. Tsellyul.-Bum. Prom.*, 1974, **64**, 152 (*Chem. Abs.*, 1975, **83**, 43 627r).
[31] D. W. Harris and M. S. Feather, *J. Amer. Chem. Soc.*, 1975, **97**, 178.
[32] W. Z. Antkowiak, W. P. Gessner, and J. Milecki, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1975, **23**, 129 (*Chem. Abs.*, 1975, **83**, 147 662q).
[33] R. Koetz and H. Neukom, *Carbohydrate Res.*, 1975, **42**, 365.
[34] S. Kawakishi, Y. Kito, and M. Namiki, *Carbohydrate Res.*, 1975, **39**, 263.
[35] M. Dizdaroglu, D. Henneberg, G. Schomburg, and C. von Sonntag, *Z. Naturforsch.*, 1975, **30b**, 416 (*Chem. Abs.*, 1975, **83**, 114 765x).



Scheme 3

principally oxalic acid, D-xylonic acid, and formic acid, respectively.[36] A radical mechanism has been proposed for the oxidation of reducing sugars by oxygen in alkaline solutions.[37]

Differential thermal and thermogravimetric analyses have been used to study the pyrolysis of pentoses.[38] An examination of the influence of agar-agar, sucrose, lactose, and sodium chloride on the rate of crystallization of D-glucose at 40 °C showed that sodium chloride has the largest retarding effect;[39] the effects of other organic and inorganic additives were also reported.[40]

In continuing their work on the transport of sugars across the human-erythrocyte membrane, Barnett and his co-workers have shown that D-glucose inhibits the transport of 6-*O*-methyl-D-galactose, indicating that both sugars utilize the same sugar-transport system.[41] The protection that various substituted hexoses afforded against deactivation of the sugar-transport system by fluorodinitrobenzene was also examined, and a mechanism for the transport of sugars across the membrane was suggested. The mechanism of inhibition of D-glucose transport in a *Neurospora* species by arsenate has also been studied.[42] The degradation of D-xylose by a pseudomonad is referred to in Chapter 17.

[36] I. I. Korol'kov and V. I. Krupenskii, *Zhur. priklad. Khim.*, 1974, **47**, 2741 (*Chem. Abs.*, 1975, **82**, 171 309c).
[37] G. L. Akim, V.-G. Ermolinskii, A. M. Kutnevich, and V. M. Nikitin, *Trudy Vses. Nauchno-Issled. Inst. Tsellyul.-Bum. Prom.*, 1974, **64**, 190 (*Chem. Abs.*, 1975, **83**, 43 639w).
[38] Y. Tanaka and T. Nakamura, *Nippon Kagaku Kaishi*, 1975, 705 (*Chem. Abs.*, 1975, **83**, 59 173a).
[39] A. Sroczynski and W. Zglinski, *Zeszyty Probl. Postepow Nauk Roln.*, 1974, **159**, 219 (*Chem. Abs.*, 1975, **83**, 10 625k).
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[41] J. E. G. Barnett, G. D. Holman, R. A. Chalkley, and K. A. Munday, *Biochem. J.*, 1975, **145**, 417.
[42] G. A. Scarborough, *Arch. Biochem. Biophys.*, 1975, **166**, 245.

3
Glycosides

O-Glycosides

Calcium and strontium salts can be used advantageously in the Fischer synthesis of methyl α-D-ribofuranoside, methyl β-D-lyxo-furanoside and -pyranoside, methyl α- and β-D-mannofuranoside, methyl α-D-talopyranoside, methyl α- and β-D-talofuranoside, methyl α-L-gulo-furanoside and -pyranoside, and the methyl D-*glycero*-D-*gulo*-heptosides.[43] The glycosides from preparative glycosidations were conveniently separated on cation-exchange resins in the Ca^{2+} or Sr^{2+} forms. The use of metal ions in glycosidations can result in the formation of significant proportions of dimethyl acetals (see Chapter 5).

Treatment of L-fucose with methanolic hydrogen chloride has been shown to yield a final equilibrium mixture containing methyl α-L-fucofuranoside (6%), methyl β-L-fucofuranoside (13%), methyl α-L-fucopyranoside (54%), and methyl β-L-fucopyranoside (27%).[44] Both L-fucopyranosides and both L-fucofuranosides are formed simultaneously in the early stages of the reaction, in contrast to the initial formation of the β-furanoside and the α-pyranoside on similar treatment of D-galactose. Methanolysis of a number of D-glucose-containing disaccharides has shown that they react initially at the reducing-end, whereafter cleavage of the inter-saccharide linkage furnishes D-gluco-furanosides and -pyranosides.[45]

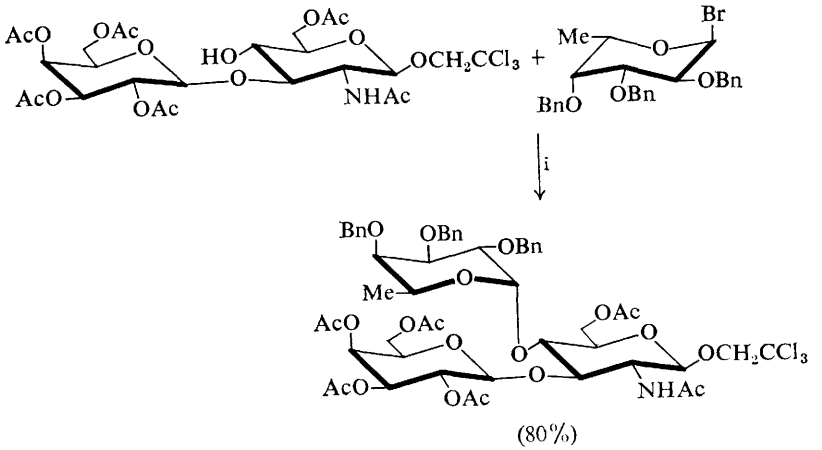
Methanolysis of α-lipomycin afforded the α- and β-furanosides and α- and β-pyranosides of 2,6-dideoxy-D-*ribo*-hexose in the proportions 12.5 : 12.5 : 25 : 50.[46]

Direct glycosidation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with alcohols in the presence of silver trifluoromethanesulphonate, 4-nitrobenzenesulphonyl chloride, and triethylamine afforded the β-glycosides in reasonable yield.[47] The use of 2,3:4,5-di-*O*-isopropylidene-D-glucose in the preparation of the methyl D-glucoseptanosides is reported in Chapter 5.

Lemieux's group have reviewed the theoretical aspects pertaining to displacements at the anomeric centre of sugars, and have defined the following criteria for successful halide-catalysed glycosidations yielding α-glycosides from derivatives of glycosyl halides: (i) the concentration of halide ion must ensure that anomerization of the glycosyl halide is faster than the displacement of the less reactive α-halide by the alcohol; (ii) the halide must react with the alcohol without assistance from metal ions or highly polar solvents; and (iii) the reaction condi-

[43] S. J. Angyal, C. L. Bodkin, and F. W. Parrish, *Austral. J. Chem.*, 1975, **28**, 1541.
[44] D. F. Mowery, jun., *Carbohydrate Res.*, 1975, **43**, 233.
[45] B. Piekarska, *Roczniki Chem.*, 1975, **49**, 1589.
[46] A. Zeeck, *Annalen*, 1975, 2079.
[47] S. Koto, Y. Hamada, and S. Zen, *Chem. Letters*, 1975, 587.

tions should minimize side-reactions involving the loss of protecting groups or the formation of glycosidic products other than the desired α-glycoside.[48] Based on these considerations, several α-linked disaccharides were synthesized in good yield and in a highly stereoselective manner by reaction of perbenzylated α-D-gluco-, α-D(L)-galacto-, and α-L-fuco-pyranosyl bromides with suitably protected derivatives of D-glucose and D-galactose in the presence of tetraethylammonium bromide; *e.g.* 6-*O*-α-D-glucopyranosyl-D-galactose, 3-*O*-α-D-glucopyranosyl-D-glucose, and 3-*O*-α-D-galactopyranosyl-D-galactose were among the disaccharides synthesized. The synthesis of the Le^a blood-group antigenic determinant, *O*-(α-L-fucopyranosyl)-(1 → 4)-[*O*-(β-D-galactopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-D-glucose, was described in the succeeding paper; the key glycosylation step is shown in Scheme 4.[49] An isomeric 6-*O*-L-fucopyranosyl analogue of the

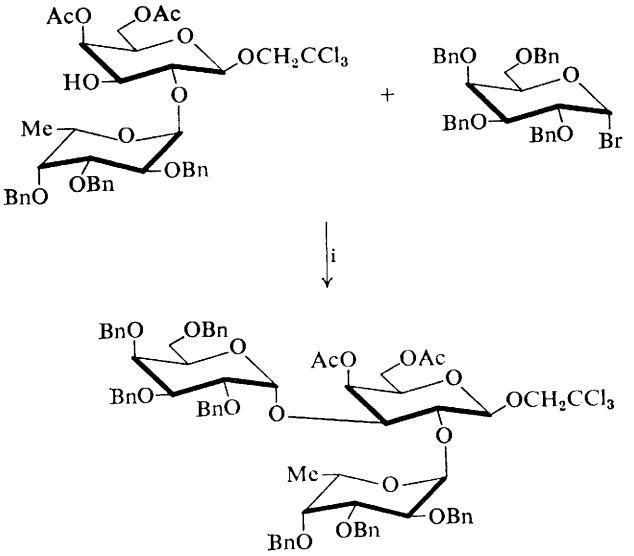


Reagent: i, $Et_4\overset{+}{N}Br^-$–Pr^i_2NEt–CH_2Cl_2–DMF

Scheme 4

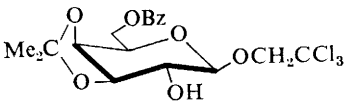
trisaccharide was also obtained in this reaction, the isomers being distinguished by ^{13}C n.m.r. spectroscopy. Another paper reported the synthesis of *O*-(α-L-fucopyranosyl)-(1 → 2)-[*O*-(α-D-galactopyranosyl)-(1 → 3)]-D-galactose, which forms part of the antigenic determinant of blood-group B substance.[50] One of the key steps in the synthesis of this trisaccharide is illustrated in Scheme 5; the D-galactopyranoside derivative (6) is an important intermediate in the synthesis of the disaccharide derivative used, since, after glycosylation, the 3,4-*O*-isopropylidene group was replaced by a 3,4-*O*-(ethoxyethylidene) group, which was converted into the corresponding axial 4-acetate on partial hydrolysis with acid. Finally, Lemieux's group have prepared β-glycosides of *O*-(α-L-fucopyranosyl)-(1 → 4)-[*O*-(β-D-galactopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-D-glucose using either 8-ethoxycarbonyl- or 8-methoxycarbonyl-octanol.[51] Semisynthetic antigens

[48] R. U. Lemieux, K. B. Hendriks, R. V. Stick, and K. James, *J. Amer. Chem. Soc.*, 1975, **97**, 4056.
[49] R. U. Lemieux and H. Driguez, *J. Amer. Chem. Soc.*, 1975, **97**, 4063.
[50] R. U. Lemieux and H. Driguez, *J. Amer. Chem. Soc.*, 1975, **97**, 4069.
[51] R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Amer. Chem. Soc.*, 1975, **97**, 4076.



Reagent: i, $Et_4\overset{+}{N}Br^-$–CH_2Cl_2–DMF

Scheme 5



(6)

were prepared from these esters by attachment to the free amino-groups in bovine serum albumin. Antibodies raised with the trisaccharide antigen precipitated blood-group Le^a substance and agglutinated Le^a red-blood cells.

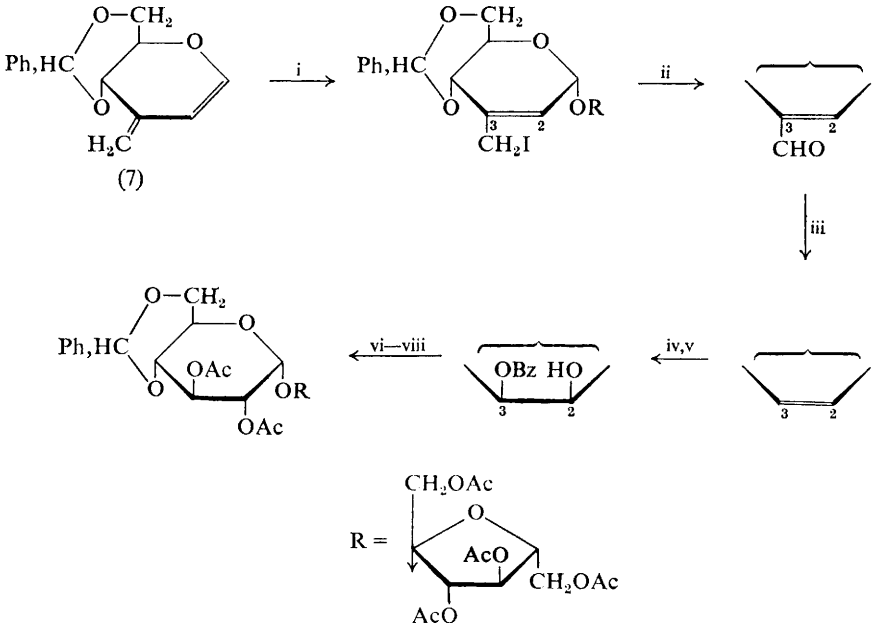
Several modified or new syntheses of α-glycosides have been reported over the past year. The mechanism of, and the effects of solvent on, the glycosidation of D-glucosyl chlorides having a non-participating group at C-2, using silver perchlorate as the principal catalyst, have been investigated.[52] It was concluded that anhydrous ether is the best solvent; silver perchlorate and *sym*-collidine are effective catalysts; β-D-glucopyranosyl chloride is superior to the α-anomer; the solvent and reagents should be as dry as possible. The above conditions were used in syntheses of α-kojibiose, β-isomaltose, β-maltose, and β-nigerose octa-acetates.[53] Methanolysis of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl bromide yielded a mixture of anomeric methyl glycosides, whether or not tetrabutyl-ammonium bromide, or mercuric cyanide, or silver tetrafluoroborate was present.[54]

Methanolysis of quaternary ammonium and triphenylphosphonium salts derived from 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranosyl bro-

[52] K. Igarashi, J. Irisawa, and T. Honma, *Carbohydrate Res.*, 1975, **39**, 213.
[53] K. Igarashi, J. Irisawa, and T. Honma, *Carbohydrate Res.*, 1975, **39**, 341.
[54] J. M. J. Fréchet and H. H. Baer, *Carbohydrate Res.*, 1975, **42**, 369.

mide has been studied to ascertain the conditions of solvent and temperature that would produce the highest yields of α-D-glucosides.[55] The quaternary ammonium salts gave the highest yields with solvents of low dielectric constant at room temperature. Even under the best conditions, the reaction of the glycosyl bromide with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside gave only a low (50%) yield of the disaccharide derivative and was not completely stereoselective. Examination of the methanolysis of some 2,3,4-tri-*O*-benzyl-D-galactopyranosyl derivatives showed that the nature of the C-6 substituent exerts only a small influence on the stereochemical outcome of the reaction compared with the effects of the solvent and the nature of the leaving group at C-1.[56] Trimethylsilylated D-glucopyranose 1-phosphate reacted with sodium 4-nitrophenate to give the 4-nitrophenyl β-glycoside initially, whereas the α-glycoside (80%) was obtained after 2 h.[57]

A multi-step approach to the synthesis of α-glycosides has been based on the highly stereoselective addition of alcohols to the conjugated diene (7) in the presence of iodonium dicollidine perchlorate.[58] This approach was used in a novel synthesis of derivatives of sucrose (Scheme 6).

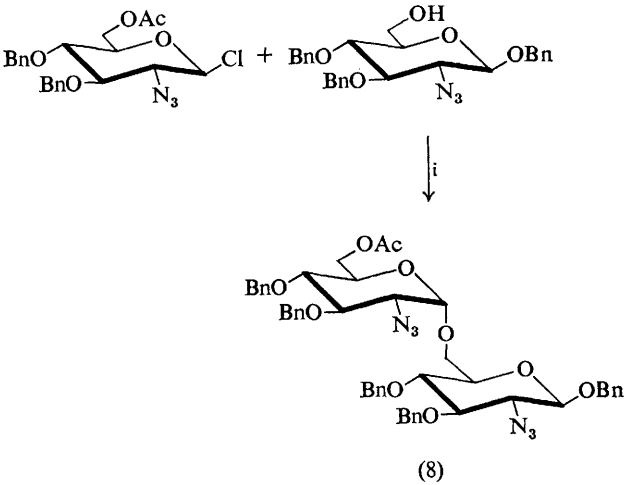


Reagents: i, $I^+(collidine)_2ClO_4^-$–ROH; ii, $AgBF_4$–DMSO–Et_3N; iii, $(Ph_3P)_2RhCOCl$; iv, OsO_4–py; v, BzCl–py; vi, CrO_3–py; vii, $NaBH_4$; viii, Ac_2O–py

Scheme 6

[55] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1975, **39**, 33.
[56] T. J. Lucas and C. Schuerch, *Carbohydrate Res.*, 1975, **39**, 39.
[57] V. N. Shibaev, Yu. Yu. Kosov, V. N. Chekunchikov, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1975, **222**, 370 (*Chem. Abs.*, 1975, **83**, 97 831w).
[58] D. E. Iley and B. Fraser-Reid, *J. Amer. Chem. Soc.*, 1975, **97**, 2563.

Paulsen and Stenzel have prepared several α-(1 → 3)-, α-(1 → 4)-, and α-(1 → 6)-linked disaccharides containing a nitrogen function at C-2 of each of the monosaccharide residues.[59] Thus, the α-(1 → 6)-linked disaccharide derivative (8) was obtained in *ca.* 70% yield by the reaction shown in Scheme 7. Deacetylation of

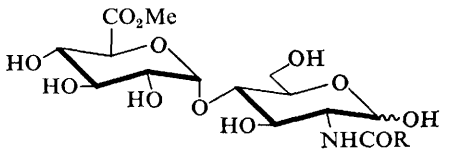


(8)

Reagent: *i*, Ag_2CO_3–$AgClO_4$–CH_2Cl_2

Scheme 7

the disaccharide derivative (8) clearly permits this approach to be extended to the synthesis of related trisaccharides and higher oligosaccharides. Other reports have described syntheses of the heparin disaccharides (9) and (10), using both



(9) R = Me
(10) R = OBn

Brigl's anhydride and the addition of nitrosyl chloride to an unsaturated sugar,[60] and 2-acetamido-2-deoxy-6-*O*-α-D-glucopyranosyl-α-D-galactopyranose and its 4-aminophenyl glycoside.[61]

Other disaccharides synthesized during the past year include α-D-glucopyranosyl α-L-sorbopyranoside (by an enzymic method),[62] gentiobiose,[63, 64] 6-*O*-β-D-

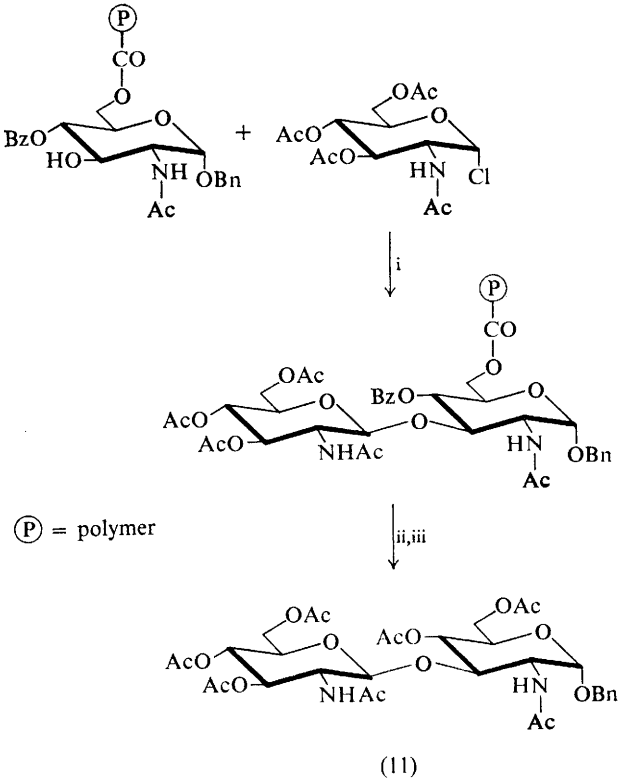
[59] H. Paulsen and W. Stenzel, *Angew. Chem. Internat. Edn.*, 1975, **14**, 558.
[60] P. C. Wyss, J. Kiss, and W. Arnold, *Helv. Chim. Acta*, 1975, **58**, 1847.
[61] M. Petitou and P. Sinaÿ, *Carbohydrate Res.*, 1975, **40**, 13.
[62] J. C. Mazza, A. Akgerman, and J. R. Edwards, *Carbohydrate Res.*, 1975, **40**, 402.
[63] R. R. King and C. T. Bishop, *Canad. J. Chem.*, 1975, **53**, 1970.
[64] E. E. Lee, A. Hartigan, and P. S. O'Colla, *Proc. Roy. Irish Acad., Sect. B*, 1975, **75**, 267 (*Chem. Abs.*, 1975, **83**, 147 666u).

galactopyranosyl-D-galactose,[64] 3- and 4-*O*-α- and -β-D-galactopyranosyl-D-galactose,[65] 4-*O*-α-L-fucopyranosyl-L-fucose,[66] and 1-*O*-β-D-fructofuranosyl-D-fructose (inulobiose; by partial acid hydrolysis of inulin).[67] Two disaccharides containing amino-sugar residues have been reported, namely 2-acetamido-2-deoxy-4-*O*-α-L-fucopyranosyl-D-glucose[68] and 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-2-deoxy-D-galactose[63] (see also ref. 59). Among disaccharide glycosides to be synthesized were 4-isothiocyanatophenyl 3-*O*-(3,6-dideoxy-β-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside,[69] 4-nitrophenyl 2-acetamido-2-deoxy-3(6)-*O*-β-D-galactopyranosyl-β-D-glucopyranoside,[70] 3-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-stearoyl-L-glycerol,[71] maltosyl, cellobiosyl, and lactosyl derivatives of hesperetin,[71a] and 4-methylumbelliferyl *N*-acetylchitoside, di-*N*-acetylchitobioside, and tri-*N*-acetylchitotrioside.[72] Modifications to disaccharide derivatives by an oxidation–reduction sequence have yielded methyl 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-α-D-glucopyranoside[73] and 4-acetamidophenyl 4-*O*-β-D-mannopyranosyl-α-L-rhamnopyranoside;[74] 4-nitrophenyl 4-*O*-α-D-mannopyranosyl-α-L-rhamnopyranoside was also prepared in the latter investigation.

The following novel approaches to the synthesis of di- and poly-saccharides have been reported. The disaccharide derivative (11) was obtained using a polymer support (Scheme 8),[75] and the disaccharide derivative (12) by glycosylation of the activated 2,3-diphenyl-2-cyclopropen-1-yl ether (13)[76] (see Vol. 8, p. 32). A (1 → 6)-linked β-D-glucan (DP ≈ 16) has been obtained by acid-catalysed condensation of the cyanoethylidene derivative (14).[77] A related study has examined the cationic polymerization of 3-*O*-acetyl-β-L-arabinofuranose 1,2,5-orthobenzoate initiated by triphenylcarbonium tetrafluoroborate, or benzoylium perchlorate,[78] or 1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranose in the presence of mercuric bromide.[79] A synthetic (1 → 6)-D-glucan, probably containing both α- and β-linkages, resulted from polycondensation of the 6′-*O*-t-butylgentiobiosyl derivative (15),[80] and a cyanoethylidene derivative analogous to (14) has been used in a new synthesis of gentiobiose.[81]

An efficient synthesis of carbohydrate 1,2-orthoesters relies on treatment of reactive, acylated aldopentofuranosyl halides with *N*,*N*-dimethylformamide

[65] M. Chacón-Fuertes and M. Martín-Lomas, *Carbohydrate Res.*, 1975, **43**, 51.
[66] M. Dejter-Jusynski and H. M. Flowers, *Carbohydrate Res.*, 1975, **41**, 308.
[67] A. G. Dickerson and J. Moor, *Carbohydrate Res.*, 1975, **39**, 162.
[68] J.-C. Jacquinet and P. Sinaÿ, *Carbohydrate Res.*, 1975, **42**, 251.
[69] G. Ekborg, P. J. Garegg, and B. Gotthammar, *Acta Chem. Scand.* (*B*), 1975, **29**, 765.
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[71] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1975, 1521.
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[73] H. B. Borén, G. Ekborg, and J. Lönngren, *Acta Chem. Scand.* (*B*), 1975, **29**, 1085.
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[75] G. Excoffier, D. Gagnaire, J.-P. Utille, and M. Vignon, *Tetrahedron*, 1975, **31**, 549.
[76] A. Ya. Khorlin, V. A. Nesmeyanov, and S. E. Zurabyan, *Carbohydrate Res.*, 1975, **43**, 69.
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[79] A. F. Bochkov, V. N. Chernetsky, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 465 (*Chem. Abs.*, 1975, **83**, 43 634r).
[80] N. K. Kochetkov and E. M. Klimov, *Carbohydrate Res.*, 1975, **44**, 138.
[81] A. F. Bochkov and N. K. Kochetkov, *Carbohydrate Res.*, 1975, **39**, 355.

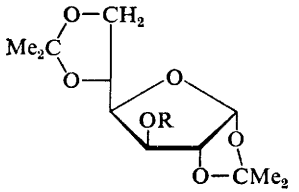


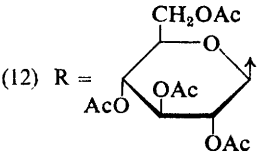
(11)

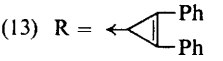
Reagents: i, $Hg(CN)_2$; ii, MeONa; iii, Ac_2O–py

Scheme 8

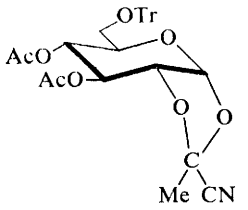
dialkyl acetals as the source of the alkoxy-group.[82] Orthoester formation with acylated aldohexopyranosyl halides was accomplished in the same way, but in the presence of silver trifluoromethanesulphonate. Lewis acid-catalysed transformation of the orthoesters yielded the corresponding β-D-glycosides (*e.g.* methyl



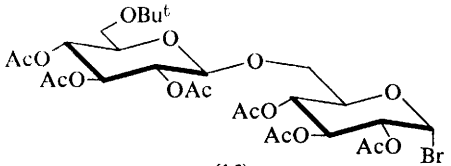




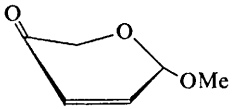
[82] S. Hanessian and J. Banoub, *Carbohydrate Res.*, 1975, **44**, C14.



(14)



(15)



(16)

2,3,5-tri-*O*-benzoyl-β-D-ribofuranoside and methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside). The orthoester procedure has also been used to prepare cholesteryl β-D-glucopyranoside.[83]

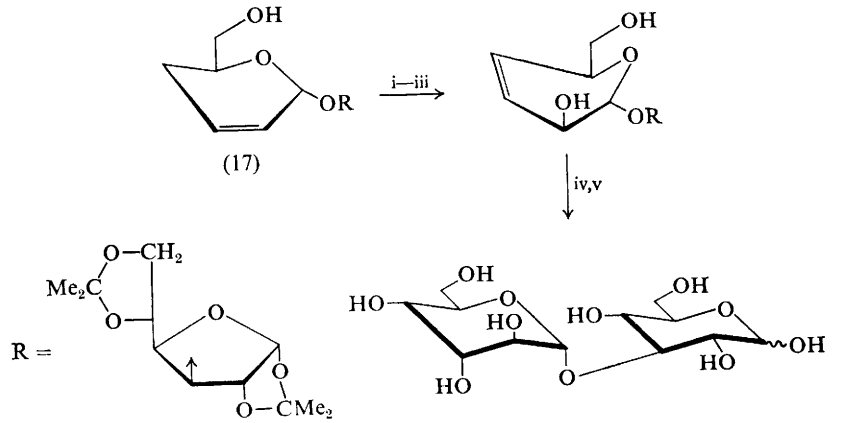
Standard procedures using oxazoline derivatives of amino-sugars have been used to prepare 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose[84] and the branched trisaccharide 3,6-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose, a hydrolysis product of the blood-group substances.[85] Conventional Koenigs–Knorr reactions have been used to prepare *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 6)-D-galactopyranose, the carbohydrate component of the flavone glycoside xanthorhamnin A,[86] and *O*-β-D-mannopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 3)-D-galactose, the trisaccharide repeating-unit of the O-specific polysaccharide from *Salmonella anatum*.[87]

Non-reducing disaccharides to be synthesized by the Koenigs–Knorr reaction include α-D-glucopyranosyl α-D-xylopyranoside, α-D-glucopyranosyl α-D-mannopyranoside, and β-D-glucopyranosyl β-D-mannopyranoside, whereas modification of αα-trehalose yielded α-D-allopyranosyl α-D-glucopyranoside.[88]

Modification of existing glycosides often provides convenient routes to new glycosides; thus, 2- and 4-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranosides were obtained by sulphonate displacements at C-4 of the D-*gluco*-counterparts.[89] Syntheses of the racemic methyl α- and β-lyxo-, β-ribo-, α-xylo-, and α-arabino-pentopyranosides have been achieved by *cis*- and *trans*-hydroxylations of the enone (16).[90] Treatment of β-gentiobiose octa-acetate with antimony pentachloride has furnished, after acetylation, 6-*O*-β-D-glucopyranosyl-α-D-altropyranose octa-acetate, the reaction presumably involving a 3,4-acetoxonium ion.[91] 1,6-Anhydromaltose has been transformed into 4-*O*-α-D-galactopyranosyl-

[83] N. I. Uvarova, G. I. Oshitok, N. F. Samoshina, and G. B. Elyakov, *Khim. prirod. Soedinenii*, 1974, 460 (*Chem. Abs.*, 1975, **82**, 17 063p).
[84] R. Kaifu, T. Osawa, and R. W. Jeanloz, *Carbohydrate Res.*, 1975, **40**, 111.
[85] S. David and A. Veyrieres, *Carbohydrate Res.*, 1975, **40**, 23.
[86] A. Lipták and P. Nánási, *Carbohydrate Res.*, 1975, **44**, 313.
[87] N. K. Kochetkov, B. A. Dmitriev, N. N. Malysheva, A. Ya. Chernyak, E. M. Klimov, N. E. Bayramova, and V. I. Torgov, *Carbohydrate Res.*, 1975, **45**, 283.
[88] E. Bar-Guilloux, J. Defaye, H. Driguez, and D. Robic, *Carbohydrate Res.*, 1975, **45**, 217.
[89] M. Petitou and P. Sinaÿ, *Carbohydrate Res.*, 1975, **42**, 180.
[90] O. Achmatowicz and P. Bukowski, *Canad. J. Chem.*, 1975, **53**, 2524.
[91] E. F. Garrido and M. J. Moragues, *Anales de Quím.*, 1974, **70**, 1088 (*Chem. Abs.*, 1975, **83**, 193 602t).

D-glucose and the related 6′-deoxy-derivative by displacements involving the 4′,6′-dimethanesulphonate.[92] 3-*O*-α-D-Altropyranosyl-D-glucose has been synthesized from the unsaturated disaccharide (17) by the reactions outlined in Scheme 9.[93] The overall synthesis is unusual in so far as it does not rely on



Reagents: i, H_2O_2–PhCN; ii, PhSeH; iii, H_2O_2; iv, OsO_4; v, H^+

Scheme 9

activation of the anomeric centre of a sugar derivative to form the disaccharide linkage (see Vol. 8, p. 19). Degradation of 1,2,4-tri-*O*-acetyl-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-*O*-methanesulphonyl-α-D-glucopyranose with alkali has afforded a route to 2-deoxy-5-*O*-β-D-glucopyranosyl-D-*erythro*-pentose, which was also prepared by selective oxidation of 3-deoxy-6-*O*-β-D-glucopyranosyl-D-*ribo*-hexose with periodate ion.[94]

Other glycosides to be prepared include 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galacto-pyranosyl)-L-glycerol,[95] 1-*O*-β-D-galacto- and -gluco-pyranosylethylene glycol,[96] 4-[*NN*-bis(2-chloroethyl)amino]phenyl β-D-gluco- and -xylo-pyranosides,[96a] 4-β-D-glucopyranosyl quercitin,[97] cirsiliol and cirsilineol β-D-glucopyranosides,[98] cholesteryl β-D-glucopyranoside (using polymeric acid-binding reagents),[99] cardenolide glycosides (using silver carbonate on Celite),[100] derivatives of 6-(trifluoroacetamido)hex-1-yl D-galactosides,[101] and the β-D-glucopyranosyl derivatives of the flavonoids fujikinin and dalpatin.[102] Conventional procedures were also used in syntheses of 3-*O*-β-D-glucuronopyranosyl morphine (*via* the lithium salt

[92] M. Mori, M. Haga, and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1480.
[93] S. David, A. Lubineau, and J. M. Vatèle, *J.C.S. Chem. Comm.*, 1975, 701.
[94] G. O. Aspinall, T. N. Krishnamurthy, and I. Furda, *Canad. J. Chem.*, 1975, **53**, 2178.
[95] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1975, 364.
[96] N. V. Prokazova, G. K. Todriya, V. A. Vaver, and L. D. Bergel'son, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2569 (*Chem. Abs.*, 1975, **82**, 171 352p).
[96a] K. Schwabe and A. Graffi, *Pharmazie*, 1975, **30**, 70 (*Chem. Abs.*, 1975, **82**, 156 632m).
[97] V. S. Batyuk, *Khim. prirod. Soedinenii*, 1974, 577 (*Chem. Abs.*, 1975, **82**, 73 389m).
[98] A. J. Kalra, M. Krishnamurti, and T. R. Seshadri, *Indian. J. Chem.*, 1974, **12**, 1040.
[99] V. Eschenfelder, R. Brossmer, and M. Wachter, *Angew. Chem. Internat. Edn.*, 1975, **14**, 715.
[100] J. Hartenstein and G. Satzinger, *Annalen*, 1974, 1763.
[101] J. E. Schneider and Y. C. Lee, *Carbohydrate Res.*, 1975, **43**, 79.
[102] S. Antus, L. Farkas, Z. Kardos-Balogh, and M. Nógrádi, *Chem. Ber.*, 1975, **108**, 3883.

of morphine)[103] and 4-acetamidophenyl β-D-glucuronopyranoside, the principal product formed on metabolism of phenacetin.[104]

Hydrolysis and Related Reactions.—The hydrolysis of 2,4-dinitrophenyl β-D-galactopyranoside at 25 °C has been found to be independent of pH between pH 1.6 and 8.4, whereas specific base-catalysis occurs above pH 8.4.[105] Studies of related glycosides of pentoses, 6-deoxyhexoses, and 6-chloro-6-deoxyhexoses furnished evidence supporting the A-1 mechanism for the hydrolysis of these glycosides with acid.

The enzymic hydrolysis of some 4-nitrophenyl 2-aroylamino-2-deoxy-β-D-glucopyranosides has been reported.[106]

A quantum mechanical study of the distribution of charge in alkyl and aryl hexopyranosides has demonstrated a correlation between the rate of acid-catalysed hydrolysis and either the inductive effects of the alkyl groups or the charge on the glycosidic oxygen atoms of aryl glycosides.[107]

2-Hydroxy-4-nitrophenyl β-D-glucopyranoside, D-glucose, 4-nitrophenol, and traces of αβ-unsaturated carbonyl compounds were identified among the products of γ-irradiation of 4-nitrophenyl β-D-glucopyranoside; mechanisms were advanced to account for the formation of these products.[108] Exchange with $H_2{}^{18}O$ during the irradiation of benzyl β-D-glucopyranoside has confirmed that D-glucose is formed by cleavage of the glycosyl-oxygen bond.[109]

Analysis of the products formed by the action of *Medicago sativa* α-D-mannosidase on 4-nitrophenyl α-D-mannopyranoside in the presence of methanol has revealed the presence of methyl α-D-mannopyranoside, signifying that a single S_N2 displacement reaction cannot take place at C-1.[110] Retention of configuration in the methyl glycoside can be explained either by a double-displacement mechanism or by attack of the alcohol on a stabilized (ion-pair) oxonium–carbonium ion from the same side as the leaving aglycone group.

Evidence has been obtained for the formation of a stable glycosyl-enzyme intermediate in the short-term reaction between α-D-glucosidase and methyl α-D-[^{14}C]glucopyranoside; the intermediate appears to be covalently linked.[111] Related studies on the enzymic hydrolysis of 2-nitrophenyl β-D-galactopyranoside at −10 °C have also been reported.[112]

Other Reactions and Features of Glycosides.—The degradation of methyl glycosides in oxygenated, aqueous 0.5 M sodium hydroxide at 120 °C has been investigated.[113] The principal products were formed by way of benzilic acid rearrangement of vicinal dicarbonyl intermediates.

[103] B. Berrang, C. E. Twine, G. L. Hennessee, and F. I. Carroll, *Synthetic Comm.*, 1975, **5**, 231.
[104] J. Kiss, K. Noack, and R. D'Souza, *Helv. Chim. Acta*, 1975, **58**, 301.
[105] D. Cocker and M. L. Sinnott, *J.C.S. Perkin II*, 1975, 1391.
[106] M. G. Vafina, N. V. Molodtsov, and L. I. Fedoreeva, *Carbohydrate Res.*, 1975, **44**, 142.
[107] I. Farkas and Z. Dinya, *Acta Chim. Acad. Sci. Hung.*, 1974, **82**, 77 (*Chem. Abs.*, 1975, **82**, 31 482f).
[108] K. Kemsley, J. S. Moore, and G. O. Phillips, *J.C.S. Perkin II*, 1975, 1638.
[109] L. I. Kudryashov, L. I. Nedoborova, O. S. Chizhov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 722 (*Chem. Abs.*, 1975, **83**, 43 626q).
[110] J. D. Prijcker, C. K. De Bruyne, M. Claeyssens, and A. De Bruyn, *Carbohydrate Res.*, 1975, **43**, 380.
[111] H.-Y. L. Lai, L. G. Butler, and B. Axelrod, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 635.
[112] A. L. Fink and K. J. Angelides, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 701.
[113] R. Malinen and E. Sjostrom, *Cellulose Chem. Technol.*, 1975, **9**, 231.

In a study of the thermal properties of di- and tri-saccharides, it has been shown that the endothermic peak (given in parentheses) is characteristic of the glycosidic linkage; *viz.* (1 → 1)-linkages (265 °C), (1 → 4)-linkages (254 °C), (1 → 3)-linkages (243 °C), (1 → 2)-linkages (233 °C), and (1 → 6)-linkages (218 °C).[114] The heat capacities of aqueous solutions of lactose (5—40%) have been measured as a function of temperature,[115] and potentiometric titration of sucrose with sodium hydroxide solution has shown that it is a very weak diprotic acid (pK_1 = 12.57, pK_2 = 13.45).[116]

Natural Products.—Biochemical aspects of αα-trehalose have been reviewed.[117]

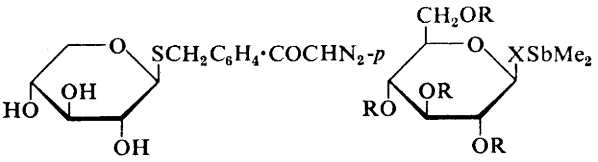
6-*O*-α-D-Mannopyranosyl-*myo*-inositol has been identified as a constituent of bakers' yeast,[118] whereas 6-*O*-β-D-galactopyranosyl-*myo*-inositol occurs in the mammary glands of rats, wherein its biosynthesis has been studied.[119] Less D-glucosyl-D-galactosylhydroxylysine was found in the collagen from rabbit corneal scar tissue than in normal collagen.[120]

Xylobiose has been obtained on hydrolysis of a hardwood xylan by a Streptomyces enzyme; D-xylose was also formed, but can be removed with a yeast.[121]

S-Glycosides

Interest in the use of thioglycosides as inhibitors and inducers of enzymes and for attachment to affinity-chromatography matrices continues. Anomeric pairs of aryl 1-thioaldosides can be separated on cation-exchange resins: this method permits the efficient separation of 1,2-*cis*-(4-nitrophenyl) 1-thio-D-aldopyranosides from reaction mixtures in which both 1,2-*cis*- and 1,2-*trans*-1-thioglycosides were present.[122] Direct alkylation of the 1-thio-sugar has led to the preparation of many alkyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranosides containing reactive groups (*e.g.* OH, NH_2, N_3, C=O, CO_2H, halogen) in the aglycone[123] and to 4-nitrobenzyl and 4-nitrophenyl 1-thioglycosides of D- and L-fucopyranose, D-mannopyranose, D-galactopyranose, and D-glucopyranose.[124] 4-Diazoacetylbenzyl 1-thio-β-D-xylopyranoside (18) has been prepared by a simple route that should be of general applicability; carbenes formed by light-induced decomposition of such diazoketones are potential photo-affinity reagents for labelling the active site of glycosidases.[125]

[114] Y. Tanaka and T. Nakamura, *Chuo Daigaku Rikogakubu Kiyo*, 1974, **17**, 169 (*Chem. Abs.*, 1975, **83**, 131 852r).
[115] Yu. A. Byval'tsev, B. G. Perelygin, and K. K. Polyanskii, *Izvest. Vyssh. Ucheb. Zaved., Pishch. Tekhnol.*, 1974, 159 (*Chem. Abs.*, 1975, **82**, 58 023u).
[116] F. Coccioli and M. Vicedomini, *Ann. Chim. (Italy)*, 1974, **64**, 369 (*Chem. Abs.*, 1975, **83**, 137 743c).
[117] A. D. Elbein, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 227.
[118] W. W. Wells, C.-H. Kuo, and W. F. Naccarato, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 644.
[119] W. W. Wells, R. E. Ray, C.-H. Kuo, and N. Iritani, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1061.
[120] C. Cintron, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 288.
[121] I. Kusakabe, T. Yasui, and T. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1355.
[122] J. Schneider, H. H. Liu, and Y. C. Lee, *Carbohydrate Res.*, 1975, **39**, 156.
[123] J. Frgala, M. Černý, and J. Staněk, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1411.
[124] K. L. Matta, R. N. Girotra, and J. J. Barlow, *Carbohydrate Res.*, 1975, **43**, 101.
[125] M. Claeyssens, F. Deleyn, E. Saman, and C. K. De Bruyne, *Carbohydrate Res.*, 1975, **42**, 352.

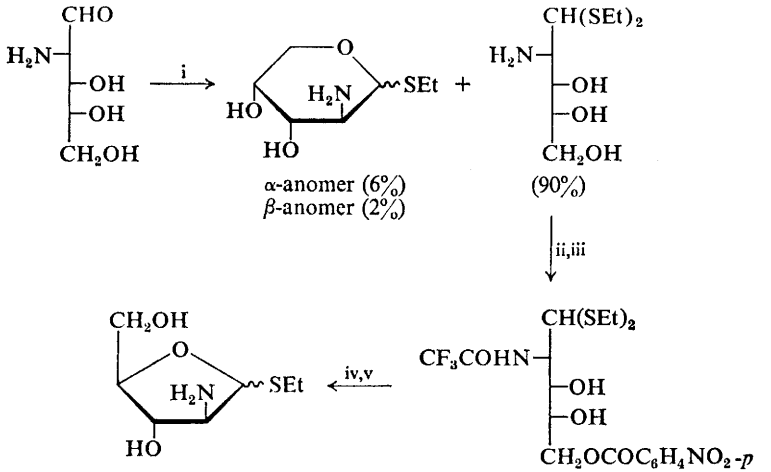


(18)

(19) X = S; R = Ac or Bz
(20) X = Se; R = Ac or Bz

The use of 1-thioglycosides in the synthesis of glycosyl halides is noted in Chapter 7.

Ethyl 2-amino-2-deoxy-1-thio-α- and -β-D-arabinopyranoside have been obtained by direct ethanethiolation of 2-amino-2-deoxy-D-arabinose, whereas ethyl 2-amino-2-deoxy-1-thio-α- and -β-D-arabinofuranoside were prepared by partial demercaptalation of a 2-amino-2-deoxy-D-arabinose diethyl dithioacetal derivative with either mercuric chloride or bromine (see Scheme 10).[126] Demer-



Reagents: i, EtSH–HCl; ii, CF_3COSEt–EtOH; iii, *p*-$NO_2C_6H_4COCl$–py; iv, $PbCO_3$–Br_2; v, basic resin

Scheme 10

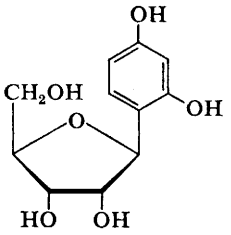
captalation of the dithioacetal with mercuric chloride gave exclusively the β-anomer, whereas a 1 : 1 mixture of α- and β-anomers was obtained with bromine. Alternatively, direct ethanethiolation of 2-amino-2-deoxy-D-arabinose in trifluoroacetic acid yielded ethyl 2-amino-2-deoxy-1-thio-α-D-arabinofuranoside (21.5%), suggesting that this reaction is thermodynamically controlled.

Esters of the disulphide and the diselenide of 1-thio- and 1-seleno-β-D-glucopyranose reacted with tetramethyldistibine ($Me_2SbSbMe_2$) to yield the derivatives (19) and (20).[127]

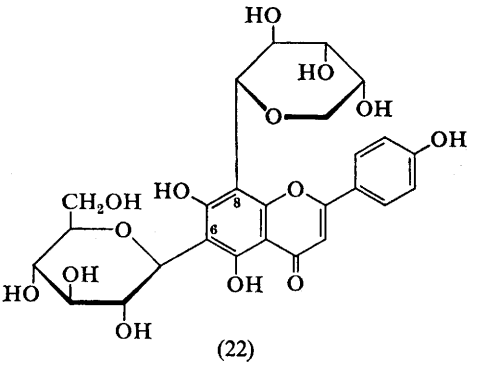
[126] M. L. Wolfrom and S. Inouye, *Carbohydrate Res.*, 1975, **41**, 117.
[127] C. L. Baimbridge, C. D. Mickey, and R. A. Zingaro, *J.C.S. Perkin I*, 1975, 1395.

C-Glycosides

The dihydroxybenzene *C*-glycoside (21) (1,3-dideazauridine) has been synthesized by a standard procedure,[128] and *C*-arabinosylation of vitexin has yielded 6-*C*-α-L-arabinopyranosyl-8-*C*-β-D-glucopyranosylapigenin,[129] which proved to be identi-



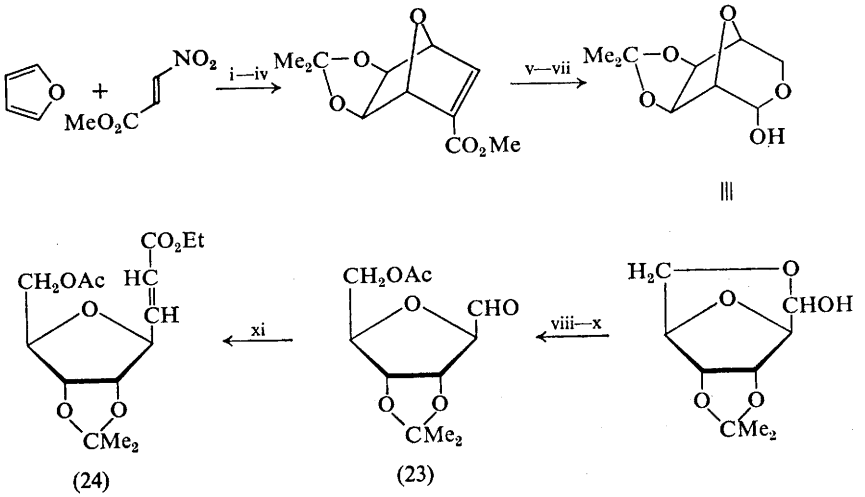
(21)



(22)

cal with one of the products obtained from acid-catalysed isomerization of schaftoside, an apigenin di-*C*-glycoside identified as (22).[130]

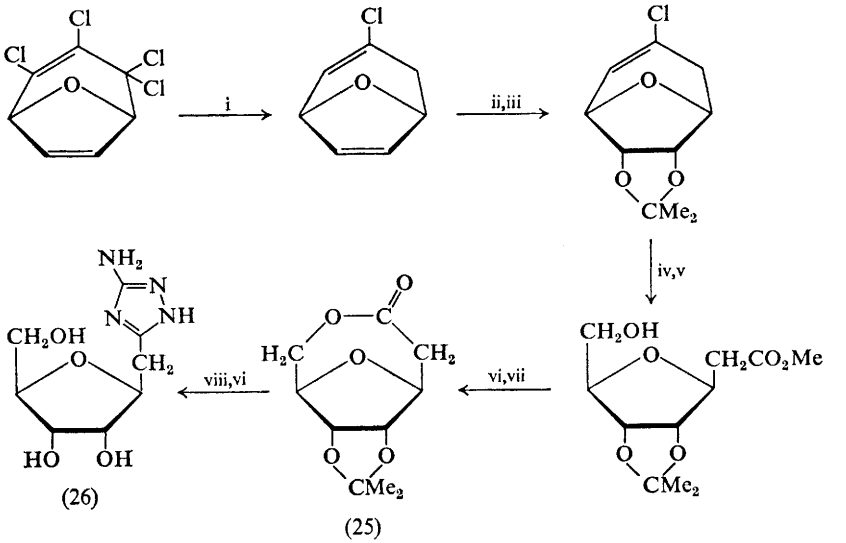
The 2,5-anhydro-DL-allose derivative (23) has been prepared by the reactions outlined in Scheme 11; although (23) is unstable, it could be converted into stable



Reagents: i, △; ii, OsO_4–H_2O_2; iii, Me_2CO–H^+; iv, DBU; v, O_3; vi, $NaBH_4$; vii, $NaIO_4$; viii, $MeNHCH_2CH_2OH$; ix, Ac_2O–py; x, H^+; xi, $Ph_3P{=}CHCO_2Et$

Scheme 11

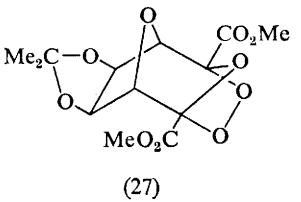
[128] R. A. Sharma, M. Bobek, and A. Bloch, *J. Medicin. Chem.*, 1975, **18**, 473.
[129] M. C. Biol, M. L. Bouillant, G. Planche, and M. J. Chopin, *Compt. rend.*, 1974, **279**, C, 409.
[130] M. J. Chopin, M. L. Bouillant, H. Wagner, and K. Galle, *Phytochemistry*, 1974, **13**, 2583.

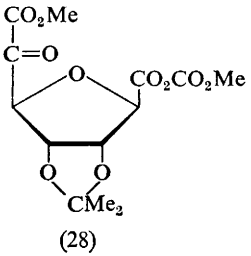


Reagents: i, $LiAlH_4$–LiH; ii, OsO_4–H_2O_2; iii, Me_2CO–H^+; iv, O_3–MeOH; v, $NaBH_4$; vi, H^+; vii, Ac_2O–py; viii, $NH_2N{=}C{=}NNH_2$

Scheme 12

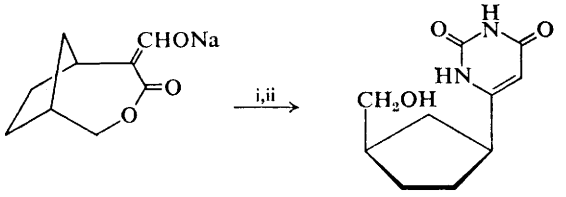
derivatives and, by a Wittig reaction, into (24).[131] A related lactone (25), prepared by the sequence of reactions shown in Scheme 12, was converted into the racemic *C*-homonucleoside (26).[132] Thermolysis of the racemic tricyclic ozonide (27) furnished mainly a hexulosonic acid derivative (see Chapter 17), but the *C*-glycoside (28) was obtained as a minor product.[133] The simple *C*-glycoside (29), a





carbocyclic derivative of 2′,3′-dideoxy-ψ-uridine, has been synthesized (Scheme 13) in order to assist in the interpretation of several sets of apparently incompatible spectroscopic data.[134] It was concluded that the 5′-hydroxymethyl group in ψ-uridine does not interact with the pyrimidine ring, but that the ring-oxygen atom influences the physicochemical properties of the pyrimidine ring of α- and β-nucleosides to different extents.

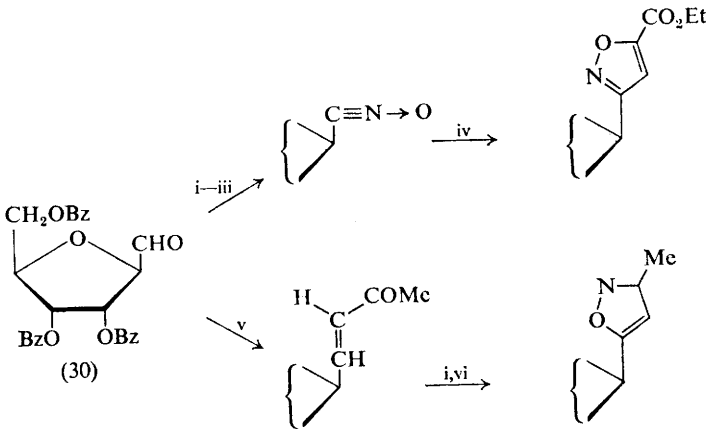
[131] G. Just, A. Martel, K. Grozinger, and M. Ramjeesingh, *Canad. J. Chem.*, 1975, **53**, 131.
[132] W. J. Gensler, S. Chan, and D. B. Ball, *J. Amer. Chem. Soc.*, 1975, **97**, 436.
[133] G. Just and K. Grozinger, *Canad. J. Chem.*, 1975, **53**, 2701.
[134] A. J. Playtis and J. D. Fissekis, *J. Org. Chem.*, 1975, **40**, 2488.



(29)

Reagents: i, NH_2CSNH_2; ii, H^+

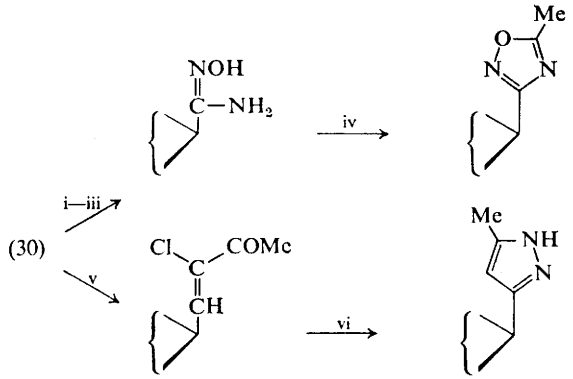
Scheme 13



(30)

Reagents: i, NH_2OH; ii, Cl_2; iii, Et_3N; iv, $HC{\equiv}CCO_2Et$; v, $Ph_3P{=}CHCOMe$; vi, I_2

Scheme 14

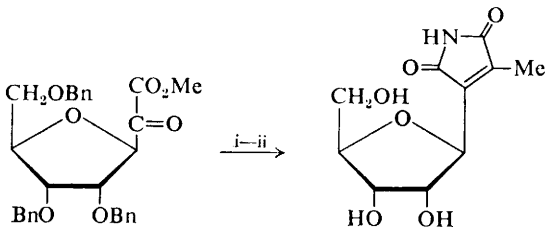


(30)

Reagents: i, NH_2OH; ii, Cl_2; iii, NH_3; iv, Ac_2O; v, $Ph_3P{=}CClCOMe$; vi, NH_2NH_2–AcOH

Scheme 15

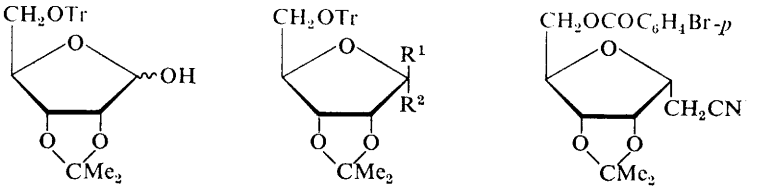
Moffatt's group has used the 2,5-anhydro-D-allose tribenzoate (30) to prepare D-ribofuranosyl-isoxazoles (Scheme 14),[135] -1,2,4-oxadiazoles, and -pyrazoles (Scheme 15).[136] Related approaches to the synthesis of *C*-nucleosides have been described; *e.g.* the final stages of a synthesis of 3-methylshowdomycin are shown in Scheme 16,[137] and the sugar derivative (31) was converted into the anomeric



Reagents: i, $Me_2PhP{=}C(Me)CONH_2$; ii, BCl_3

Scheme 16

C-glycosides (32) and (33) using a Wittig reaction.[138] The β-glycoside (32) is formed initially, but it anomerizes to the α-glycoside (33) under the basic conditions of the reaction. Similar reactions were carried out with 2,3:5,6-di-*O*-isopropylidene-D-manno- and -allo-furanose. Condensation of 2,3,5-tri-*O*-benzyl-D-ribofuranose with carbomethoxymethylenetriphenylphosphorane produced a

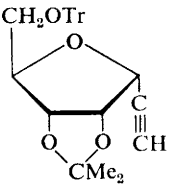


(31) (32) $R^1 = CH_2CO_2Me$; $R^2 = H$ (34)
(33) $R^1 = H$; $R^2 = CH_2CO_2Me$

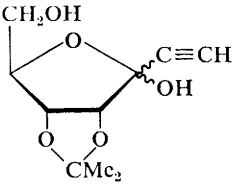
mixture of *cis*- and *trans*-olefins, which rapidly cyclized to furanosyl *C*-glycosides on treatment with base. The structures of the cyclized products assigned by ^1H and ^{13}C n.m.r. spectroscopy were confirmed by comparison with compound (34) of established α-configuration.

Buchanan's group has published details of the reaction of 2,3-*O*-isopropylidene-D-ribofuranose with ethynylmagnesium bromide to yield 1,2-dideoxy-4,5-*O*-isopropylidene-D-*allo*-hept-1-ynitol, which was converted into its 7-trityl ether.[139] Treatment of this trityl ether with toluene-*p*-sulphonyl chloride in pyridine furnished 2,3-*O*-isopropylidene-5-*O*-trityl-α-D-ribofuranosylethyne (35).

[135] H. P. Albrecht, D. B. Repke, and J. G. Moffatt, *J. Org. Chem.*, 1975, **40**, 2143.
[136] D. B. Repke, H. P. Albrecht, and J. G. Moffatt, *J. Org. Chem.*, 1975, **40**, 2481.
[137] G. Trummlitz, D. B. Repke, and J. G. Moffatt, *J. Org. Chem.*, 1975, **40**, 3352.
[138] H. Ohrui, G. H. Jones, J. G. Moffatt, M. L. Maddox, A. T. Christensen, and S. K. Byram, *J. Amer. Chem. Soc.*, 1975, **97**, 4602.
[139] J. G. Buchanan, A. D. Dunn, and A. R. Edgar, *J.C.S. Perkin I*, 1975, 1191.

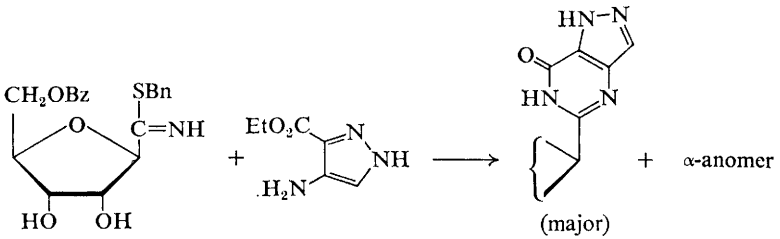


(35)



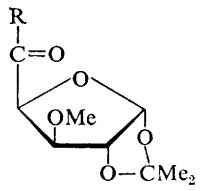
(36)

Other transformations yielding 2,3-*O*-isopropylidene-5-*O*-trityl-α- and -β-L-lyxo- and -β-D-ribo-furanosylethynes and (36) were reported. This group has also described the synthesis of D-ribofuranosyl derivatives of methyl propiolate (*e.g.* methyl 4,7-anhydro-5,6,8-tri-*O*-benzyl-2,3-dideoxy-D-*allo*-oct-2-ynoate), which

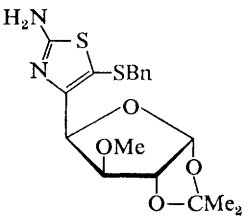


(major)

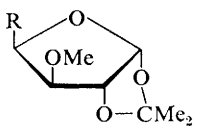
Scheme 17



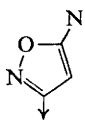
(37) R = CH_2SBn
(38) R = H



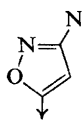
(39)



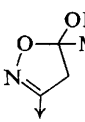
(40) R =



(41) R =



(42) R =



should readily undergo cycloadditions (*e.g.* with benzylazide) and thus make available a range of *C*-nucleosides [139a] (see also Chapter 21).

Structural analogues of the formycins have been reported in a paper describing a general route to 2-glycosylated pyrimidinones (see Scheme 17).[140] Treatment of formycin with *NN*-dimethylformamide dineopentyl acetal gave the 2,5′-cyclonucleoside as one of the products, and 2′,3′-*O*-isopropylideneformycin was converted into the 4,5′-cyclonucleoside by way of the 5′-toluene-*p*-sulphonate.[141] Adenosine deaminase caused deamination of the 2,5′-cyclonucleoside only, indicating that the *anti*-conformation of a substrate is utilized by the enzyme.

Tronchet's group have synthesized a number of aldofuranosyl derivatives containing a heterocyclic residue at C-4; for example, (37)[142] and (38)[143] were transformed into (39) and into (40)—(42), respectively, in simple, two-step sequences.

Structural investigations on the *C*-β-D-glucuronides of sulphinpyrazone and phenylbutazone have been reported.[144] Other compounds related to the *C*-glycosides are mentioned in Chapters 4 and 17.

[139a] J. G. Buchanan, A. R. Edgar, M. J. Power, and G. C. Williams, *J.C.S. Chem. Comm.*, 1975, 501.
[140] T. Huynh-Dinh, A. Kolb, C. Gouyette, and J. Igolen, *J. Org. Chem.*, 1975, **40**, 2825.
[141] J. Žemlička, *J. Amer. Chem. Soc.*, 1975, **97**, 5896.
[142] J. M. J. Tronchet and H. Eder, *Helv. Chim. Acta*, 1975, **58**, 1507.
[143] J. M. J. Tronchet, O. Martin, J.-B. Zumwald, N. Le-Hong, and F. Perret, *Helv. Chim. Acta*, 1975, **58**, 1735.
[144] W. J. Richter, K. O. Alt, W. Dieterle, J. W. Faigle, H.-P. Kriemler, H. Mory, and T. Winkler, *Helv. Chim. Acta*, 1975, **58**, 2512.

4
Ethers and Anhydro-sugars

Ethers

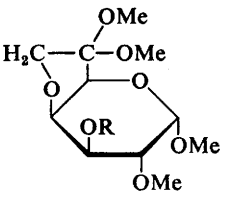
Methyl Ethers.—The methylation of partially acetylated sugars (*e.g.* methyl 2,3-di-*O*-acetyl-α-D-glucopyranoside, cellulose acetate) has been accomplished with methyl trifluoromethanesulphonate in the presence of a sterically hindered, weak base (*e.g.* 2,6-di-t-butylpyridine).[145] Another novel procedure for methylation has used methyl iodide and anhydrous aluminium oxide in DMF.[146] This procedure is claimed to be better than the modified Purdie procedure for the methylation of free sugars, disaccharides, and aldobiouronic acids.

The following methyl ethers of monosaccharides have been synthesized during the past year: 2,4-di-*O*-methyl-D-lyxose (*via* methylation of methyl 3-*O*-benzoyl-α-D-lyxopyranoside with diazomethane–boron trifluoride etherate),[147] 2,3,4-tri-*O*-methyl-D-allose (by solvolysis of 1,6-anhydro-2,4-di-*O*-benzoyl-3-*O*-methanesulphonyl-β-D-glucopyranose, saponification of the product, methylation, and hydrolysis),[148] 2-*O*-methyl-D-mannose [*via* methylation of methyl 4,6-*O*-benzylidene-3-*O*-(2,6-dimethoxybenzoyl)-α-D-mannopyranoside],[149] 2-*O*-methyl-D-galactose [*via* partial methylation of benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside with diazomethane and either stannous chloride dihydrate or boron trifluoride etherate(methanolate) as catalyst],[150] and 2,4-di-*O*-methyl-D-galactose (*via* methylation of methyl 3-*O*-benzyl-2-*O*-methyl-6-*O*-trityl-α-D-galactopyranoside).[151] The following methyl ethers have been synthesized in an investigation of the action of emulsin on carbohydrate derivatives: 2-, 3-, and 4-mono-, 4,6-di-, and 2,4,6- and 2,3,4-tri-*O*-methyl phenyl β-D-glucopyranoside.[152] Selected methyl ethers of sucrose, including 4- and 6′-mono-, 1′,6′-, 4,6-, 4,6′-, and 6,6′-di-, and 1′,4,6′- and 1,6,6′-tri-*O*-methylsucrose, have been prepared by the methylation of partially acylated sucroses using diazomethane–boron trifluoride etherate.[153]

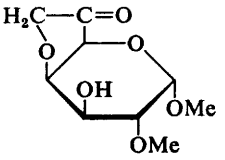
An improved synthesis of methyl (methyl 2,4-di-*O*-methyl-α-D-galactopyranosid)uronate has been achieved from the corresponding 2-*O*-methyl ether, which was converted into methyl [methyl 2-*O*-methyl-3-*O*-(4-nitrobenzoyl)-α-D-galactopyranosid]uronate before methylation with diazomethane–boron tri-

[145] J. Arnap, L. Kenne, B. Lindberg, and J. Lönngren, *Carbohydrate Res.*, 1975, **44**, C5.
[146] S. Bose and P. L. Soni, *Sci. Cult.* (*Calcutta*), 1974, **40**, 368 (*Chem. Abs.*, 1975, **82**, 58 029a).
[147] S. A. Abbas, A. H. Haines, and A. G. Wells, *Carbohydrate Res.*, 1975, **42**, 362.
[148] J. S. Brimacombe and L. C. N. Tucker, *Carbohydrate Res.*, 1975, **40**, 387.
[149] S. A. Abbas and A. H. Haines, *Carbohydrate Res.*, 1975, **41**, 298.
[150] G. J. F. Chittenden, *Carbohydrate Res.*, 1975, **43**, 366.
[151] P. Kováč and R. Brežný, *Chem. Zvesti*, 1975, **29**, 544.
[152] P. Nanasi and A. Liptak, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 63 (*Chem. Abs.*, 1975, **82**, 98 325a).
[153] M. G. Lindley, G. G. Birch, and R. Khan, *Carbohydrate Res.*, 1975, **43**, 360.

fluoride etherate.[154] The by-products of the methylation step were identified as derivatives of methyl 4,7-anhydro-α-D-*galacto*-heptopyranosid-6-ulose dimethyl acetal [*e.g.* (43)]; debenzoylation of (43) followed by hydrolysis with acid afforded



(43) R = $COC_6H_4NO_2$-*p*



(44)

the heptopyranosid-6-ulose (44). Methyl (methyl 2,4-di-*O*-methyl-α-D-galactopyranosid)uronate has also been synthesized *via* oxidation of methyl 3-*O*-benzyl-2,4-di-*O*-methyl-α-D-galactopyranoside,[151] and unambiguous syntheses of the 2-, 3-, and 4-*O*-methyl ethers of this uronic acid have been reported.[154a]

Adenosine reacted with diazomethane in 1,2-dimethoxyethane to give methylated and ethylated derivatives, the methyl groups originating from the solvent.[155] The partial methylation of methyl 4,6-dideoxy-α-D-*xylo*- and -L-*lyxo*-hexopyranoside using methyl iodide and sodium hydroxide in acetonitrile–DMF has been examined.[156] Differences in the rates of methylation of the hydroxy-groups were interpreted in terms of steric factors and intramolecular hydrogen-bonding.

Lindley and Birch have attempted to ascertain why certain sugars are so sweet, recognizing that sweetness is a function of the hydroxy-groups.[157] Using partially methylated derivatives of αα-trehalose, they were able to show that only half of the αα-trehalose molecule participates in the sensory response. It was also found that methyl ethers of methyl α-D-glucopyranoside are more bitter than the parent glycoside, presumably because of their increased lipophilicity.

The action of 6-*O*-alkyl-D-glucoses and -galactoses in inhibiting the transport of sugars across human erythrocyte membranes has been investigated.[41]

Equilibrated aqueous solutions of 3-*O*-methyl-D-xylose and -glucose have been shown to contain about 15% of furanoid forms, whereas none is found in those of the parent aldoses.[15]

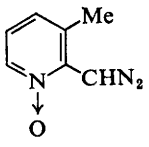
The characterization of methyl tri-*O*-methyl-D-glucopyranosides (obtained by methanolysis of methylated polysaccharides) is referred to in Chapter 26, and the reduction of methylthiomethyl ethers (formed during DMSO-based oxidations) to methyl ethers is noted in Chapter 22.

Other Alkyl and Aryl Ethers.—A temporary, non-participating 2-*O*-allyl group has been used in the synthesis of benzyl 6-*O*-(2-*O*-allyl-3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-α-D-galactopyranoside; its removal was effected by isomerization and hydrolysis.[158]

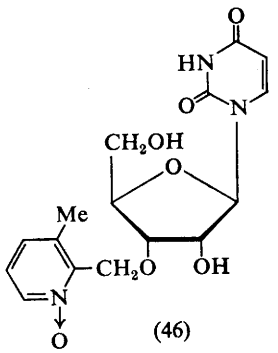
[154] P. Kováč, R. Palovčík, V. Kováčik, and J. Hirsch, *Carbohydrate Res.*, 1975, **44**, 205.
[154a] J. Hirsch, P. Kováč, and V. Kováčik, *Chem. Zvesti*, 1975, **29**, 833.
[155] L. M. Pike, M. K. A. Khan, and F. Rottman, *J. Org. Chem.*, 1974, **39**, 3674.
[156] K. Kefurt, J. Stanek, jun., Z. Kefurtová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 300.
[157] M. G. Lindley and G. G. Birch, *J. Sci. Food Agric.*, 1975, **26**, 117.
[158] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1975, 361.

Partial benzylation of methyl 2,3-di-*O*-benzyl-α-D-galactopyranoside gave principally methyl 2,3,6-tri-*O*-benzyl-α-D-galactopyranoside, whereas the isomeric 2,6-di-*O*-benzyl ether gave a mixture of methyl 2,4,6- and 2,3,6-tri-*O*-benzyl-α-D-galactopyranosides in the ratio of *ca.* 4 : 1.[159] In both methyl 2,6-di-*O*-benzyl-α- and -β-D-galactopyranosides, the axial hydroxy-group at C-4 is more reactive than that at C-3 towards benzylation. A combination of allylation, benzylation, and standard deprotection procedures has been used to prepare all the possible mono-, di-, and tri-*O*-benzylated derivatives of methyl α-D-glucopyranoside.[160] Reductive cleavage of benzylidene acetals to give benzyl ethers is noted in Chapter 5, and photolysable 2-nitrobenzyl ethers of nucleosides are referred to in Chapter 21.

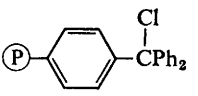
The use of 1-oxido-3-methyl-2-pyridyldiazomethane (45) has been recommended for the synthesis of dinucleotides; the reagent reacts with ribonucleosides to give a mixture of the corresponding 2′- and 3′-ethers [*e.g.* (46)] that can be



(45)



(46)



(47)

separated by either fractional crystallization or chromatography.[161] Such ethers are unaffected by esterification reactions (notably phosphorylation) and can be removed by treatment with ammonia in methanol. 4-Methoxytrityl ethers of nucleotides have been cleaved using the naphthalene radical-anion in HMPT.[162]

A 'polymer-bound' tritylating reagent (47) has been used to protect the primary hydroxy-group of methyl α-D-glucopyranoside, which was then benzoylated, *etc.*, to give methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside.[163]

Several 2,4-dinitrophenyl ethers of D-glucose have been prepared; these ethers are stable towards acids but not towards bases, and are liable to migrate under very weakly basic conditions.[164] The use of 2,3-diphenyl-2-cyclopropenyl ethers in the synthesis of disaccharides has been noted in Chapter 3.

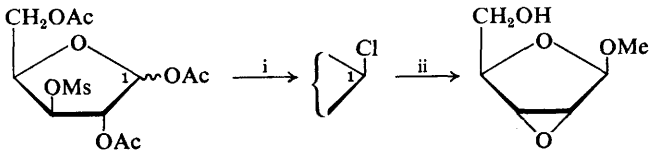
Silyl Ethers.—t-Butyldiphenylsilyl chloride reacts preferentially with the primary hydroxy-group of sugar derivatives (*e.g.* methyl α-D-glucopyranoside), although secondary hydroxy-groups also react if sufficient reagent is used.[165] t-Butyl-

[159] H. M. Flowers, *Carbohydrate Res.*, 1975, **39**, 245.
[160] J. M. Küster and I. Dyong, *Annalen*, 1975, 2179.
[161] Y. Mizuno, T. Endo, and K. Ikeda, *J. Org. Chem.*, 1975, **40**, 1385.
[162] G. L. Greene and R. L. Letsinger, *Tetrahedron Letters*, 1975, 2081.
[163] J. M. J. Fréchet and K. E. Haque, *Tetrahedron Letters*, 1975, 3055.
[164] A. Klemer and L. Kleefeldt, *Annalen*, 1975, 1658.
[165] S. Hanessian and P. Lavallee, *Canad. J. Chem.*, 1975, **53**, 2975.

diphenylsilyl ethers are more stable than other silyl ethers and are unaffected by conditions that hydrolyse trityl, t-butyldimethylsilyl, and tetrahydropyranyl ethers. They were also shown to be stable to the usual conditions of hydrolysis and hydrogenolysis used in the removal of acetal and benzyl groups, respectively. The protection of polyhydroxy-compounds by t-butyldimethylsilylation has serious limitations where there is the possibility of acyl migration.[166] Thus, migration of one of the acyl groups occurred when tetrabutylammonium fluoride in THF was used to desilylate 2-*O*-(t-butyldimethylsilyl)-1,3-di-*O*-stearoyl-glycerol. Cleavage of this silyl group can also occur during hydrogenolysis of benzyl ethers.

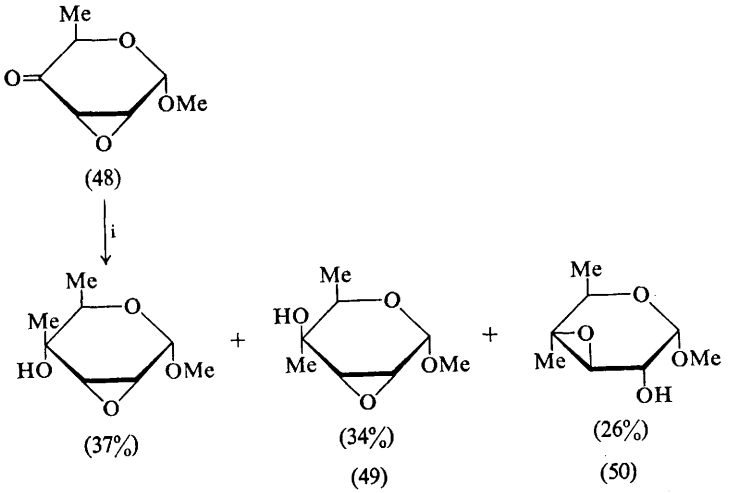
Intramolecular Ethers (Anhydro-sugars)

Oxirans.—A convenient synthesis of methyl 2,3-anhydro-β-D-ribofuranoside is shown in Scheme 18.[167] Several oxirans of branched-chain sugars have been obtained by reaction of methyl-lithium with the hexopyranosid-4-ulose (48) (Scheme 19).[168] Equilibration of either (49) or (50) with base gave a mixture



Reagents: i, HCl; ii, MeONa–MeOH

Scheme 18

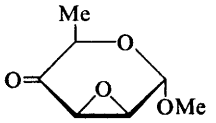


Reagent: i, MeLi

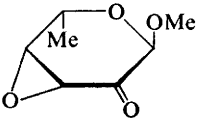
Scheme 19

[166] G. H. Dodd, B. T. Golding, and P. V. Ioannou, *J.C.S. Chem. Comm.*, 1975, 249.
[167] J. A. Montgomery and S. D. Clayton, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 147.
[168] H. Paulsen and K. Eberstein, *Tetrahedron Letters*, 1975, 1495.

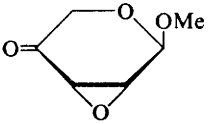
containing (49) (8%) and (50) (92%); thus, the more substituted oxiran is preferred. Similar studies on (51)—(53) were also reported. Other related derivatives and α-nitro-oxirans are mentioned in Chapters 16 and 10, respectively.



(51)



(52)



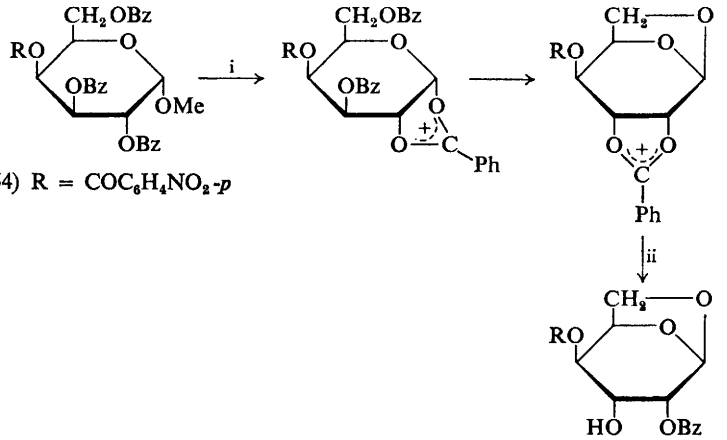
(53)

Methyl 6-deoxy-β-D-glucopyranoside 2- and 4-toluene-*p*-sulphonates have been converted into the expected 2,3- and 3,4-oxirans, respectively, using a basic resin; neither oxiran underwent migration under the reaction conditions used.[169]

An ^{18}O-labelling study of the deamination of 2-amino-2-deoxy-D-mannose has shown that at least 62% of the D-glucose formed arises from 1,2-anhydro-D-glucopyranose, whereas the rest results from nucleophilic attack of water at C-2 of the diazonium ion.[170]

Dioxirans derived from hexitols and tetritols have been used as cross-linking reagents for DNA.[170a]

Other Anhydrides.—The sequence shown in Scheme 20 has provided a novel route



(54) R = $COC_6H_4NO_2$-*p*

Reagents: i, HF; ii, H_2O

Scheme 20

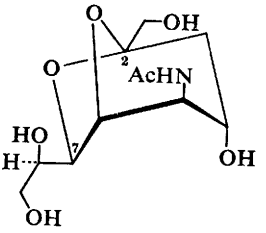
to derivatives of 1,6-anhydro-β-D-gulopyranose from the D-galactoside tetra-ester (54); ^{13}C n.m.r. spectroscopy was used to identify the intermediate benzoxonion ions.[171] Thermolysis of 3-*O*-acetyl-α-D-xylopyranose 1,2,4-orthoacetate in

[169] J. Staněk, K. Čapek, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3370.
[170] J. W. Llewellyn and J. M. Williams, *Carbohydrate Res.*, 1975, **42**, 168.
[170a] L. Ötvös and I. Elekes, *Tetrahedron Letters*, 1975, 2477, 2481.
[171] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1975, **29**, 181.

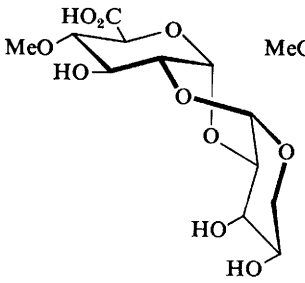
chlorobenzene has been found to yield 2,3-di-*O*-acetyl-1,5-anhydro-β-D-xylofuranose (13%),[7] while treatment of 3,4,6-tri-*O*-benzyl-α-D-glucopyranose 1,2-(2-ethyl orthoacetate) with mercuric bromide in nitromethane furnished 2-*O*-acetyl-1,6-anhydro-3,4-di-*O*-benzyl-β-D-glucopyranose by intramolecular glycosidation.[172]

Opening of the anhydro-ring of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose with titanium tetrachloride and alkoxytitanium trichlorides has been examined.[173, 174] Data have been presented to show that 1,6-anhydro-3,4-dideoxy-β-D-*glycero*-hex-3-enopyranos-2-ulose(levoglucosenone) is not principally derived from 1,6-anhydro-β-D-glucopyranose on acid-catalysed pyrolysis of cellulose.[175] It appears that 1,6-anhydro-β-D-glucopyranose itself is not a primary product of cellulose pyrolysis, so that the principal mechanism of its formation must involve a precursor still to be identified.

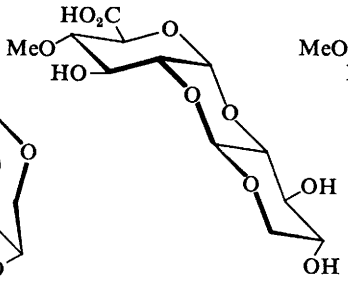
5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranose has been shown to exist in equilibrium with the 2,7-anhydride (55) in the presence of dilute



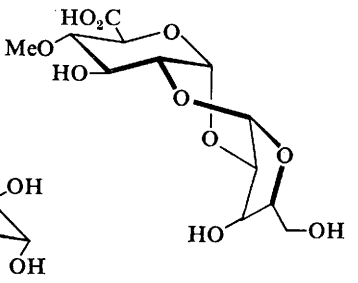
(55)



(56)



(57)



(58)

aqueous acids.[176] Three previously unknown acids present in hydrolysates of 4-*O*-methylglucuronoxylan have been isolated and identified as 4-*O*-methyl-α-D-glucopyranosyluronic acid α- and β-D-xylopyranose 1,2′:2,1′-dianhydrides [(56)

[172] N. N. Malysheva, V. I. Torgov, E. M. Klimov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2153.

[173] L. Fenichel, G. Deak, P. Bako, S. Holly, and Z. Csuros, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 313 (*Chem. Abs.*, 1975, **83**, 164 445p).

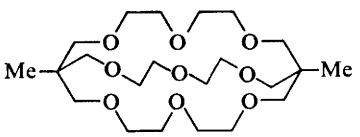
[174] L. Fenichel, G. Deak, S. Holly, P. Bako, and Z. Csuros, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 299 (*Chem. Abs.*, 1975, **83**, 131 850p).

[175] A. Broido, M. Evett, and C. C. Hodges, *Carbohydrate Res.*, 1975, **44**, 267.

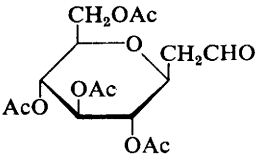
[176] S. K. Gross, J. E. Evans, V. N. Reinhold, and R. H. McCluer, *Carbohydrate Res.*, 1975, **41**, 344.

and (57), respectively] and 4-*O*-methyl-α-D-glucopyranosyluronic acid α-D-xylofuranose 1,2′:2,1′-dianhydride (58).[177] These anhydrides are in equilibrium with 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylose in acid media.

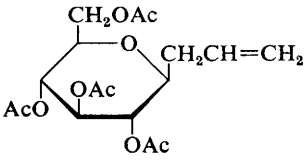
1,4-Anhydroerythritol has been converted into 1,4-anhydro-DL-threitol by chloride displacement of a toluene-*p*-sulphonyloxy-group.[178] The dimethyl [2]-cryptand (59) has been obtained in a five-step synthesis from pentaerythritol, and was shown to form strong complexes with potassium ions in deuteriochloroform-carbon disulphide solution.[179]



(59)



(61)



(60)

The *C*-allyl β-D-glucopyranoside tetra-acetate (60) has been converted into 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-D-*glycero*-D-*gulo*-octose (61) by sequential oxidations with osmium tetroxide and periodic acid.[180] Compound (61) closely resembles a number of the *C*-glycosides referred to in Chapter 3.

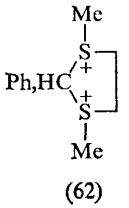
[177] K. Larsson, *Carbohydrate Res.*, 1975, **44**, 199.
[178] J. C. Goodwin and J. E. Hodge, *Carbohydrate Res.*, 1975, **44**, 106.
[179] A. C. Coxon and J. F. Stoddart, *Carbohydrate Res.*, 1975, **44**, C1.
[180] Yu. A. Zhdanov, L. A. Uzlova, and Z. I. Glebova, *Zhur. obshchei Khim.*, 1974, **44**, 2049.

5
Acetals

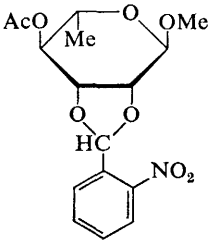
Synthesis, Properties, and Reactions

Benzylidene acetals can be formed under essentially neutral conditions using the bis-sulphonium ion (62) obtained by methylation of benzaldehyde ethylene dithioacetal with methyl fluorosulphonate (Magic Methyl®).[181] Thus, methyl α-D-glucopyranoside afforded a 4,6-*O*-benzylidene derivative (25%) when treated with (62) in pyridine. In the absence of an acid catalyst, it is unlikely that the thermodynamically favoured acetal would be obtained in high yield.

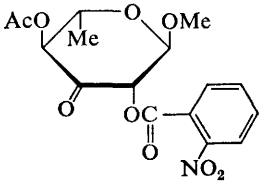
Collins *et al.* have reported details of their interesting work on the photochemical rearrangement of 2-nitrobenzylidene acetals. Derivatives of methyl 4,6-*O*-(2-nitrobenzylidene)glycopyranosides rearranged upon u.v.-irradiation in methanol to give the corresponding 4- and 6-(2-nitrosobenzoates), which were converted into the 4- and 6-(2-nitrobenzoates) on oxidation with trifluoroperacetic acid.[182] Thus, methyl 2,3-di-*O*-acetyl-4,6-*O*-(2-nitrobenzylidene)-α- and -β-D-



(62)



(63)



(64)

galactopyranosides afforded the corresponding 4- and 6-(2-nitrobenzoates) in the ratio of *ca.* 7 : 3, whereas the diacetates of methyl α- and β-D-glucopyranoside and methyl α-D-mannopyranoside 4,6-*O*-(2-nitrobenzylidene) acetals gave the 4- and 6-(2-nitrobenzoates) in the ratio of *ca.* 3 : 7. Related irradiations, *etc.*, were carried out on 2,3- and 3,4-*O*-(2-nitrobenzylidene)-glycopyranosides, which usually gave one of the possible isomers in high yield.[183] Thus, the 2,3-*O*-(2-nitrobenzylidene) derivatives of methyl 4-*O*-acetyl-α-L-rhamnopyranoside [*i.e.* (63)] and methyl 4,6-di-*O*-methyl-β-D-allopyranoside gave the 2- and 3-(2-nitrobenzoates), respectively. Identification of the photo-

[181] R. M. Munavu and H. H. Szmant, *Tetrahedron Letters*, 1975, 4543.
[182] P. M. Collins, N. N. Oparaeche, and V. R. N. Munasinghe, *J.C.S. Perkin I*, 1975, 1700.

products [*e.g.* from (63)] was accomplished by oxidation with ruthenium tetraoxide to the pyranosiduloses [*e.g.* (64)], whose structures could be assigned by ^{1}H n.m.r. spectroscopy.

Acetal-ring cleavage of 3-*O*-benzyl- and 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-gluco- and -manno-pyranoside derivatives with a mixture of lithium aluminium hydride and aluminium trichloride afforded the corresponding 4-*O*-benzyl ethers.[183a] The direction of cleavage is determined by steric factors imposed by the substituent at C-3; with 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-galacto-pyranoside derivatives, for example, the ratio of the resulting 4- and 6-*O*-benzyl ethers was *ca.* 9 : 1, whereas substitution at O-3 with a group (*e.g.* methyl) less bulky than benzyl favoured the formation of 6-*O*-benzyl ethers.

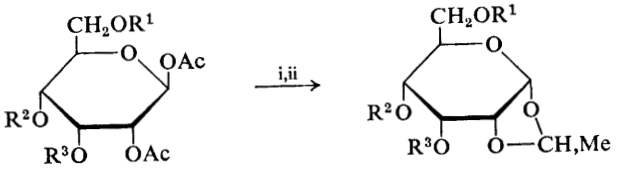
The ozonolysis of benzylidene acetals to give benzoylated compounds is discussed in Chapter 6.

The concentrations of acetic acid and acetic anhydride used in the acid-catalysed acetolysis of 1,2-*O*-isopropylidenealdofuranoses have been shown to influence the proportions of acyclic by-products formed (see Vol. 7, p. 46). The formation of acyclic compounds can be suppressed by decreasing the concentration of acetic anhydride, although the configuration of the starting material also affects the outcome of acetolysis.[184]

Acetonation of D-ribose and D-arabinose using ethyl (or methyl) isopropenyl ether in DMF containing a trace of toluene-*p*-sulphonic acid has been reported.[185] D-Ribose yielded principally 3,4-*O*-isopropylidene-β-D-ribopyranose, together with other minor products such as 2,3-*O*-isopropylidene-D-ribofuranose, and D-arabinose gave mostly 3,4-*O*-isopropylidene-β-D-arabinopyranose and some 1,2:3,4-diacetal. D-Fucose and 6-*O*-methyl-D-galactose afforded 3,4-*O*-isopropylidene derivatives, in 40—60% yield, on treatment with acetone and anhydrous cupric sulphate.[186] D-Ribose also yielded a 3,4-*O*-isopropylidene derivative (15—20%) on similar treatment, whereas 4,6-*O*-isopropylidene-D-glucose was obtained in low yield when D-glucose reacted in the presence of DMF.

Two of the four possible diastereoisomeric 2,3:5,6-di-*O*-ethylidene-β-D-allofuranoses have been isolated following acid-catalysed condensation of D-allose with acetaldehyde.[187] Examination of the diastereoisomers and derivatives thereof by ^{1}H n.m.r. spectroscopy established the configuration of the C-2′ atom in the 2,3-*O*-ethylidene ring as *R* and as either *R* or *S* in the other acetal ring. Condensation of 3-*O*-benzyl-D-allose with acetaldehyde gave, after acetylation, 1,2-di-*O*-acetyl-3-*O*-benzyl-4,6-*O*-ethylidene-β-D-allopyranose, 5,6-di-*O*-acetyl-3-*O*-benzyl-1,2-*O*-(*R*)-ethylidene-α-D-allofuranose, and two diastereoisomeric 3-*O*-benzyl-1,2(*R*):5,6-di-*O*-ethylidene-α-D-allofuranoses.[188] An alternative route to related diastereoisomeric 1,2-*O*-ethylidene-α-D-allopyranoses, *via* reduction of acetoxonium-ion intermediates, is shown in Scheme 21, while acid-catalysed condensation of methyl 4,6-*O*-benzylidene-α-D-allopyranoside

[183] P. M. Collins and N. N. Oparaeche, *J.C.S. Perkin I*, 1975, 1695.
[183a] A Lipták, I. Jodál, and P. Nánási, *Carbohydrate Res.*, 1975, **44**, 1.
[184] K. Bischofberger and R. H. Hall, *Carbohydrate Res.*, 1975, **42**, 175.
[185] J. Gelas and D. Horton, *Carbohydrate Res.*, 1975, **45**, 181.
[186] S. Morgenlie, *Carbohydrate Res.*, 1975, **41**, 77.
[187] W. E. Dick, jun. and D. Weisleder, *Carbohydrate Res.*, 1975, **39**, 87.
[188] W. E. Dick, jun., D. Weisleder, and J. E. Hodge, *Carbohydrate Res.*, 1975, **42**, 55.



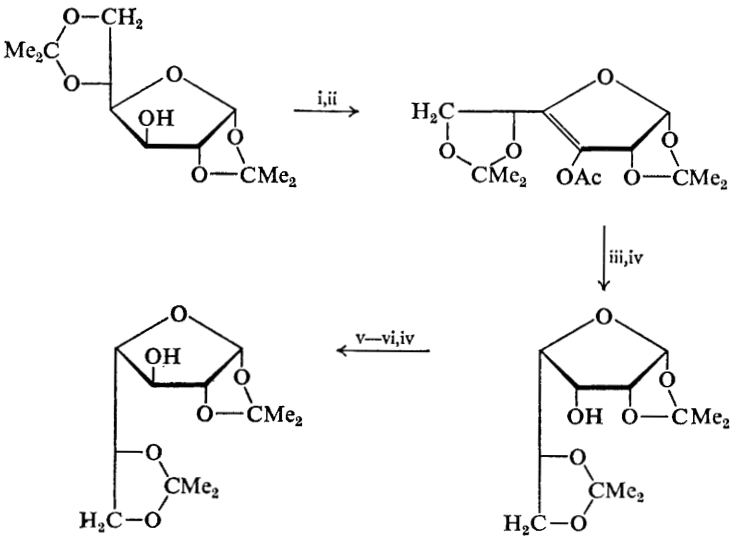
$R^1 = R^2 = R^3 = Ac$
$R^1 = R^2 = Ac, R^3 = Bn$
$R^1,R^2 = CHMe, R^3 = Bn$

Reagents: i, $AlCl_3$; ii, $NaBH_4$

Scheme 21

with acetaldehyde furnished diastereoisomeric 2,3:4,6-di-*O*-ethylidene derivatives.[189]

Stevens has reported details of his work on the acetonation of D-glucose using 4% sulphuric acid as the catalyst [190] (see Vol. 3, p. 38). In addition to 1,2:5,6- and 1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose, the reaction yielded 1,2:3,4- and 2,3:4,5-di-*O*-isopropylidene-D-glucoseptanoses. D-Glucose reacted with acetone–cupric sulphate in the presence of 20% of DMF to give several products, including 4,6- and 5,6-*O*-isopropylidene-D-glucose.[191] Similar treatment of D-galactose gave 1,2:5,6- and 1,2:3,4-di- and 5,6-, 3,4-, and 4,6-*O*-isopropylidene derivatives.[192]



Reagents: i, RuO_2–IO_4^-; ii, Ac_2O–py; iii, H_2–Pd–C; iv, HO^-; v, TsCl–py; vi, $Bu_4\overset{+}{N}{}^-OBz$

Scheme 22

[189] W. E. Dick, jun., D. Weisleder, and J. E. Hodge, *Carbohydrate Res.*, 1975, **42**, 65.
[190] J. D. Stevens, *Austral. J. Chem.*, 1975, **28**, 525.
[191] S. Morgenlie, *Acta Chem. Scand.* (*B*), 1975, **29**, 278.
[192] S. Morgenlie, *Acta Chem. Scand.* (*B*), 1975, **29**, 367.

An alternative route affording 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose is illustrated in Scheme 22.[193]

Various *N*-substituted 2-amino-2-deoxy-D-glucoses reacted with 2,2-dimethoxypropane (or 2,2-dibenzyloxypropane)–DMF in the presence of toluene-*p*-sulphonic acid at room temperature to give 4,6-*O*-isopropylidene derivatives.[194] However, treatment of 2-acetamido-2-deoxy-D-glucose with 2,2-dibenzyloxypropane at 80 °C also afforded benzyl 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside. Acid-catalysed acetonation of D-*glycero*-D-*galacto*-heptose yielded the 1,2:3,4:6,7-tri-*O*-isopropylidene pyranose derivative only, whereas D-*glycero*-L-*gluco*- and D-*glycero*-L-*manno*-heptose reacted in the furanose form to give 1,2:5,6- and 1,2:6,7-di-*O*-isopropylidene-D-*glycero*-L-*gluco*-heptofuranose (53% and 11%, respectively) and 2,3:5,6- and 2,3:6,7-di-*O*-isopropylidene-D-*glycero*-L-*manno*-heptofuranose (77% and 18%, respectively).[195]

Unsaturated aldehydes (acrylaldehyde, methacrolein, crotonaldehyde, cinnamaldehyde, *etc.*) reacted with methyl α-D-mannopyranoside to form methyl 2,3:4,6-di-*O*-alkenylidene-α-D-mannopyranosides.[196] Only in two cases were *exo*- and *endo*-isomers of the five-membered ring detected and isolated.

The use of polystyrene resins incorporating vinylbenzaldehyde (*i.e.* polymer-bound benzylaldehyde) for the acetalation of glycopyranosides has been examined.[197] Thus, the polymer reacted with methyl α-D-glucopyranoside to give a 4,6-arylidene derivative, which could be benzoylated and then cleaved (trifluoroacetic acid) from the polymer to give methyl 2,3-di-*O*-benzoyl-α-D-glucopyranoside in good yield. Benzylation of the polymer-bound glycoside was less successful.

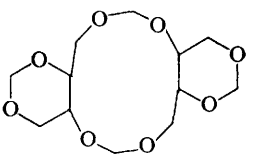
Methyl 2,3:6,7- and 2,3:4,7-di-*O*-cyclohexylidene-β-D-*glycero*-L-*manno*-heptopyranoside[198] and 1′,2:4,6-di-*O*-isopropylidenesucrose[199] have been prepared by standard procedures.

Derivatives of Acyclic Sugars

Acid-catalysed methylenation of erythritol afforded 1,3:2,4- and 1,4:2,3-di-*O*-methylene derivatives and some 1,4-anhydro-2,3-*O*-methylene-erythritol.[200] ^{1}H N.m.r. spectroscopy and m.s. of the diacetals furnished unequivocal evidence for the constitutional assignments. Acid-catalysed equilibration of the two diacetals indicated that there is a free-energy difference of 1.37 kcal mol^{-1} in favour of the 1,3:2,4-isomer; associated electronic and steric factors were discussed. Methylenation of dimethyl galactarate, methyl D-arabinonate, and methyl D-ribonate has also been examined.[201] The structures of the resulting bicyclic methylene diacetals did not support the view that *gauche* oxygen–oxygen interactions are an important stabilizing feature in *cis*-fused 3,5,8,10-tetraoxabicyclo-

[193] R. U. Lemieux and R. V. Stick, *Austral. J. Chem.*, 1975, **28**, 1799.
[194] A. Hasegawa, T. Sakurai, and N. Hasegawa, *Carbohydrate Res.*, 1975, **45**, 19.
[195] B. A. Dmitriev, A. Ya. Chernyak, O. S. Chizhov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1213 (*Chem. Abs.*, 1975, **83**, 147 660n).
[196] Z. Jedlinski, J. Maslinska-Solich, and A. Dworak, *Carbohydrate Res.*, 1975, **42**, 227.
[197] J. M. J. Fréchet and G. Pellé, *J.C.S. Chem. Comm.*, 1975, 225.
[198] A. Chiron and P. Szabo, *Carbohydrate Res.*, 1975, **45**, 85.
[199] R. Khan and K. S. Mufti, *Carbohydrate Res.*, 1975, **43**, 247.
[200] I. J. Burden and J. F. Stoddart, *J.C.S. Perkin I*, 1975, 666.
[201] I. J. Burden and J. F. Stoddart, *J.C.S. Perkin I*, 1975, 675.

[5,3,0]decanes (*cf.* Vol. 7, p. 198). Acid-catalysed methylenation of erythritol in an aqueous medium has yielded 1,3-*O*- and 1,3:2,4-, 1,4:2,3-, and 1,2:3,4-di-*O*-methylene-erythritol, whereas the macrocyclic derivative (65) was obtained when



(65)

the reaction was carried out in benzene.[201a] The conformations of the seven-membered rings in 2,5-*O*- and 1,3:2,5-di-*O*-methylenemannitol [202] and 1,6-di-*O*-benzyl-2,5-*O*-methylene-D-*arabino*-hex-3-ulose [203] have been discussed.

1:2:4,5:6,7-Tri-*O*-isopropylidene-D-*glycero*-D-*galacto*-heptitol has been synthesized and converted into D-*gluco*-hept-3-ulose (see Scheme 2).[12]

Acetals Derived from Aldehydo-sugars

The dimethyl acetals of D-glucose, D-xylose, and L-idose were obtained in 15—25% yield by Fischer glycosidation of the aldose in the presence of strontium or calcium chloride, whereas they were not detected in the absence of these salts.[204] This increase must be due to complex formation with these cations: whereas the ring form of the sugars lacks the steric arrangement (*ax.*, *eq.*, *ax.*) required for complex formation with the cations, the acyclic form is able to adopt a suitable conformation for complexation.

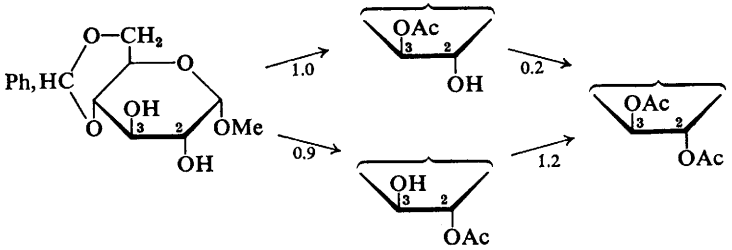
The 2,3:5,6- and 3,4:5,6-di-*O*-isopropylidene derivatives of D-glucose dimethyl acetal were obtained when D-glucose was treated at room temperature first with acidified methanol and then with 2,2-dimethoxypropane (or acetone).[205]

[201a] R. B. Jensen, O. Buchardt, S. E. Jorgensen, J. U. R. Nielsen, G. Schroll, and C. Altona, *Acta Chem. Scand.* (*B*), 1975, **29**, 373.
[202] T. B. Grindley and W. A. Szarek, *Canad. J. Chem.*, 1974, **52**, 4062.
[203] T. B. Grindley and W. A. Szarek, *Canad. J. Chem.*, 1974, **52**, 4072.
[204] F. W. Parrish, S. J. Angyal, M. E. Evans, and J. A. Mills, *Carbohydrate Res.*, 1975, **45**, 73.
[205] J. D. Stevens, *Carbohydrate Res.*, 1975, **45**, 143.

6
Esters

Carboxylic Esters

In a paper similar in content to one published in 1974 (see Vol. 8, p. 28), Lehrfeld has pointed out that selective acylations cannot be considered only in terms of the relative reactivities of the hydroxy-groups of the starting material in multistep reactions, since the reactivities of the hydroxy-groups in partially acylated materials must also be considered if erroneous conclusions are to be avoided.[206] This point is illustrated in Scheme 23, which shows experimentally determined



Reagent: 0·43 mol l^{-1} Ac_2O–py

Scheme 23

values for the relative rates of partial acetylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside: it can be seen that substitution of an acetyl group at O-3 appreciably *decreases* the reactivity of the hydroxy-group at C-2, whereas similar substitution at O-2 slightly *increases* the reactivity of the hydroxy-group at C-3.

Selective acetylation of 1,6-anhydro-β-D-galactopyranose and its 2-acetate with acetic anhydride in pyridine yielded principally the 2,4-diacetate, which isomerized on passage through a column of silica gel to give a 1 : 1-mixture of the 2,3- and 2,4-diacetates.[207] Selective acetylation of D-galactose using 6.2 molar equivalents of acetic anhydride in the presence of sodium acetate gave the β-penta-acetate (7.5%), a tetra-acetate fraction (16%), a triacetate fraction (3%), and a diacetate fraction (3%); methylation with diazomethane–boron trifluoride etherate was then used to determine the composition of the various fractions.[208]

Benzoyl cyanide has been found to be superior to *N*-benzoylimidazole as a selective benzoylating reagent for carbohydrates. Thus, mono-benzoylation of

[206] J. Lehrfeld, *Carbohydrate Res.*, 1975, **39**, 364.
[207] M. E. Chacón-Fuertes and M. Martín-Lomas, *Carbohydrate Res.*, 1975, **42**, C4.
[208] E. E. Lee and E. O'Brien, *Carbohydrate Res.*, 1975, **41**, 313.

methyl 4,6-*O*-benzylidene-α-D-mannopyranoside with benzoyl cyanide afforded the 2- and 3-acetates in the ratio 7 : 3, whereas a 1 : 1-mixture of these acetates was obtained with *N*-benzoylimidazole.[209] The hydrogen-bonded *endo*-hydroxy-group at C-5 in 1,4:3,6-dianhydro-D-glucitol was preferentially benzoylated using benzoyl chloride in pyridine, whereas neither *N*-benzoylimidazole nor benzoyl cyanide showed an appreciable selectivity for either of the hydroxy-groups. These observations are in keeping with an *N*-benzoylpyridinium intermediate interacting with oxygen atoms capable of acting as hydrogen-bond acceptors, whereas both benzoyl cyanide and *N*-benzoylimidazole, which lack an atom with a formal positive charge, would not experience this attraction. In comparing benzoyl chloride–pyridine, benzoyl chloride–triethylamine, and *N*-benzoylimidazole as selective benzoylating reagents for a number of methyl 4,6-*O*-benzylidene-D-hexopyranosides, Honig and Weidmann concluded that benzoyl chloride–triethylamine is as good as, if not better than, *N*-benzoylimidazole.[210] Acceptable yields of the following benzoates were obtained on selective benzoylation of the corresponding monosaccharide aldoses: α-D-glucopyranose and α-D-mannopyranose 1,2,3,6-tetrabenzoates, β-L-arabinopyranose 1,2,3-tribenzoate, and α-D-xylopyranose 1,2,4-tribenzoate.[211] Selective benzoylation of 1,6-anhydro-4′,6′-*O*-benzylidene-β-lactose, using 2.1 molar equivalents of benzoyl chloride in pyridine, yielded five benzoates, which were identified as the 2,2′,3,3′-tetrabenzoate (3%), 2,3,3′-tribenzoate (11%), 2,2′,3-tribenzoate (5%), 2,3′-dibenzoate (30%), and 3′-benzoate (22%).[212] Mono-benzoylation of methyl α-D-lyxopyranoside has afforded the 3-benzoate in 46% yield.[147]

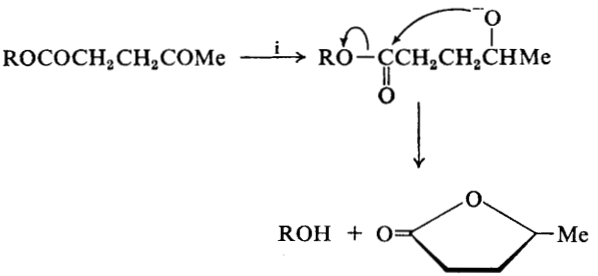
Ozonolysis of 2,3-di-*O*-acetyl, -methyl, and -toluene-*p*-sulphonyl derivatives of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside afforded the corresponding 6(or 4)-benzoates, the regiospecificity depending on the particular derivative examined.[213] The formation of 2-nitrosobenzoates *via* photolysis of 2-nitrobenzylidene derivatives is referred to in Chapter 5. The use of DMF as a solvent in a Koenigs–Knorr reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with methyl 18-β-glycyrrhetate resulted in the formation of 2,3,4,6-tetra-*O*-acetyl-1-*O*-formyl-β-D-glucopyranose (24%), but no glycoside was obtained.[214] The ratio of acyclic to cyclic products formed in acetolyses of 1,2-*O*-isopropylidenealdofuranoses depends on the relative concentrations of acetic anhydride and acetic acid used; a low concentration of acetic anhydride furnished principally the cyclic acetates.[184] An improved yield of α-D-mannopyranose penta-acetate resulted when the reagents (acetic anhydride and pyridine) were removed under reduced pressure before crystallization of the product from cold water.[215]

Two reports have described similar syntheses of αα-trehalose 6,6′-diesters of either long-chain fatty acids or mycolic acids by the reaction of 6,6′-dideoxy-6,6′-diodo(or dibromo)-2,2′,3,3′,4,4′-hexa-*O*-trimethylsilyltrehalose with the potassium

[209] S. A. Abbas and A. H. Haines, *Carbohydrate Res.*, 1975, **39**, 358.
[210] H. Honig and H. Weidmann, *Carbohydrate Res.*, 1975, **39**, 374.
[211] J. F. Batey, C. Bullock, E. O'Brien, and J. M. Williams, *Carbohydrate Res.*, 1975, **43**, 43.
[212] T. Chiba, M. Haga, and S. Tejima, *Carbohydrate Res.*, 1975, **45**, 11.
[213] P. Deslongchamps, C. Moreau, D. Fréhel, and R. Chênevert, *Canad. J. Chem.*, 1975, **53**, 1204.
[214] L. Hough and A. W. Lewis, *Carbohydrate Res.*, 1975, **42**, 173.
[215] R. K. Irani and J. L. Bose, *Indian J. Chem.*, 1975, **13**, 529 (*Chem. Abs.*, 1975, **83**, 79 497n).

(or sodium) salt of the acids.[216, 216a] 2,3,4,6-Tetra-*O*-acetyl-1-*O*-acyl-β-D-glucopyranoses have also been obtained by treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the silver salts of long-chain (C_3–C_{18}) fatty acids.[217]

2,6-Dimethoxybenzoyl esters did not undergo acyl migration under conditions used for methylation.[149] Levulinic acid esters have been recommended as protecting groups for alcohols.[218] Levulinic anhydride (prepared from the acid using DCC) reacted with alcohols in pyridine to give the esters in high yield. Cleavage of these esters was achieved by reduction of the carbonyl group with sodium borohydride, which was followed by an intramolecular ester exchange (see Scheme 24). Levulinates are moderately stable towards acids, and can be



Reagent: i, $NaBH_4$–dioxan–H_2O

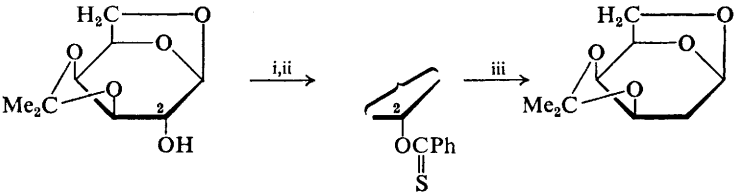
Scheme 24

prepared and cleaved in the presence of acetals, alkenic linkages, other esters, and nitro-groups. Hydrazine selectively cleaved the 1-acetyl group of peracetylated mono- and di-saccharides, and it can also be used to detach acetylated sugars bound to polymeric supports by a 1-*O*-succinoyl linkage.[219] The reaction of ammonia with acylated sugars has been reviewed.[220]

Esters of 4-hydroxy- and 3,4-dihydroxy-cinnamic acid have been found in two new D-glucosides isolated from the leaves of *Nandina domestica* Thunb.[221] The synthesis of optically active derivatives of truxinic acids by photolysis of cinnamoyl esters of D-mannitol is reported in Chapter 28.

A new mild reaction for the deoxygenation of secondary alcohols has been applied successfully to sugars; the reaction, which is radical in character, involves the reduction of a thiobenzoate with tributylstannane (see Scheme 25).[222] Other references to the use of thioesters and the photochemical transformation of dithiobis(thioformates) into thiols are reported in Chapter 11, the reaction of cyclic thiocarbonates with methyl iodide to give ω-iodo-*S*-methyl thiocarbonates

[216] J.-F. Tocanne, *Carbohydrate Res.*, 1975, **44**, 301.
[216a] R. Toubiana, B. C. Das, J. Defaye, B. Mompon, and M.-J. Toubiana, *Carbohydrate Res.*, 1975, **44**, 308.
[217] Y. Nishikawa, K. Yoshimoto, G. Kurono, and K. Michishita, *Chem. Pharm. Bull. (Japan)*, 1975, **23**, 597.
[218] A. Hassner, G. Strand, M. Rubinstein, and A. Patchornik, *J. Amer. Chem. Soc.*, 1975, **97**, 1614.
[219] G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydrate Res.*, 1975, **39**, 368.
[220] M. E. Gelpi and R. A. Cadenas, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 81.
[221] N. Morita, M. Shimizu, M. Arisawa, and Y. Shirataki, *Chem. Pharm. Bull. (Japan)*, 1974, **22**, 2752.
[222] D. H. R. Barton and S. W. McCombie, *J.C.S. Perkin I*, 1975, 1574.



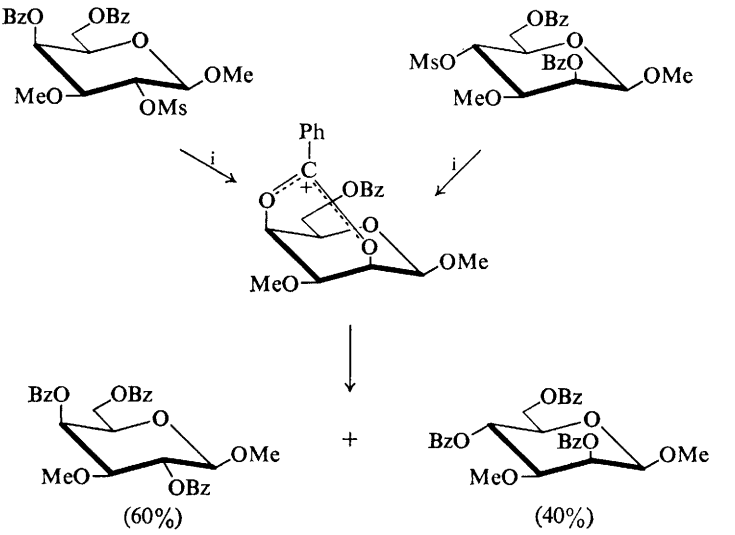
Reagents: i, $PhCCl{:}\overset{+}{N}Me_2Cl^-$; ii, H_2S–py; iii, Bu_3SH–PhMe

Scheme 25

in Chapter 13, and the thermolysis of 6-*S*-allyl- and 6-*S*-benzyl-dithiocarbonates in Chapter 14.

Acyloxonium Ions and Orthoesters

The preparation and transformation of acyloxonium ions containing 1,3-dioxolenium or 1,3-dioxenium rings have been reviewed.[223] MO calculations (MINDO/2) relating to the mechanism of the reactions of α-D-xylopyranose 1,2,4-orthoacetate have been reported.[224] The 1,2- and 1,4-acyloxonium ions are considered to be the principal product-determining intermediates in acid-catalysed reactions, with attack taking place preferentially at C-1. Isomerization of the 1,2-acyloxonium ion to the 2-*O*-acetylglycosyl cation was found to be unlikely energetically. The predicted reaction pathways are generally in good agreement with experimental results.

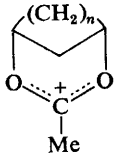


Reagent: i, KOBz–DMF

Scheme 26

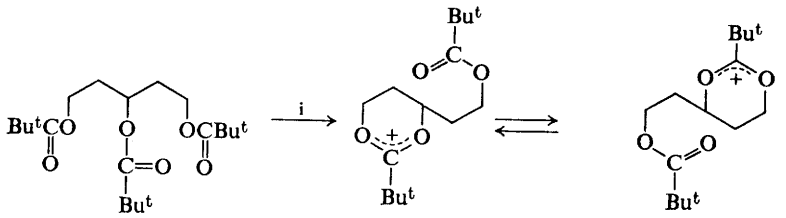
[223] H. Paulsen, *Pure Appl. Chem.*, 1975, **41**, 69.
[224] K. Ya. Burschtein, I. N. Fundiler, and A. F. Bochkov, *Tetrahedron*, 1975, **31**, 1303.

The formation of six-membered acyloxonium ions in displacement reactions of carbohydrate sulphonates is comparatively rare, but recent examples of such participation by a β-benzoyloxy-group are shown in Scheme 26.[225] Paulsen and his co-workers have also studied six-membered acyloxonium ions in simple model systems; *e.g.* (66) and (67) were obtained from the corresponding *cis*- or



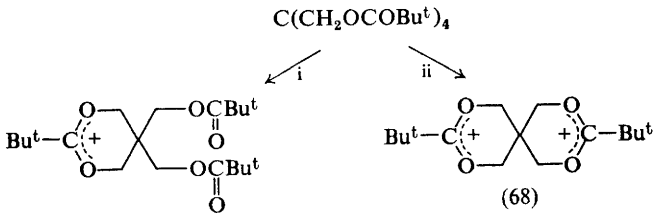
(66) $n = 2$
(67) $n = 3$

trans-1,3-diacetates using trifluoromethanesulphonic acid.[226] The systems depicted in Schemes 27 and 28, which include the novel spirodiacyloxonium ion (68), were also examined.[227]



Reagent: i, $SbCl_5$

Scheme 27



Reagents: i, $SbCl_5$; ii, CF_3SO_3H

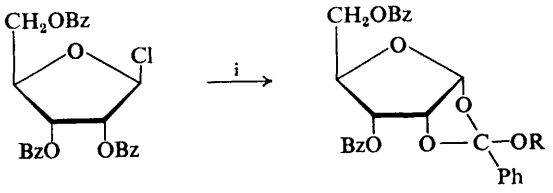
Scheme 28

Syntheses of 1,2-orthoesters of 4,6-*O*-benzylidene-α-D-galactopyranose have been reported.[228] 1,2-Orthoesters of D-glucopyranose and several steroid or triterpenoid alcohols have been prepared *via* acetylated glycosyl nitrates; *e.g.* 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(cholesteryl orthoacetate) was obtained when a mixture of cholesterol, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide,

[225] M. Miljković, D. Glišin, and M. Gligorijević, *J. Org. Chem.*, 1975, **40**, 1054.
[226] H. Paulsen and H. Meyborg, *Chem. Ber.*, 1975, **108**, 3176.
[227] H. Paulsen, H. Meyborg, and H. Behre, *Chem. Ber.*, 1975, **108**, 3190.
[228] A. F. Bochkov, V. M. Dashunin, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 632 (*Chem. Abs.*, 1975, **83**, 43 648y).

silver nitrate, and collidine was stirred in THF at room temperature.[229] Related 1,2-orthoesters of steroid and triterpenoid alcohols were also obtained from 1,2-(1-t-butyl orthoesters) by transesterification (*e.g.* with cholesterol) in the absence of a catalyst.[230] A new approach to 1,2-orthoesters involved the reaction of acylated glycosyl halides with an appropriate *NN*-dimethylformamide acetal (see, *e.g.*, Scheme 29).[82] '1,2-Orthoamides' [*e.g.* (69)] have been synthesized and used to prepare β-glycosides of 2-benzamido-2-deoxy-sugars (Scheme 30).[231]

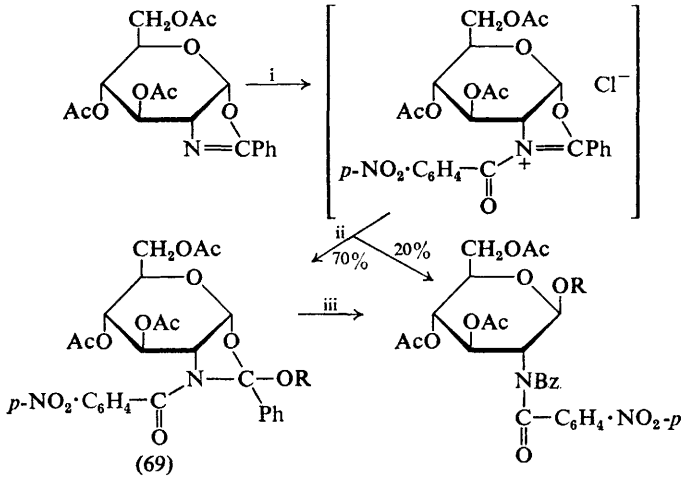
1,2,4-Orthoesters have been obtained from 1,2-orthoesters under catalysis by bases [232] or by acids in the presence of molecular sieves (Scheme 31).[233] Molecular



R = Me, Pr^i, C_6H_{11}

Reagent: i, $Me_2NCH(OR)_2$–CH_2Cl_2

Scheme 29

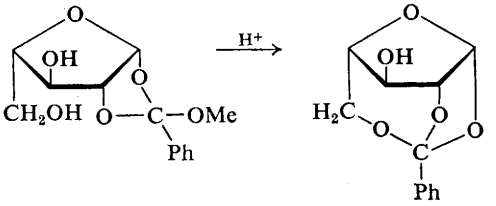


R = Me, Pr^i, or 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose

Reagents: i, *p*-$NO_2C_6H_4COCl$–CH_2Cl_2; ii, ROH–py; iii, TsOH

Scheme 30

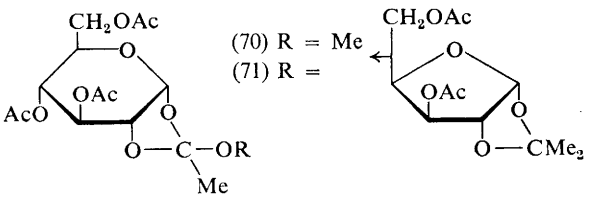
[229] N. I. Uvarova, N. F. Samoshina, and G. B. Elyakov, *Carbohydrate Res.*, 1975, **39**, 351.
[230] N. I. Uvarova, N. F. Samoshina, L. E. Novikova, and G. B. Elyakov, *Carbohydrate Res.*, 1975, **42**, 165.
[231] S. Hanessian, G. Cassinelli, and M. Casey, *Carbohydrate Res.*, 1975, **44**, C18.
[232] A. F. Bochkov, Ya. V. Voznyi, V. M. Kalinevich, A. S. Shashikov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 415 (*Chem. Abs.*, 1975, **83**, 28 469x).
[233] A. F. Bochkov, Ya. V. Voznyi, V. N. Chernetskii, V. M. Dashunin, and A. V. Rodionov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 420 (*Chem. Abs.*, 1975, **83**, 28 479a).



Scheme 31

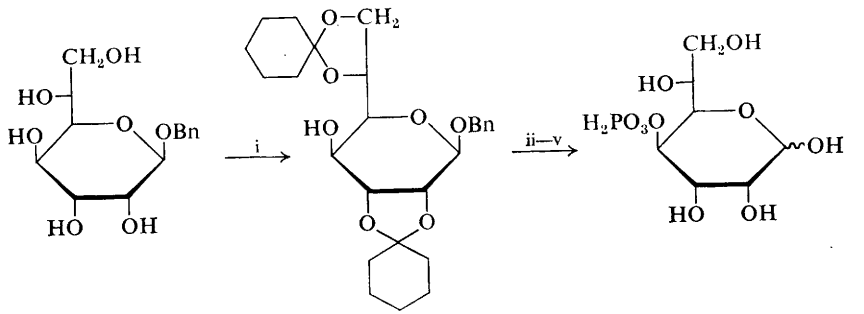
sieves were also used in other acid-catalysed transesterifications of 1,2-orthoesters [*e.g.* (70) → (71)].

Examples of the use of 1,2-orthoesters in the synthesis of glycosides, glycans, acetals, and anhydrides are noted in Chapters 3 and 5.



Phosphates

Syntheses of the 4-phosphates of D-*glycero*-D-*gulo*-heptose [234] (Scheme 32) and D-*glycero*-L-*manno*-heptose [235] have been reported by similar routes. Phosphorylation of *muco*-inositol with polyphosphoric acid gave the hexakisphosphate, whereas *epi*-inositol afforded a tetrakisphosphate only.[236] The contents of inositol cyclic 1,2-phosphate and D-glucopyranose 1- and 6-phosphates in extracts from brain tissues have been determined by g.l.c. of the trimethylsilylated

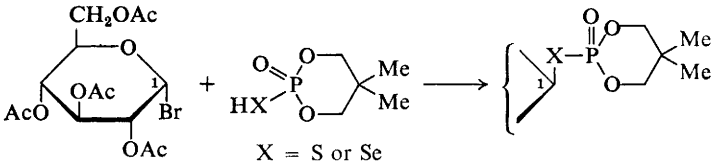


Reagents: i, cyclohexanone–H^+; ii, PCl_3–py; iii, $Ba(OH)_2$; iv, H_3O^+; v, H_2–Pd

Scheme 32

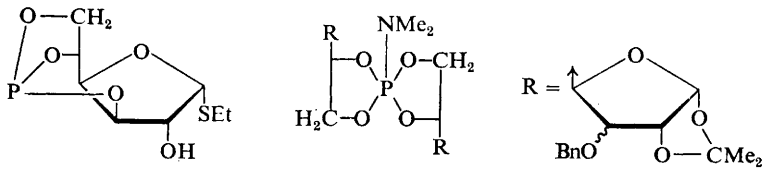
[234] A. Chiron and P. Szabo, *J.C.S. Perkin I*, 1975, 603.
[235] B. A. Dmitriev, A. Ya. Chernyak, O. S. Chizhov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1406 (*Chem. Abs.*, 1975, **83**, 179 428j).
[236] D. J. Cosgrove, *Carbohydrate Res.*, 1975, **40**, 380.

derivatives.[237] Syntheses of P^1-dolichyl P^2-α-D-mannopyranosyl pyrophosphate and the analogous P^1-citronellyl derivative have been reported; the D-mannopyranosyl-phosphate linkage in both compounds was most susceptible to hydrolysis by acid.[238] 2-Amino-2-deoxy-α-D-glucopyranosyl phosphate has been obtained by hydrazinolysis of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate).[238a] The reactions of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with monothio- or monoseleno-phosphoric acid derivatives gave the β-thio- and β-seleno-glycosyl phosphates (Scheme 33), whereas a mixture of



Scheme 33

β-thio- and β-seleno-glycosyl phosphates was obtained with thioselenophosphoric acid derivatives.[239] Tris(dialkylamino)phosphines have been used in the synthesis of such novel phosphites as (72) from ethyl 1-thio-α-D-glucofuranose [240] and of the

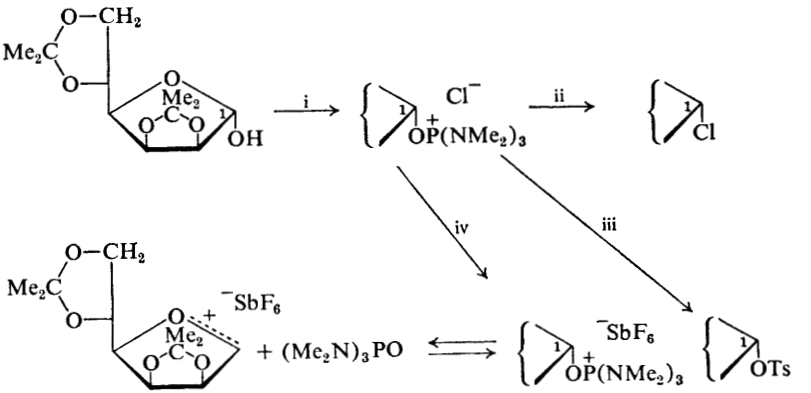


(72) (73)

spirophosphoranes (73) from 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allo- or -glucofuranose.[241] Tris(dimethylamino)phosphine in carbon tetrachloride has also been used to prepare various 1-substituted derivatives of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (Scheme 34).[242]

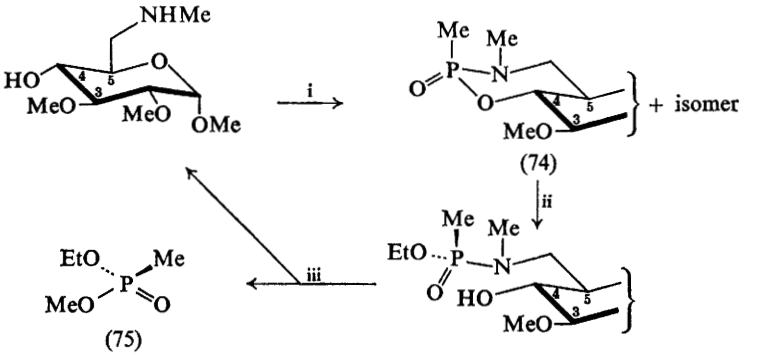
The enthalpies for the hydrolysis of a number of glycoside and nucleoside cyclic phosphates have been measured.[243] Photolysis of the disodium salt of α-D-glucopyranose 6-phosphate in aqueous solution furnished a number of products, including D-gluconic acid 6-phosphate, D-arabinofuranose 5-phosphate, D-glyceric acid 3-phosphate, glycolic acid phosphate, phosphoric acid, and

[237] A. L. Majumder and F. Eisenberg, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 133.
[238] C. D. Warren and R. W. Jeanloz, *Biochemistry*, 1975, **14**, 412.
[238a] M. Simon and O. Luderitz, *Carbohydrate Res.*, 1975, **40**, 7.
[239] M. Michalska, J. Michalski, and I. Orlich, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1974, **22**, 1053 (*Chem. Abs.*, 1975, **82**, 140 430h).
[240] E. E. Nifant'ev, M. P. Koroteev, Z. K. Zhane, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1462.
[241] R.-A. Boigegrain and B. Castro, *Tetrehedron Letters*, 1975, 3459.
[242] R.-A. Boigegrain, B. Castro, and B. Gross, *Tetrahedron Letters*, 1975, 3947.
[243] J. A. Gerlt, F. H. Westheimer, and J. M. Sturtevant, *J. Biol. Chem.*, 1975, **250**, 5059.



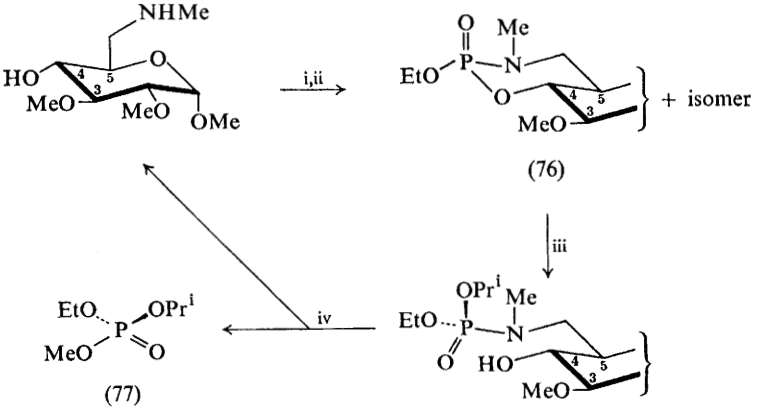
Reagents: i, $(Me_2N)_3P$–CCl_4; ii, Δ; iii, AgOTs; iv, $AgSbF_6$

Scheme 34



Reagents: i, $MeP{:}OCl_2$; ii, NaOEt; iii, MeOH–HCl

Scheme 35

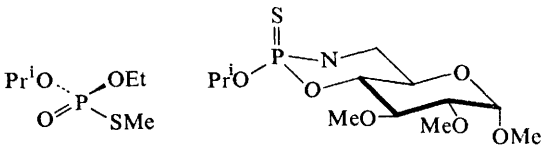


Reagents: i, $POCl_3$; ii, NaOEt; iii, $NaOPr^i$; iv, MeOH–HCl

Scheme 36

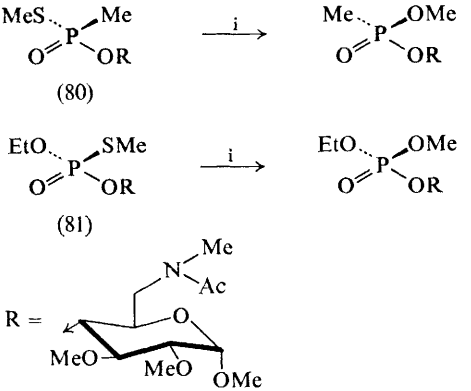
carbon dioxide; D-glucose was not formed.[244] Carbohydrate phosphorodiamidates [ROP:O(NMe_2)$_2$] were reductively cleaved by lithium and ethylamine in t-butanol yielding the corresponding deoxy-sugar.[245]

Inch and his co-workers continue to use carbohydrate derivatives in the synthesis of chiral esters of phosphorus acids and for studies of the displacement reactions of these esters. For example, the tetrahydro-1,3,2-oxazaphosphorine-2-ones (74) and (76) were synthesized and cleaved to give the chiral phosphonate (75) and the chiral phosphate (77), as illustrated in Schemes 35 and 36.[246, 247] 2-Thiones related to (74) and (76) were also prepared and used to obtain such chiral thiophosphates as (78) from (79). Attack on the acyclic *S*-methyl phos-



(78) (79)

phonothioate (80) and phosphorothioate (81) carbohydrate derivatives with methoxide occurred with inversion and with retention of configuration at phosphorus, respectively (Scheme 37). Although the results imply that a five-co-



(80)

(81)

Reagent: i, NaOMe

Scheme 37

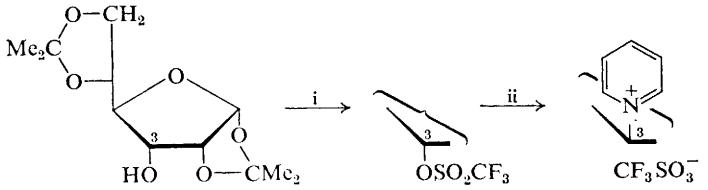
ordinate intermediate is formed in displacements in the phosphorothioate series, there is no need to invoke such intermediates in the phosphonothioate series, where the displacements may proceed by an S_N2 (P) mechanism.[248, 249]

Other references to phosphates are to be found in Chapter 21.

[244] C. Triantaphylides and M. Halmann, *J.C.S. Perkin II*, 1975, 34.
[245] S. Oida, H. Saeki, Y. Ohashi, and E. Ohki, *Chem. Pharm. Bull. (Japan)*, 1975, **23**, 1547.
[246] J. M. Harrison, T. D. Inch, and G. J. Lewis, *J.C.S. Perkin I*, 1975, 1892.
[247] C. R. Hall, T. D. Inch, G. J. Lewis, and R. A. Chittenden, *J.C.S. Chem. Comm.*, 1975, 720.
[248] T. D. Inch, G. J. Lewis, R. G. Wilkinson, and P. Watts, *J.C.S. Chem. Comm.*, 1975, 500.

Sulphonates

Carbohydrate trifluoromethanesulphonates (triflates) have been prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-allo- and -gluco-furanose. On heating in pyridine, the *allo*-triflate yielded the pyridinium salt with inversion of the configuration at C-3 (Scheme 38), while 1,2:3,4-di-*O*-isopropylidene-α-D-galacto-



Reagents: i, $(CF_3SO_2)_2O$–py; ii, py

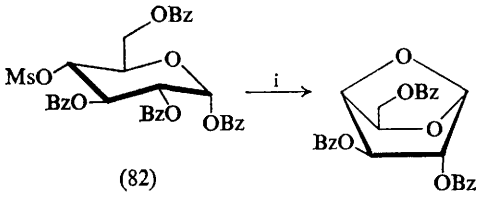
Scheme 38

pyranose gave the 6-pyridinium salt following reaction with triflic anhydride in pyridine at −15 °C.[250] Studies on the selective toluene-*p*-sulphonylation of methyl 6-deoxy-β-D-glucopyranoside have shown that all possible mono- and di-sulphonates are obtained using a molar equivalent of the reagent; it was concluded that further sulphonylation is retarded by the sulphonate group already present in the molecule, especially if it is vicinal to the reacting hydroxy-group.[169]

Treatment of alditols with a bulky sulphonyl chloride, *viz.* 2,4,6-tri-isopropylbenzenesulphonyl chloride, afforded αω-disulphonates with erythritol, L-arabinitol, and D-mannitol, whereas 1,4-anhydro-ω-sulphonates were formed with ribitol, xylitol, allitol, and D-glucitol.[251] Selective mesitylenesulphonylation of sucrose gave the 1′,6,6′-trisulphonate (40% yield), which was converted into 1′,6,6′-trichloro- and 1′,6,6′-trideoxy-sucrose.[252] Related displacements on acetylated sucrose 4- and 1′,4-dimethanesulphonates with benzoate, azide, bromide, and chloride ions have furnished the corresponding derivatives of β-D-fructofuranosyl α-D-galactopyranoside (*galacto*-sucrose).[253] Attempted azide displacement of the sulphonate group in the D-glucopyranose derivative (82) resulted in rearrangement to 1,4-anhydro-β-D-galactopyranose 2,3,6-tribenzoate (Scheme 39), but normal displacement occurred for 1,2,3-tri-*O*-benzoyl-4-*O*-methanesulphonyl-β-L-arabinopyranose.[211]

Deuterium-labelling studies have revealed a 1,2-hydride shift when methyl 5,6-*O*-cyclohexylidene-3-*O*-methanesulphonyl-α- and -β-D-allofuranosides react with methylmagnesium iodide; a plausible mechanism for this hydride shift is shown for the 2-deuteriated β-anomer in Scheme 40.[254] The reduction of hexofuranosyl 5-sulphonates with intramolecular participation by groups at either C-3 or C-6 is referred to in Chapter 13.

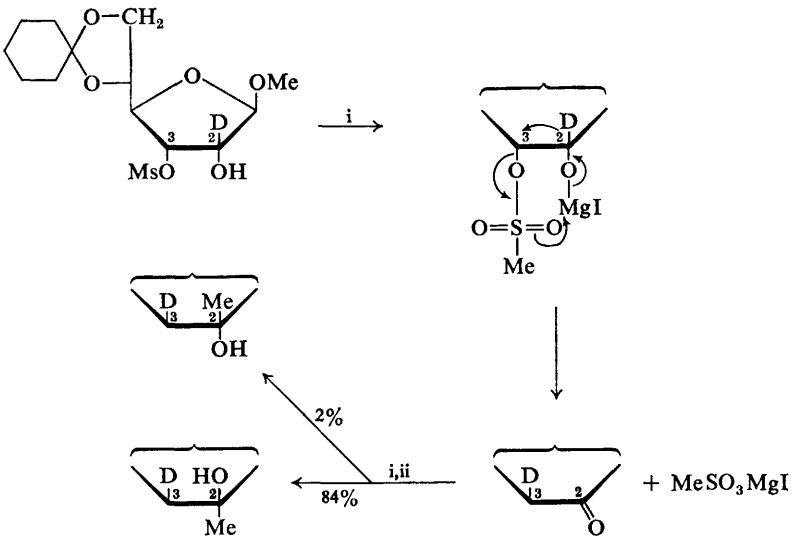
[249] T. D. Inch and G. J. Lewis, *Carbohydrate Res.*, 1975, **45**, 65.
[250] L. D. Hall and D. C. Miller, *Carbohydrate Res.*, 1975, **40**, C1.
[251] M. Mort, B. E. Stacey, and B. Tierney, *Carbohydrate Res.*, 1975, **43**, 183.
[252] L. Hough, S. P. Phadnis, and E. Tarelli, *Carbohydrate Res.*, 1975, **44**, C12.
[253] P. H. Fairclough, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1975, **40**, 285.
[254] M. Kawana and S. Emoto, *Tetrahedron Letters*, 1975, 3395.



(82)

Reagent: i, NaN_3–DMF

Scheme 39



Reagents: i, MeMgI; ii, H_2O

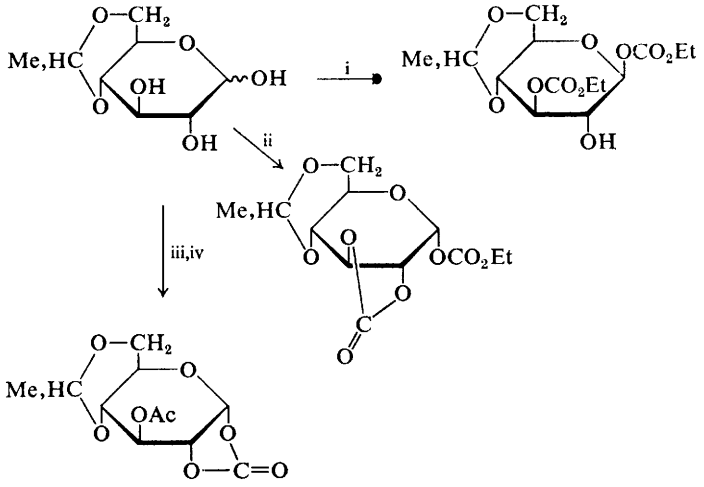
Scheme 40

Other Esters

Several carbonylating reagents used in the preparation of cyclic carbonates of sugar derivatives have been compared; the results are summarized in Scheme 41.[255]

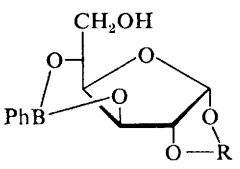
Russian workers have reported the synthesis of a number of benzeneboronates by the reaction of aldoses and alditols with benzeneboronic acid.[256, 257] Thus, glycerol gave a mixture of the 1,2- and 1,3-benzeneboronates, and D-glucose afforded the 1,2:3,5-bis(benzeneboronate) (83), whose structure was deduced by spectroscopic comparison with the 3,5-benzeneboronate (84) prepared from 1,2-*O*-isopropylidene-α-D-glucofuranose. On treatment with two molar

[255] H. Komura, T. Yoshino, and Y. Ishido, *Carbohydrate Res.*, 1975, **40**, 391.
[256] M. G. Edelev, T. M. Filippova, V. N. Robos, I. K. Shmyrev, A. S. Guseva, S. G. Verenikina, and A. M. Yurkevich, *Zhur. obshchei Khim.*, 1974, **44**, 2321 (*Chem. Abs.*, 1975, **82**, 73 320g).
[257] S. A. Guseva, S. G. Verenikina, S. F. Dymova, and A. M. Yurkevich, *Zhur. obshchei Khim.*, 1974, **44**, 2327 (*Chem. Abs.*, 1975, **82**, 73 323k).

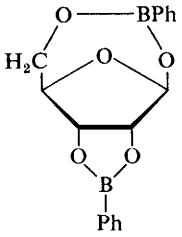


Reagents: i, EtOCOCl–py; ii, EtOCOCl–Et_3N; iii, 1,1′-carbonyldi-imidazole–THF; iv, Ac_2O–py

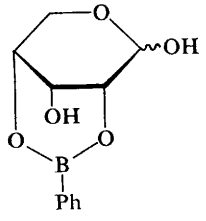
Scheme 41



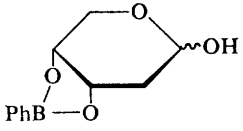
(83) R = BPh
(84) R = CMe_2



(85)



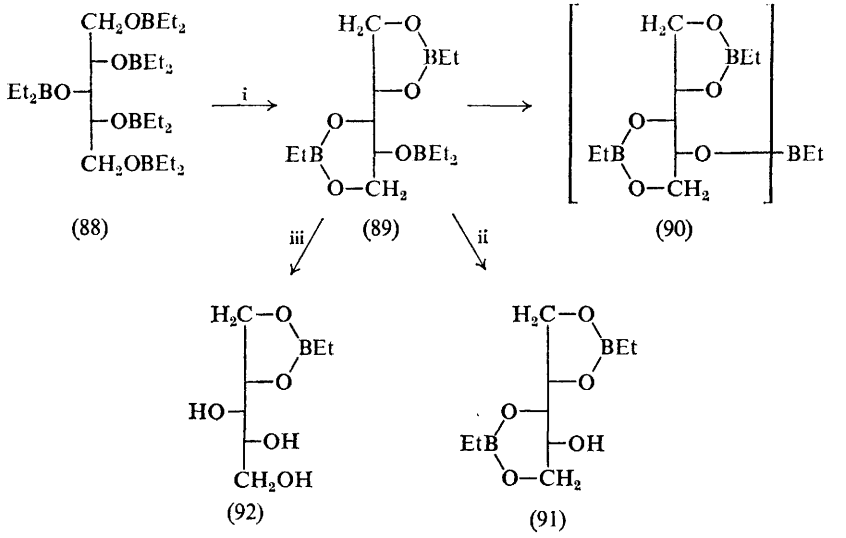
(86)



(87)

equivalents of benze neboronic acid, D-ribose yielded the furanoid 1,5:2,3-bis-(benzeneboronate) (85), whereas the pyranoid 2,4-benzeneboronate (86) was obtained with a molar equivalent of the reagent. 2-Deoxy-D-*erythro*-pentose gave only the pyranoid 3,4-benzeneboronate (87). The structures and conformations of all the benzeneboronates were examined by ^{11}B n.m.r. spectroscopy. D-Glucono-1,4-lactone reacted with benzeneboronic acid in methyl cellosolve to give the 3,5-benzeneboronate.[258] Treatment of xylitol with an excess of tri-

[258] K. Murase and M. Murakami, *Yamanouchi Seiyaku Kenkyu Hokoku*, 1974, **2**, 62 (*Chem. Abs.*, 1975, **83**, 179 446p).

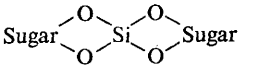


Reagents: i, Δ; ii, $MeCOCH_2COMe$; iii, MeOH

Scheme 42

ethylborane gave the pentakis derivative (88), which yielded the cyclic boronates (89) and (90) on heating (Scheme 42).[259] Selective de-esterification of the boronate (89) with either acetylacetone or methanol gave (91) and (92), respectively, which were used to prepare 2-*O*- and 1,2,3-tri-*O*-acetyl-DL-xylitol. The conversion of butaneboronic acid esters of carbohydrates into trimethylsilylated derivatives for analysis by g.l.c.–m.s. is noted in Chapter 26.

The orthosilicates (93) of D-glucose, D-fructose, and D-galactose were obtained in 30—40% yields by treatment of two molar equivalents of the sugar with silicon disulphide in DMF, whereas the orthosilicates (94) were obtained when four molar equivalents of the sugar were used.[260]



(93)

$Si(O\text{-sugar})_4$

(94)

Sugar = D-glucose, D-galactose, or D-fructose

[259] W. V. Dahlhoff and R. Köster, *Annalen*, 1975, 1926.

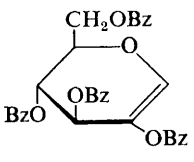
[260] M. G. Voronkov, G. P. Vasyanovich, and A. I. Polyakov, *Zhur. obshchei Khim.*, 1975, **45**, 480 (*Chem. Abs.*, 1975, **82**, 171 319h).

7
Halogenated Sugars

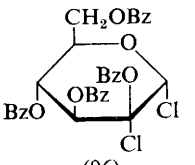
Glycosyl Halides

The cleavage of acetylated alkyl and aryl 1-thio-β-D-glucopyranosides and -galactosides with dichloromethyl methyl ether and zinc chloride gave principally the corresponding α-glycopyranosyl chlorides.[261] Methyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose both reacted with dibromomethyl methyl ether and zinc bromide to yield 2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl bromide and other products containing a bromine atom at C-2.[262] 2,3-*O*-Isopropylidene-5-*O*-trityl-β-D-ribofuranosyl chloride has been prepared by selective tritylation of 2,3-*O*-isopropylidene-D-ribofuranose and treatment of the tritylated product with triphenylphosphine in carbon tetrachloride.[263] 2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose reacted with titanium tetrachloride in the presence of ethoxytitanium trichloride to give 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl chloride.[174]

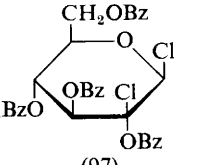
Re-investigation of the chlorination of the hydroxyglycal tetrabenzoate (95) has shown that the *cis*-adducts (96) and (97) are formed in the ratio of 3 : 7.[264] Chlorination of (95) at low temperatures furnished the orthoacid chloride



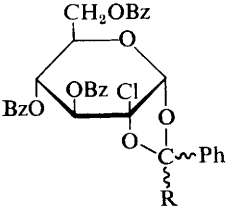
(95)



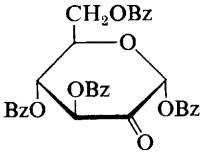
(96)



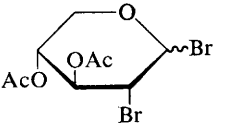
(97)



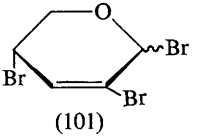
(98)



(99)



(100)



(101)

[261] I. Farkas, R. Bognar, M. M. Menyhart, A. K. Tarnai, M. Bihari, and J. Tamas, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 325 (*Chem. Abs.*, 1975, **83**, 10 707p).
[262] K. Bock, C. Pedersen, and R. Rasmussen, *Acta Chem. Scand.* (*B*), 1976, **29**, 185.
[263] R. S. Klein, H. Ohrui, and J. J. Fox, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 265.
[264] I. Lundt and C. Pedersen, *Acta Chem. Scand.* (*B*), 1975, **29**, 70.

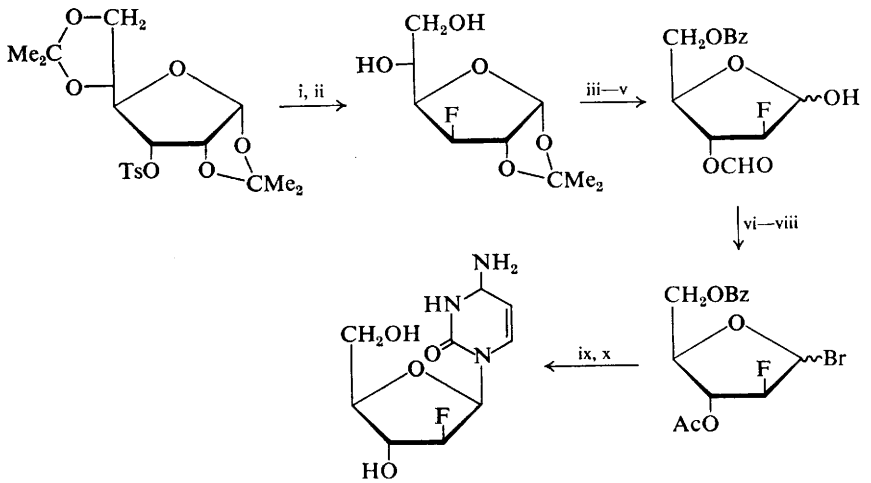
(98, R = Cl), which could be transformed into the corresponding orthoester (98, R = OMe), orthoacid fluoride (98, R = F), and benzylidene acetal (98, R = H). Hydrolysis of (98, R = Cl) gave the hexopyranos-2-ulose tetrabenzoate (99). Analogous results were obtained with the D-*lyxo*-isomer of (95). 1,2,3,4-Tetra-*O*-acetyl-β-D-xylopyranose reacted with acetyl bromide and zinc bromide to give the α-glycosyl bromide initially and then the dibromo-derivative (100) and the unsaturated tribromo-derivative (101).[264a] However, no comparable reactions occurred with the corresponding arabino-, ribo-, and lyxo-tetrabenzoates. The mechanisms of the reactions yielding (100) and (101) were discussed.

The bromide-exchange reactions and hydrolyses of a series of acetylated glycosyl halides have been examined in a variety of solvents.[265] 1,2-*trans*-Glycosyl halides showed a greater variation of rate in the solvents examined than did the *cis*-isomers. It was concluded that both hydrolysis and exchange reactions of 1,2-*trans*-glycosyl halides involve a two-step process, with the formation of carbonium ion intermediates. The observed order of reaction depends on whether or not the formation of these ions is rate determining.

Glycosyl halides used in the synthesis of glycosides have been mentioned in Chapter 3.

Other Halogenated Derivatives

Fox and his co-workers have reported a practical synthesis of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine by the sequence of reactions outlined in Scheme 43.[266] 4-Deoxy-4-fluoro-α-D-sorbose (103) has been prepared by the

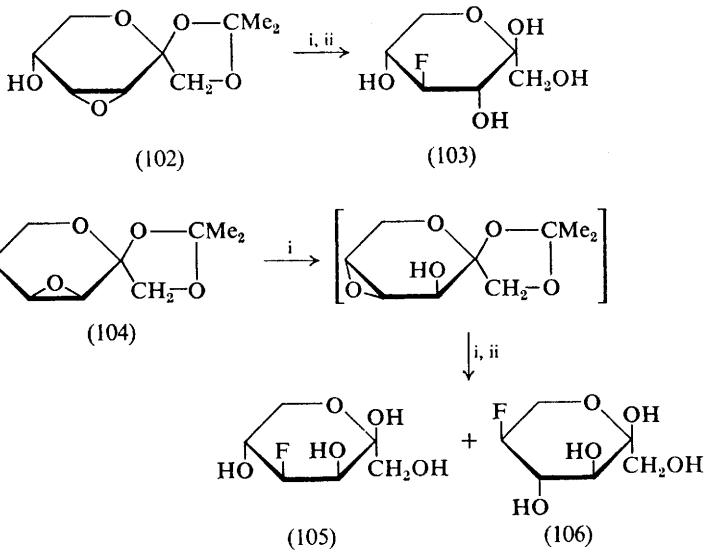


Reagents: i, KF–$MeCONH_2$; ii, H_3O^+; iii, BzCl–py; iv, *aci*-resin; v, KIO_4; vi, NaOMe–MeOH; vii, Ac_2O–py; viii, HBr–AcOH; ix, N^4-acetyl-bis(trimethylsilyl)cytosine; x, NH_3–MeOH

Scheme 43

[264a] K. Bock, C. Pedersen, and P. Rasmussen, *Acta Chem. Scand.* (*B*), 1975, **29**, 389.
[265] M. J. Duffy, G. Pass, G. O. Phillips, and A. Samee, *J.C.S. Perkin II*, 1975, 1138.
[266] U. Reichmann, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1975, **42**, 233.

action of potassium hydrogen fluoride on 3,4-anhydro-1,2-*O*-isopropylidene-β-D-psicopyranose (102).[267] Under identical conditions, 3,4-anhydro-1,2-*O*-isopropylidene-β-D-tagatopyranose (104) underwent epoxide migration yielding both 4-deoxy-4-fluoro-D-tagatose (105) and 5-deoxy-5-fluoro-α-L-sorbose (106) after deacetonation (Scheme 44). The 1-fluoro-, 1-chloro-, and 1-bromo-



(102) (103) (104) (105) (106)

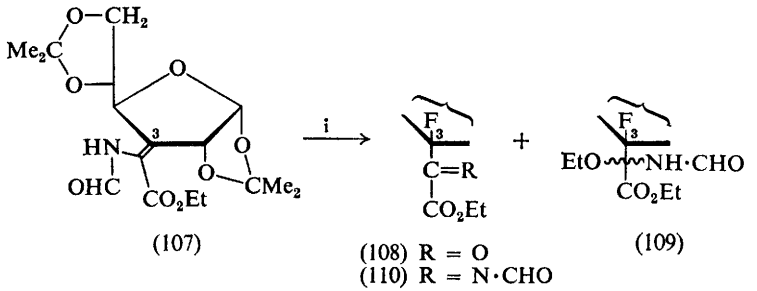
Reagents: i, KHF_2–$HOCH_2CH_2OMe$; ii, H_3O^+

Scheme 44

derivatives of dihydroxyacetone 3-phosphate have been synthesized.[268] Treatment of (*E*)-3-deoxy-3-*C*-ethoxycarbonyl(formylamino)methylene-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (107) with trifluoro(fluoroxy)methane in methylene chloride–ethanol afforded the fluorinated branched-chain sugars (108) and (109), whereas the fluorinated *N*-formylimine (110) was formed exclusively when dry, ethanol-free methylene chloride was used as solvent (Scheme 45).[269]

1,2-*O*-Isopropylidene-α-D-ribofuranose has been used as the starting material in a convenient synthesis of 1,3,5-tri-*O*-acetyl-2-chloro-2-deoxy-α-D-arabinofuranose (111), which was subsequently transformed into 1-(2-chloro-2-deoxy-β-D-arabinofuranosyl)cytosine and its α-anomer (Scheme 46).[270] The reaction of 1,2-*O*-isopropylidene-α-D-glucofuranose with sulphuryl chloride at room temperature yielded 6-chloro-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose 3,5-di(chlorosulphate), whereas 5,6-dichloro-5,6-dideoxy-1,2-*O*-isopropylidene-β-L-idofuranose 3-chlorosulphate was obtained if the reaction mixture was heated at 50 °C before being worked up.[271] Similar treatment of 1,2-*O*-isopropylidene-

[267] G. V. Rao, L. Que, jun., L. D. Hall, and T. P. Fondy, *Carbohydrate Res.*, 1975, **40**, 311.
[268] J. B. Silverman, P. S. Babiarz, K. P. Mahajan, J. Buschek, and T. P. Fondy, *Biochemistry*, 1975, **14**, 2252.
[269] K. Bischofberger, A. J. Brink, and A. Jordaan, *J.C.S. Perkin I*, 1975, 2457.
[270] G. Ritzmann, R. S. Klein, D. H. Hollenberg, and J. J. Fox, *Carbohydrate Res.*, 1975, **39**, 227.
[271] H. Parolis, *Carbohydrate Res.*, 1975, **43**, C1.

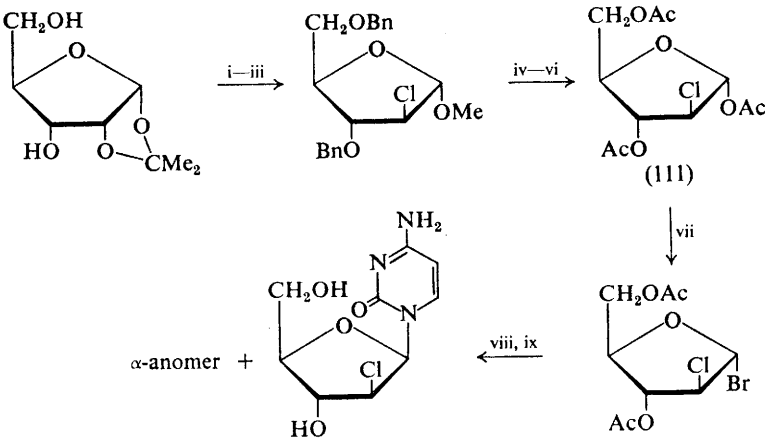


(107) (108) R = O (110) R = N·CHO (109)

Reagent: i, CF_3OF–CH_2Cl_2–EtOH

Scheme 45

α-D-glucofuranurono-6,3-lactone at room temperature furnished 5-chloro-5-deoxy-1,2-*O*-isopropylidene-β-L-idofuranurono-6,3-lactone. Sucrose has been converted into 6-chloro-6-deoxy-α-D-glucopyranosyl 6-chloro-6-deoxy-β-D-fructofuranoside or 4,6-dichloro-4,6-dideoxy-α-D-galactopyranosyl 6-chloro-6-deoxy-β-D-fructofuranoside by selective reaction with sulphuryl chloride.[272] The chlorinated derivatives are precursors of the anhydrosucrose derivatives noted in

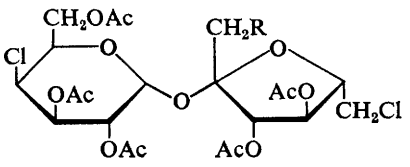


Reagents: i, BnCl–KOH; ii, MeOH–H^+; iii, Ph_3P–CCl_4; iv, Pd–C–H_2; v, Ac_2O–NaOAc; vi, Ac_2O–H_2SO_4; vii, HBr–CH_2Cl_2; viii, N^4-acetyl-bis(trimethylsilyl)cytosine; ix, NH_3–MeOH

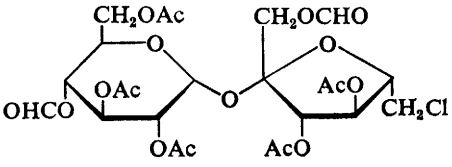
Scheme 46

Chapter 4. Other chlorinated derivatives of sucrose have been prepared by treatment of partially esterified derivatives of sucrose with methanesulphonyl chloride–DMF.[273] Thus, sucrose 2,3,3′,4′,6-penta-acetate reacted with this reagent at 85 °C to give a mixture of products from which the 1′,4,6′-trichloride (112), the 1′-*O*-formyl-4,6′-dichloride (113), the 4,6′-dichloride (114), and the

[272] L. Hough, S. P. Phadnis, and E. Tarelli, *Carbohydrate Res.*, 1975, **44**, 37.
[273] R. Khan, M. R. Jenner, and K. S. Mufti, *Carbohydrate Res.*, 1975, **39**, 253.



(112) R = Cl
(113) R = OCHO
(114) R = OH

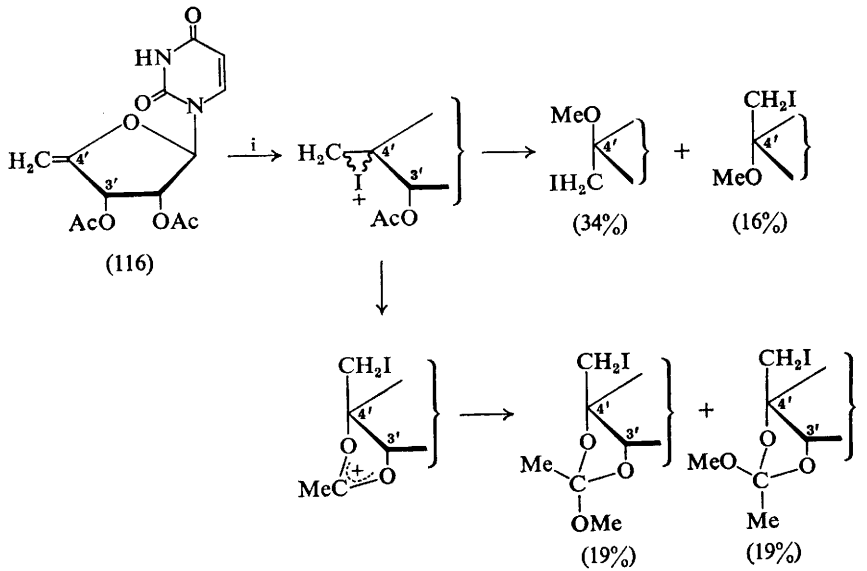


(115)

1′,4-di-*O*-formyl-6′-chloride (115) were isolated. Treatment of sucrose 1′,2,3,3′,-4,4′-hexabenzoate with methanesulphonyl chloride–DMF gave the expected 6,6′-dichloro-6,6′-dideoxysucrose hexabenzoate.

The reaction of methyl 2,3-anhydro-β-D-ribofuranoside with hydrogen bromide in acetic acid–acetic anhydride led to the formation of methyl 2,3-di-*O*-acetyl-5-bromo-5-deoxy-αβ-D-xylofuranoside, presumably *via* the intermediacy of the 3,5-acetoxonium ion.[274] Similar treatment of methyl 2,3-anhydro-5-*O*-benzoyl-β-D-ribofuranoside furnished methyl 2-*O*-acetyl-3-*O*-benzoyl-5-bromo-5-deoxy-αβ-D-xylofuranosides.

Improved yields of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside have been recorded in a synthesis that involved heating the corresponding 6-toluene-*p*-sulphonate with sodium iodide in butanone.[275] Moffatt's group



(116)

(34%) (16%)

(19%) (19%)

Reagent: i, I_2–MeOH–AgOAc

Scheme 47

[274] D. H. Hollenberg, K. A. Watanabe, and J. J. Fox, *Carbohydrate Res.*, 1975, **42**, 241.
[275] T.-S. Lin and R. E. Harmon, *Org. Prep. Proc. Internat.*, 1975, **7**, 11 (*Chem. Abs.*, 1975, **83**, 59 175c).

has examined the reactions of iodine in methanol with a number of substituted derivatives of 1-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)uracil.[276] Thus, the 2′,3′-di-*O*-acetyl derivative (116) gave 5′-deoxy-5′-iodo-4′-methoxy-nucleosides with the β-D-*ribo*- and the α-L-*lyxo*-configurations in addition to diastereoisomeric 3′,4′-orthoacetates (Scheme 47). Similar treatment of deacetylated-(116) or the derived cyclic 2′,3′-carbonate afforded 5′-iodo-4′-methoxy adducts possessing only the β-D-*ribo*-configuration.

The reaction of cyclic thiocarbonates with methyl iodide (Chapter 13) and the use of 5′-deoxy-5′-iodo-nucleosides in the synthesis of unsaturated nucleosides (Chapter 14) are reported elsewhere.

[276] J. P. H. Verheyden and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1975, **97**, 4386.

8
Amino-sugars

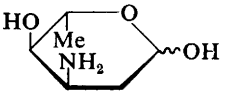
Natural Products

A 2,3-diamino-2,3-dideoxyhexose isolated from the lipopolysaccharides of *Rhodopseudomonas viridis* and *R. palustris* has been tentatively assigned a *gluco*-configuration.[277]

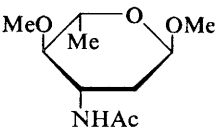
Synthesis

Approaches to the synthesis of glycosaminides have been reviewed (in Russian).[278]

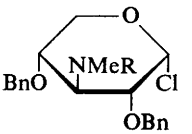
Several reports on the synthesis of naturally occurring amino-sugars have appeared during the past year. Standard procedures – *via* azido-sugars prepared from either sulphonate or oxiran derivatives – have been used in syntheses of 3-amino-2,3,6-trideoxy-L-*ribo*-hexose (ristosamine) (117)[279] and its *N*-benzoate,[280] methyl 3-acetamido-2,3,6-trideoxy-4-*O*-methyl-α-L-*arabino*-hexopyranoside (methyl *N*-acetylactinosaminide) (118),[281] the gentosaminyl chloride (119),[282] and a derivative (120) of ezoaminuroic acid, a component of the specific antifungal antibiotic ezomycin.[283] Ammonolysis of the mixture of dianhydro-D-



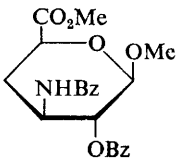
(117)



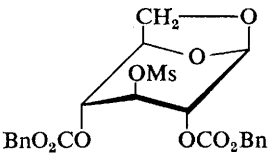
(118)



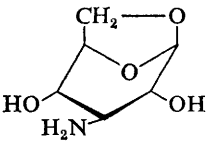
(119) R = $C_6H_3 \cdot (NO_2)_2$



(120)



(121)

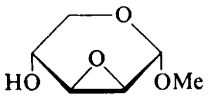


(122)

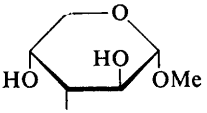
[277] J. Roppel, H. Mayer, and J. Weckesser, *Carbohydrate Res.*, 1975, **40**, 31.
[278] S. E. Zurabyan and A. Ya. Khorlin, *Uspekhi Khim.*, 1974, **43**, 1865.
[279] W. W. Lee, H. Y. Wu, J. J. Marsh, jun., C. W. Mosher, E. M. Acton, L. Goodman, and D. W. Henry, *J. Medicin. Chem.*, 1975, **18**, 767.
[280] F. Sztaricskai, I. Pelyvas, R. Bognar, and G. Bujtas, *Tetrahedron Letters*, 1975, 1111.
[281] W. W. Lee, H. Y. Wu, J. E. Christensen, L. Goodman, and D. W. Henry, *J. Medicin. Chem.*, 1975, **18**, 768.
[282] A. Hasegawa and M. Kiso, *Carbohydrate Res.*, 1975, **44**, 121.
[283] T. Ogawa, M. Akatsu, and M. Matsui, *Carbohydrate Res.*, 1975, **44**, C22.

allopyranoses obtained on treatment of the 1,6-anhydro-β-D-glucopyranose methanesulphonate (121) with alkali gave 3-amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (122), which ^1H n.m.r. spectroscopy indicated to assume a boat conformation.[284] Hydrolysis of (122) yielded kanosamine (3-amino-3-deoxy-D-glucose).

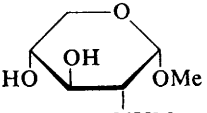
The synthesis of both α- and β-anomers of methyl gentosaminide (methyl 3-deoxy-3-methylamino-D-xylopyranoside) has been described.[285] A number of novel 3-alkylamino-analogues of gentosamine were also obtained by ammonolysis, *etc.* of methyl 2,3-anhydro-β-D-ribopyranoside, and the synthesis of L-gentosamine was discussed. Ring-opening of methyl 2,3-anhydro-α-D-lyxopyranoside (123) (prepared from an epoxy-ketone intermediate, see Chapter 16)



(123)

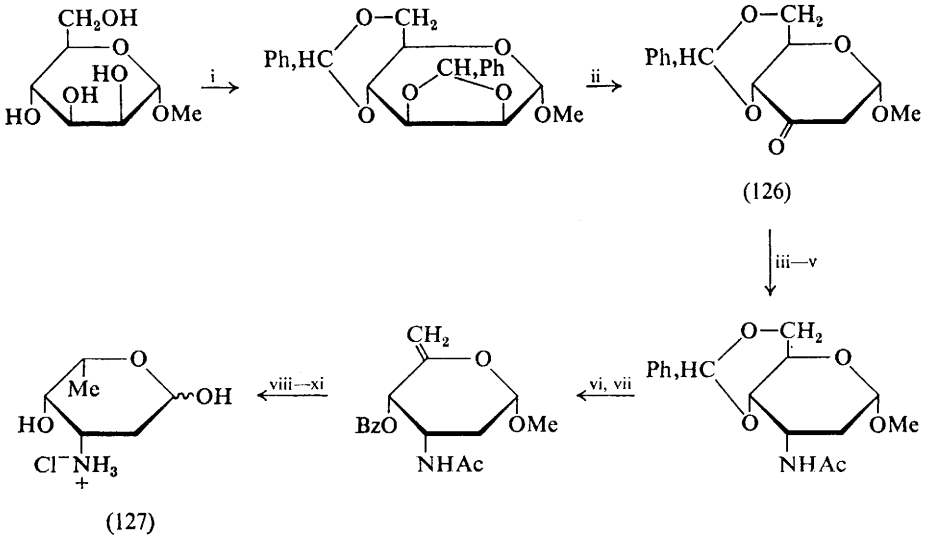


(124)



(125)

with methylamine yielded a 4 : 3 mixture of methyl 3-deoxy-3-methylamino-α-D-arabinopyranoside (124) and methyl 2-deoxy-2-methylamino-α-D-xylopyranoside (125). The L-enantiomer of (124), which has recently been found as a component of the gentamycins and other amino-glycoside antibiotics, was also prepared.



(126)

(127)

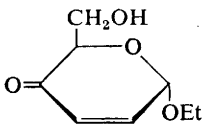
Reagents: i, $PhCH(OMe)_2$; ii, BuLi; iii, NH_2OH; iv, $LiAlH_4$; v, Ac_2O; vi, NBS–CCl_4; vii, AgF; viii, NaOMe–MeOH; ix, H_2–Pd; x, $Ba(OH)_2$; xi, aq. HCl

Scheme 48

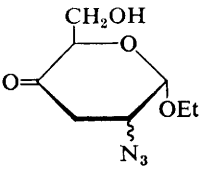
[284] T. Trnka, M. Černý, M. Buděšínsky, and J. Pacák, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3038.
[285] D. J. Cooper, D. H. Davies, A. K. Mallams, and A. S. Yehaskel, *J.C.S. Perkin I*, 1975, 785.

Daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) hydrochloride (127) has been prepared in 40% yield overall from methyl α-D-mannopyranoside by the route shown in Scheme 48.[286] A key step in this sequence is the conversion of methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside into the 2-deoxyhexopyranosid-3-ulose (126) using butyl-lithium in hexane.

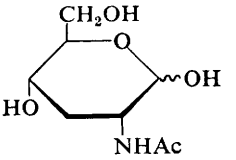
On treatment with sodium azide in aqueous acetic acid, the glycenosidulose (128) afforded a mixture of *erythro*- and *threo*-azido-ketones (129), which were separated after reduction (sodium borohydride) to ethyl 2-azido-2,3-dideoxy-α-D-*ribo*-hexopyranoside and the corresponding D-*lyxo*-isomer.[286a] The *ribo*-isomer was converted into *N*-acetyl-lividosamine (2-acetamido-2,3-dideoxy-D-*ribo*-hexose) (130) by a standard series of transformations.



(128)

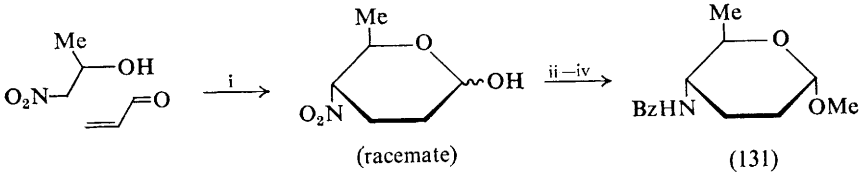


(129)



(130)

Derivatives of DL-tolyposamine (4-amino-2,3,4,6-tetradeoxy-*erythro*-hexose) (131) have been prepared *via* condensation of acrolein with 1-nitropropan-2-ol (Scheme 49),[287] and DL-daunosamine triacetate (133) resulted when the oxazole (132) was subjected to the novel sequence of reactions shown in Scheme 50.[288]



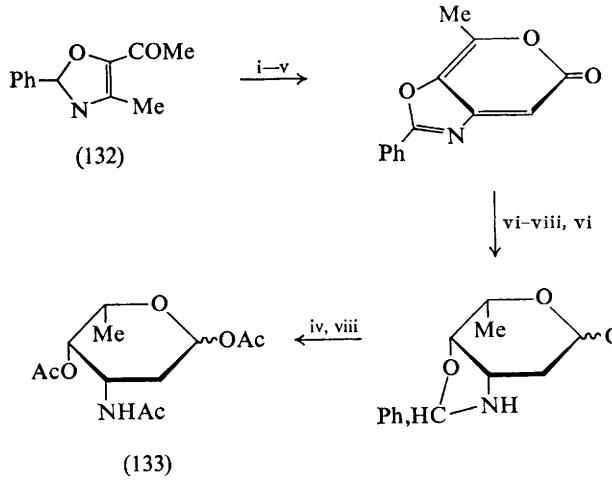
(racemate) (131)

Reagents: i, $NHEt_2$–HCO_2H; ii, H^+–MeOH; iii, H_2–Ni; iv, Bz_2O

Scheme 49

A new route to vicinal amino-dideoxy-sugars has been reported.[289] Thus, catalytic reduction of the keto-hydrazone (135) obtained by treatment of the glycosidulose (134) with alkali gave methyl 3-amino-3,4,6-trideoxy-α-L-*ribo*-hexopyranoside (Scheme 51). Stevens and his co-workers have extended their work on 4-amino-sugars by synthesizing methyl 4-amino-4,6-dideoxy-α-D-altropyranoside[290] and the isomeric D-idose compound.[291] 3-Amino-3-deoxy-D-altrosides [*e.g.* (136)] derived from tryptamines have been prepared from methyl

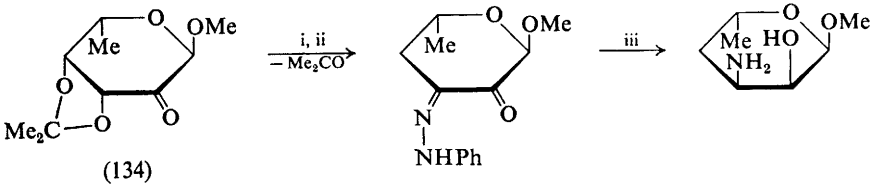
[286] D. Horton and W. Weckerle, *Carbohydrate Res.*, 1975, **44**, 227.
[286a] E. Jegou, J. Cleophax, J. Leboul, and S. D. Gero, *Carbohydrate Res.*, 1975, **45**, 323.
[287] S. Zen, E. Kaji, and H. Kohno, *Chem. Letters*, 1974, 1029.
[288] C. M. Wong, T.-L. Ho, and W. P. Niemczura, *Canad. J. Chem.*, 1975, **53**, 3144.
[289] P. M. Collins, W. G. Overend, and V. M. Racz, *J.C.S. Chem. Comm.*, 1975, 181.
[290] C. L. Stevens, D. Chitharanjan, K. G. Taylor, and P. M. Pillai, *J. Org. Chem.*, 1975, **40**, 2471.
[291] C. L. Stevens, J. P. Dickerson, K. G. Taylor, P. Blumbergs, and P. M. Pillai, *J. Org. Chem.*, 1975, **40**, 2468.



(132) (133)

Reagents: i, NBS; ii, HCN–KCN; iii, MeOH–HCl; iv, H^+; v, $SOCl_2$; vi, H_2–$Rh \cdot Al_2O_3$; vii, $NaAlH_2(OCH_2CH_2OMe)_2$; viii, Ac_2O–py

Scheme 50

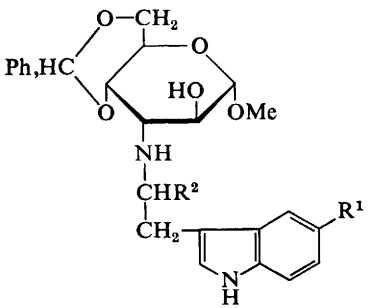


(134) (135)

Reagents: i, aq. NaOH; ii, $PhNHNH_2$; iii, H_2–PtO_2–HOAc

Scheme 51

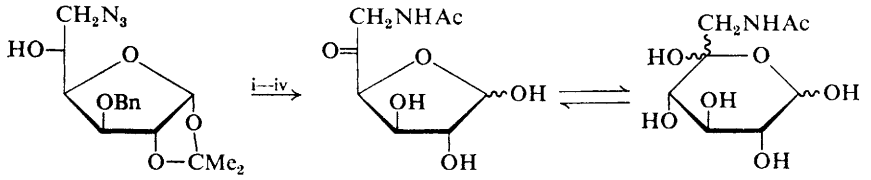
2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside.[292] This anhydro-sugar also reacted with liquid ammonia to give a 9 : 1 mixture of the 3-amino-3-deoxy-D-altroside and the 2-amino-2-deoxy-D-glucoside derivatives, whereas it reacted



(136) R^1 = H, OH, OMe, OBn; R^2 = H, Me

[292] O. V. Lukin, E. N. Samshina, N. P. Solov'eva, K. F. Turchin, M. M. Vigdorchik, and N. N. Suvorov, *Zhur. org. Khim.*, 1975, **11**, 1109.

with ammonium carbonate in methanol to give equal proportions of the isomers.[293] 6-Acetamido-6-deoxy-D-*xylo*-hexos-5-ulose (138) – prepared from the azide (137) as shown in Scheme 52 – exists in aqueous solution as a mixture of the



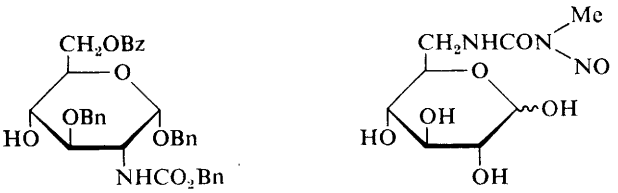
(137) (138)

Reagents: i, CrO_3–Me_2CO; ii, H_2–Pd–C; iii, Ac_2O–MeOH; iv, H_3O^+

Scheme 52

furanoid and pyranoid forms in equilibrium.[294] Many derivatives of 2-amino-2-deoxy-D-glucose with O-4 unsubstituted [*e.g.* (139)] have been prepared for use in the synthesis of disaccharide components of heparin.[295]

Interest in the synthesis of disaccharides containing amino-sugar residues has increased over the past year (see also Chapter 3). Standard transformations on naturally occurring disaccharides have been used in the preparation of 6′-amino-6′-deoxy- and 1′,6′-diamino-1′,6′-dideoxy-sucrose,[296] 6′-acetamido-6′-deoxy-lactose,[297] and 4′,6′-diacetamido-4′,6′-dideoxycellobiose;[298] in the last synthesis, treatment of methyl 4′,6′-di-*O*-methanesulphonyl-β-lactoside pentabenzoate with sodium azide in HMPT also gave an elimination product, methyl 4-*O*-(6-azido-2,3-di-*O*-benzoyl-4,6-dideoxy-α-L-*threo*-hex-4-enopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside, and methyl 2,3,6-tri-*O*-benzoyl-β-D-glucopyranoside by interglycosidic cleavage. 2-Mono-, 2,6′-di-, and 2,6,6′-tri-acetamido-deoxy derivatives



(139) (140)

of neotrehalose have been synthesized using the Koenigs–Knorr procedure with appropriate monosaccharide derivatives.[299] 2-Acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-mannose and -D-glucose have been prepared by addition of nitromethane to 3-*O*-β-D-galactopyranosyl-D-arabinose, followed by acetylation,

[293] I. Ježo, *Chem. Zvesti*, 1975, **29**, 124.
[294] D. E. Kiely and L. Benzing-Nguyen, *J. Org. Chem.*, 1975, **40**, 2630.
[295] P. C. Wyss and J. Kiss, *Helv. Chim. Acta*, 1975, **58**, 1833.
[296] T. Suami, T. Ikeda, S. Nishiyama, and R. Adachi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1953.
[297] T. Chiba, M. Haga, and S. Tejima, *Chem. Pharm. Bull. (Japan)*, 1975, **23**, 1283.
[298] R. S. Bhatt, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1975, **43**, 57.
[299] I. Ježo, *Chem. Zvesti*, 1975, **29**, 126.

ammonolysis, and application of the Nef reaction.[300] This route was also used to convert 3-*O*-β-D-mannopyranosyl-D-arabinose into 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-mannose and -D-glucose. Details of syntheses of dihydrostreptobiosamine[301] (see Vol. 8, p. 133) and 2-amino-5-*O*-carbamoyl-2-deoxy-L-xylonic acid,[302] a constituent of polyoxin J (see Vol. 7, p. 150), have been published.

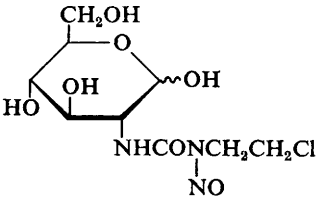
The reaction of D-glucose with serotonin oxalate has furnished 1-deoxy-1-(5-hydroxytryptamino)-D-fructose, the product of Amadori rearrangement.[303]

Other references to amino-sugars appear in Chapters 3, 14, 15, 17, and 21.

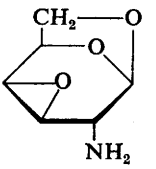
Reactions

The deamination of amino-sugars and related compounds has been comprehensively reviewed,[304] while more recent work on the deamination of 2-amino-2-deoxy-D-mannose is discussed in Chapter 4.

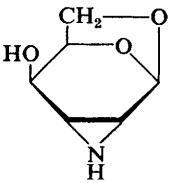
Analogues [*e.g.* (140)] of the streptozotocins (Vol. 3, p. 154; Vol. 4, p. 130) have been prepared by treatment of 6-amino-6-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucitol, and 1-amino-1-deoxy-D-glucitol, -D-arabinitol, -D-mannitol, and -D-galactitol with *N*-methyl-*N*-nitrosocarbamoylazide.[305] 2-Amino-2-deoxy-D-glucose has been converted into the nitrosated mustard derivative (141) by treatment with 2-chloroethyl isocyanate and then with nitrous acid.[306] The selective degradation of compounds containing amino-groups by thermal decomposition of *N*-acyl-*N*-nitroso derivatives has been



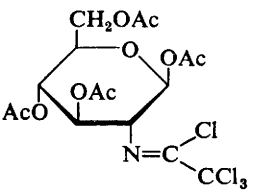
(141)



(142)



(143)



(144)

[300] G. Johnson, R. I. Lee, and Y. C. Lee, *Carbohydrate Res.*, 1975, **39**, 271.
[301] S. Umezawa, H. Sano, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1975, **48**, 556.
[302] H. Kuzuhara, M. Kimura, and S. Emoto, *Carbohydrate Res.*, 1975, **45**, 245.
[303] L. Mester and M. Mester, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 141.
[304] J. M. Williams, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 9.
[305] N. Gassmann, F. Stoos, A. Meier, G. Büyük, S. E. Helali, and E. Hardegger, *Helv. Chim. Acta*, 1975, **58**, 182.
[306] H. D. Burns and N. D. Heindel, *Org. Prep. Proc. Internat.*, 1974, **6**, 259 (*Chem. Abs.*, 1975, **82**, 112 213w).

discussed by Llewellyn and Williams [307] (see also, Vol. 5, p. 65). Methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*N*-nitrosoacetamido)-β-D-glucopyranoside, for example, decomposed in aqueous solution to give 3,4,6-tri-*O*-acetyl-2,5-anhydro-D-mannose, a product of ring-contraction, with concomitant release of the aglycone. The reaction can, however, only be used when the *N*-nitrosoamide is sufficiently stable for it to be prepared and transferred to an aqueous medium prior to any decomposition.

A study of the reaction of 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α- and -β-D-glucopyranoses with hydrogen bromide in chloroform by i.r. spectroscopy has demonstrated that the β-anomer rapidly yields the oxazolinium bromide; it also underwent deuterium-exchange faster than the α-anomer in chloroform–deuterium oxide.[308]

Hydrolysis of 2-amino-1,6:3,4-dianhydro-2-deoxy-β-D-galactopyranose (142) with alkali gave, in addition to 2-amino-1,6-anhydro-2-deoxy-β-D-glucopyranose, the isomeric epimino-sugar (143), although this isomerization did not occur at room temperature [309] (*cf.* 1,6:3,4-dianhydro-β-D-galactopyranose).

The γ-radiolysis of 2-amino-2-deoxy-D-glucose in the presence of nitrogen or nitrous oxide has been examined; although 2-deoxyaldoses were formed in the presence of nitrogen, their formation was suppressed in the presence of nitrous oxide.[310] A mechanism involving attack of hydrated electrons on the amino-group – resulting in the formation of ammonia – was proposed. A related study has shown that D-arabinose and glyceraldehyde are primary products of γ-radiolysis of aqueous solutions of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose, presumably in the absence of radical scavengers.[311]

The chlorination of such amino-sugars as 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose and 6-amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose yielded relatively unstable *NN*-dichloro-derivatives.[312] 2-Acetamido-2-deoxyaldohexopyranose acetates (and *N*-acetylglycosamines) reacted with phosphorus pentachloride to give 2-(tetrachloroethylidene)amino derivatives [*e.g.* (144)] instead of the expected 2-trichloroacetamidoderivatives.[313]

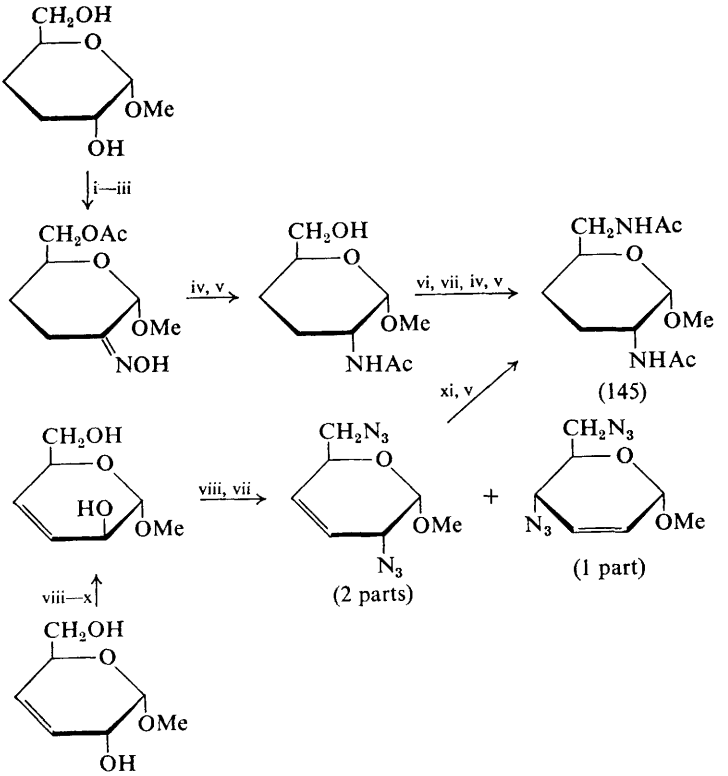
Dehydration of 1-deoxy-1-dibenzylamino-D-fructuronic acid at 100 °C and pH 7 yielded 4-hydroxy-5-methyl-3(2*H*)-furanone, a flavouring component of beef.[313a] The mechanism of formation of the product was examined using labelled substrates and deuterium oxide.

Di- and Poly-amino-sugars

Several syntheses of derivatives of purpurosamine C (2,6-diamino-2,3,4,6-tetradeoxy-D-*erythro*-hexose) have been reported during the past year. A 45% yield

[307] J. W. Llewellyn and J. M. Williams, *J.C.S. Perkin I*, 1975, 1428.
[308] P. Stoeckl, H. Hoenig, and H. Weidmann, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 225.
[309] M. Černý, O. Juláková, J. Paćak, and M. Buděšínský, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2116.
[310] L. I. Kudryashov and T. M. Senchenkova, *Zhur. obshchei Khim.*, 1975, **45**, 439 (*Chem. Abs.*, 1975, **82**, 125 543d).
[311] L. I. Kudyrashov and T. M. Senchenkova, *Zhur. obshchei Khim.*, 1974, **4**, 1389 (*Chem. Abs.*, 1975, **82**, 9936k).
[312] J. M. J. Tronchet and J. Poncet, *Carbohydrate Res.*, 1975, **42**, 347.
[313] A. M. Dempsey and L. Hough, *Carbohydrate Res.*, 1975, **41**, 63.
[313a] K. B. Hicks and M. S. Feather, *J. Agric. Food Chem.*, 1975, **23**, 957 (*Chem. Abs.*, 1975, **83**, 164 458v).

overall is claimed for a synthesis of methyl purpurosaminide C from 2-amino-2-deoxy-D-glucose *via* successive treatments of methyl 2-(benzyloxycarbonyl)-amino-2-deoxy-3,4,6-tri-*O*-methanesulphonyl-α-D-glucopyranoside with sodium azide, zinc dust and sodium iodide, and hydrogen.[314] Two approaches to the synthesis of methyl di-*N*-acetylpurpurosaminide C (145), in which the amino-groups at C-2 and C-6 are introduced successively and simultaneously, are outlined in Scheme 53.[315] In the approach used by Gero's group, ethyl 2-azido-4-



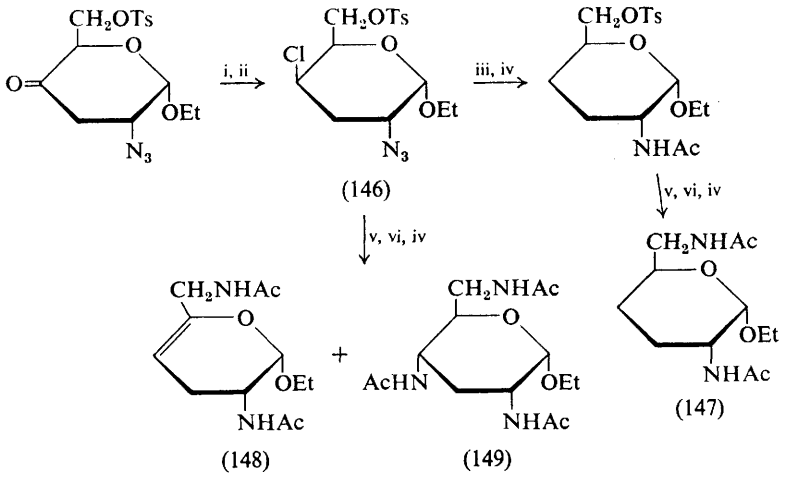
Reagents: i, Ac_2O–py; ii, RuO_4–CCl_4; iii, NH_2OH; iv, H_2–Pd; v, Ac_2O–MeOH; vi, TsCl–py; vii, NaN_3–DMF; viii, MsCl–py; ix, NaOBz–DMF; x, HO^-; xi, H_2–Pt

Scheme 53

chloro-2,3,4-trideoxy-6-*O*-toluene-*p*-sulphonyl-α-D-*xylo*-hexopyranoside (146) could be converted either into ethyl di-*N*-acetylpurpurosaminide C (147) or into a mixture of ethyl di-*N*-acetylsisosaminide (148) and ethyl 2,4,6-triacetamido-2,3,4,6-tetradeoxy-α-D-*ribo*-hexopyranoside (149) (Scheme 54).[316]

A large-scale synthesis of 2,6-diamino-2,6-dideoxy-D-glucose, a constituent of the neomycin group of antibiotics, has been based on a route previously used to

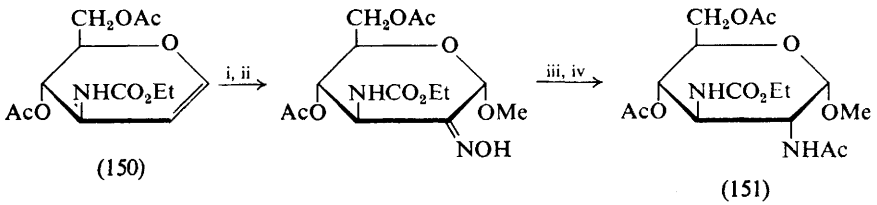
[314] S. Rakhit and M. Georges, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 153.
[315] J. S. Brimacombe, I. Da'Aboul, and L. C. N. Tucker, *J.C.S. Perkin I*, 1975, 979.
[316] J. Cleophax, S. D. Gero, E. Jegou-Aumont, J. Leboul, and D. Mercier, *J.C.S. Chem. Comm.*, 1975, 11.



Reagents: i, $NaBH_4$; ii, SO_2Cl_2; iii, N_2H_4–Ni; iv, Ac_2O–MeOH; v, NaN_3–DMF; vi, H_2–cat.

Scheme 54

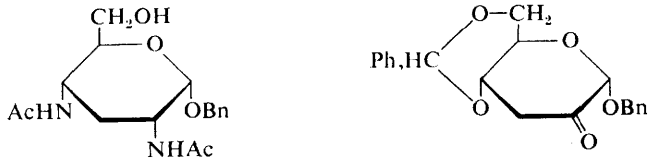
prepare 2,3-diamino-2,3-dideoxy-D-glucose.[317] A derivative (151) of the latter diamino-sugar has been obtained from 4,6-di-*O*-acetyl-3-(ethoxycarbonyl)-amino-3-deoxy-D-glucal (150) *via* the addition of nitrosyl chloride (Scheme 55).[318]



Reagents: i, NOCl–EtOAc; ii, MeOH–py; iii, H_2–Pd–C–N_2H_4; iv, Ac_2O

Scheme 55

The 2,4-diamino-2,3,4-trideoxy-D-*ribo*-hexose derivative (152) and series of unsaturated amino-sugar derivatives have been synthesized from benzyl 4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hexopyranosidulose (153).[319]



[317] W. Meyer zu Reckendorf, L. Rolf, and N. Wassiliadou-Micheli, *Carbohydrate Res.*, 1975, **45**, 307.

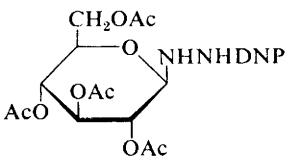
[318] A. J. Brink, R. H. Hall, and A. Jordaan, *J. S. African Chem. Inst.*, 1974, **27**, 124 (*Chem. Abs.*, 1975, **82**, 86 522z).

[319] W. Meyer zu Reckendorf, U. Kamprath-Scholz, E. Bischof, and N. Wassiliadou-Micheli, *Chem. Ber.*, 1975, **108**, 3397.

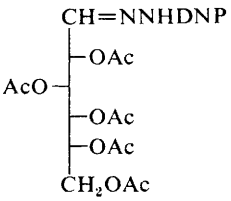
9

Hydrazones, Osazones, and Related Compounds

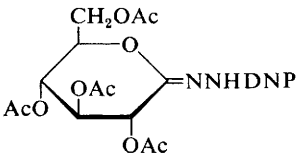
The reaction of D-glucose phenylhydrazone with glacial acetic acid has been examined; *N*-fructosyl-*N*-glucosylphenylhydrazine was formed at 30 °C, whereas 1,2-dideoxy-1-phenylimino-2-phenylhydrazono-D-*arabino*-hexose was obtained after brief treatment at 100 °C.[320] 2-(D-*arabino*-Tetrahydroxybutyl)quinoxaline was also formed. The acetolysis of the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl 2,4-dinitrophenylhydrazine (154) yielded a mixture of acyclic and cyclic hydrazones



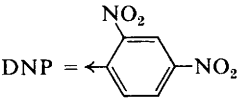
(154)



(155)



(156)

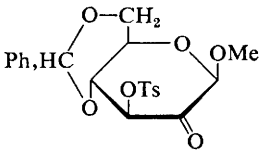


[(155) and (156), respectively].[321] Degradation of 4-*O*-dichloroisoeverninyl-2,6-dideoxy-D-*arabino*-hexose (curacin) semicarbazone with alkali gave 2,3,6-trideoxy-D-*erythro*-hex-2-enose semicarbazone.[322]

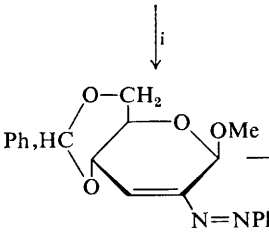
Treatment of the glycosidulose (157) with phenylhydrazine in pyridine furnished the phenylazoalkene (158), which underwent 1,4-addition with such nucleophiles as methoxide, azide, and hydride ions to give *arabino*(equatorial) adducts (159) (Scheme 56).[323] The synthesis and reactions of related furanoid azoalkenes are noted in Chapter 14.

Carbohydrates containing a carbonyl group reacted with 1-dimethylaminonaphthalene-5-sulphonylhydrazine (dansylhydrazine) to give fluorescent hydrazones.[324]

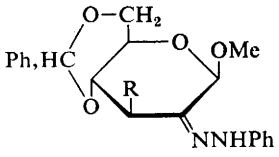
[320] N. A. Chursina, I. F. Strel'tsova, and V. A. Afanas'ev, *Izvest. Akad. Nauk Kirg. S.S.R.*, 1975 64 (*Chem. Abs.*, 1975, **83**, 147 661p).
[321] A. Gerecs, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 467 (*Chem. Abs.*, 1975, **83**, 28 476x).
[322] V. Deulofeu, E. G. Gros, and O. L. Galmarini, *Anales de Quim.*, 1974, **70**, 1062 (*Chem. Abs.*, 1975, **83**, 164 474x).
[323] P. M. Collins, W. G. Overend, and V. H. Racz, *Carbohydrate Res.*, 1975, **45**, 127.
[324] P. Weber and L. Hof, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1298.



(157)



(158)



(159) R = H, N_3, OMe

Reagents: i, $PhNHNH_2$–py; ii, $NaBH_4$ or NaN_3 or NaOMe

Scheme 56

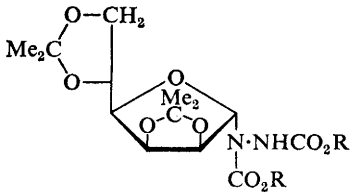
10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines

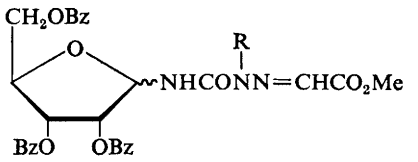
The kinetics of the reaction between D-glucose and several alkylamines have been examined by a potentiometric method,[325] and the rate of formation of *N*-glycosides [*e.g.* 1-*N*-(4-aminobenzoyl)-D-xylopyranosylamine] has been measured by determining the decrease of free amino-groups using 1,2-naphthoquinone-4-sulphonic acid.[326] Rate constants for the Maillard reaction of glycine with D-xylose, D-glucose, and D-fructose have also been reported.[327]

The reaction of reducing monosaccharides (*e.g.* 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose) with an equimolar mixture of phthalimide, dialkyl azodicarboxylate, and triphenylphosphine yielded both anomeric *N*-glycosylphthalimides,[328] whereas *N*-glycosyl-1,2-dialkoxycarbonylhydrazines [*e.g.* (160)] were obtained in the absence of phthalimide.[329]

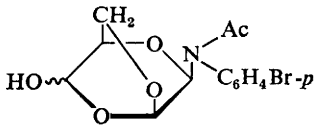
2,3,5-Tri-*O*-benzoyl-α-D-ribofuranosyl bromide reacted with the metal salts of a series of semicarbazones to give D-ribofuranosylamine derivatives having the general structure (161).[330]



(160) R = Et or Bu



(161) R = alkyl or H



(162)

[325] S. Kolka, *Roczniki Chem.*, 1975, **49**, 965.
[326] A. Fukuda, T. Ishizaki, and T. Ito, *J. Faculty Agric., Tottori Univ.*, 1974, **9**, 20 (*Chem. Abs.*, 1975, **83**, 10 702h).
[327] P. A. Bobbio, F. O. Bobbio, and L. M. V. Trevisan, *Anais. Acad. brasil. Cienc.*, 1973, **45**, 419 (*Chem. Abs.*, 1975, **83**, 79 493h).
[328] J. Jurczak, G. Grynkiewicz, and A. Zamojski, *Carbohydrate Res.*, 1975, **39**, 147.
[329] G. Grynkiewicz and J. Jurczak, *Carbohydrate Res.*, 1975, **43**, 188.
[330] P. Fiedler, M. Hřebabecký, and J. Beránek, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2378.

Glycosylamines linked through the aliphatic amino-group were formed when oligosaccharides were treated with 2-(4-aminophenyl)ethylamine.[331] Reduction of these glycosylamines afforded 1-amino-1-deoxyalditol derivatives, which after linking to Sepharose were used in the purification of antibodies specific for the oligosaccharide. Jeanloz's group have reported syntheses of 1-*N*-(L-aspart-1- and -4-oyl)-4-*O*-β-D-galactopyranosyl-β-D-glucopyranosylamine, *via* condensation of 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosylamine with *N*-(benzyloxycarbonyl)-L-aspartic anhydride,[332] and several free and protected glycodipeptides and one glycotripeptide containing the 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyanosylamine residue.[333]

The selective benzoylation of *N*-acetyl-*N*-aryl-β-D-ribopyranosylamines has been investigated.[333a] The relative reactivity of the hydroxy-groups was HO-4 > HO-2 > HO-3, whereas that for analogous β-D-xylopyranosylamines (Z. Smiatacz, *Carbohydrate Res.*, 1974, **38**, 117) and α-L-arabinopyranosylamines (Z. Smiatacz, *Roczniki Chem.*, 1974, **48**, 947) was HO-3 ≈ HO-4 > HO-2. Methyl ethers of *N*-acetyl-*N*-aryl-β-D-xylo- and -α-L-arabino-pyranosylamines have been prepared by Kuhn methylation.[334]

The relative stability to ammonolysis of a series of 2,3,4,6-tetra-*O*-acetyl-*N*-nitrobenzoyl- and -dinitrobenzoyl-β-D-glucopyranosylamines has been determined.[335] *N*-Acetyl-2,3,4,6-tetra-*O*-acetyl-*N*-nitroso-β-D-glucopyranosylamine decomposed on heating in pyridine–methylene chloride to give only β-D-glucopyranose penta-acetate, whereas methyl 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosides were obtained in the ratio of 3 : 1 when the reaction was conducted in methanol–pyridine.[336] Investigations of the products resulting from oxidation of *N*-acetyl-*N*-(4-bromophenyl)-β-D-glucopyranosylamine and its 4,6-*O*-benzylidene derivative with periodate have indicated that they consist of a mixture of aldehydic and hemialdal forms [*e.g.* (162)].[337]

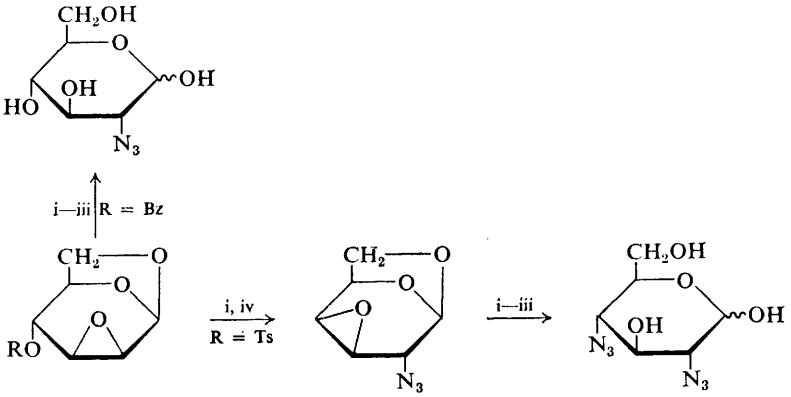
1-Phenylethylamine has been resolved following conversion into diastereoisomeric glycosylamines by reaction with 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose.[338]

N-Ribofuranosyl derivatives of nitrogen-containing heterocyclic compounds are referred to in Chapter 21, and the conversion of Schiff-base derivatives of *aldehydo*-sugars into 1-aminophosphonates is noted in Chapter 18.

Azides

2-Azido-2-deoxy- and 2,4-diazido-2,4-dideoxy-D-glucose have been synthesized from derivatives of 1,6:2,3-dianhydro-β-D-mannopyranose as indicated in Scheme 57.[339] Other azido-sugars and a 5′-azido-5′-deoxynucleoside are mentioned in Chapters 8 and 21, respectively.

[331] A. M. Jeffrey, D. A. Zopf, and V. Ginsburg, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 608.
[332] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1975, **43**, 371.
[333] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1975, **43**, 281.
[333a] Z. Smiatacz and E. Paszkiewicz, *Roczniki Chem.*, 1975, **49**, 909.
[334] Z. Smiatacz, *Carbohydrate Res.*, 1975, **39**, 344.
[335] S. Delpy and A. S. Cerezo, *Anales Asoc. quim. argentina*, 1975, **63**, 49 (*Chem. Abs.*, 1975, **83**, 193 610u).
[336] O. Larm, *Carbohydrate Res.*, 1975, **43**, 192.
[337] Z. Fialkiewiczowa, *Roczniki Chem.*, 1975, **49**, 81.
[338] P. Axerio and M. Bemporad, *Afinidad*, 1975, **32**, 469 (*Chem. Abs.*, 1975, **83**, 178 465g).
[339] H. Paulsen, H. Koebernick, W. Stenzel, and P. Köll, *Tetrahedron Letters*, 1975, 1493.

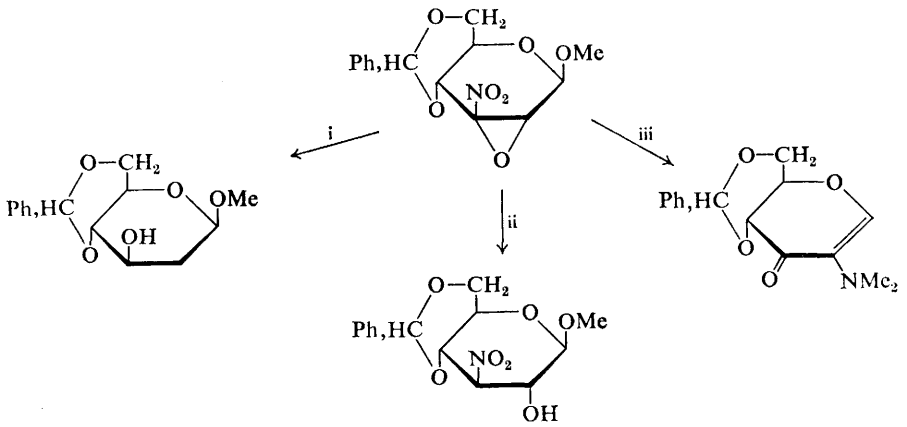


Reagents: i, NaN_3; ii, Ac_2O–H_2SO_4; iii, H_3O^+; iv, NaOMe–MeOH

Scheme 57

Nitro-sugars

Baer and Madumelu have described a number of the reactions of methyl 2,3-anhydro-4,6-*O*-benzylidene-3-nitro-β-D-allopyranoside (Scheme 58) and related

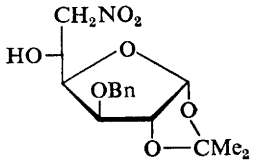


Reagents: i, $NaBH_4$; ii, H_2–Pd; iii, $NHMe_2$

Scheme 58

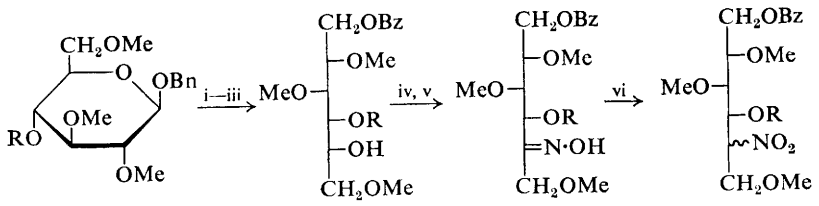
α-nitro-oxirans having the β-D-*talo*, α-D-*manno*, and β-D-*gulo* configurations.[340] Base-catalysed addition of nitromethane to 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose gave 3-*O*-benzyl-6-deoxy-1,2-*O*-isopropylidene-6-nitro-α-D-glucofuranose (163) as the kinetically favoured product.[341] Subsequent epimerization led to a 2 : 1 mixture of the α-D-*gluco* and β-L-*ido* isomers. The cyclization of the free nitro-hexoses to nitro-inositols is discussed in Chapter 19.

[340] H. H. Baer and C. B. Madumelu, *Carbohydrate Res.*, 1975, **39**, C8.
[341] J. Kovář and H. H. Baer, *Carbohydrate Res.*, 1975, **39**, 19.



(163)

Onodera and his co-workers have reported a method for the selective cleavage of the terminal glycosidic bond of (1 → 4)-linked oligo- and poly-saccharides that depends on the introduction of an electron-withdrawing nitro-group at C-5 of the reducing-end residue (*i.e.* in a β-position to the glycosidic bond to be cleaved).[342] Thus, the reactions shown in Scheme 59 were used to transform benzyl hepta-*O*-



(164)

R = 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl

Reagents: i, H_2–Pd–C; ii, $NaBH_4$; iii, BzCl–py; iv, Ac_2O–DMSO; v, NH_2OH; vi, H_2O_2–$(CF_3CO)_2O$

Scheme 59

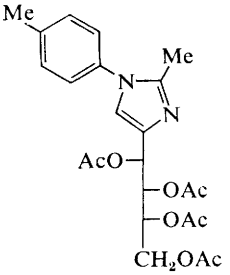
methyl-β-maltoside into the epimeric 5-nitro-derivatives (164), which underwent cleavage of the glycosidic bond in aqueous sodium hydroxide.

Other references to nitro-sugars appear in Chapters 14 and 21.

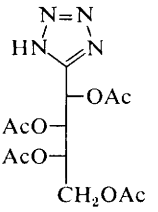
Heterocyclic Derivatives

The reaction of 2-amino-2-deoxyheptoses with ethyl acetoacetate – leading to the formation of pyrroles – has been reported.[342a] The novel imidazole (165) has been produced from 2-acetamido-2-deoxy-*N*-*p*-tolyl-β-D-gluco-pyranosylamine under acetolysing conditions.[343] Acetylated aldononitriles reacted with ammonium azide in DMF to give 5-(polyacetoxyalkyl)tetrazoles [*e.g.* (166)], which afforded the corresponding 5-(polyhydroxyalkyl)tetrazoles on deacetylation.[344] Treatment of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose with hydrazine hydrate has been found to give the pyridazine derivative (167) in addition to the expected hydrazino-compound (168), particularly at high temperatures.[345, 346] A mechanism to account for the

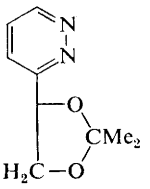
[342] N. Kashimura, K. Yoshida, and K. Onodera, *Carbohydrate Res.*, 1975, **40**, 375.
[342a] F. Garcia Gonzalez, J. Fernandez-Bolanos, and J. Galbis Perez, *Anales de Quim.*, 1974, **70**, 1082 (*Chem. Abs.*, 1975, **83**, 193 598w).
[343] S. Hirano and R. Yamasaki, *Carbohydrate Res.*, 1975, **43**, 377.
[344] A. M. Seldes, I. M. E. Thiel, and J. O. Deferrari, *Carbohydrate Res.*, 1975, **39**, 47.
[345] P. Smit, G. A. Stork, and H. C. van der Plas, *J. Heterocyclic Chem.*, 1975, **12**, 957.
[346] P. Smit, G. A. Stork, and H. C. van der Plas, *J. Heterocyclic Chem.*, 1975, **12**, 75.



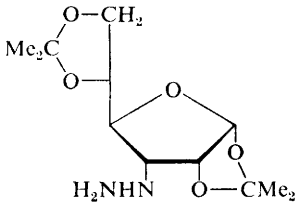
(165)



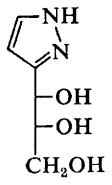
(166)



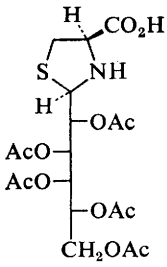
(167)



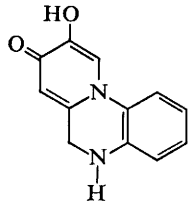
(168)



(169)



(170)

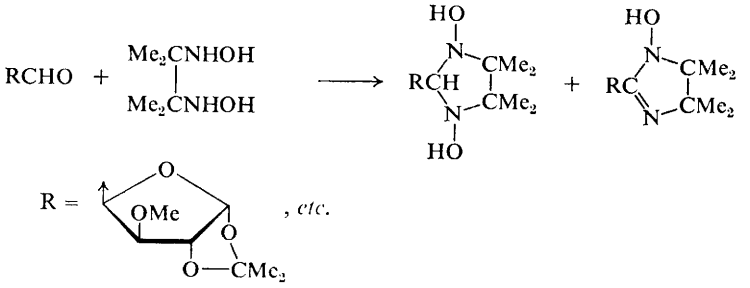


(171)

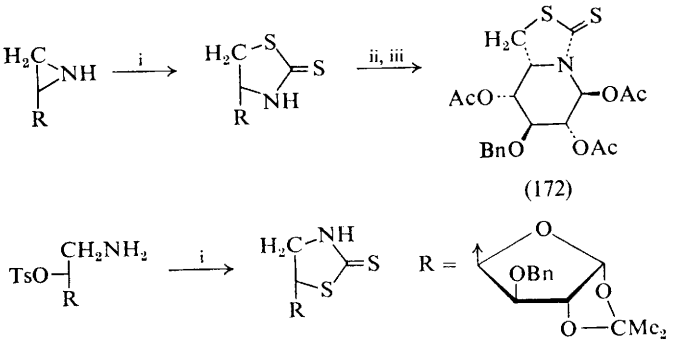
formation of (167) was proposed. Both (168) and the isomeric D-*allo* compound yielded the trihydroxypropylpyrazole (169) quantitatively on treatment with concentrated hydrochloric acid.[346] The reactions of 2,3:5,6-di-*O*-cyclohexylidene-D-mannono-1,4-lactone with the carbanions of some heterocyclic compounds (*e.g.* 2-picoline and 2,4,6-collidine) have been investigated.[347] The thiazolidine derivative formed when 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-galactose reacts with L-cysteine has been reported to have the stereochemistry shown in (170).[348] The reaction of D-*threo*-2,5-hexodiulose with *o*-phenylenediamine resulted in the formation of a tricyclic oxopyrido[1,2*a*]quinoxaline (171).[349]

Imidazolidine and imidazoline derivatives were formed when dialdose derivatives reacted with 2,3-bis(hydroxylamino)-2,3-dimethylbutane (Scheme 60); oxidation of these derivatives afforded stable free radicals that were examined by e.s.r. spectroscopy.[350] Several oxazole and thiazolidine derivatives having C-5 and C-6 of 3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-idofuranose as part of the heterocyclic ring, and a new class of bicyclic thiazolidine derivative (172) have been prepared by the reactions shown in Scheme 61.[351] The bis(1,3,4-oxadiazole) derivative (173) has been prepared by condensative cyclization of 2,3,4,5-tetra-*O*-acetylgalactaric acid bis(benzoylhydrazide) with triethyl orthoformate in *p*-dioxan.[352] Other heterocyclic derivatives are referred to in Chapters 14 and 15.

[347] Yu. A. Zhdanov, V. G. Alekseeva, and V. N. Fomina, *Doklady Akad. Nauk S.S.S.R.*, 1974, **218**, 339.
[348] R. Bognár, Z. Györgydeák, L. Szilágyi, and L. Somogyi, *Annalen*, 1975, 1637.
[349] K. Imada, *Carbohydrate Res.*, 1975, **39**, 379.
[350] J. M. J. Tronchet, E. Mihaly, and M. Geoffroy, *Helv. Chim. Acta*, 1975, **58**, 1187.
[351] M. Iwakawa and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1975, **48**, 610.
[352] M. A. E. Shaban, E. S. H. El Ashry, M. A. M. Nassr, and V. N. Reinhold, *Carbohydrate Res.*, 1975, **42**, C1.

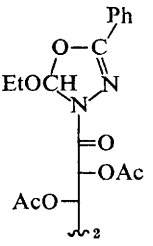


Scheme 60



Reagents: i, CS_2; ii, AcOH; iii, Ac_2O–py

Scheme 61



(173)

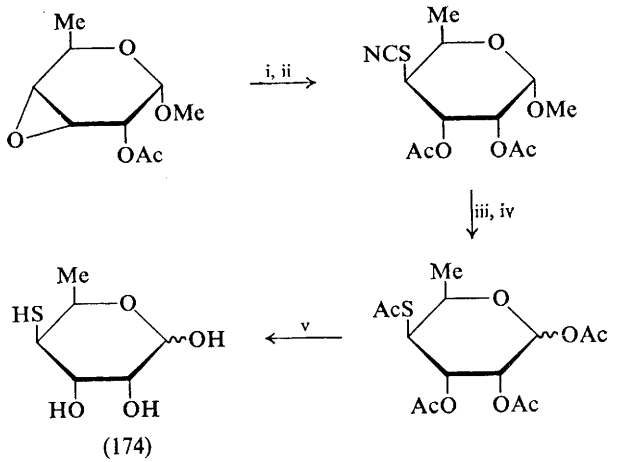
Oximes

The structures of D-arabinose and D-glucose oximes have been determined by means of various physical techniques.[353] D-Arabinose oxime was shown to exist in the *anti*(*Z*) acyclic form in the solid state and to isomerize to a 4 : 1 mixture of the *syn*(*E*) and *anti*(*Z*) forms in aqueous solution; D-glucose oxime exists in the cyclic β-pyranose form in the solid state and isomerizes to a mixture of the β-pyranose (23%), α-pyranose (7%), *anti*(*Z*) (13.5%), and *syn*(*E*) (56.5%) forms in aqueous solution.

[353] P. Finch and Z. Merchant, *J.C.S. Perkin I*, 1975, 1682.

11
Thio- and Seleno-sugars

6-Deoxy-4-thio-D-gulose (174) has been synthesized by the route shown in Scheme 62.[354] This thio-sugar showed little tendency to adopt the five-membered ring form containing the sulphur atom, either as the free sugar or under acetolysing conditions. An unsuccessful attempt to synthesize derivatives of 6-deoxy-4-thio-D-allose was also reported. Treatment of ethyl 3,5,6-tri-*O*-acetyl-2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside (175) with bromine furnished an unstable



(174)

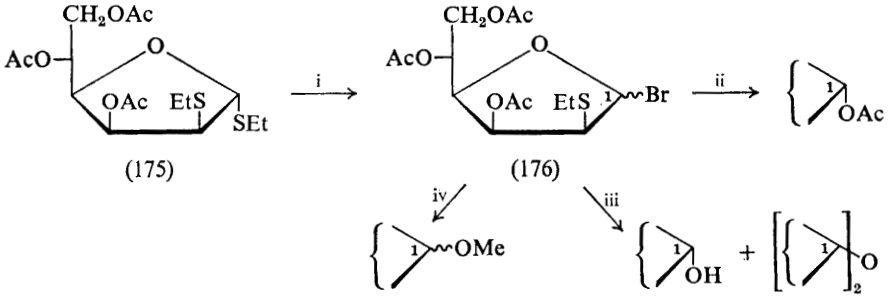
Reagents: i, KSCN–NH_4Cl; ii, Ac_2O–py; iii, Zn–Ac_2O–AcOH; iv, Ac_2O–H_2SO_4; v, MeONa–MeOH

Scheme 62

glycosyl bromide (176) from which several derivatives of 2-*S*-ethyl-2-thio-D-mannofuranose were obtained (Scheme 63).[355] An excess of chlorine converted the dithio-derivative (175) into a dichloro-derivative (177), which reacted with methanol to give a mixture of three glycosides, one retaining the chlorine atom at C-2 and the others resulting from replacement of both chlorine atoms by methoxy-groups (Scheme 64).

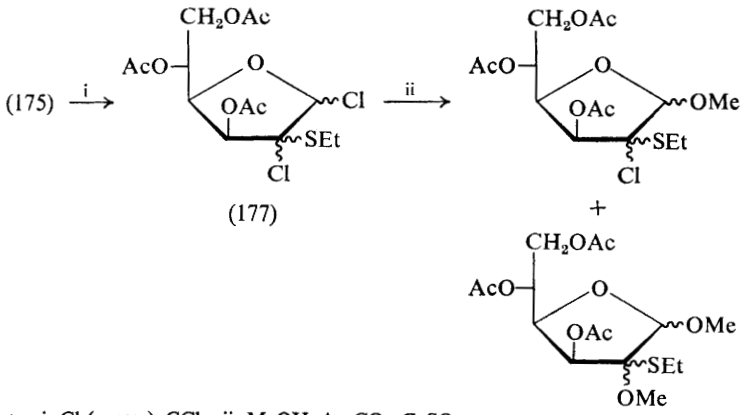
A novel method for the selective and direct replacement of the primary hydroxy-group of nucleosides by a thiol ether group is shown in Scheme 65,

[354] R.-A. Boigegrain and B. Gross, *Carbohydrate Res.*, 1975, **41**, 135.
[355] D. Horton and M. Sakata, *Carbohydrate Res.*, 1975, **39**, 67.



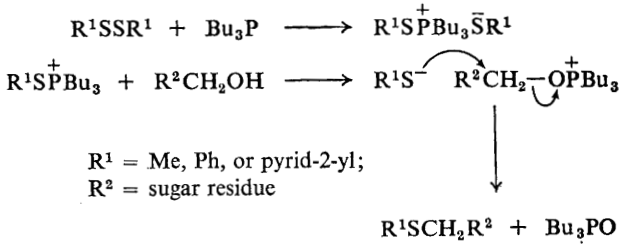
Reagents: i, Br_2–CCl_4; ii, $Hg(OAc)_2$–AcOH; iii, H_2O; iv, MeOH–Ag_2CO_3

Scheme 63



Reagents: i, Cl_2(excess)–CCl_4; ii, MeOH–Ag_2CO_3–$CaSO_4$

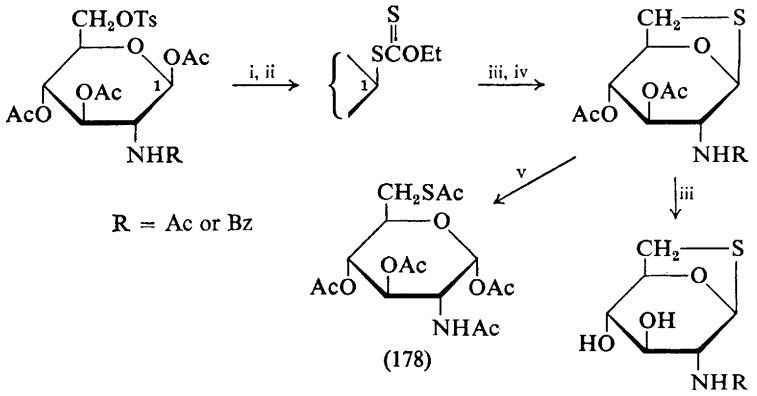
Scheme 64



Scheme 65

together with the mechanism suggested for the reaction.[356] This method should be of general applicability. A synthesis of 2-amino-2-deoxy-6-thio-α-D-gluco-pyranose penta-acetate (178) has been accomplished (Scheme 66) by a route that involves migration of the sulphur atom from C-1 to C-6.[357] A related migration,

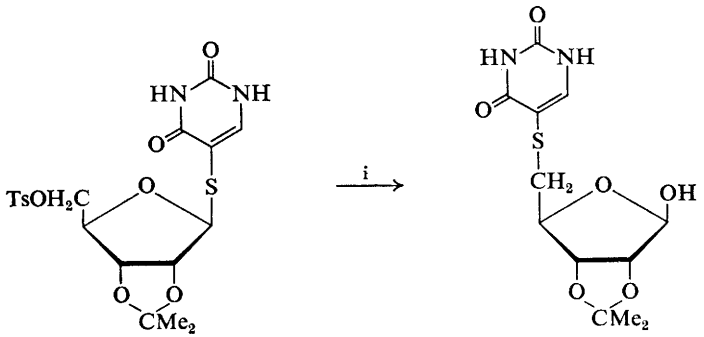
[356] I. Nakagawa and T. Hata, *Tetrahedron Letters*, 1975, 1409.
[357] K. Yamamoto, M. Haga, and S. Tejima, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 233.



Reagents: i, $TiCl_4$; ii, KCS_2OEt; iii, MeONa–MeOH; iv, Ac_2O–py; v, Ac_2O–AcOH–H_2SO_4

Scheme 66

presumably involving a 1′,5′-episulphonium ion, took place when *S*-(2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-β-D-ribofuranosyl)-5-thiouracil (179) was treated with a base (Scheme 67).[358] Direct displacement of the 6-sulphonate



Reagent: i, $NaHCO_3$–DMF

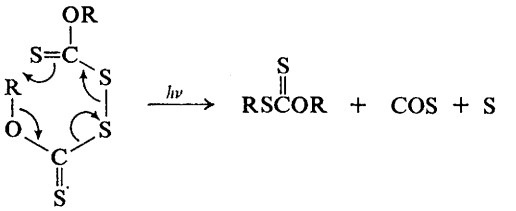
Scheme 67

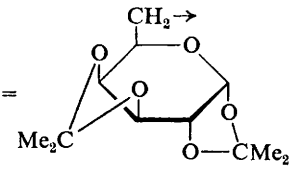
group of several D-glucopyranoside derivatives with sodium ethyl xanthate yielded the expected 6-ethoxythiocarbonyl-6-thio-derivatives, which were converted into the corresponding 6-thiols by alkaline hydrolysis; however, 1,2:3,4-di-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranose reacted with sodium ethyl xanthate only with difficulty to give the 6,6′-sulphide.[359] In this case, photolysis of the dithiobis(thioformate) (180) was used to introduce a

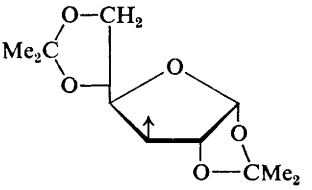
[358] E. J. Eustace, M. V. Pickering, T. E. Stone, and G. D. Daves, jun., *J. Heterocyclic Chem.*, 1974, **11**, 1113.

[359] D. Trimnell, E. I. Stout, W. M. Doane, C. R. Russell, V. Beringer, M. Saul, and G. Van Gessel, *J. Org. Chem.*, 1975, **40**, 1337.

sulphur-containing substituent at C-6 (Scheme 68).[360] The photolysis of the secondary dithiobis(thioformate) (181) gave a product retaining the original configuration at C-3.



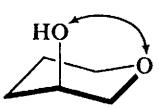
(180) R = 

(181) R = 

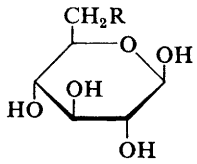
Scheme 68

The product ratios of the radical addition of sodium hydrogen [^{35}S]sulphite to eight different methyl 6-deoxyhex-5-enopyranosides have been determined.[361] Conformational analysis of the results enabled a direct comparison of diastereoisomeric monosaccharides for the first time; for one set of products (D-*allo* and L-*talo*), the agreement between the experimental results and theoretical predictions was improved if a value of 0.45 kcal mol^{-1} was assigned to the ring oxygen–O_{ax}-3 interaction (182).

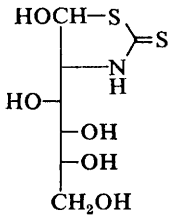
6-*S*-Dimethylarsino-6-thio-β-D-glucopyranose (183) and 6-*Se*-dimethylarsino-6-seleno-β-D-glucopyranose (184) have been synthesized by methods similar to



(182)



(183) R = $SAsMe_2$
(184) R = $SeAsMe_2$



(185)

those used to prepare the related 1-substituted derivatives[362] (see Vol. 7, p. 27). The seleno-ester (184) was hydrolysed at the C—Se bond in an alkaline medium about six and a half times faster than was the thio-ester (183) at the C—S bond. 2-Amino-2-deoxyaldoses reacted with carbon disulphide in the presence of DCC to give thiazolidine-2-thiones [*e.g.* (185)].[363]

Other references to thio-sugars are noted in Chapters 3 and 12.

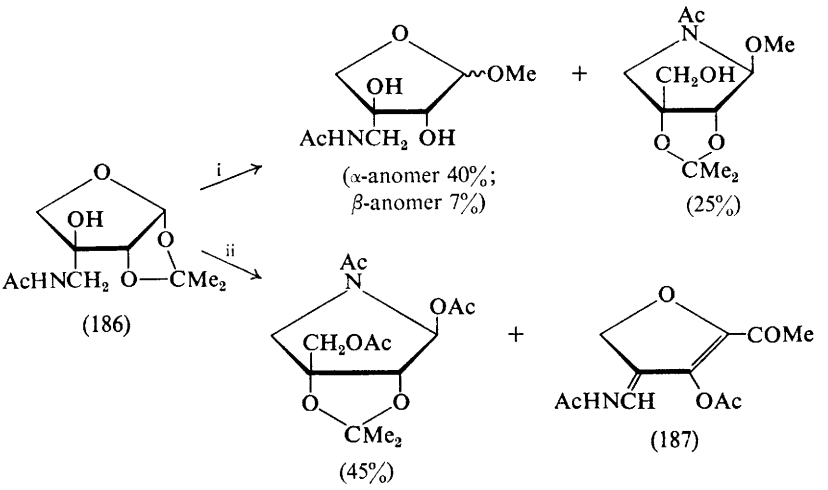
[360] E. I. Stout, W. M. Doane, C. R. Russell, and L. B. Jones, *J. Org. Chem.*, 1975, **40**, 1331.
[361] J. Lehmann and W. Weckerle, *Carbohydrate Res.*, 1975, **42**, 275.
[362] G. C. Chen, R. A. Zingaro, and C. R. Thompson, *Carbohydrate Res.*, 1975, **39**, 61.
[363] J. C. Jochims, *Chem. Ber.*, 1975, **108**, 2320.

12

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

Nitrogen Derivatives

Acid-catalysed methanolysis of the apiose derivative (186) gave a mixture of oxygen- and nitrogen-containing ring forms, the latter arising with migration of the isopropylidene group during the reaction (Scheme 69).[364] The principal



Reagents: i, MeOH–H^+; ii, Ac_2O–AcOH–H_2SO_4

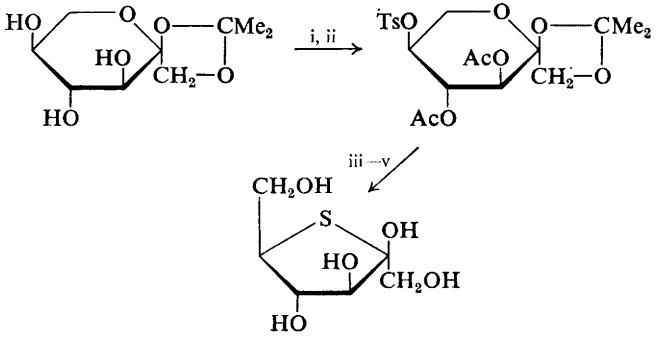
Scheme 69

product from the acetolysis of (186) was also a pyrrolidine derivative, namely 4-acetamido-3-*C*-(acetoxymethyl)-1-*O*-acetyl-4-deoxy-2,3-*O*-isopropylidene-β-D-erythrofuranose, although a small proportion of the unsaturated compound (187) was also formed.

Sulphur Derivatives

5-Thio-D-fructose has been synthesized by three routes in which a thioacetyl group was introduced by direct displacements on L-sorbopyranose 5-sulphonates; the best route is illustrated in Scheme 70.[365] ^1H N.m.r. spectroscopy indicated

[364] D. H. Ball, F. H. Bissett, M. H. Halford, and L. Long, jun., *Carbohydrate Res.*, 1975, **45**, 91
[365] M. Chmielewski and R. L. Whistler, *J. Org. Chem.*, 1975, **40**, 639.

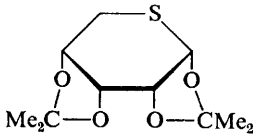


Reagents: i, TsCl–py; ii, Ac_2O–py; iii, MeC(O)SK–DMF; iv, MeONa–MeOH; v, CF_3CO_2H–H_2O

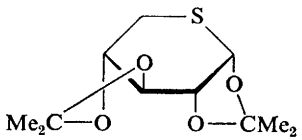
Scheme 70

that 5-thio-D-fructose exists principally in the β-furanose form in aqueous solution. Acetylation of 5-thio-D-fructose with acetic anhydride in pyridine yielded the α- and β-thiofuranose penta-acetates, whereas the acyclic penta-acetate was also formed in the presence of zinc chloride. 6-Deoxy-4-thio-D-gulose (see p. 77) is unusual in that it does not adopt the five-membered ring form containing the sulphur atom.[354]

Hughes and his co-workers have reported the preparation of three types of thio-sugar derivatives. The strong preference of 5-thio-D-ribose and 5-thio-D-xylose for ring-closure on to sulphur is shown by the formation of the strained 1,2:3,4-diacetals (188) and (189) in the reactions with acetone and 2,2-dimethoxy-



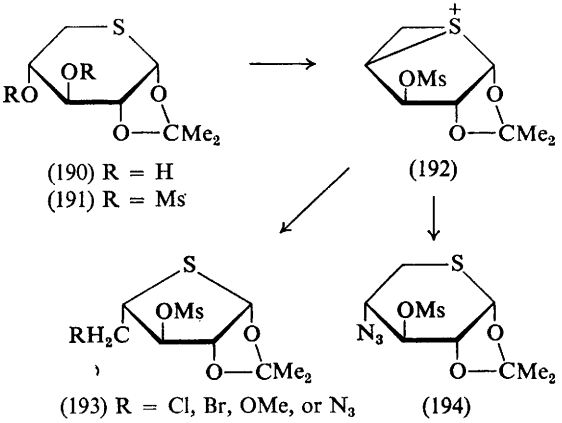
(188)



(189)

propane, respectively, whereas D-ribose and D-xylose yield mainly isopropylidene-furanoses.[366] The *trans*-fused 3,4-*O*-isopropylidene group of (189) can be removed selectively to give the monoacetal (190), whereas selective hydrolysis of (188) could not be achieved, in contrast to previous experience with the oxygen analogue. Methanesulphonylation of the monoacetal (190) furnished an unstable dimethanesulphonate (191) that readily reacted with nucleophiles (*e.g.* chloride, bromide, methoxide, and azide ions), by way of an intermediate episulphonium ion (192), to give 4-thio-L-arabinofuranosyl derivatives (193) and, in one case (with azide ion), an additional product (194) that retained the original stereochemistry (Scheme 71).[367] Several sulphoxides and sulphones in the 5-thio-D-ribopyranose series [*e.g.* (195) and (196)] have been obtained by oxidation of the appropriate glycoside or 1-acetate with either hydrogen peroxide–acetic acid or sodium periodate.[368] Sulphoxide configurations and conformations were assigned

[366] N. A. Hughes and C. J. Wood, *J.C.S. Chem. Comm.*, 1975, 294.
[367] W. Clegg, N. A. Hughes, and C. J. Wood, *J.C.S. Chem. Comm.*, 1975, 300.
[368] C. J. Clayton, N. A. Hughes, and T. D. Inch, *Carbohydrate Res.*, 1975, **45**, 55.



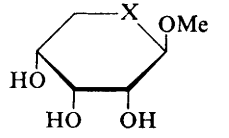
(190) R = H
(191) R = Ms

(192)

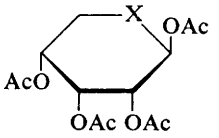
(193) R = Cl, Br, OMe, or N_3

(194)

Scheme 71

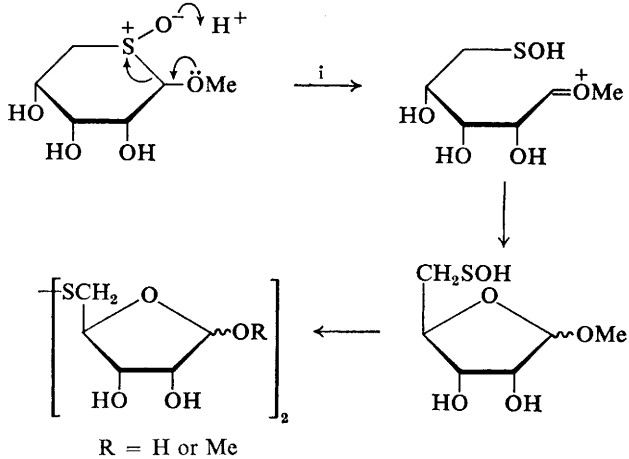


(195) X = $\overset{+}{S}—\bar{O}$ or SO_2



(196) X = $\overset{+}{S}—\bar{O}$ or SO_2

by ^1H n.m.r. spectroscopy. The sulphoxide (195; X = $\overset{+}{S}—\bar{O}$) was stable to base, but was converted by aqueous or methanolic acid into bis(5-deoxy-D-ribofuranose) 5,5′-disulphide and glycosides thereof, respectively (Scheme 72); the sulphone (195; X = SO_2) was stable to acid, but it decomposed in dilute sodium hydroxide into a complex mixture of products.



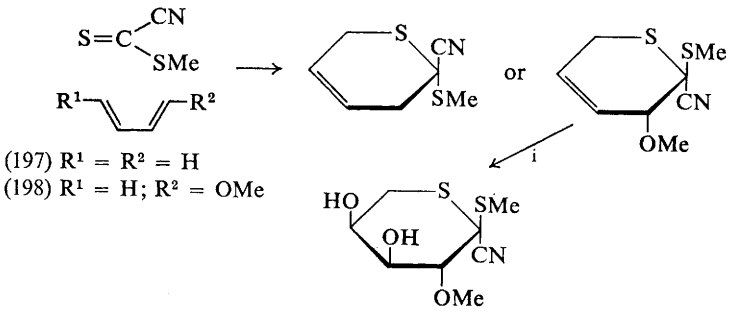
R = H or Me

Reagent: i, MeOH–H^+ or H_3O^+

Scheme 72

Whistler's group have continued their investigations of the biological aspects of thio-sugars. 5-Thio-D-glucose was found to be a substrate for yeast hexokinase, and it competitively inhibited the phosphorylation of D-glucose.[369] 5-Thio-D-glucose 1-phosphate is a substrate for rat-muscle phosphoglucomutase and it competitively inhibited the isomerization of D-glucopyranose 1-phosphate into the 6-phosphate. ^{14}C-Labelling techniques have been used to investigate the metabolism of 5-thio-D-glucose and 6-thio-D-fructose in rats; 5-thio-D-glucose was largely (~ 90%) excreted, whereas 6-thio-D-fructose was efficiently metabolized, part of the label being exhaled and the remainder retained.[369a] 5-Thio-D-glucose elevated the level of D-glucose in the blood of rats, whereas 6-thio-D-fructose did not.

Several novel monosaccharide derivatives containing a sulphur atom in the ring have been obtained *via* Diels–Alder reactions of methyl cyanodithioformate with buta-1,3-diene (197) and *trans*-1-methoxybuta-1,3-diene (198) (Scheme 73); the



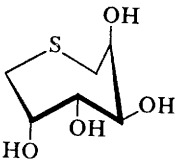
(197) $R^1 = R^2 = H$
(198) $R^1 = H$; $R^2 = OMe$

Reagent: i, OsO_4

Scheme 73

regioselectivity of the reagent is opposite to that of carbonyl dienophiles with (198).[370] *cis*-Hydroxylation of the Diels–Alder adducts afforded such compounds as (199).[371]

The 1,6-dimethanesulphonates of D-mannitol or galactitol reacted with sodium sulphide at pH 8—10 to give the corresponding 1,6-thioanhydrohexitols [*e.g.* (200)].[372]

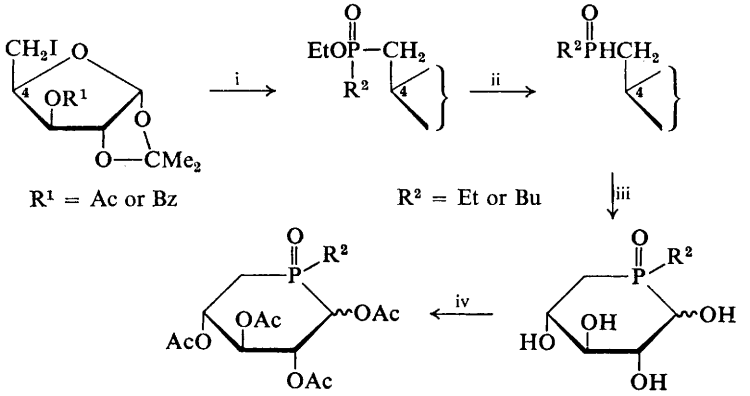


(200)

[369] M. S. Chen and R. L. Whistler, *Arch. Biochem. Biophys.*, 1975, **169**, 392.
[369a] M. J. Pitts, M. Chmielewski, M. S. Chen, M. M. A. Abd El-Rahman, and R. L. Whistler, *Arch. Biochem. Biophys.*, 1975, **169**, 384.
[370] D. M. Vyas and G. W. Hay, *J.C.S. Perkin I*, 1975, 180.
[371] D. M. Vyas and G. W. Hay, *Canad. J. Chem.*, 1975, **53**, 1362.
[372] M. Jarman and L. J. Griggs, *Carbohydrate Res.*, 1975, **44**, 317.

Phosphorus Derivatives

Seo and Inokawa have reported syntheses of the 5-alkylphosphinyl-5-deoxy-D-xylopyranoses shown in Scheme 74, although the stereochemistry at the phosphorus atom has still to be established.[373]



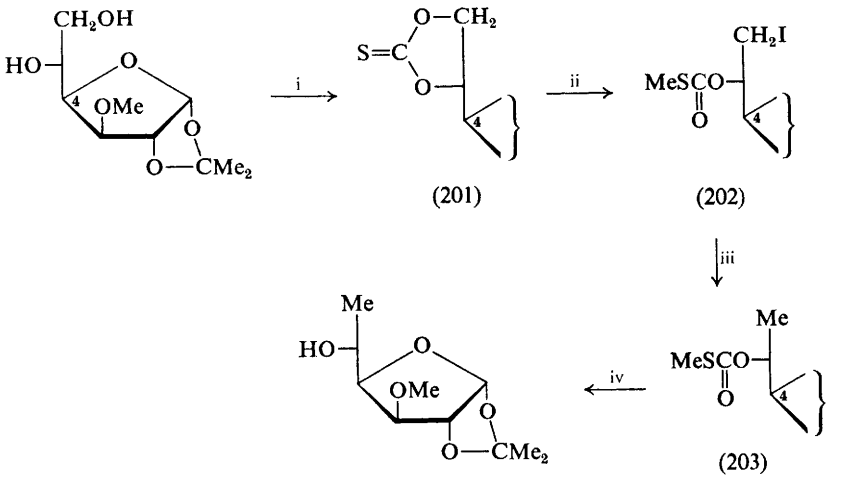
Reagents: i, Et(or Bu)P(OEt)$_2$; ii, NaAlH$_2$(OCH$_2$CH$_2$OMe)$_2$; iii, H_3O^+; iv, Ac$_2$O–py

Scheme 74

[373] K. Seo and S. Inokawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 1237.

13
Deoxy-sugars

New methods have been reported for the deoxygenation of carbohydrate derivatives. One such procedure reported by Barton's group has been noted in Chapter 6 (see Scheme 25).[222] This group has also shown that ring-opening reactions of diol thiocarbonates can be usefully applied to the synthesis of 6-deoxy-sugars (Scheme 75).[374] Thus, the diol thiocarbonate (201) was transformed into the iodo-thio-



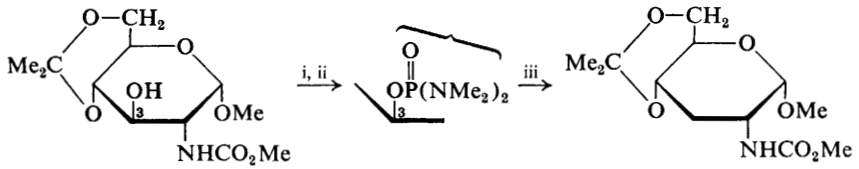
Reagents: i, di-imidazol-1-yl thione; ii, MeI; iii, $Cr(OAc)_2$–BuSH–DMF; iv, MeONa–MeOH

Scheme 75

carbonate (202) by refluxing in methyl iodide, and (202) into the 6-deoxy-thiocarbonate (203) on reduction with chromium(II) acetate in refluxing DMF in the presence of butane-1-thiol. Finally, hydrolysis of (203) with sodium methoxide in methanol gave 6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose. The reduction of phosphorodiamidates with lithium and ethylamine in t-butyl alcohol has also been used to prepare deoxy-sugars (Scheme 76); however, the reaction of phosphorodiamidates derived from 1,2:5,6-di-*O*-isopropylidene-α-D-gluco- or -D-allo-furanose yielded 2,3-dideoxy-derivatives (Scheme 77).[245]

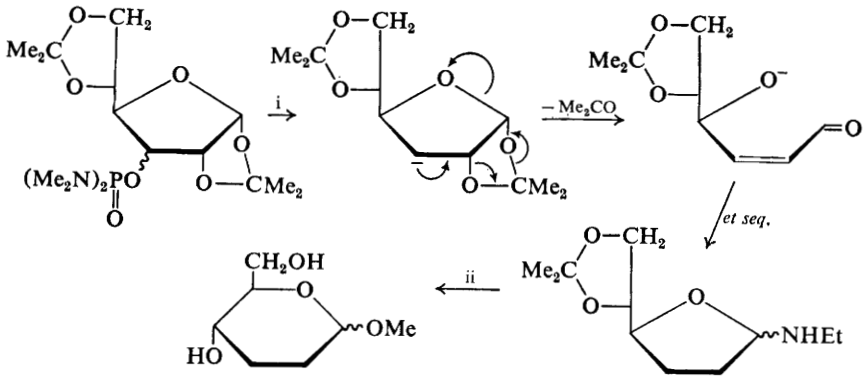
2-Deoxy-DL-*erythro*-pentose has been synthesized by the route shown in Scheme 78.[375] The products obtained by pyrolysis of 2-deoxy-D-*arabino*-hexose

[374] D. H. R. Barton and R. V. Stick, *J.C.S. Perkin I*, 1975, 1773.
[375] V. B. Mochalin and A. N. Kornilov, *Zhur obshchei Khim.*, 1974, **44**, 2334.



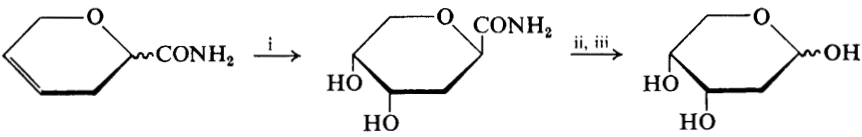
Reagents: i, NaH; ii, $(Me_2N)_2P(O)Cl$; iii, Li–$EtNH_2$

Scheme 76



Reagents: i, Li–$EtNH_2$; ii, MeOH–H^+

Scheme 77



Reagents: i, $KMnO_4$; ii, NaOBr; iii, H_3O^+

Scheme 78

have been examined,[375a] and this deoxy-sugar has been found to be incorporated into the glycolipids of normal and transformed cells.[376] Several derivatives of 2-deoxy-D-*arabino*-hexose, including the 3-*O*-methyl ether, the 3,6-anhydride, and a steroidal glycoside thereof, have been detected in *Pteris inaequalis*.[377]

A series of 1,6-anhydro-2- and -3-deoxy-D-hexopyranoses has been prepared by reductive ring-opening of appropriate oxirans.[378] In a procedure for the synthesis of 3-deoxyaldoses, the monosaccharide benzoylosazone was converted into the 3-deoxyaldosulose by treatment with an aromatic amine; base-catalysed

[375a] Y. Tanaka and T. Nakamura, *Chuo Daigaku Rikogakuba Kiyo*, 1974, **17**, 175 (*Chem. Abs.*, 1975, **83**, 131 849v).

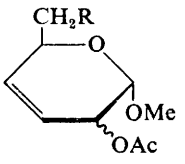
[376] M. R. Steiner, K. Somers, and S. Steiner, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 795.

[377] T. Murakami, N. Tanaka, T. Tezuka, and C.-M. Chen, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1634.

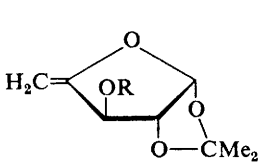
[378] N. M. Merlis, E. A. Andrievskaya, L. I. Kostelian, and O. P. Golova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 139 (*Chem. Abs.*, 1975, **82**, 125 529d).

isomerization of the 3-deoxyaldosulose afforded the corresponding meta-saccharinic acid, which, after lactonization, was reduced to the 3-deoxyaldose.[379] Oxymercuration–demercuration of the 2-methoxy-5,6-dihydropyran derivatives (204) has led to syntheses of racemic 3-deoxy-, 3,4- and 3,6-dideoxy-, and 3,4,6-trideoxy-hexopyranosides.[380]

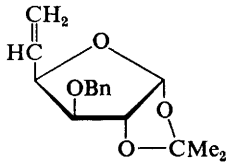
5-Deoxy-L-arabinose has been prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by way of the alkenic derivative (205),[381] and an oxymercuration–



(204) R = H or Ac



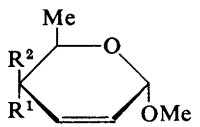
(205) R = tetrahydropyranyl



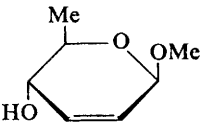
(206)

demercuration sequence was used to obtain derivatives of 5-deoxy-D-*xylo*-hexose from the terminal alkene (206);[382] such alcohols as propan-2-ol added to the intermediate mercurinium ion mainly at C-5, whereas sterically hindered t-butyl alcohol added mainly at C-6. The products obtained from reduction of hexofuranosyl 5-sulphonates (*e.g.* 6-deoxy-1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose and the corresponding 3,6-dideoxy-derivative) with lithium aluminium hydride have been shown to depend on the type of substituent at C-3 and C-6.[383] Thus, reduction of either 3- or 6-deoxy-sulphonates gave principally the 3(6),5-dideoxy-derivative by C—O bond scission, whereas the 3,6-dideoxy-sulphonate underwent S—O bond scission.

cis-Hydroxylation of the 2,3-unsaturated sugars (207), (208), and (209) has been used in the total synthesis of methyl 6-deoxy-α-DL-manno-, -β-DL-allo-,



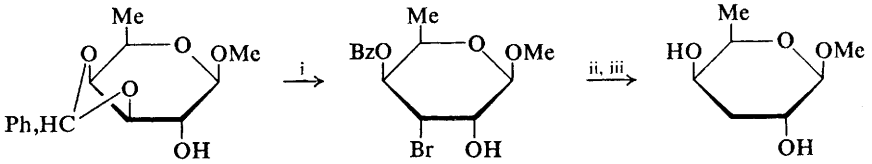
(207) R^1 = OH; R^2 = H
(209) R^1 = H; R^2 = OH



(208)

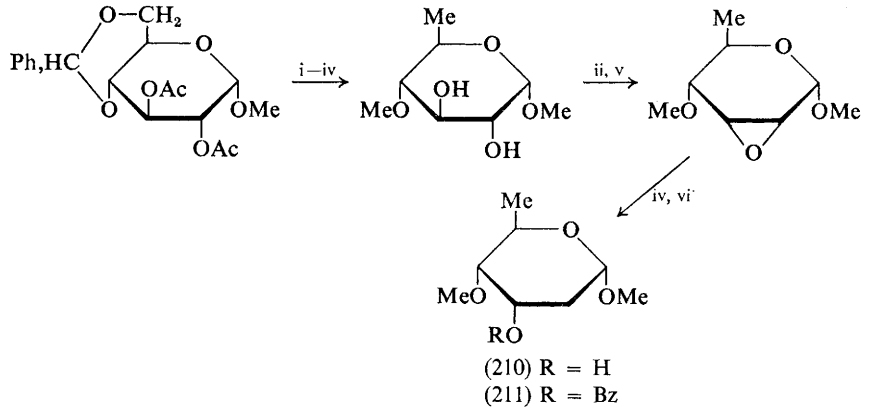
-α-DL-talo-, and -α-DL-gulo-hexopyranosides.[384] Convenient syntheses of 6-deoxy-D-gulose (antiarose)[385] and 6-deoxy-2,3-di-*O*-methyl-D-glucopyranose[386] have been described from D-gulono-1,4-lactone and phenyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-β-D-glucopyranoside, respectively.

[379] Š. Kučár, J. Zámocký, and Š. Bauer, *Coll. Czech. Chem. Comm.*, 1975, **40**, 457.
[380] A. Banaszek, *Bull. Acad. polon. Sci., Sér. Sci. khim.*, 1974, **22**, 1045 (*Chem. Abs.*, 1975, **82**, 156 638t).
[381] J. Kiss, R. D'Souza, and P. Taschner, *Helv. Chim. Acta*, 1975, **58**, 311.
[382] S. Czernecki, C. Georgoulis, and C. Provelenghiou, *Tetrahedron Letters*, 1975, 2623.
[383] A. Zobáčová, V. Heřmánková, Z. Kefurtová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3505.
[384] O. Achmatowicz and B. Szechner, *Roczniki Chem.*, 1975, **49**, 1715.
[385] L. M. Lerner, *Carbohydrate Res.*, 1975, **44**, 116.
[386] L. Kiss, A. Liptak, and P. Nanasi, *Acta Chim. Acad. Sci. Hung.*, 1974, **83**, 201 (*Chem. Abs.*, 1975, **82**, 43 667h).



Reagents: i, NBS–CCl_4; ii, H_2–Pd–C; iii, $Ba(OMe)_2$

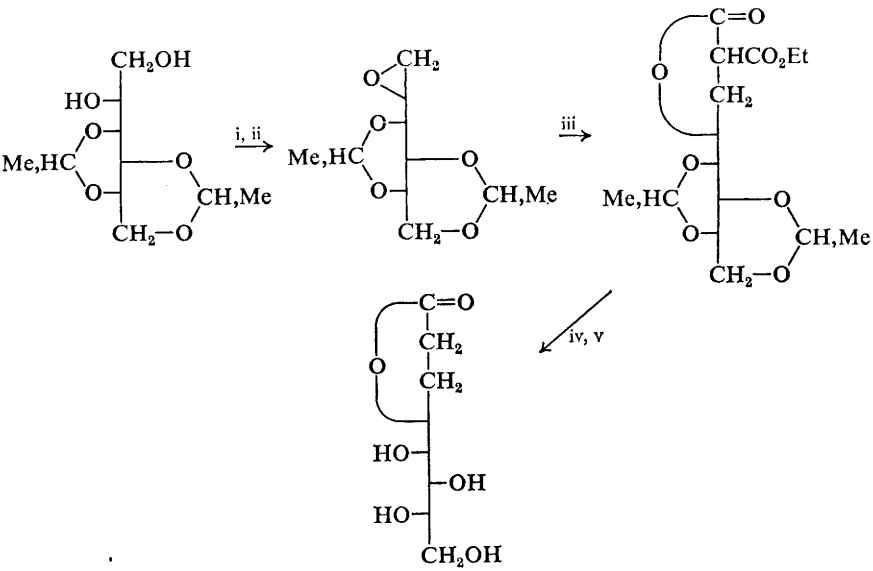
Scheme 79



(210) R = H
(211) R = Bz

Reagents: i, H^+; ii, TsCl–py; iii, CH_2N_2–BF_3; iv, $LiAlH_4$–THF; v, MeONa–MeOH; vi, BzCl–py

Scheme 80



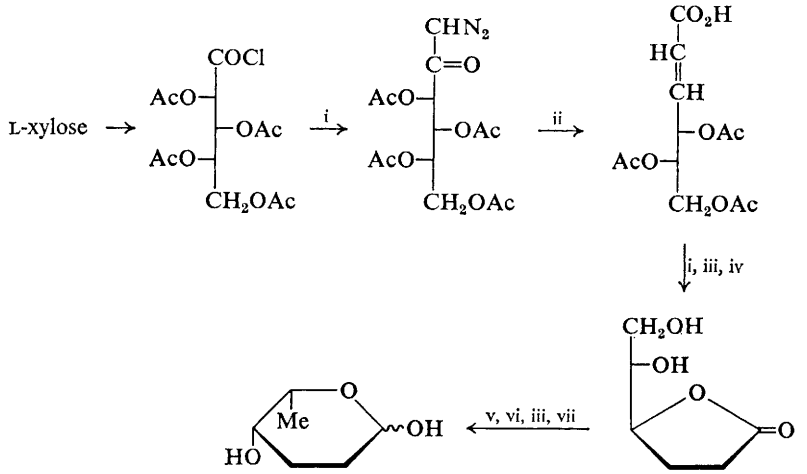
Reagents: i, TsCl–py; ii, EtONa; iii, $NaCH(CO_2Et)_2$; iv, HO^-; v, H^+

Scheme 81

A new synthesis of the methyl β-glycoside of abequose (3,6-dideoxy-D-*xylo*-hexose) is shown in Scheme 79.[387] Methyl 2,6-dideoxy-4-*O*-methyl-α-D-*ribo*-hexopyranoside (210) has been synthesized (Scheme 80), but the derived 3-benzoate (211) was clearly distinguishable from that obtained from methyl α-varioside, which is claimed to have the same structure [388] (see Vol. 8, p. 91). It appears that variose is more likely to be a 2,6-dideoxy-3-*O*-methylhexose.

2,3-Dideoxyoctono-1,4-lactones can be prepared by way of the opening of terminal oxirans with the carbanion formed from dimethyl malonate (Scheme 81).[389]

Jarý's group have prepared 4,6-dideoxy-D-*arabino*-hexose, 3,4,6-trideoxy-D-*erythro*- and -D-*threo*-hexose, and 2,4,6-trideoxy-D-*erythro*-hexose by conventional ring-opening reactions of oxirans.[390] New syntheses of 2,3,6-trideoxy-DL-*erythro*-hexose (DL-amicetose) [391] and 2,3,6-trideoxy-L-*threo*-hexose (rhodinose) (Scheme 82) [392] have been reported.



Reagents: i, CH_2N_2–Et_2O; ii, Ag_2O–H_2O; iii, H_2–Pd–C; iv, 1M-NaOH; v, TsCl–py; vi, NaI–Me_2CO; vii, $LiAlH_2(OBu^i)$

Scheme 82

1,6,6′-Trideoxysucrose is available by reduction of the 1,6,6′-trichloro-derivative [252] (see Chapter 6).

[387] K. Eklind, P. J. Garegg, and B. Gotthammer, *Acta Chem. Scand.* (*B*), 1975, **29**, 633.
[388] H. Takai, H. Yuki, and K. Takiura, *Tetrahedron Letters*, 1975, 3647.
[389] V. I. Kornilov, Yu. A. Zhdanov, and S. V. Turik, *Zhur. obshchei Khim.*, 1974, **44**, 2057 (*Chem. Abs.*, 1975, **82**, 17 031b).
[390] K. Kefurt, Z. Kefurtová, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 164.
[391] J. Bastard and M. Fetizon, *Bull. Soc. chim. France*, 1975, 1617.
[392] R. Knollmann and I. Dyong, *Chem. Ber.*, 1975, **108**, 2021.

14
Unsaturated Derivatives

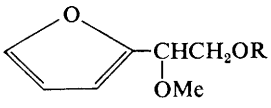
Fraser-Reid has summarized his own contributions to the chemistry of unsaturated sugars.[393]

Glycals

3,4,6-Tri-*O*-benzyl-D-glucal has been prepared by direct benzylation of D-glucal or its triacetate and by the reaction of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide with zinc dust.[394] The ^{1}H n.m.r. spectra of 3,4,6-tri-*O*-acetyl- and -benzyl-D-glucal were examined in the presence of lanthanide shift reagents. References to the use of glycal derivatives in the synthesis of α-glycosides and diamino-sugars are included in Chapters 3 and 8, respectively.

Details of the reductive rearrangement of glyc-2-enopyranosides to give 3-deoxyglycal derivatives and acyclic vinyl ethers have been reported (see Vol. 4, p. 95).[395] The work was extended to other allylic derivatives, and the reaction pathways were investigated by means of deuterium-labelling techniques.

A reinvestigation of the acid-catalysed addition of methanol to D-glucal has shown that the products are methyl 2-deoxy-α-D-*arabino*-hexopyranoside, the furans (212) and (213), and the acyclic derivatives (214) and (215).[396] An analogous



(212) R = H
(213) R = Me

$MeOCH_2CH_2COCH_2\underset{\displaystyle OMe}{\underset{|}{C}}HCH(OMe)_2$

(214)

$MeOCH_2CH_2COCH_2CH_2CO_2Me$

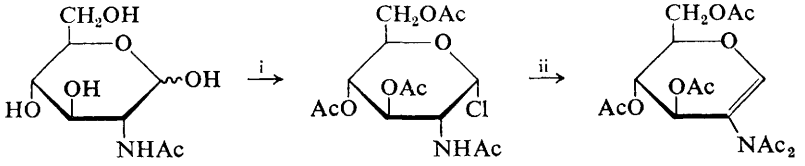
(215)

reaction with 3,4,6-tri-*O*-acetyl-D-glucal gave first methyl 4-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside, which reacted further to give the compounds (213)—(215).

An improved synthesis of the 2-acetamido-D-glucal derivative (216) has been described (Scheme 83).[397] The chlorination of 2,3,4,6-tetra-*O*-benzoyl-2-hydroxy-D-glucal is discussed in Chapter 7.

On hydrolysis in refluxing aqueous *p*-dioxan, 3,4,6-tri-*O*-acetyl-D-glucal, 3,4,6-tri-*O*-acetyl-D-galactal, and 3,4-di-*O*-acetyl-D-arabinal were each converted into a

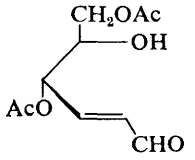
[393] B. Fraser-Reid, *Accounts Chem. Res.*, 1975, **8**, 192.
[394] P. Boullanger, J.-C. Martin, and G. Descotes, *J. Heterocyclic Chem.*, 1975, **12**, 91.
[395] B. Fraser-Reid, S. Y.-K. Tam, and B. Radatus, *Canad. J. Chem.*, 1975, **53**, 2005.
[396] Z. Zwierzchowska-Nowakowska and A. Zamojski, *Roczniki Chem.*, 1974, **48**, 1019 (*Chem. Abs.*, 1974, **81**, 169 717x).
[397] N. Pravdić, I. Franjić-Mihalić, and B. Danilov, *Carbohydrate Res.*, 1975, **45**, 302.



(216)

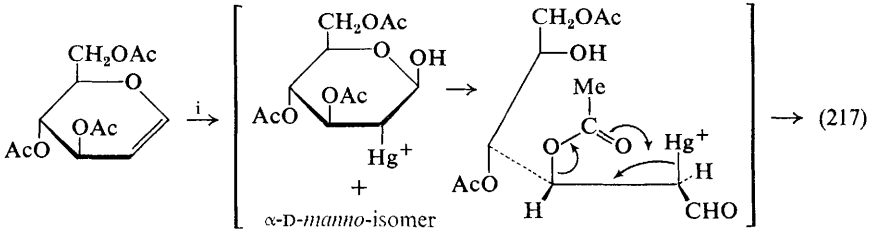
Reagents: i, AcCl–HCl; ii, $MeC(OAc)=CH_2-H^+$

Scheme 83



(217)

partially acetylated *aldehydo*-hex-2-enopyranose [*e.g.* (217)] *via* the related pseudoglycal, which is the principal product obtained after brief reaction[398] (see also Vol. 4, p. 95). The conversion of pseudoglycals into (217) and related *trans*-$\alpha\beta$-unsaturated aldehydes occurs by photochemically induced isomerization of the corresponding *cis*-aldehyde, which exists in equilibrium with the pseudoglycal. 3,4,6-Tri-*O*-acetyl-D-glucal was quantitatively converted into (217) when treated with acidified *p*-dioxan in the presence of mercuric sulphate (Scheme 84);

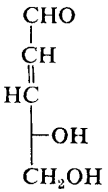


Reagent: i, $HgSO_4-H_2O-H^+$

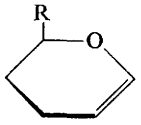
Scheme 84

analogous $\alpha\beta$-unsaturated aldehydes were also formed from 3,4,6-tri-*O*-acetyl-D-galactal and 3,4-di-*O*-acetyl-D-arabinal.[399] However, D-glucal and D-galactal underwent elimination catalysed by mercuric ion and acid to yield 2-(D-*glycero*-1,2-dihydroxyethyl)furan. Freshly prepared aqueous solutions of 2-deoxy-D-*erythro*-pentose were found to contain no detectable amounts of the $\alpha\beta$-unsaturated aldehyde (218) at room temperature, although a maximum yield (~ 13%) of (218) was obtained at 120 °C, when it is probably formed by β-elimination of water from the open-chain form of the deoxypentose.[400]

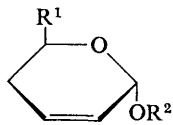
[398] S. Y.-K. Tam and B. Fraser-Reid, *Carbohydrate Res.*, 1975, **45**, 29.
[399] F. Gonzalez, S. Lesage, and A. S. Perlin, *Carbohydrate Res.*, 1975, **42**, 267.
[400] H. Esterbauer, E. B. Sanders, and J. Schubert, *Carbohydrate Res.*, 1975, **44**, 126.



(218)

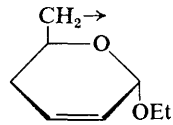


(219) R = H, Me, or CH_2OH



(220) R^1 = H, Me, or CH_2OH;

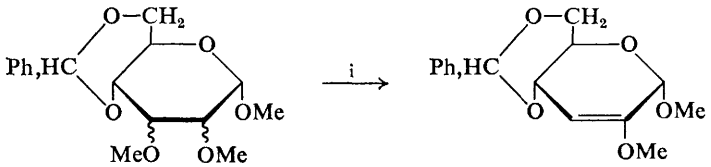
R^2 = Me, Et, Bn, or



The racemic glycals (219) reacted with NBS in the presence of alcohols to give 2-alkoxy-3-bromo-tetrahydropyrans, which yielded unsaturated glycosides (220) on dehydrobromination.[401]

Other Unsaturated Derivatives

Treatment of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-gluco-, altro-, and -allo-pyranosides with butyl-lithium afforded the vinyl ether derivative shown in Scheme 85 in yields of 30, 45, and 55%, respectively.[402] Substituted 2,3-unsatu-



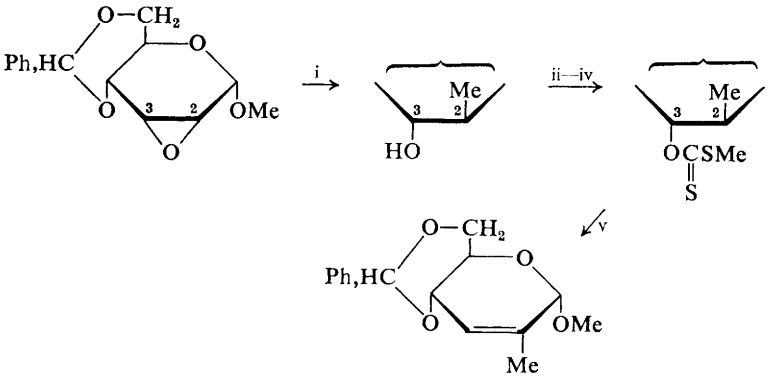
Reagent: i, BuLi

Scheme 85

rated derivatives [*e.g.* (221)] resulted from Chugaev pyrolysis of xanthates obtained by the reactions outlined in Scheme 86.[403] Several of the minor components have been identified from the reaction between ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside and chlorosulphonyl isocyanate in either acetonitrile or ether; they include a 3-(alkoxycarbonyl)amino-3-deoxy-D-allal, 2-(D-*glycero*-1,2-diacetoxyethyl)furan, two hex-2-enopyranosylamines, and the *C*-linked disaccharides (222) and (223).[404]

Overend's group has described the synthesis and reactions of some 2- and 3-arylazo-derivatives of methyl 2,3-dideoxy-D-pent-2-enofuranoside [*e.g.* (224)—(228)].[405] These arylazoalkenes (Ar = Ph or *p*-$NO_2C_6H_4$) are useful synthetic

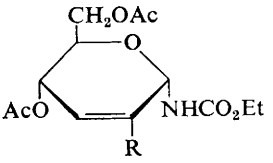
[401] V. B. Mochalin and A. N. Kornilov, *Khim. geterotsikl. Soedinenii*, 1975, 171 (*Chem. Abs.*, 1975, **83**, 43 623m).
[402] A. Klemer and G. Rodemeyer, *Chem. Ber.*, 1975, **108**, 1896.
[403] D. R. Hicks and B. Fraser-Reid, *Canad. J. Chem.*, 1975, **53**, 2017.
[404] R. H. Hall, A. Jordaan, and O. G. de Villiers, *J.C.S. Perkin I*, 1975, 626.
[405] P. M. Collins, J. R. Hurford, and W. G. Overend, *J.C.S. Perkin I*, 1975, 2163.

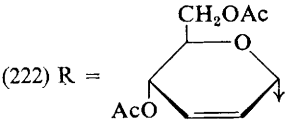


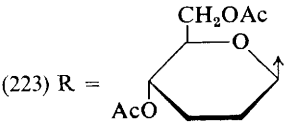
(221)

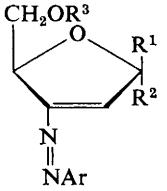
Reagents: i, $LiMe_2Cu$; ii, NaH; iii, CS_2; iv, MeI; v, heat

Scheme 86

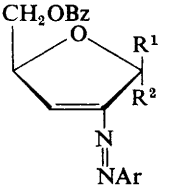




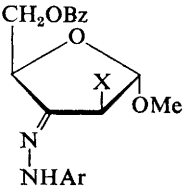




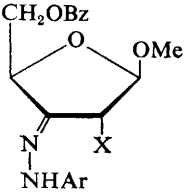
(224) R^1 = H; R^2 = OMe; R^3 = Bz
(225) R^1 = OMe; R^2 = H; R^3 = Bz
(226) R^1 = H; R^2 = OMe; R^3 = Tr



(227) R^1 = H; R^2 = OMe
(228) R^1 = OMe; R^2 = H

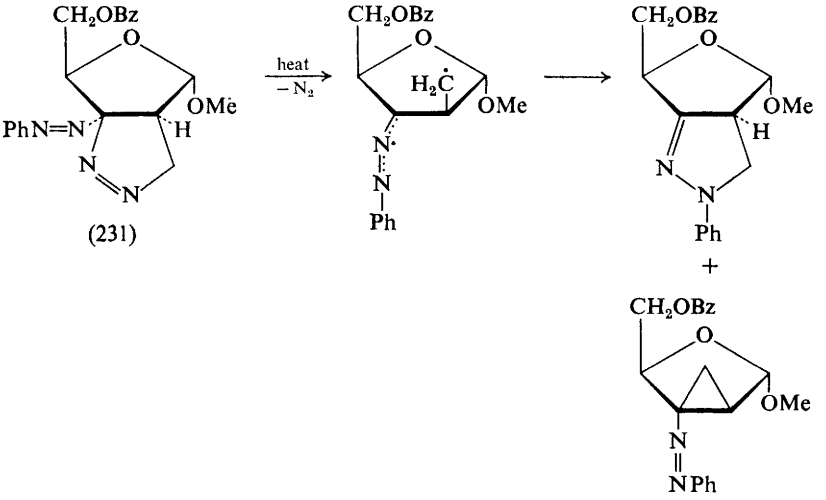


(229) X = NH_2, N_3, OMe, *etc.*



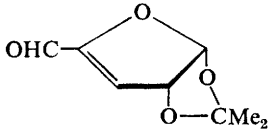
(230) X = NH_2, N_3, OMe, *etc.*

intermediates: for example, (224) and (225) underwent 1,4-addition reactions in a highly stereoselective way with nucleophiles (*e.g.* ammonia, dimethylamine, water, acetic acid, methoxide ion, and azide ion, *etc.*) to give (229) and (230), respectively, as products. Novel heterocyclic structures were obtained from 1,4-cycloadditions to the arylazoalkenes; for example the *cis*-adduct (231) was formed when methyl 5-*O*-benzoyl-2,3-dideoxy-3-phenylazo-α-D-*glycero*-pent-2-enofuranoside (224; Ar = Ph) reacted with diazomethane in ether.[405a] This adduct evolved nitrogen on pyrolysis to give two products, presumably formed by cyclization of a biradical intermediate (Scheme 87).

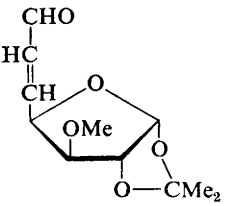


Scheme 87

The αβ-unsaturated aldehydes (232) and (233) have been converted into imidazoline and imidazolidine derivatives by reaction with bis(hydroxylamino)-2,3-dimethylbutane [350] (see also Scheme 60).



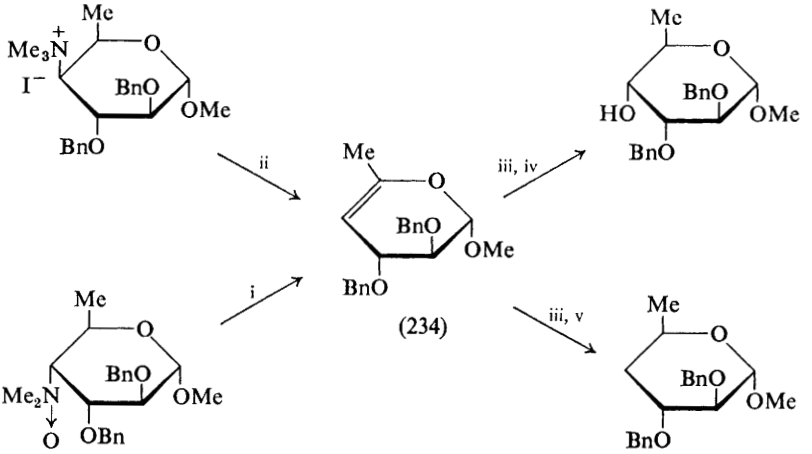
(232)



(233)

Syntheses of methyl 2,3-di-*O*-benzyl-4,6-dideoxy-α-D-*threo*-hex-4-enopyranoside (234) have been achieved by either Cope or Hofmann eliminations on appropriate derivatives of 4-amino-4-deoxy-sugars (Scheme 88).[406]

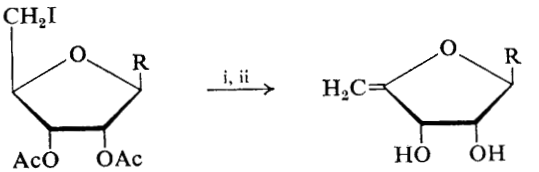
[405a] P. M. Collins, J. R. Hurford, and W. G. Overend, *J.C.S. Perkin I*, 1975, 2178.
[406] C. L. Stevens and D. Chitharanjan, *J. Org. Chem.*, 1975, **40**, 2474.



Reagents: i, heat; ii, Ag_2O; iii, B_2H_6; iv, H_2O_2; v, HOAc

Scheme 88

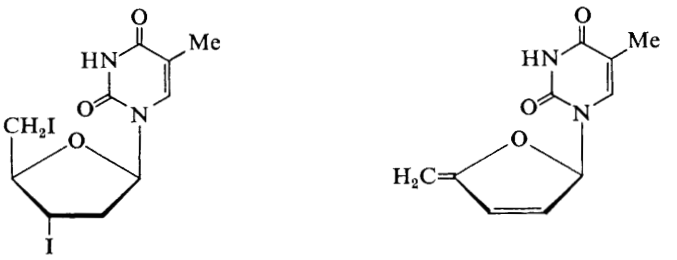
Verheyden and Moffatt have prepared 1-(5-deoxypent-4-enofuranosyl) derivatives of uridine, thymidine, cytidine, and 2′-deoxycytidine by dehydrohalogenation of the acetylated 5′-deoxy-5′-iodo-nucleoside with either silver fluoride in pyridine or DBN (Scheme 89).[407] Similar treatment of 3′,5′-dideoxy-3′,5′-diiodothymidine (235) furnished the dihydrofuran derivative (236). Addition reactions of 4′,5′-unsaturated derivatives of nucleosides are mentioned in Chapter 21.



R = pyrimidine base

Reagents: i, AgF–py or DBN; ii, NH_3–MeOH

Scheme 89

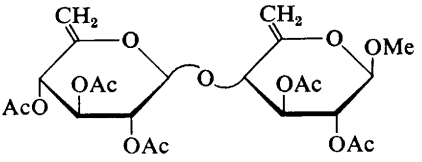


(235) (236)

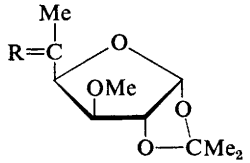
[407] J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, 1974, **39**, 3573.

The thermal degradation of 6-*S*-allyl and 6-*S*-benzyl dithiocarbonates derived from methyl cellobioside, sucrose, and cellulose has yielded such unsaturated sugars as (237) with juxtaposed double bonds.[408]

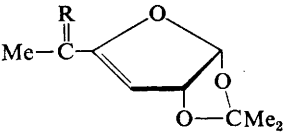
Wittig methylenation of the keto-sugar (238) gave the unsaturated derivatives (239) and (240) in addition to the expected product (241).[409] 1,2:5,6-Di-*O*-isopropylidene-α-D-*ribo*- and -*xylo*-hexofuranos-3-uloses reacted with stabilized



(237)

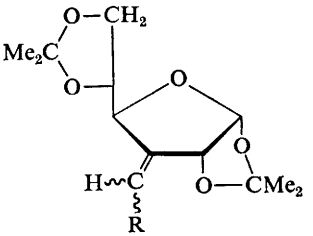


(238) R = O
(241) R = CH_2

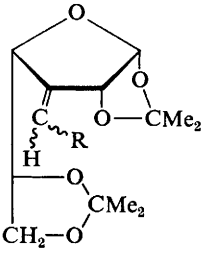


(239) R = O
(240) R = CH_2

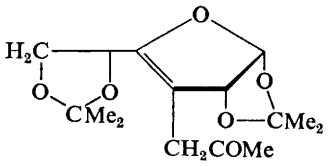
phosphorus ylides to give a series of 3-*C*-(acylmethylene) derivatives (242) and (243).[410] The keto-sugar having the *xylo*-configuration gave a mixture of the geometrical isomers of the expected enones or αβ-unsaturated esters, whereas the



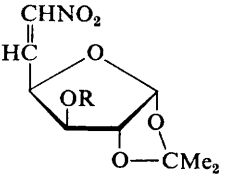
(242) R = CO_2Et, COMe, COPh, or CHO



(243) R = CO_2Et, COMe, COPh, *etc.*



(244)



(245) R = Me, Bn, or Ac

[408] G. Descotes, A. Faure, and J.-C. Martin, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 189.
[409] J. M. J. Tronchet, C. Cottet, and F. Barbalat-Rey, *Helv. Chim. Acta*, 1975, **58**, 1501.
[410] J. M. J. Tronchet and B. Gentile, *Carbohydrate Res.*, 1975, **44**, 23.

corresponding *ribo*-compound also gave side-products originating from epimerization at C-4 or from allylic protrotropy. Both *cis*-(242) (R = COMe) and *cis*-(243) (R = COMe) underwent rapid allylic transposition to yield the 3-*C*-(2-oxopropyl) derivative (244) on heating in DMSO.

The stereoselectivity of nucleophilic additions to the nitro-olefin (245) has been shown to be influenced more by the solvent and temperature than by the type of substituent at O-3.[411]

5′,6′-Unsaturated derivatives of hexofuranosyl nucleosides are mentioned in Chapter 21.

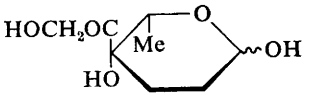
[411] M. Funabashi, Y. Okamura, K. Mikami, and J. Yoshimura, *Chem. Letters*, 1974, 1453.

15
Branched-chain Sugars

Compounds with an $R^1—C—OR^2$ Branch

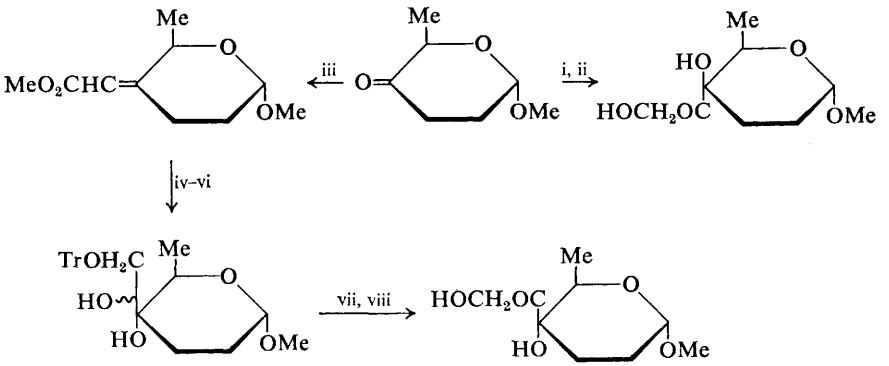
The chemistry and biochemistry of apiose have been reviewed.[412]

X-Ray analysis of the antibiotic pillaromycin A has shown that the constituent branched-chain sugar (pillarose) has the structure (246),[413] although it was



(246)

previously suggested [M. Asai, *Chem. Pharm. Bull.* (*Japan*), 1970, **18**, 1699] that pillarose is a 2,3,6-trideoxy-2-*C*-glycolylhexos-4-ulose. Confirmatory evidence for the structure of pillarose was provided by syntheses of the D-enantiomer of methyl pillaroside and its C-4 epimer (Scheme 90).[414] Isomeric structures based

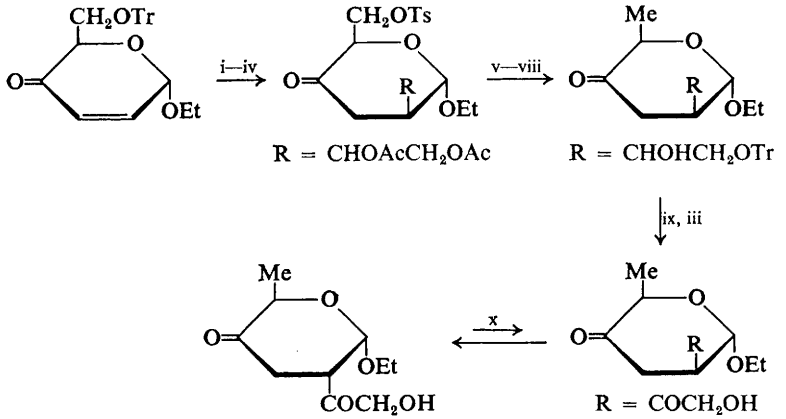


Reagents: i, CH_2=$CHMgBr$; ii, OsO_4–H_2O_2–Bu^tOH; iii, Ph_3P=$CHCO_2Me$; iv, $LiAlH_4$–Et_2O; v, TrCl–py; vi, OsO_4–py; vii, DMSO–DCC–H^+; viii, HCl–$CHCl_3$

Scheme 90

on 2,3,6-trideoxy-2-*C*-glycolylhexos-4-ulose (see Scheme 91) were also prepared during the course of this investigation so that they could be compared with natural pillarose.

[412] R. R. Watson and N. S. Orenstein, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 135.
[413] J. O. Pezzanite, J. Clardy, P.-Y. Lau, G. Wood, D. L. Walker, and B. Fraser-Reid, *J. Amer. Chem. Soc.*, 1975, **97**, 6250.
[414] D. L. Walker and B. Fraser-Reid, *J. Amer. Chem. Soc.*, 1975, **97**, 6251.

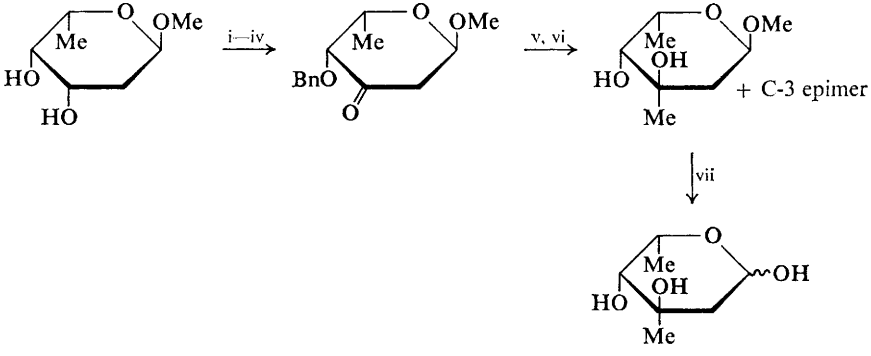


Reagents: i, $(CH_2OH)_2$–Ph_2CO–*hν*; ii, Ac_2O–py; iii, HCl–$CHCl_3$; iv, TsCl–py; v, NaI–MeCOEt; vi, H_2; vii, MeOH–H_2O–Et_3N; viii, TrCl–py; ix, CrO_3,2py; x, DBU

Scheme 91

The details of two closely related syntheses of vinelose (6-deoxy-3-*C*,2-*O*-dimethyl-L-talose) [415, 416] and of a synthesis of methyl D-aldgaroside (methyl 4,6-dideoxy-3-*C*-[(*S*)-1-hydroxyethyl]-β-D-*ribo*-hexopyranoside 3,3¹-cyclic carbonate)[417] have appeared (see Vol. 8, p. 103).

Axenose (2,6-dideoxy-3-*C*-methyl-L-*xylo*-hexose) (247) has been synthesized from a 2,6-dideoxy-sugar glycoside, although the route yielded the 3-*C*-epimer as the principal product (Scheme 92).[418] Preferential formation of the *lyxo*-isomer in the Grignard reaction is unexpected in view of previous experience gained with



(247)

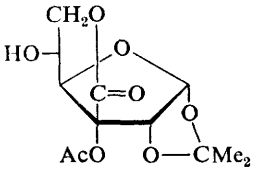
Reagents: i, BzCl–py; ii, CF_3SO_3Bn; iii, BaO; iv, RuO_4–CCl_4; v, MeMgCl; vi, H_2–Pd; vii, H_3O^+

Scheme 92

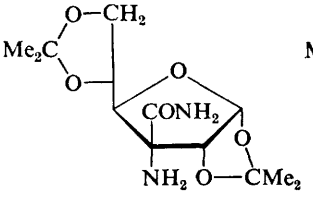
[415] J. S. Brimacombe, S. Mahmood, and A. J. Rollins, *J.C.S. Perkin I*, 1975, 1292.
[416] M. Funabashi, S. Yamazaki, and J. Yoshimura, *Carbohydrate Res.*, 1975, **44**, 275.
[417] J. S. Brimacombe, J. Minshall, and C. W. Smith, *J.C.S. Perkin I*, 1975, 682.
[418] P. J. Garegg and T. Norberg, *Acta Chem. Scand.* (*B*), 1975, **29**, 507.

methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*erythro*-hexopyranosid-3-ulose (see Vol. 2, p. 139).

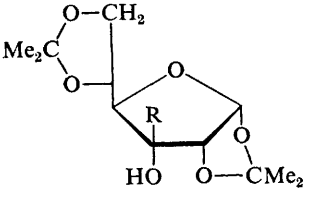
It has been established that the kinetically favoured product formed on the addition of cyanide ion to 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose has the D-*allo*-configuration, whereas the thermodynamically favoured product has the D-*gluco*-configuration.[419] Conversion of the D-*allo*-isomer into the lactone derivative (248) was used to establish its stereochemistry. Other branched-chain derivatives, including the amino-sugar (249), were also prepared.



(248)

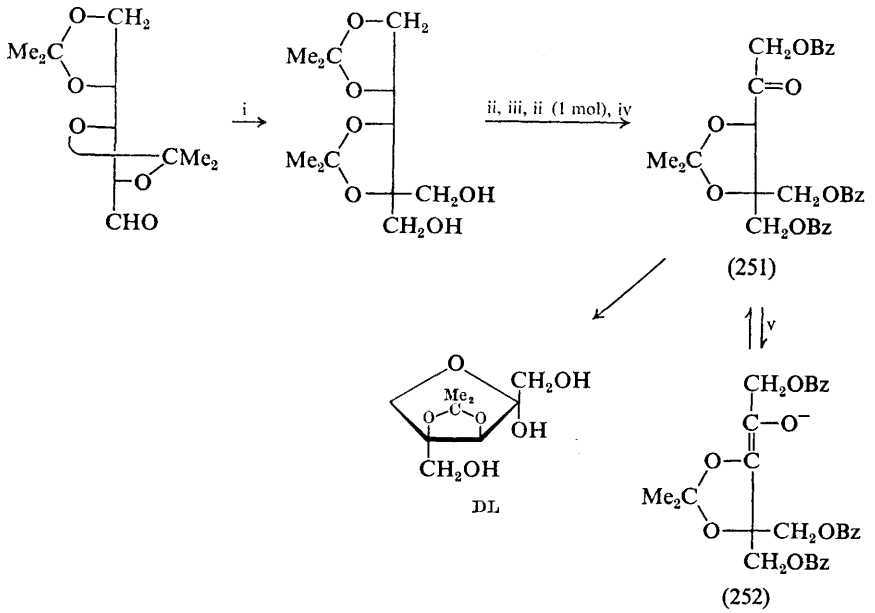


(249)



(250) R = C≡CH, $CH_2C{\equiv}CH$, *etc.*

The branched-chain acetylenic sugars (250) were obtained when 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose reacted with appropriate organometallic derivatives.[420]



(251)

(252)

Reagents: i, HCHO–NaOH; ii, BzCl–py; iii, H_3O^+; iv, RuO_4; v, NaOMe

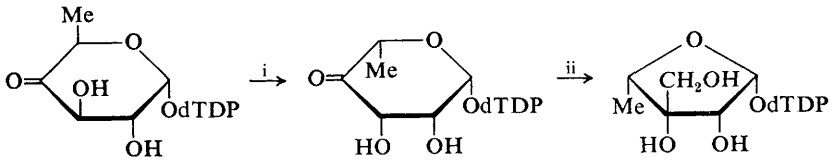
Scheme 93

[419] J.-M. Bourgeois, *Helv. Chim. Acta*, 1975, **58**, 363.

[420] A. Gonzalez, M. Orzaez, E. Martinez, V. Custardoy, and R. Mestres, *Anales de Quim.*, 1974, **70**, 1073 (*Chem. Abs.*, 1975, **83**, 164 447r).

An attempt to synthesize L-dendroketose [4-*C*-(hydroxymethyl)-L-*glycero*-pentulose] from 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose failed because one of the intermediates (251) racemized when treated with methanolic sodium methoxide.[421] Deuterium-exchange studies indicated that racemization occurs by way of the enolate (252) prior to debenzoylation (Scheme 93); had debenzoylation occurred initially, the debenzoylated product would have cyclized and enolization involving C-3 might not have been observed. Although base-catalysed adolization of dihydroxyacetone yielded racemic dendroketose, a similar reaction with glyceraldehyde gave mainly hexuloses and little dendroketose.[422]

Grisebach's group have isolated the enzymes that convert 2′-deoxythymidine 5′-(6-deoxy-α-D-*xylo*-hexopyranosyl-4-ulose pyrophosphate) into the corresponding L-dihydrostreptose derivative (Scheme 94).[423] A simple method for the



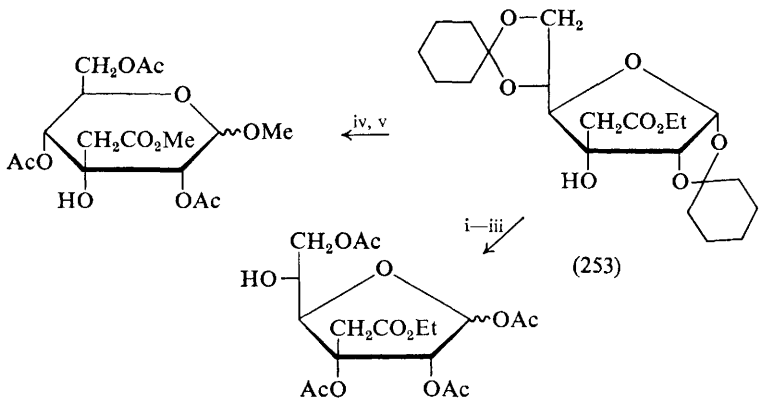
dTDP = 2′-deoxythymidine pyrophosphate

Reagents: i, TDP–4-keto-L-rhamnose-3,5-epimerase; ii, TDP–'dihydrostreptose synthase'

Scheme 94

isolation of crystalline D-hamamelose in good yield from the bark of witch-hazel has been reported.[424]

The hydrolysis and methanolysis of the branched-chain sugar derivative (253) gave the products shown in Scheme 95 on acetylation; hydrolysis of (253) with an



Reagents: i, H_2O–*aci*-resin; ii, Ac_2O–TsOH; iii, EtOH; iv, MeOH–*aci*-resin; v, Ac_2O–py

Scheme 95

[421] H. C. Jarrell, W. A. Szarek, J. K. N. Jones, A. Dmytraczenko, and E. B. Rathbone, *Carbohydrate Res.*, 1975, **45**, 151.
[422] J. Konigstein, D. Anderle, and F. Janecek, *Chem. Zvesti*, 1974, **28**, 701.
[423] H. P. Wahl, U. Matern, and H. Grisebach, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1041.
[424] H. Glick, A. Thanbichler, J. Sellmair, and E. Beck, *Carbohydrate Res.*, 1975, **39**, 160.

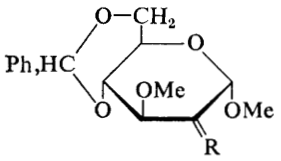
acid resin yielded 3-*C*-carboxymethyl-D-allose as an equilibrium mixture of the free acid and its lactone.[425]

The ^{13}C n.m.r. spectra of methyl hexopyranosides having methyl groups attached at either C-2 or C-4 have been discussed; the ^{13}C resonances for axial *C*-methyl groups were found *ca.* 6 p.p.m. upfield from those of the corresponding equatorial groups.[426]

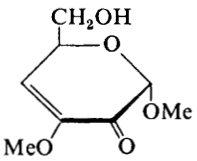
References to related branched-chain sugars are also contained in Chapters 4, 21, and 23.

Compounds with an $R^1—C—R^2$ Branch

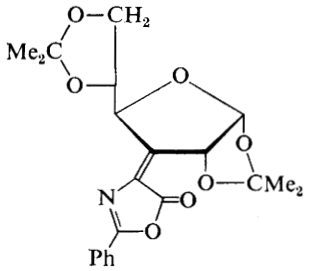
The glycosidulose (254) reacted with methylene triphenylphosphorane in toluene to give the 2-*C*-methylene derivative (255), which was converted into methyl 4,6-*O*-benzylidene-2-deoxy-2-*C*,3-*O*-dimethyl-α-D-glucopyranoside on catalytic hydrogenation over Raney nickel.[427] However, the enone (256) (58%) preponderated when the Wittig reaction was carried out in more polar solvents such as DMSO.



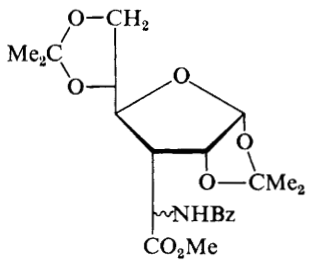
(254) R = O
(255) R = CH_2



(256)



(257)



(258)

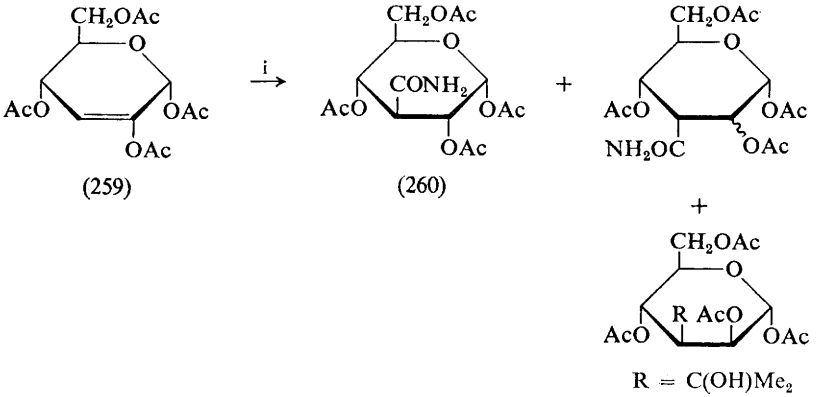
2-Phenyloxazol-5-one has been used to prepare the unsaturated derivative (257; *cis* : *trans* isomers 1 : 1) from 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose.[428] Hydrogenation of (257) in methanol in the presence of sodium acetate afforded the glycyl derivative (258). The acetone-initiated addition of formamide to the glycal tetra-acetate (259) gave a mixture of branched-chain products (Scheme 96), one (260) of which was used to prepare thymine and cytosine

[425] Yu. A. Zhdanov, Yu. E. Alekseev, and G. E. Guterman, *Doklady Akad. Nauk S.S.S.R.*, 1974, **218**, 1359 (*Chem. Abs.*, 1975, **82**, 86 527e).

[426] M. Miljković, M. Gligorijević, T. Satoh, D. Glišin, and R. G. Pitcher, *J. Org. Chem.*, 1974, **39**, 3847.

[427] M. Miljković and D. Glišin, *J. Org. Chem.*, 1975, **40**, 3357.

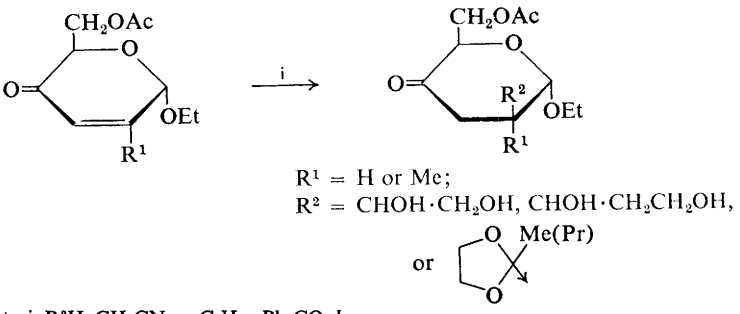
[428] A. Rosenthal and K. Dooley, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 61 (*Chem. Abs.*, 1975, **82**, 140 418k).



(259) (260)

Reagent: i, $HCONH_2$–Me_2CO–*hν*

Scheme 96

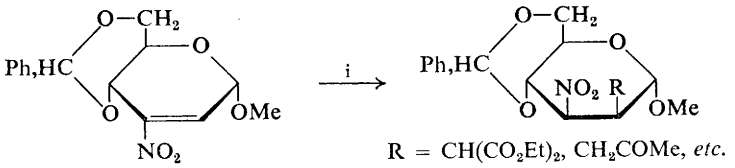


Reagent: i, R^2H–CH_3CN or C_6H_{12}–Ph_2CO–*hν*

Scheme 97

analogues of gougerotin.[429] The photochemically induced addition of alcohols and dioxolans to carbohydrate enones has also been investigated (Scheme 97).[430]

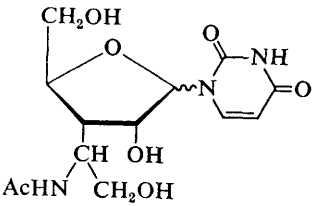
A phase-transfer process has been used to obtain the kinetically favoured D-*manno*-derivatives in the reactions of methyl 4,6-*O*-benzylidene-2,3-dideoxy-



Reagent: i, $CH_2(CO_2Et)_2$, *etc.*–aq.NaOH–$C_{16}H_{33}\overset{+}{P}Bu_3Br^-$

Scheme 98

[429] A. Rosenthal and M. Ratcliffe, *Carbohydrate Res.*, 1975, **39**, 79.
[430] B. Fraser-Reid, D. R. Hicks, D. L. Walker, D. E. Iley, M. B. Yunker, S. Y.-K. Tam, R. C. Anderson, and J. Saunders, *Tetrahedron Letters*, 1975, 297.

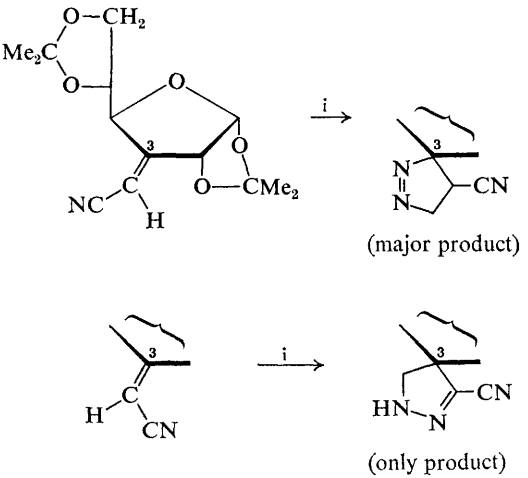


(261)

3-*C*-nitro-α-D-*erythro*-hex-2-enopyranoside with some active methylene compounds (Scheme 98).[431]

The formation of fluorinated branched-chain sugars has been mentioned already in Chapter 7 (see Scheme 45).[269] The hydrogenation product of (107) has been converted into nucleoside derivatives (261) containing a branch at C-3 of the sugar residue.[432]

The regioselectivity of the addition of diazomethane to a series of 3-*C*-methylene derivatives (*e.g.* Scheme 99) has been examined, particularly with regard to the stereochemistry and nature of the substituents at C-3[1].[433]



Reagent: i, CH_2N_2

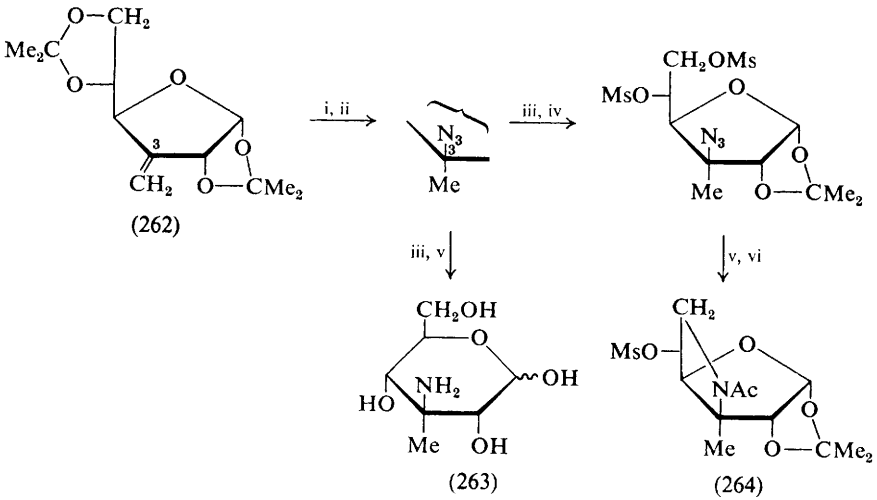
Scheme 99

Details of the base-catalysed rearrangement of methyl 3-acetamido-3,6-dideoxy-2-*O*-methanesulphonyl-α-L-gluco- and -L-galacto-pyranosides into methyl 2,5-dideoxy-2-*C*-(methoxyacetamidomethyl)-α-L-arabinofuranosides have been published [434] (see Vol. 3, p. 73).

[431] T. Sakakibara and R. Sudoh, *J. Org. Chem.*, 1975, **40**, 2823.
[432] A. J. Brink and A. Jordaan, *Carbohydrate Res.*, 1975, **41**, 355.
[433] J. M. J. Tronchet, B. Gentile, and J. Tronchet, *Helv. Chim. Acta*, 1975, **58**, 1817.
[434] K. Čapek, J. Jarý, and Z. Samek, *Coll. Czech. Chem. Comm.*, 1975, **40**, 149.

Compounds with an R—C—N Branch

Brimacombe's group have used the addition of mercuric azide to the 3-*C*-methylene derivative (262) to prepared branched-chain amino-sugars [*e.g.* (263)] as outlined in Scheme 100.[435, 436] The conversion of the branched-chain azide



Reagents: i, $Hg(OAc)_2$–NaN_3; ii, $NaBH_4$; iii, H_3O^+; iv, MsCl–py; v, H_2–Pt; vi, Ac_2O

Scheme 100

into the 3,6-epimino-derivative (264) established that it has the D-*gluco*-configuration, thus reversing a previous assignment.

[435] J. S. Brimacombe, J. A. Miller, and U. Zakir, *Carbohydrate Res.*, 1975, **44**, C9.
[436] J. S. Brimacombe, J. A. Miller, and U. Zakir, *Carbohydrate Res.*, 1975, **41**, C3.

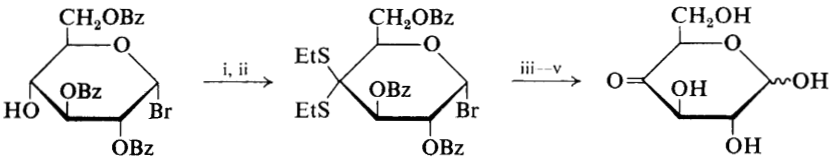
16

Aldehydo-sugars, Aldosuloses, Dialdoses, and Diuloses

The use of aldosuloses as synthetic intermediates is mentioned in Chapters 8, 13, 14, 15, and 19.

Pillarose, a branched-chain component of the antibiotic pillaromycin A, has been shown by *X*-ray crystallography [413] and synthesis [414] to be a 2,3,6-trideoxy-4-*C*-glycolylhexose (246) and not a 2,3,6-trideoxy-2-*C*-glycolylhexos-4-ulose as originally proposed (see Chapter 15).

The reactions outlined in Scheme 101 were used to prepare D-*xylo*-hexos-4-



Reagents: i, RuO_4–CCl_4; ii, EtSH–BF_3; iii, H_2O–Ag_2CO_3; iv, MeONa–MeOH; v, $HgCl_2$–$CdCO_3$

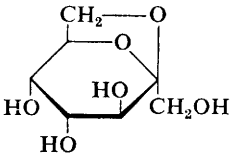
Scheme 101

ulose, which afforded a mixture of D-glucitol and galactitol on reduction with sodium borohydride.[437] A related sequence of reactions on methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside gave methyl α-D-*xylo*-hexopyranosid-4-ulose. An attempt to obtain the glycosyl phosphate of the 4-ulose was unsuccessful since the phenyl groups could not be removed from 2,3,6-tri-*O*-benzoyl-α-D-hexopyranosyl-(4-ulose diethyl dithioacetal) (diphenyl phosphate).

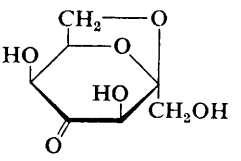
The selective catalytic oxidation of several 2-ulose derivatives has been studied; *e.g.*, the 2,7-anhydroheptulose (265) was selectively oxidized at the axial hydroxy-group to give the corresponding 5-ulose, which rapidly isomerized to the 2,7-anhydrohept-2,4-diulose (266).[438] 2,3-*O*-Isopropylidene-β-D-fructopyranose and 2,7-anhydro-4,5-*O*-isopropylidene-β-D-*allo*-hept-2-ulose were selectively oxidized at HO-4 and HO-3, respectively, whereas the furanose derivative (267) furnished the aldulosonic acid derivative (268).[438a] The reduction of these diuloses was also investigated.

Factors governing the reduction of a number of 2- and 3-uloses derived from methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranosides with sodium borohydride

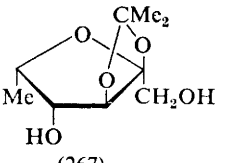
[437] J. F. Batey, C. Bullock, J. Hall, and J. M. Williams, *Carbohydrate Res.*, 1975, **40**, 275.
[438] K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, 1975, **108**, 3611.
[438a] K. Heyns, W.-D. Soldat, and P. Köll, *Chem. Ber.*, 1975, **108**, 3619.



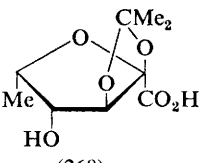
(265)



(266)



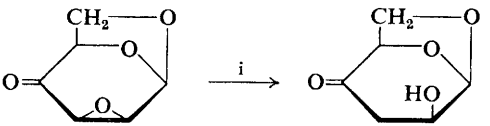
(267)



(268)

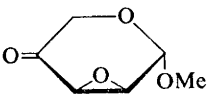
have been examined.[439] In the presence of α- and β-axial groups attack from the *trans* direction – *i.e.* the axial or equatorial direction, respectively – is favoured, whereas adjacent equatorial substituents reduce the marked preference for attack from the axial direction exhibited by the corresponding unsubstituted cyclohexanone.

α-Keto-oxirans have been further studied. Thus, the four diastereoisomeric 1,6:2,3- and 1,6:3,4-dianhydro-D-hexopyranosuloses have been prepared by oxidation of the corresponding alcohols with either ruthenium tetraoxide or oxygen in the presence of platinum.[440] The resulting dianhydro-D-hexopyranosuloses reacted with sodium iodide in acetone-acetic acid to give the corresponding 1,6-anhydro-3-deoxyglycosulose, electronic factors evidently outweighing steric factors in the opening of the oxiran rings (see Scheme 102).[441]



Reagent: i, NaI–Me_2CO–AcOH

Scheme 102



(269)

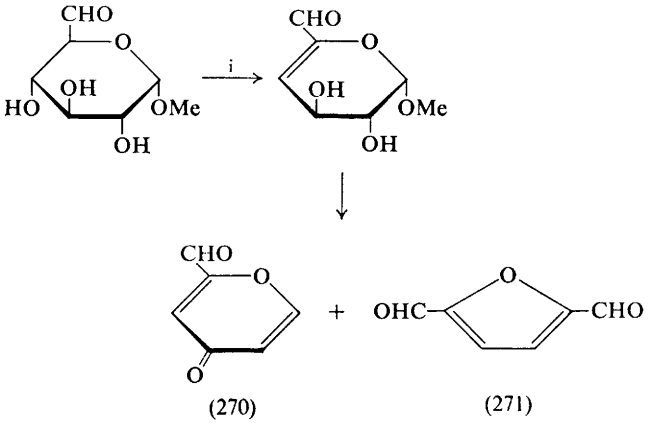
Reduction of the α-keto-oxiran (269) with sodium borohydride yielded methyl 2,3-anhydro-β-L-ribo- and -α-D-lyxo-pyranosides in the ratio of 3 : 2.[285]

3-Deoxy-D-*erythro*-hexosulose has been shown to be less stable than normal carbohydrates and to polymerize within the range 100—200 °C.[442] The poly-

[439] Y. Kondo, N. Kashimura, and K. Onodera, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2553.
[440] K. Heyns, R.-W. Rennecke, and P. Köll, *Chem. Ber.*, 1975, **108**, 3645.
[441] R.-W. Rennecke, K. Eberstein, and P. Köll, *Chem. Ber.*, 1975, **108**, 3652.
[442] F. Shafizadeh and Y.-Z. Lai, *Carbohydrate Res.*, 1975, **40**, 263.

merization was accompanied by dehydration, scission, and rearrangement reactions which produced water, furan derivatives, carbonyl compounds of low molecular weight, saccharinic acid, carbon dioxide, and carbon monoxide.

Theander's group has studied the reactions of several dialdose derivatives. Methyl α-D-*gluco*-hexodialdo-1,5-pyranoside underwent β-elimination and then conversion into the dienone (270) and the furan dicarboxaldehyde (271) on treatment with aqueous alkali (Scheme 103).[443] The degradation of methyl α-D-



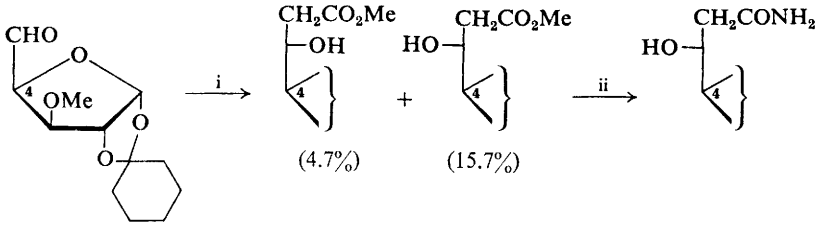
Reagent: i, HO^-

Scheme 103

galacto-, α- and β-D-*gluco*-, α- and β-D-*gulo*-, and α-L-*manno*-hexodialdo-1,5-pyranosides has been investigated at 90 °C and pH 1.5—6.[443a] Acid hydrolysis of the glycosidic linkage predominated below pH ~ 3.5, whereas β-elimination predominated above this pH. The D-*gulo* compounds isomerized to the corresponding L-*manno* compounds at pH 4—6. A photolytic method has been used in the selective oxidation of the primary hydroxy-groups of methyl β-cellobioside.[444] Thus, photolysis of methyl 6,6′-diazido-6,6′-dideoxy-β-cellobioside pentaacetate and hydrolysis of the di-imino-derivative afforded methyl 6,6′-dialdehydo-β-cellobioside, a model for cellulose fully oxidized at C-6. The degradation of the oxidized methyl β-cellobioside was also examined: at 30 °C, no reaction was observed at pH 1.2 and 4.5, some β-elimination was indicated at pH 7, and rapid reaction occurred at pH ⩾ 9; at 80 °C, some reaction was indicated at pH 2, significant β-elimination was observed at pH 4.5, and at pH ⩾ 6 the reaction was rapid.

The principal product obtained from a Reformatsky reaction on 1,2-*O*-cyclohexylidene-3-*O*-methyl-α-D-*xylo*-pentodialdo-1,4-furanose has been shown to have the D-*gluco*-configuration (see Scheme 104)[445] (*cf.* the addition of Grignard

[443] H. F. G. Beving and O. Theander, *Acta Chem. Scand.* (*B*), 1975, **29**, 577.
[443a] H. F. G. Beving and O. Theander, *Acta Chem. Scand.* (*B*), 1975, **29**, 641.
[444] H. F. G. Beving, A. F. Luetzow, and O. Theander, *Carbohydrate Res.*, 1975, **41**, 105.
[445] Yu. A. Zhdanov, Yu. E. Alekseev, G. E. Levitan, and N. A. Dolgopolova, *Zhur. obshchei Khim.*, 1975, **45**, 1327 (*Chem. Abs.*, 1975, **83**, 179 427h).

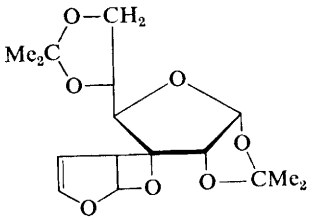


Reagent: i, $BrCH_2CO_2Me$–Zn; ii, NH_3

Scheme 104

reagents where the L-*ido*-isomer usually predominates). The configuration of the Reformatsky product was established by its conversion into the amide, which proved to be identical with that obtained by partial hydrolysis of the corresponding nitrile (see also Vol. 4, p. 91).

The photochemically induced 1,2-addition of furan to 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose and related derivatives yielded 2,7-dioxabicyclo[3,2,0]hept-3-enes [*e.g.* (272)].[446]



(272)

Other references to aldosuloses and dialdoses can be found in the following chapters: the synthesis of 6-acetamido-6-deoxy-D-*xylo*-hexos-5-ulose (Chapter 8); the formation of heterocyclic derivatives from dialdoses (Chapter 10); the reactions of dialdoses with Wittig reagents (Chapter 3) and Ivanov's reagent (Chapter 17); the base-catalysed cyclization of D-*xylo*-hexos-5-ulose 6-phosphate (Chapter 19); the oxidation of αα-trehalose derivatives to glycosiduloses (Chapter 22).

[446] J. M. J. Tronchet and B. Baehler, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 449.

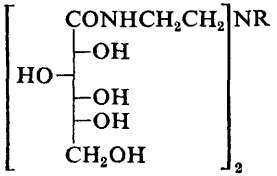
17
Sugar Acids and Lactones

Aldonic Acids

The properties of aldonolactones in aqueous solution and as enzyme inhibitors have been reviewed.[447]

Heating of 2-deoxy-D-*arabino*-hexonic acid resulted in appreciable lactonization on melting.[448] Subsequent decomposition of the lactone involved dehydration, decarboxylation, and scission to yield water, carbon dioxide, carbon monoxide, 5-(2-hydroxyethylidene)-2(5*H*)-furanone, acrylaldehyde, and acetic acid. 3-Deoxy-D-*ribo*-hexono-1,4-lactone – which cannot undergo β-elimination – is thermally more stable and gives products resulting from scission and dehydration.

Treatment of D-glucono-1,4-lactone with triphenylboroxole afforded a 3,5-benzeneboronate, which was converted into 6-*O*-dimethylglycyl-D-glucono-1,4-lactone (kangamolactone).[258] D-Glucono-1,5-lactone reacted with bis(2-aminoethyl)amine in refluxing methanol to give the amide (273), which was transformed into a series of surfactants (274) and (275) when treated with long-chain alkyl cyanates or carboxylic acids.[449]

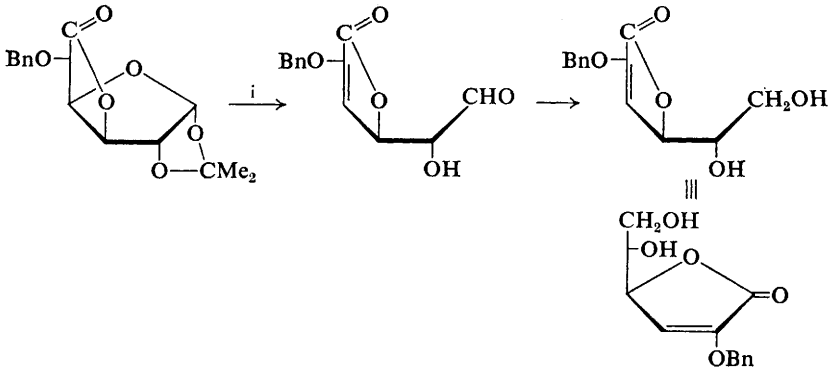


(273) R = H
(274) R = $CONH(CH_2)_nMe$; n = 6—11, 16
(275) R = $CO(CH_2)_nMe$; n = 6—11, 16

Treatment of D-fructose 6-phosphate with oxygen and alkali resulted in loss of C-1 and the formation of D-arabinonic acid 5-phosphate.[450]

The yield of 2-*O*-benzyl-3-deoxy-L-*threo*-hex-2-enono-1,4-lactone obtained when 5-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone reacted with sodium borohydride (Scheme 105) has been improved by variation of the dipolar aprotic solvent and temperature.[451] Five-membered 2-acetamido-2,3-dideoxy-D-hex-2-enonolactones have been synthesized from 2-acetamido-2-deoxy-D-aldono-1,4-lactones by treatment with dicyclohexylamine in ethanolic

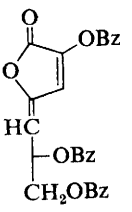
[447] S. K. Dutta, *Bangladesh Pharm. J.*, 1975, **4**, 5 (*Chem. Abs.*, 1975, **83**, 79 484f).
[448] F. Shafizadeh and Y.-Z. Lai, *Carbohydrate Res.*, 1975, **42**, 39.
[449] G. Czichocki, G. Engler, and E. Ulsperger, *J. prakt. Chem.*, 1974, **316**, 895 (*Chem. Abs.*, 1975, **82**, 73 363y).
[450] J. M. Chirgwin and E. A. Noltmann, *J. Biol. Chem.*, 1975, **250**, 7272.
[451] W. Timpe, K. Dax, N. Wolf, and H. Weidmann, *Carbohydrate Res.*, 1975, **39**, 53.



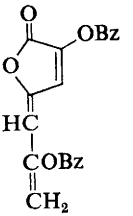
Reagent: i, $NaBH_4$–HMPT at 45 °C

Scheme 105

solution, while an unsaturated 1,5-lactone, namely 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,5-lactone, was obtained by oxidation of 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-galactose with silver carbonate on Celite followed by mild hydrolysis.[452] D-*glycero*-D-*gulo*-Heptono-1,4-lactone reacted with an excess of benzoyl chloride in pyridine to give, in addition to the pentabenzoate, 2,5,6,7-tetra-*O*-benzoyl-3-deoxy-D-*arabino*-hept-2-enono-1,4-lactone and the unsaturated derivatives (276) and (277).[453]



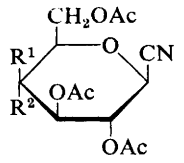
(276)



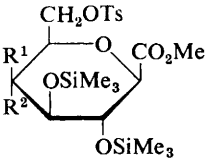
(277)

The compounds discussed in the following section closely resemble some of the compounds mentioned in the section on *C*-glycosides in Chapter 3. The glycosyl cyanides (278) and (279) have been converted into the amino-acids (282) and (283) by way of the sulphonates (280) and (281), respectively.[454] The amino-acids (282) and (283) were used to prepare novel polymers which exhibited the properties both of polysaccharides and proteins. Investigations on the synthesis of puromycin analogues have led to the preparation of the 2,5-anhydro-D-allonic acid derivative (284) from 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofura-

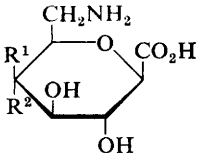
[452] M. Pokorny, E. Zissis, H. G. Fletcher, jun., and N. Pravdić, *Carbohydrate Res.*, 1975, **43**, 345.
[453] M. I. Litter and R. M. De Lederkremer, *Anales Asoc. quím. argentina*, 1974, **62**, 147 (*Chem. Abs.*, 1975, **82**, 58 034g).
[454] E.-F. Fuchs and J. Lehmann, *Chem. Ber.*, 1975, **108**, 2254.



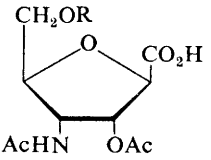
(278) R^1 = H; R^2 = OAc
(279) R^1 = OAc; R^2 = H



(280) R^1 = H; R^2 = $OSiMe_3$
(281) R^1 = $OSiMe_3$; R^2 = H

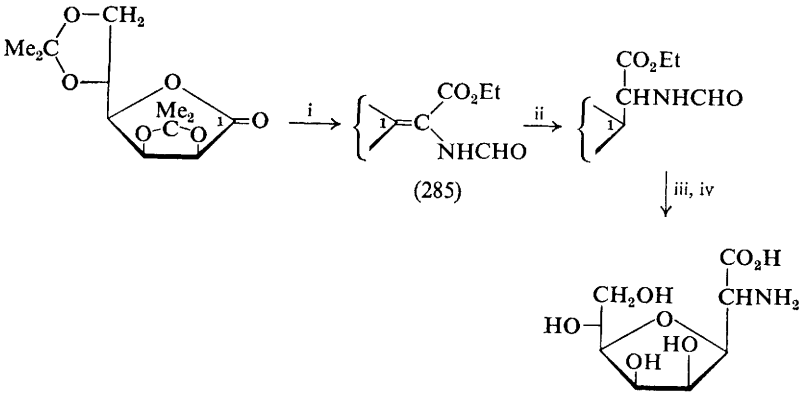


(282) R^1 = H; R^2 = OH
(283) R^1 = OH; R^2 = H



(284) R = $COC_6H_4NO_2$-*p*

nose.[455] The major (*E*)-isomer (285) obtained by the reaction of α-metallated ethyl isocyanoacetate with 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone gave 2-L- (and 2-D-) (β-D-mannofuranosyl)glycines on hydrogenation and hydrolysis (see Scheme 106).[456] The reaction of 2,3:5,6-di-*O*-cyclohexylidene-D-



Reagents: i, EtO_2CCH_2NCO–KH–THF; ii, H_2; iii, H_3O^+; iv, HO^-

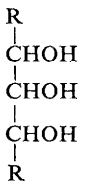
Scheme 106

mannono-1,4-lactone with an equimolar mixture of phthalimide, dialkyl azodicarboxylate, and triphenylphosphine to yield *N*-glycosylphthalimides is noted in Chapter 10, while a synthesis of 2,3-dideoxyoctono-1,4-lactones is outlined in Chapter 13 (see Scheme 81).

[455] H. S. El Khadem, T. D. Audichya, E. S. H. El Ashry, and R. Sindric, *Carbohydrate Res.*, 1975, **41**, 318.
[456] K. Bischofberger, R. H. Hall, and A. Jordaan, *J.C.S. Chem. Comm.*, 1975, 806.

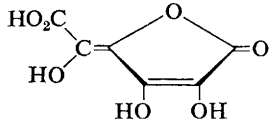
Aldaric Acids

The ^1H n.m.r. spectra of a series of trihydroxyglutaric acid derivatives (286) have shown that the hydrogens attached to C-2 and C-4 in the D- and L-*arabino* isomers are non-equivalent, whereas those of the symmetrical *ribo* and *xylo* isomers are equivalent.[457] Vicinal coupling constants were used to predict the following major conformations: *ribo*-(286) (sickle), *xylo*-(286) (sickle), and *arabino*-(286) (planar zig-zag).

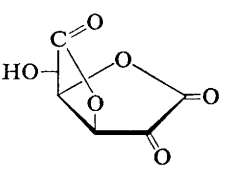


(286) R = CO_2H, $CONH_2$, CONHNHPh, *etc.*

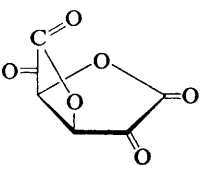
Oxidation of D-mannaric acid 1,4:6,3-dilactone with potassium permanganate furnished the dienone (287), which was transformed into a series of pyrrole and other derivatives.[458] The hydroxy-groups at C-2 and C-5 of D-mannaric acid 1,4:6,3-dilactone could also be oxidized in turn to give (288) and (289); treatment of these products with hydrogen bromide in acetic acid–acetic anhydride introduced geminal bromo and acetyl groups at C-2 and at C-5, respectively.[458a]



(287)

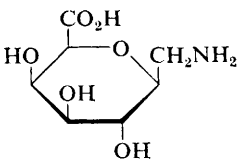


(288)

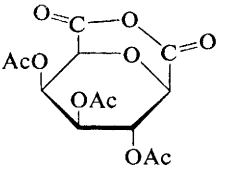


(289)

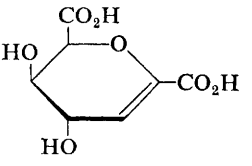
7-Amino-2,6-anhydro-7-deoxy-L-*glycero*-L-*galacto*-heptonic acid (290) has been synthesized by way of selective ring-opening of 3,4,5-tri-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptaric anhydride (291) with ammonia.[459] Daucic acid, a sugar acid present in wheat, sugar beet, and tobacco, *etc.*, has been identified as 2,6-anhydro-3-deoxy-D-*xylo*-hept-2-enaric acid (292).[460]



(290)



(291)



(292)

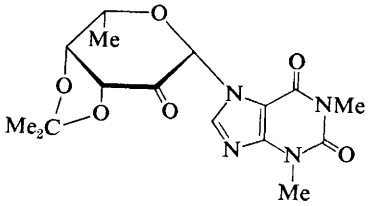
[457] R. E. Gall and L. Tarasoff, *Austral. J. Chem.*, 1975, **28**, 687.
[458] K. Heyns and A. Linkies, *Chem. Ber.*, 1975, **108**, 3637.
[458a] K. Heyns and A. Linkies, *Chem. Ber.*, 1975, **108**, 3633.
[459] E.-F. Fuchs and J. Lehmann, *Carbohydrate Res.*, 1975, **45**, 135.
[460] D. H. R. Barton, B. D. Brown, D. D. Ridley, D. A. Widdowson, A. J. Keys, and C. J. Leaver, *J.C.S. Perkin I*, 1975, 2069.

Aldaric acids obtained by treatment of 4-*O*-methyl-D-glucuronic acid with alkali are reported in the following section.

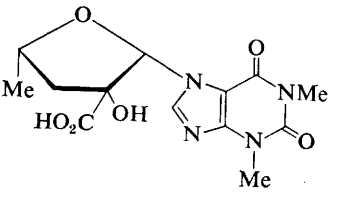
Saccharinic Acids

Aspinall's group have reported that the alkaline degradation of 6-*O*-β-D-glucopyranosyl-3-*O*-methyl-D-glucose yields the epimeric 6-*O*-β-D-glucopyranosyl metasaccharinic acids, which were characterized by reduction to 3-deoxy-6-*O*-β-D-glucopyranosyl-D-*ribo*- and -D-*arabino*-hexoses.[461] This procedure did not give completely satisfactory results when applied to a branched β-glucan, although it appears to hold promise for the analysis of oligosaccharides. 2-Deoxy-5-*O*-β-D-glucopyranosyl-D-*erythro*-pentose was obtained by selective oxidation of 3-deoxy-6-*O*-β-D-glucopyranosyl-D-*ribo*-hexose with periodate.[94]

On treatment with alkali, the β-L-fucopyranosyltheophylline derivative (293) rearranged to the saccharinic acid (294), presumably *via* an α-dicarbonyl inter-



(293)



(294)

mediate.[462] Methyl β-D-glucopyranoside and methyl β-cellobioside were also converted into saccharinic acids when subjected to oxygen–alkali treatment at 120 °C.[113] Both 3-deoxy-2-*C*-(hydroxymethyl)-*erythro*- and -*threo*-pentaric acids were formed when 4-*O*-methyl-D-glucuronic acid was treated with limewater in the absence of oxygen.[463] However, in the presence of air, formation of the 3-deoxy-2-*C*-(hydroxymethyl)pentaric acids was suppressed, and these acids were minor products in sodium hydroxide under an oxygen atmosphere when much larger proportions of 3-deoxy-*threo*-pentaric, deoxytetraric(malic), 3-*O*-methylarabinaric, and *O*-methyl erythraric acids were obtained. The structures of the isosaccharinic acids were determined from the mass spectra of their TMS derivatives.[464]

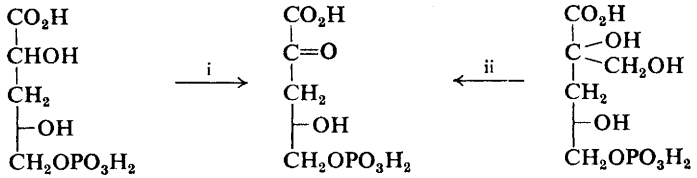
The formation of glucometasaccharinic acids by thermal degradation of 3-deoxy-D-*erythro*-hexosulose is noted in Chapter 16.

Ulosonic Acids

3-Deoxy-D-*glycero*-pentulosonic acid 5-phosphate has been obtained both by oxidation of a mixture of 3-deoxy-D-*erythro*- and -D-*threo*-pentonic acid 5-phosphates with potassium chlorate–vanadium(v) oxide and by treatment of a mixture

[461] G. O. Aspinall, T. N. Krishnamurthy, I. Furda, and R. Khan, *Canad. J. Chem.*, 1975, **53**, 2171.
[462] T. Halmos, J. Herscovici, and K. Antonakis, *Compt. rend.*, 1974, **279**, *C*, 855.
[463] L. Lowendahl, G. Petersson, and O. Samuelson, *Carbohydrate Res.*, 1975, **43**, 355.
[464] G. Petersson, *Carbohydrate Res.*, 1975, **43**, 1.

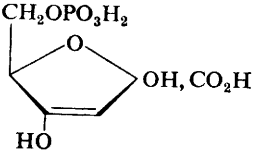
of 3-deoxy-2-*C*-(hydroxymethyl)-D-*erythro*- and -D-*threo*-pentonic acid 5-phosphates with a molar equivalent of periodate (Scheme 107).[465] 3-Deoxy-D-*glycero*-pentulosonic acid has been identified as an intermediate in the metabolism of D-xylose by a Pseudomonad.[466] Oxidation with vanadium(v) oxide–potassium



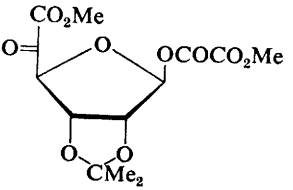
Reagents: i, V_2O_5–$KClO_3$; ii, $NaIO_4$

Scheme 107

chlorate was used to convert the 6-phosphates of glucometasaccharinic acid and 3-deoxy-D-*xylo*-hexonic acid into 3-deoxy-D-*erythro*- and -D-*threo*-hexulosonic acid 6-phosphates, respectively (*cf.* Scheme 107).[467] 3-Deoxy-D-*erythro*-hexulosonic acid 6-phosphate was rapidly degraded by decimolar hydrochloric acid at 95 °C, and it underwent aldol cleavage between C-3 and C-4 with bases to give pyruvic acid and D-glyceraldehyde 3-phosphate. The enol (295) was also produced on oxidation of glucometasaccharinic acid 6-phosphate with vanadium(v) oxide–potassium chlorate.[468]



(295)



(296)

L-*xylo*-Hexulosonic acid has been prepared *via* oxidation of 3,4:5,6-di-*O*-isopropylidene-L-sorbose[469] and the racemic derivative (296) was produced on thermolysis of the ozonide (27) (see Chapter 3).[133]

3-Deoxy-D-*arabino*-[1-^{14}C]heptulosonic acid 7-phosphate has been synthesized by addition of potassium [^{14}C]cyanide to 2-deoxy-D-*arabino*-hexose 6-phosphate, hydrolysis, and selective oxidation of the resulting 3-deoxyheptonic acid 7-phosphates at C-2 with potassium chlorate–vanadium(v) oxide.[470] A one-step synthesis of a mixture of 3-deoxy-D-*ribo*- and -D-*arabino*-heptulosonic acid 7-phosphates is based on a metal-ion-catalysed reaction of D-erythrose 4-phosphate with oxalacetate.[471]

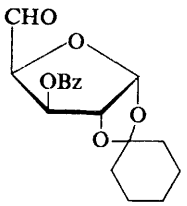
[465] F. Trigalo and L. Szabo, *J.C.S. Perkin I*, 1975, 604.
[466] A. S. Dahms, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1433.
[467] F. Trigalo, W. Jachymczyk, J. C. Young, and L. Szabo, *J.C.S. Perkin I*, 1975, 593.
[468] F. Trigalo and L. Szabo, *J.C.S. Perkin I*, 1975, 598.
[469] N. Gospodinov, Sh. Levi, E. Pencheva, and P. Lazarova, *Trudy Nauchroizsled. Khim.-Farm. Inst.*, 1974, **9**, 187 (*Chem. Abs.*, 1975, **83**, 59 191e).
[470] F. Trigalo, M. Level, and L. Szabo, *J.C.S. Perkin I*, 1975, 600.
[471] K. M. Herrmann and M. D. Poling, *J. Biol. Chem.*, 1975, **250**, 6817.

3-Deoxy-D-*manno*-octulosonic acid has been identified as the principal component (53%) of the capsular polysaccharide antigen of *Neisseria meningitidis* serogroup 29-e.[472]

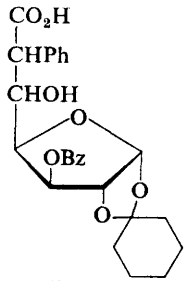
Uronic Acids

The Reformatsky reaction has been applied to a dialdose derivative (see Scheme 104),[445] while a related derivative (297) reacted with Ivanov's reagent[PhCH(MgCl)(CO_2MgCl)] to give the hepturonic acid derivatives (298).[473] A novel approach to uronic acids involves the bromination of CH_2NO_2 groups and the conversion of the resulting CBr_2NO_2 group into the nitrile by triphenylphosphine.[474]

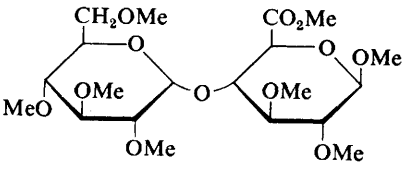
Methyl (methyl α- and β-D-glucopyranosid)uronate[475] and benzyl 2,3,4-tri-*O*-(chloroacetyl)-β-D-glucopyranosiduronic acid and its methyl ester[476] have been



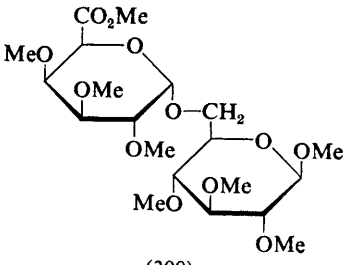
(297)



(298)



(299)



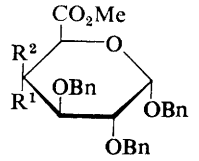
(300)

synthesized using conventional transformations. C.d. and ^{1}H and ^{13}C n.m.r. spectroscopy have been used to determine the configurations and conformations of methyl (methyl 3,4-*O*-benzylidene-α- and -β-D-galactopyranosid)uronate.[477]

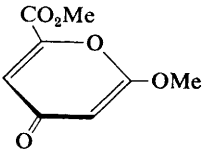
The disaccharide hexuronates (299) and (300) underwent smooth elimination of the substituents at C-4 and C-4′ on treatment with DBU in benzene–acetic

[472] A. K. Bhattacharjee, H. J. Jennings, and C. P. Kenny, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 489.
[473] Yu. A. Zhdanov, G. V. Bogdanova, and O. V. Doron'kina, *Doklady Akad. Nauk S.S.S.R.*, 1975, **222**, 104 (*Chem. Abs.*, 1975, **83**, 97 745w).
[474] O. G. De Villiers, A. Jordaan, and G. J. Lourens, *J.S. African Chem. Inst.*, 1975, **28**, 27 (*Chem. Abs.*, 1975, **83**, 97 763a).
[475] P. Kováč, J. Alföldi, and M. Košík, *Chem. Zvesti*, 1975, **29**, 820.
[476] N. Roy and C. P. J. Glaudemans, *Carbohydrate Res.*, 1975, **45**, 299.
[477] C. Peciar, R. Palovčík, J. Alföldi, T. Sticzay, and P. Kováč, *Chem. Zvesti*, 1975, **29**, 397.

anhydride; *e.g.*, (299) yielded 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucopyranose and methyl (methyl 4-deoxy-2,3-di-*O*-methyl-α-L-*threo*-hex-4-enopyranosid)-uronate.[478] A methylated glucuronoxylomannan was also degraded by this procedure to give, after mild acidic hydrolysis and deacetylation, 3,4,6-tri-*O*-methyl-D-mannose and 3,4-di-*O*-methyl-6-*O*-(2,3,4-tri-*O*-methyl-β-D-xylopyranosyl)-D-mannose; certain structural features of the polysaccharide could be deduced from the formation of these fragments.[479] The *gluco*-methanesulphonate (301) underwent both β-elimination and decarboxylative elimination with certain bases, whereas the *galacto*-methanesulphonate (302) and a related L-*ido*-sulphonate underwent β-elimination only.[480] Methyl (methyl 4-deoxy-α-L-*threo*-hex-4-enopyranosid)uronate was transformed into the pyrone derivative (303)



(301) R^1 = OMs; R^2 = H
(302) R^1 = H; R^2 = OMs



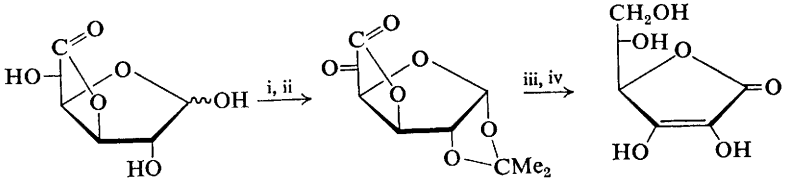
(303)

when treated with sodium hydride in dimethoxyethane.[480a] Methylation of dibenzoylated derivatives of methyl (methyl α-D-galactopyranosid)uronate has led to simple preparations of the 2-,3-, and 4-*O*-methyl ethers.[154a]

Other uronic acid derivatives are referred to in Chapters 3, 4, and 21.

Ascorbic Acids

A straightforward synthesis of L-ascorbic acid from D-glucurono-6,3-lactone is shown in Scheme 108 (*cf.* Vol. 5, p. 119).[481] The mechanism of oxidation of L-ascorbic acid by mercuric chloride in aqueous acid has been deduced from kinetic studies.[482] E.s.r. spectroscopy has been used to examine the radicals



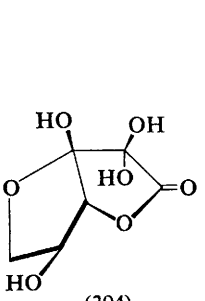
Reagents: i, Me_2CO–H^+; ii, MnO_2; iii, H^+; iv, $NaBH_4$

Scheme 108

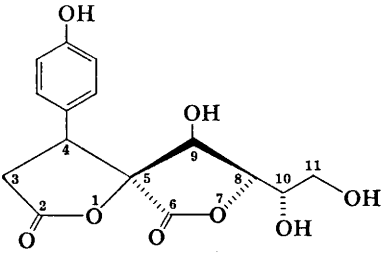
[478] G. O. Aspinall, T. N. Krishnamurthy, W. Mitura, and M. Funabashi, *Canad. J. Chem.*, 1975, **53**, 2186.
[479] G. O. Aspinall and A. S. Chaudhari, *Canad. J. Chem.*, 1975, **53**, 2189.
[480] J. Kiss and P. Taschner, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 279.
[480a] J. Hirsch, P. Kováč, and V. Kováčik, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 431.
[481] T. Kitahara, T. Ogawa, T. Naganuma, and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2189.
[482] R. Senehi, R. M. Mehrotra, and S. P. Mushran, *J. Inorg. Nuclear Chem.*, 1975, **37**, 1753 (*Chem. Abs.*, 1975, **83**, 147 677y).

formed when L-ascorbic acid reacts with hydrazines in oxygenated alkaline solutions.[483] The structures and formation of the radicals were discussed.

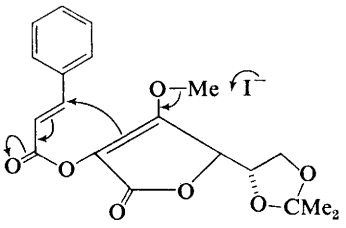
Evidence has been presented to show that dehydro-L-ascorbic acid (obtained by oxidation of L-ascorbic acid) exists mainly as a bicyclic hydrated species (304) (*i.e.* 3,6-anhydro-L-*xylo*-hexulono-1,4-lactone hydrate) in aqueous solution.[484]



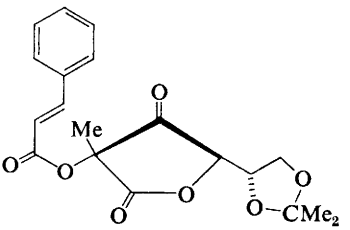
(304)



(305) 4*R*, 5*S*, 8*R*, 9*R*, 10*S*
(306) 4*S*, 5*S*, 8*R*, 9*R*, 10*S*



(307)



(308)

Brimacombe's group has described an attempt to prepare relatives of leucodrin (305) and conocarpin (306) by intramolecular Michael cyclization of the enolate anion obtained by demethylation of 2-*O*-(*E*)-cinnamoyl-5,6-*O*-isopropylidene-3-*O*-methyl-L-ascorbic acid (307).[485] However, treatment of (307) with lithium iodide in DMSO furnished the isomeric 2-*C*-methyl derivative (308) by remethylation of the enolate anion by the liberated methyl iodide.

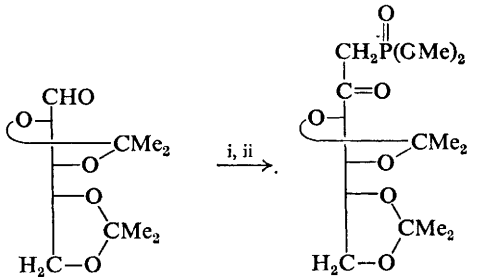
The formation of dicarboxylic acids from L-ascorbic acid is noted in Chapter 2.

[483] H. Utsumi, Y. Kirino, and T. Kwan, *Chem. Pharm. Bull. (Japan)*, 1975, **23**, 1516, 1632.
[484] K. Pfeilsticker, F. Marx, and M. Bockish, *Carbohydrate Res.*, 1975, **45**, 269.
[485] J. S. Brimacombe, A. W. Murray, and Zahur-ul-Haque, *Carbohydrate Res.*, 1975, **45**, 45.

18
Inorganic Derivatives

Carbon-bonded Compounds

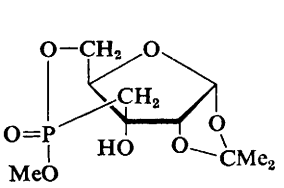
Derivatives of 1-deoxy-D-fructose 1-phosphonate have been prepared *via* the reaction of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose with the lithium salt of dimethyl methanephosphonate (Scheme 109).[486] Analogous reactions



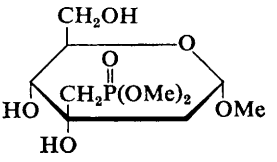
Reagents: i, $LiCH_2P{:}O(OMe)_2$; ii, RuO_4

Scheme 109

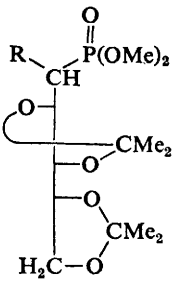
yielded such branched-chain phosphonates as (309) and (310). Dimethyl phosphite also reacted with *aldehydo*-sugars or Schiff bases derived from them to give α-hydroxy- and α-amino-phosphonates [*e.g.* (311) and (312), respectively].[487]



(309)



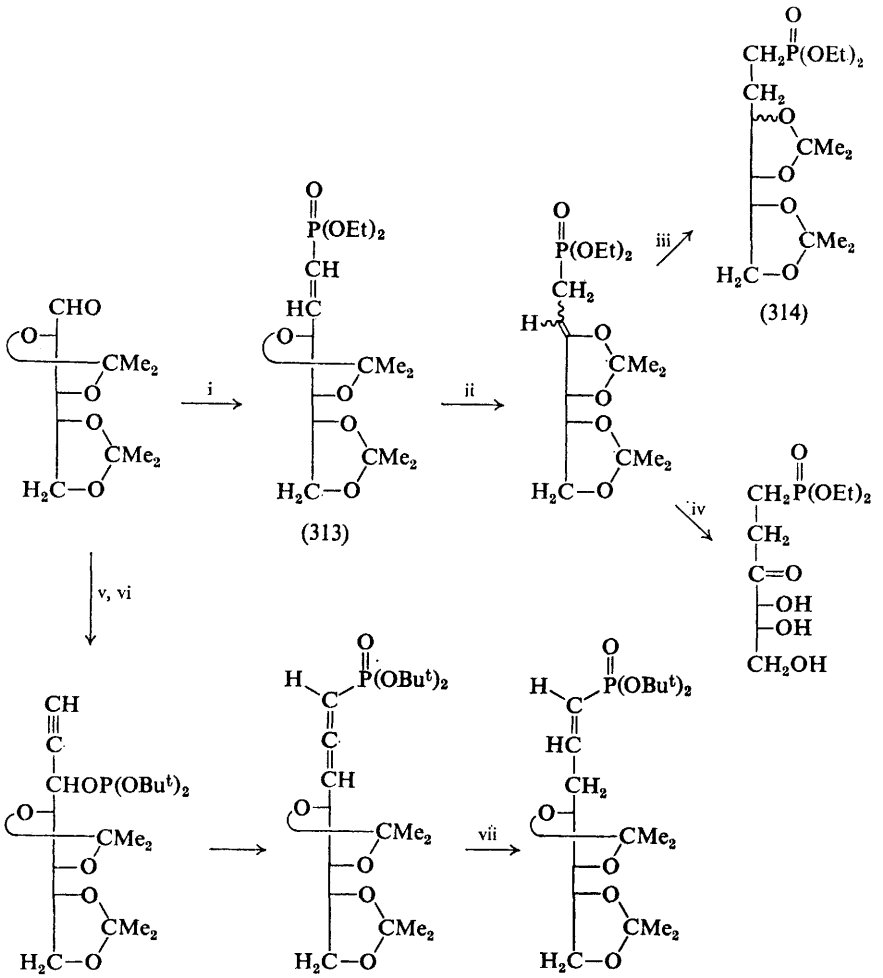
(310)



(311) R = OH
(312) R = NHBn

[486] H. Paulsen and W. Bartsch, *Chem. Ber.*, 1975, **108**, 1229.
[487] H. Paulsen and H. Kuhne, *Chem. Ber.*, 1975, **108**, 1239.

Unsaturated-sugar phosphonates have been obtained by the routes outlined in Scheme 110;[488] the *trans*-alkene (313) underwent an allylic rearrangement on treatment with sodium ethoxide in ethanol to give the 1,2-dideoxy-sugar phosphonates (314) after hydrogenation.[489]



Reagents: i, $NaCH[P{:}O(OEt)_2]_2$; ii, EtONa–EtOH; iii, H_2–Pd; iv, H^+; v, $HC{\equiv}CMgBr$; vi, $(Bu^tO)_2PCl$; vii, H_2–Pt

Scheme 110

The synthesis of 5-alkylphosphinyl-5-deoxy-D-xylopyranose and its derivatives has been noted in Chapter 12.[373]

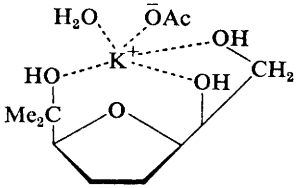
[488] H. Paulsen and W. Bartsch, *Chem. Ber.*, 1975, **108**, 1732.
[489] H. Paulsen and W. Bartsch, *Chem. Ber.*, 1975, **108**, 1745.

Oxygen-bonded Compounds

Studies on the interactions between polyhydroxy-compounds and metal ions are also reported in Chapters 3 and 19.

A conductimetric investigation of the complexation between carbohydrates (*e.g.* D-arabinose, D-fructose, D-glucose, L-sorbose, sucrose, *etc.*) and the hydroxides of alkali and alkaline-earth metals has shown that reducing sugars bind the bases more strongly than non-reducing sugars.[490] D-Glucitol and D-mannitol did not form complexes (see also Vol. 8, p. 123), whereas 1 : 1-complexes of the other carbohydrates and bases were formed in solution.

The chemical shift of the H-1 resonance in the ^{1}H n.m.r. spectrum of methyl 2,3-*O*-isopropylidene-4-*O*-methyl-β-L-rhamnopyranoside was significantly affected by the addition of sodium ions.[491] Since the H-1 resonance of the corresponding α-glycoside was unaffected by sodium ions, the β-glycoside is considered to form a tridentate complex involving O-1, O-2, and the ring-oxygen atom. The complexes formed between sodium ions and 1,4-anhydroerythritol and 5-methoxy-2-phenyl-1,3-dioxans were also discussed. A specific complex (315) (m.p. 55—57 °C) was formed by the addition of potassium acetate to a solution of the free triol in aqueous methanol.[492]



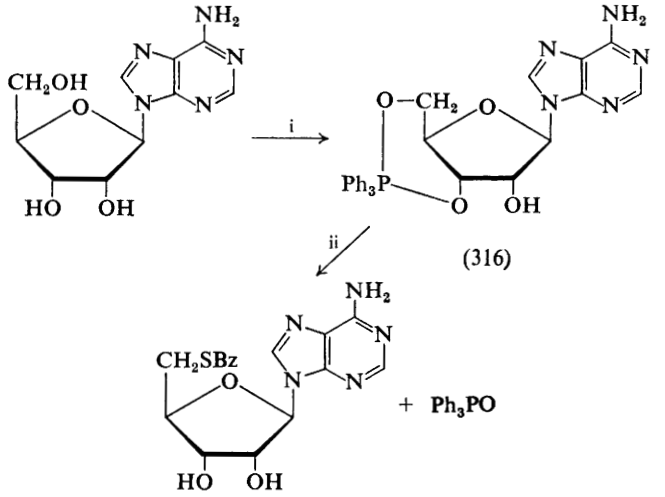
(315)

The interactions between nucleosides (cytidine and guanosine) and various halides of metals from Groups 2A and 2B in DMSO have been examined by ^{1}H and ^{13}C n.m.r. spectroscopy.[493] Both the metal ions and the anions were found to influence the chemical shifts of the amino-protons and C-2 and C-4 of the bases in a complicated way (see also Vol. 8, p. 125). ^{1}H N.m.r. spectroscopic studies have shown that methyl α-D-gulopyranoside forms 1 : 1-complexes with the lanthanide ions Eu^{3+} and Pr^{3+} in aqueous solution.[494] The ratios of the induced chemical shifts for these ions were not equal for all protons, suggesting either appreciable contact shifts or the complex formed has non-axial symmetry. The best fit between the calculated and observed shifts was obtained when the lanthanide ion was placed near the *ax.*, *eq.*, *ax.* sequence of oxygen functions at C-1—C-3. Spectroscopic methods have also been used to assign structures to the 3 : 1-complexes formed in aqueous solution between the sodium salt of methyl α-D-galactopyranosiduronate and lanthanide ions.[495] Magnesium D-gluconate

[490] S. P. Moulik and D. P. Khan, *Carbohydrate Res.*, 1975, **41**, 93.
[491] A. H. Haines, K. C. Symes, and A. G. Wells, *Carbohydrate Res.*, 1975, **41**, 85.
[492] R. E. Hackler, *J. Org. Chem.*, 1975, **40**, 2978.
[493] T. Yokono, S. Shimokawa, and J. Sohma, *J. Amer. Chem. Soc.*, 1975, **97**, 3827.
[494] H. Grasdalen, T. Anthonsen, B. Larsen, and O. Smidsrod, *Acta Chem. Scand.* (*B*), 1975, **29**, 17.
[495] H. Grasdalen, T. Anthonsen, B. Larsen, and O. Smidsrod, *Acta Chem. Scand.* (*B*), 1975, **29**, 99.

dihydrate has been recommended as a standard for the determination of serum magnesium by atomic absorption spectrometry.[496]

The 3′,5′-triphenylphosphorane (316) was obtained when adenosine reacted with triphenylphosphine in the presence of ethyl azodicarboxylate (Scheme 111).[497] Thiobenzoic and benzoic acids cleaved the phosphorus-containing ring to give 5′-substituted adenosines.



Reagents: i, Ph_3P–$EtO_2CN{=}NCO_2Et$; ii, BzSH

Scheme 111

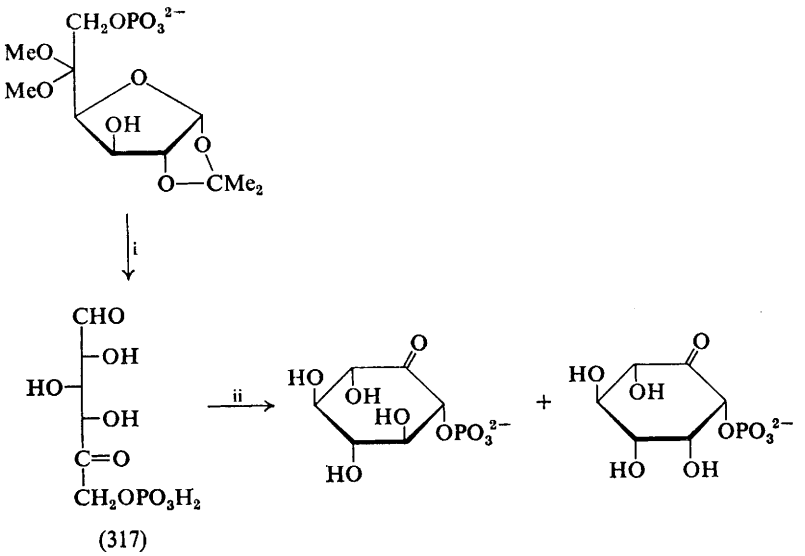
Methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranosides and other sugars containing a vicinal *trans*-diol grouping reacted with dibutyltin oxide to give *O*-(dibutylstannylene) derivatives[498] (see Vol. 8, p. 121).

Conductimetric, electrophoretic, and polarimetric measurements have been used to investigate the complexation of borate anions with sugars and related polyhydroxy-compounds.[499] The results were used to optimize the separation of sugars on anion-exchange resins in the borate form.

[496] M. Zief and A. J. Barnard, jun., *Carbohydrate Res.*, 1975, **45**, 320.
[497] J. Kimura, Y. Hashimoto, and O. Mitsunobu, *Chem. Letters*, 1974, 1473.
[498] S. David and A. Thieffry, *Compt. rend.*, 1974, **279**, *C*, 1045.
[499] W. Woelter, *Chem.-Ztg.*, 1974, **98**, 493 (*Chem. Abs.*, 1975, **82**, 31 466d).

19
Cyclitols

D-*xylo*-Hexos-5-ulose 6-phosphate (317) cyclized in base to give two inosose phosphates (Scheme 112), thus simulating the cyclization step that occurs in the biosynthesis of inositols.[500]



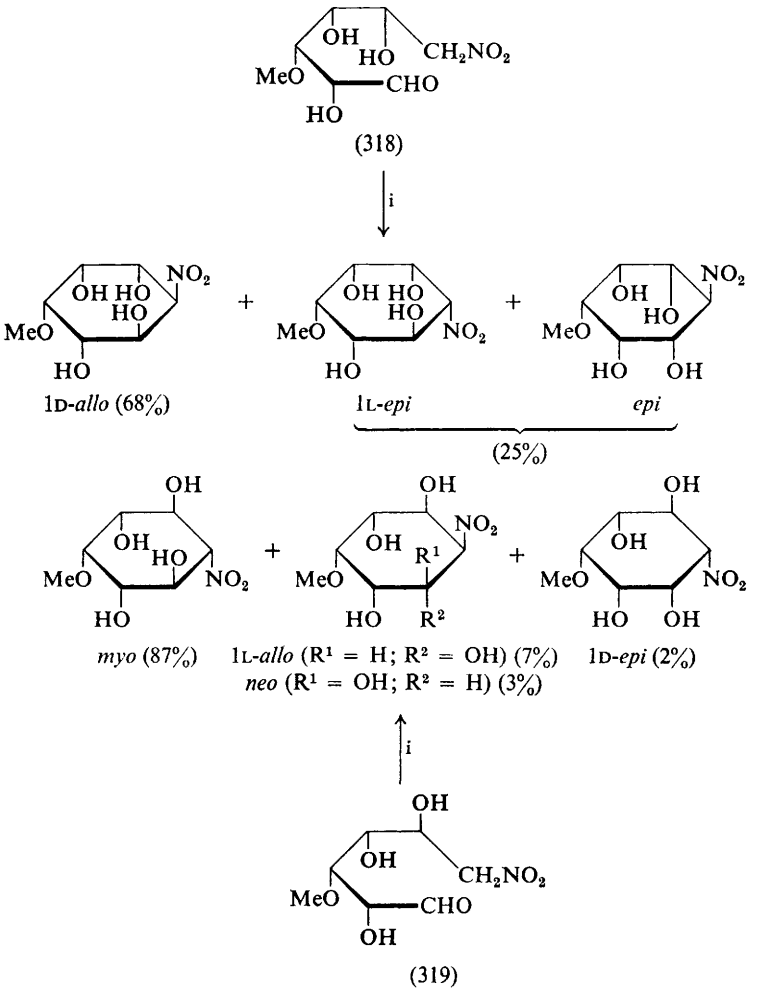
(317)

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

Scheme 112

The synthesis of deoxynitroinositols by the base-catalysed cyclization of 6-deoxy-3-*O*-methyl-6-nitro-D-allose (318) and -L-talose (319) has been investigated.[501] The products shown in Scheme 113 were formed under conditions (pH 8—9) favouring kinetic control, whereas similar equilibrium mixtures of stereoisomers were obtained from (318) and (319) in strongly alkaline solutions (pH > 12) that favour thermodynamic control. A related base-catalysed cyclization of 3-*O*-benzyl-6-deoxy-6-nitro-D-glucose and -L-idose gave mixtures of *O*-benzyldeoxynitroinositols.[341] Under conditions of kinetic control, the D-*gluco*-derivative furnished mainly 6-*O*-benzyl-3-deoxy-3-nitro-*muco*-inositol

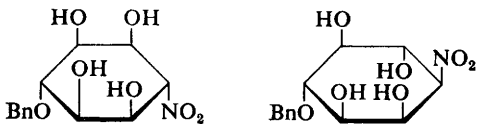
[500] D. E. Kiely and W. R. Sherman, *J. Amer. Chem. Soc.*, 1975, **97**, 6810.
[501] J. Kovář and H. H. Baer, *Carbohydrate Res.*, 1975, **45**, 161.



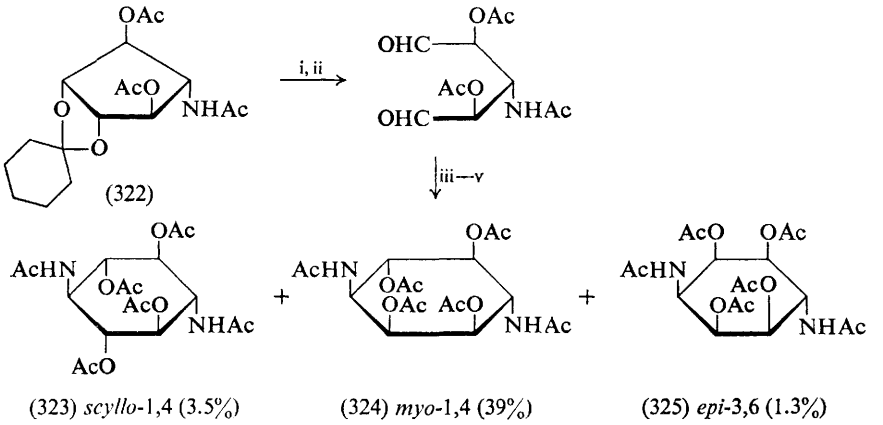
Reagent: i, $Ba(OH)_2$ at pH 7.9–8.7 and 0 °C

Scheme 113

(320) and 4-*O*-benzyl-1-deoxy-1-nitro-L-*myo*-inositol (321) in a ratio of 3 : 1; the L-*ido*-derivative gave the D-*myo*-compound D-(321) and 4-*O*-benzyl-1-deoxy-1-nitro-*scyllo*-inositol in a similar ratio. Epimerization of each individual nitro-inositol under conditions favouring thermodynamic control led to mixtures of the



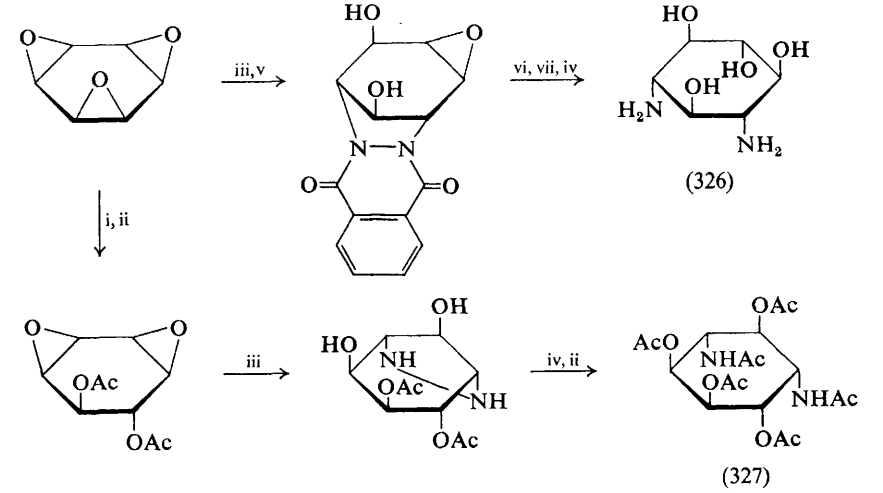
(320) (321)



Reagents: i, AcOH–H_2O; ii, $NaIO_4$; iii, $MeNO_2$–MeONa; iv, H_2–Ni; v, Ac_2O–py

Scheme 114

same composition containing the DL-*myo*- (68%), *muco*- (18%), and *scyllo*- (12%) isomers. The condensation of a dialdehydo-compound with nitromethane has been used to convert the aminocyclopentanetetraol derivative (322) into the new diaminoinositol derivatives (323)—(325) (Scheme 114).[502] *cis,cis*-1,2:3,4:5,6-Tri-anhydroinositol ('*cis*-benzene trioxide') has been used in total syntheses of streptamine (326)[503] and the racemic *chiro*-2,4-diaminoinositol hexa-acetate

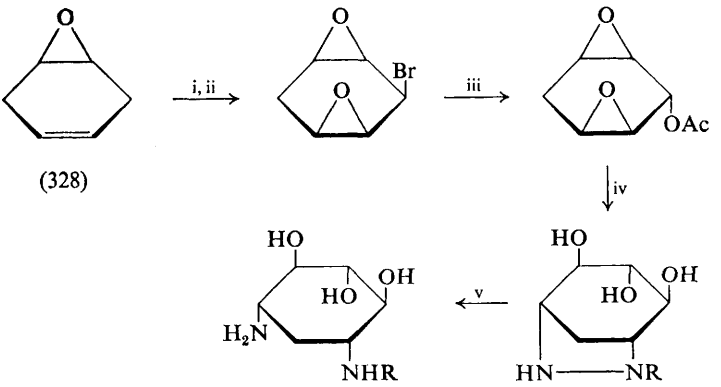


Reagents: i, AcOH–AcONa; ii, Ac_2O–py; iii, N_2H_4; iv, H_2–Ni; v, phthalic anhydride; vi, H_3O^+; vii, NaOH

Scheme 115

[502] J. Suami, K. Tadano, and S. Horiuchi, *Bull. Chem. Soc. Japan*, 1975, **48**, 2895.
[503] R. Schwesinger and H. Prinzbach, *Angew. Chem. Internat. Edn.*, 1975, **14**, 630.

(327) [504] (Scheme 115). The cyclohexadiene oxide (328) has been converted into 2-deoxystreptamine (329) and (±)-hyosamine (330) (Scheme 116),[505] and 1,2:3,4:5,6-trianhydro-*allo*-inositol ('*trans*-benzene trioxide').[505a]



(328)

(329) R = H
(330) R = Me

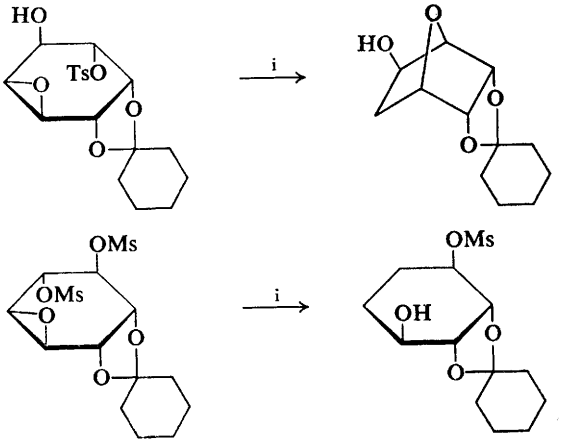
Reagents: i, NBS; ii, CF_3CO_3H; iii, $Me_4\overset{+}{N}\overset{-}{O}Ac$; iv, N_2H_3R; v, H_2–Ni

Scheme 116

Suami's group has synthesized the isomeric 3,4-, 2,5-, 4,5-, and 4,6-dideoxystreptamines using standard reactions of either azide ion or hydrazine on dideoxyinositol sulphonates or oxirans.[506] The reduction of 1,2:3,4-dianhydroinositols and 1,2-anhydroinositol sulphonates with lithium aluminium hydride has furnished a series of dideoxy- or 1,4-anhydrodeoxy-inositols (*e.g.*, see Scheme 117).[507] 1-Amino-1-deoxy-*myo*-inositol has been synthesized by way of an azide displacement on 1L-1,2:3,4-di-*O*-cyclohexylidene-5-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-*chiro*-inositol.[508]

The rates of displacement of the sulphonate group by azide ion in DMF at 110 °C have been measured for a series of toluene-*p*-sulphonyl and *p*-bromobenzenesulphonyl derivatives of 3-*O*-methyl-*chiro*-inositol and 4-*O*-methyl-*allo*-inositol; in these compounds, the arylsulphonate group was located at position 4 (or 3), and the substituents at positions 1, 2, 5, and 6 were isopropylidene, acetyl, methyl, and cyclic carbonate.[509] All of the resulting azides (except the unreactive penta-*O*-methyl-*chiro* derivative) could be converted into one of two inosamine penta-acetates, *viz.* 1L-2-amino-2-deoxy-1-*O*-methyl-*allo*-inositol and 1D-3-amino-3-deoxy-4-*O*-methyl-*chiro*-inositol penta-acetates. The results were discussed in terms of currently held views on displacement reactions with carbohydrate and related sulphonates.

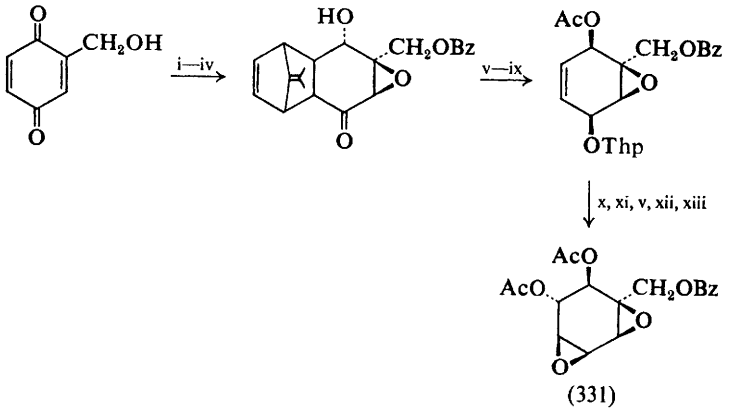
[504] H. Prinzbach and H.-W. Schneider, *Tetrahedron Letters*, 1975, 3073.
[505] H. Prinzbach, R. Keller, and R. Schwesinger, *Angew. Chem. Internat. Edn.*, 1975, **14**, 632.
[505a] H. Prinzbach, R. Keller, and R. Schwesinger, *Angew. Chem. Internat. Edn.*, 1975, **14**, 633.
[506] T. Suami, S. Ogawa, H. Uchino, and Y. Funaki, *J. Org. Chem.*, 1975, **40**, 456.
[507] T. Suami, S. Ogawa, and Y. Funaki, *Bull. Chem. Soc. Japan*, 1975, **48**, 1545.
[508] A. Olesker, D. Mercier, S. D. Gero, C. J. Pearce, and J. E. G. Barnett, *J. Antibiotics*, 1975, **28**, 490.
[509] M.-C. Wu and L. Anderson, *Carbohydrate Res.*, 1975, **44**, 53.



Reagent: i, $LiAlH_4$

Scheme 117

A racemic derivative (331) of crotepoxide, a naturally occurring branched-chain dianhydroinositol exhibiting cancerostatic activity, has been synthesized from 2-hydroxymethyl-1,4-benzoquinone by the route outlined in Scheme 118.[510]



Reagents: i, dimethylfulvene; ii, epoxidation; iii, $NaBH_4$(1 eq.); iv, BzCl–py; v, MsCl–py; vi, Δ; vii, Zn $(BH_4)_2$; viii, AcOH–AcOK; ix, dihydropyran; x, OsO_4; xi, Ac_2O(1 eq.)–py; xii, H^+; xiii, Al_2O_3

Scheme 118

The acetylated *myo*-inositol phosphate (332) has been synthesized and incorporated into fatty-acid derivatives of glycerol phosphate (*i.e.* phosphoinositides).[511, 512] 1,4,5,6-Tetra-*O*-benzyl-*sn-myo*-inositol has been transformed into

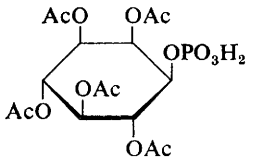
[510] K. Oda, A. Ichihara, and S. Sakamura, *Tetrahedron Letters*, 1975, 3187.

[511] A. I. Lyutik, M. S. Sadovnikova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1974, **44**, 2573 (*Chem. Abs.*, 1975, **82**, 86 519d).

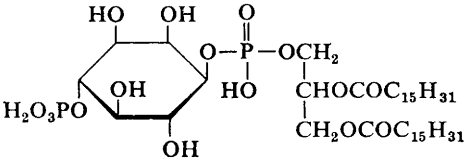
[512] A. I. Lyutik, V. I. Sukhanov, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1974, **44**, 2595 (*Chem. Abs.*, 1975, **82**, 86 520x).

1,2:4,5-[513] and other di-*O*-cyclohexylidene-*sn-myo*-inositols;[514] an optically pure form of 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol was used to prepare several diphosphoinositides [*e.g.* (333)].[515]

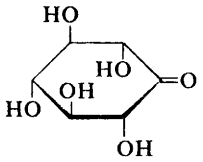
Umezawa's group has published details of the conversion of streptidine into its di-*N*-acetyl-di-*N*-benzyloxycarbonyl-*O*-cyclohexylidene derivative, which is an important intermediate in their total synthesis of dihydrostreptomycin.[516]



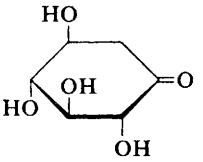
(332)



(333)



(334)



(335)

The products of γ-radiolysis of *scyllo*-inositol in deaerated aqueous solution included the inosose (334), *myo*-inositol, and the deoxyinosose (335), whereas only (334) was formed from γ-radiolysis in the presence of oxygen.[517] A reaction scheme was proposed to account for the formation of these products.

The stereochemistry of the tritiation of L-*chiro*-inositol and its hexa-*O*-methyl derivative and *myo*-inositol by self-radiation-induced exchange with tritiated water of high specific activity has been investigated.[518] Tritium-labelling of the two inositols took place mainly with retention of configuration, whereas appreciable stereochemical inversion of configuration occurred in the methylated derivative. Tritiation occurred mainly at C-1 of L-*chiro*-inositol, with slight inversion of configuration at this position only.

Angyal's group has extended its investigations on the association of polyhydroxy-compounds with cations in solution. ^{1}H N.m.r. spectroscopic studies showed that those cyclitols containing an *ax.*, *eq.*, *ax.* sequence of three hydroxy-groups (*e.g. epi*-inositol) form complexes with cations (*e.g.* Ca^{2+}, Sr^{2+}, and Ba^{2+}); addition of these divalent cations produced a downfield shift of all the proton resonances.[519] 1 : 1-Complexes of *epi*-inositol and calcium or strontium chloride

[513] M. S. Sadovnikova, Z. P. Kuznetsova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1974, **44**, 2593 (*Chem. Abs.*, 1975, **82**, 86 521y).
[514] M. S. Sadovnikova, Z. P. Kuznetsova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1975, **11**, 1211 (*Chem. Abs.*, 1975, **83**, 179 445n).
[515] M. S. Sadovnikova, V. I. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 17 (*Chem. Abs.*, 1975, **83**, 131 855u).
[516] S. Umezawa, Y. Takahashi, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1975, **48**, 560.
[517] A. Kirsch, C. von Sonntag, and D. Schulte-Frohlinde, *J.C.S. Perkin II*, 1975, 1334.
[518] D. H. T. Fong, C. L. Bodkin, M. A. Long, and J. L. Garnett, *Austral. J. Chem.*, 1975, **28**, 1981.
[519] S. J. Angyal and R. J. Hickman, *Austral. J. Chem.*, 1975, **28**, 1279.

could be isolated, and the calcium chloride complex proved useful in the separation of *epi*-inositol from *myo*-inositol used in its synthesis.

The phosphorylation of *muco*- and *epi*-inositols, and the g.l.c. of TMS derivatives of inositol phosphates are noted in Chapter 6. ^{13}C N.m.r. spectroscopic studies on cyclitols are referred to in Chapter 23.

20
Antibiotics

The structures and syntheses of amino-glycoside antibiotics [520, 521] and the mode of action and of inactivation of these antibiotics [522] have been reviewed. Reviews have also appeared on the chemistry and conformation of tobramycin,[523] and on the structure of, and clinical resistance to, sisomycin and the gentamycin group of antibiotics.[524]

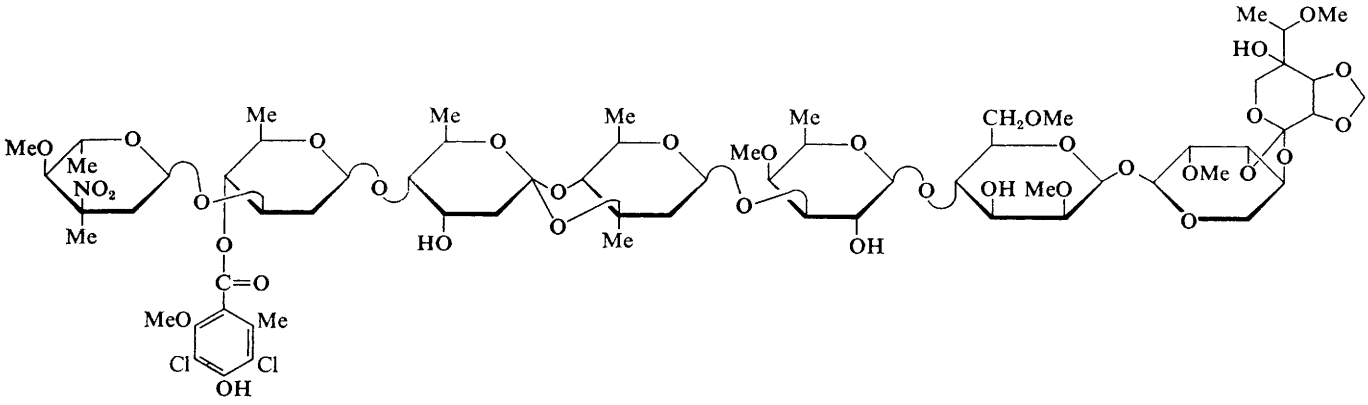
Aspiculamycin is not a tripeptidyl amino-sugar derivative (see Vol. 8, p. 130), but is identical with gougerotin.[525] Following last year's report on the structure of ezomycin A_1 (see Vol. 8, p. 130), the structures of related complexes (B_1, B_2, C_1, C_2, D_1, and D_2) have been elucidated, and a mechanism has been suggested for their interconversion.[526]

The structure (336) of the octasaccharide antibiotic everninomycin D produced by *Micromonospora carbonaceae* has been reported by Ganguly and his co-workers.[527] Orthoester linkages akin to that present in everninomycin D are also found in destomycins A, B,[528] and C,[529] which chemical and spectroscopic evidence indicated to have the structures (337); each antibiotic contains a 6-amino-6-deoxyheptopyranosylidene residue attached to a D-hexose residue, which is linked in turn to streptamine. D-Talose is the hexose component of destomycins A and C.

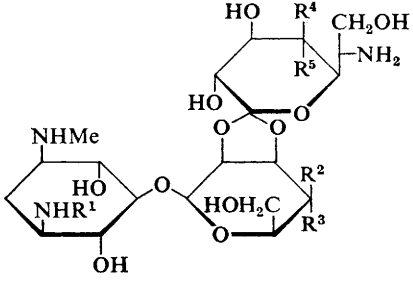
A combination of chemical and spectroscopic methods has been elegantly used to establish the complete structures of aldgamycins E and F (338), two closely related macrolide antibiotics isolated from *Streptomyces lavendulae*.[530] Each antibiotic contains mycinose (6-deoxy-2,3-di-*O*-methyl-D-allose) and aldgarose (see p. 100) as component sugars.

The structures (339) of the gentamycin antibiotics A_1, A_3, A_4,[531] and A_2 [531a] have been elucidated by ^1H and ^{13}C n.m.r. spectroscopy in conjunction with mass

[520] S. Umezawa, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 111.
[521] S. Umezawa, *Drug Action Drug Resist. Bact.*, 1975, **2**, 3 (*Chem. Abs.*, 1975, **83**, 168 344c).
[522] H. Umezawa, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 183.
[523] K. F. Koch, *Drug Action Drug Resist. Bact.*, 1975, **2**, 113 (*Chem. Abs.*, 1975, **83**, 168 347f).
[524] P. J. L. Daniels, *Drug Action Drug Resist. Bact.*, 1975, **2**, 77 (*Chem. Abs.*, 1975, **83**, 168 346e).
[525] F. W. Lichtenthaler, T. Morino, and H. M. Menzel, *Tetrahedron Letters*, 1975, 665.
[526] K. Sakata, A. Sakurai, and S. Tamura, *Tetrahedron Letters*, 1975, 3191.
[527] A. K. Ganguly, O. Z. Sarre, D. Greeves, and J. Morton, *J. Amer. Chem. Soc.*, 1975, **97**, 1982.
[528] S. Kondo, K. Iinuma, H. Naganawa, M. Shimura, and Y. Sekizawa, *J. Antibiotics*, 1975, **28**, 79.
[529] M. Shimura, Y. Sekizawa, K. Iinuma, H. Naganawa, and S. Kondo, *J. Antibiotics*, 1975, **28**, 83.
[530] H. Achenbach and W. Karl, *Chem. Ber.*, 1975, **108**, 759, 772, 780.
[531] T. L. Nagabhushan, W. N. Turner, P. J. L. Daniels, and J. B. Morton, *J. Org. Chem.*, 1975, **40**, 2830.
[531a] T. L. Nagabhushan, P. J. L. Daniels, R. S. Jaret, and J. B. Morton, *J. Org. Chem.*, 1975, **40**, 2835.

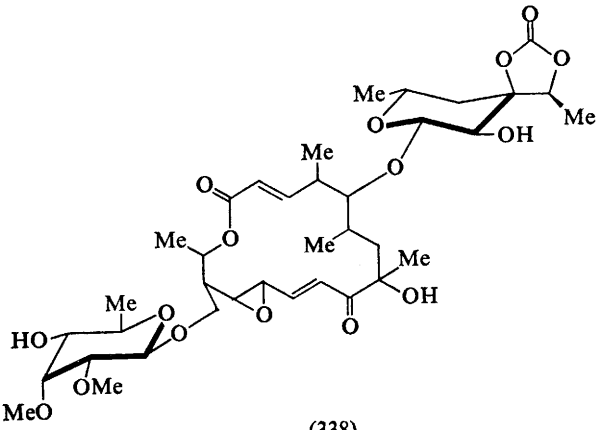


(336)

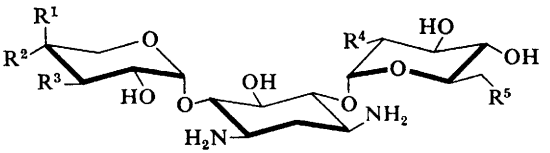


(337)

destomycin	R^1	R^2	R^3	R^4	R^5
A	H	OH	H	OH	H
B	Me	H	OH	H	OH
C	Me	OH	H	OH	H

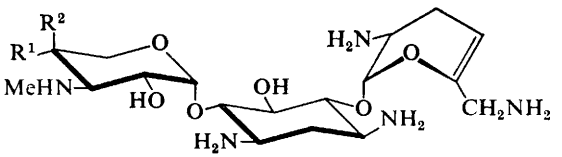


(338)

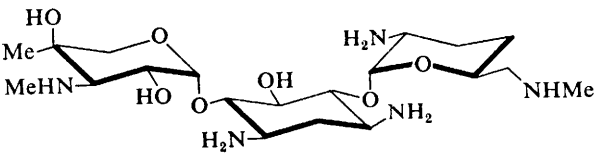


(339)

gentamycin	R^1	R^2	R^3	R^4	R^5
A_1	OH	H	NHMe	NH_2	OH
A_2	H	OH	OH	NH_2	OH
A_3	OH	H	NHMe	OH	NH_2
A_4	H	OH	NMe·CHO	NH_2	OH



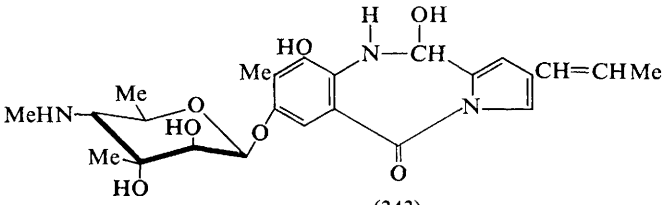
(340) R^1 = OH; R^2 = H
(341) R^1 = H; R^2 = OH



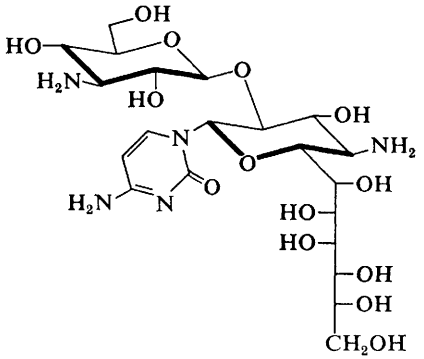
(342)

spectrometry. The broad-spectrum amino-glycoside antibiotics 66-40B and 66-40D produced as minor components by fermentation of *Micromonospora inyoensis* have been shown to be (340) and (341), respectively;[532] these antibiotics are closely related to sisomycin (341; R^1 = Me, R^2 = OH), whose structure was reported last year (Vol. 8, p. 132). The antibiotic 66-40D (341) contains 3-deoxy-3-methylamino-L-arabinose (see also p. 61) as one of the constituent sugars. ^{1}H N.m.r. spectroscopy and mass spectrometry have revealed that the antibiotic XK-62-2 is C^1-*N*-methylgentamycin C_{1a} (342).[533] The absolute configurations of glycoses linked axially to O-4 and O-6 of deoxystreptamine can be determined from the ^{13}C chemical shifts of C-4, C-6, C-1′, and C-1″ at different pH values.[534]

The anti-tumour antibiotic sibiromycin has been assigned the structure (343); the component sugar, sibirosamine, is 4,6-dideoxy-3-*C*-methyl-4-methylamino-D-altropyranose [535] (see Vol. 7, p. 148).



(343)



(344)

The effect of the intrinsic helicity due to the ansa ring on the biological activities of the streptovaricins has been examined.[536]

The structure (344) of the nucleoside antibiotic hikizimycin has been supported by comparison of its ^{13}C n.m.r. spectrum with those of 3- and 4-aminodeoxy-D-

[532] D. H. Davies, D. Greeves, A. K. Mallams, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1975, 814.

[533] R. S. Egan, R. L. DeVault, S. L. Mueller, M. I. Levenberg, A. C. Sinclair, and R. S. Stanaszek, *J. Antibiotics*, 1975, **28**, 29.

[534] T. L. Nagabhushan and P. J. L. Daniels, *Tetrahedron Letters*, 1975, 747.

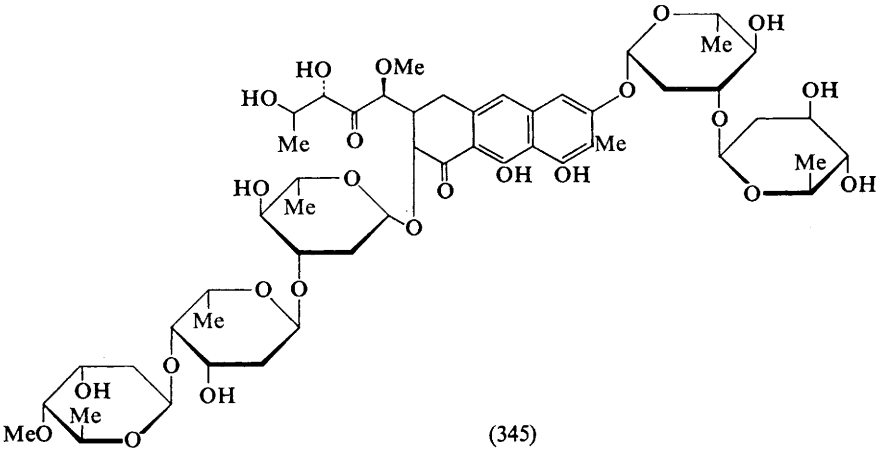
[535] A. S. Mesentsev, V. V. Kulyaeva, and L. M. Rubasheva, *J. Antibiotics*, 1974, **27**, 866.

[536] K. L. Rinehart, W. M. J. Knoll, K. Kakinuma, F. J. Antosz, I. C. Paul, A. H.-J. Wang, F. Reusser, L. H. Li, and W. C. Krueger, *J. Amer. Chem. Soc.*, 1975, **97**, 196.

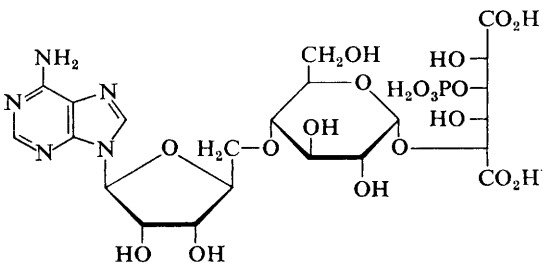
glucose derivatives and galactitol.[537] Spectroscopic methods have also been used to determine the structure of narbomycin, a macrolide antibiotic isolated from cultures of *Streptomyces narbonensis*; the sugar component of this antibiotic is desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose).[538]

Structural investigations on ristomycin A, an antibiotic related to vancomycin, have been reported.[539] This antibiotic comprises D-mannose and a tetrasaccharide {*O*-D-arabinofuranosyl-(1 → 2)-*O*-D-mannopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 6)]-D-glucopyranose} linked to the aglycone, part of whose structure was also elucidated.[540] Chemical and spectroscopic evidence has been presented to support the presence of three oxygenated phenylglycol residues in the aglycone of the antibiotic vancomycin.[541]

The complete structure (345) of the chromomycinone antibiotic variamycin has been reported.[542]



(345)



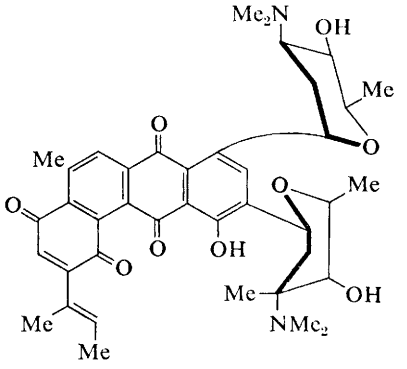
(346)

[537] K. Uchida, E. Breitmaier, and W. A. Koenig, *Tetrahedron*, 1975, **31**, 2315.
[538] K. Noguchi and A. Watarai, *Kagaku To Yakugaku No Kyoshitsu*, 1974, **43**, 99 (*Chem. Abs.*, 1975, **83**, 120 704t).
[539] F. Sztaricskai, R. Bognar, and M. M. Puskas, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 75 (*Chem. Abs.*, 1975, **82**, 125 539g).
[540] R. Bognar, F. Sztaricskai, M. G. Brazhnikova, N. N. Lomakina, and V. A. Zenkova, *Magyar Kém. Folyóirat*, 1974, **80**, 503 (*Chem. Abs.*, 1975, **82**, 58 024v).
[541] G. A. Smith, K. A. Smith, and D. H. Williams, *J.C.S. Perkin I*, 1975, 2108.
[542] G. B. Lokshin, Yu. V. Zhdanovich, and A. D. Kuzovkov, *Bioorg. Khim.*, 1975, **1**, 479 (*Chem. Abs.*, 1975, **83**, 193 632c).

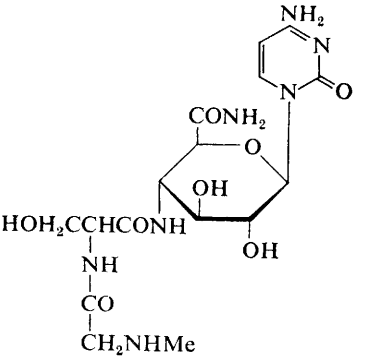
The insecticidal exotoxin (346) isolated from *Bacillus thuringiensis* has been identified as a 5′-adenosyl derivative of 5-*O*-α-D-glucopyranosylallaric acid 3-phosphate.[543]

The constitution and absolute configuration of the polyether antibiotic Ro21-6150 from *Streptomyces hygroscopicus* have been established by *X*-ray crystallographic analysis of the silver salt.[544] Ro21-6150 is the fourth polyether antibiotic shown to contain 2,3,6-trideoxy-4-*O*-methyl-D-*erythro*-hexopyranose; this sugar is β-linked in Ro21-6150 and septamycin, whereas it is α-linked in the antibiotics A-204A and dianemycin. Spectroscopic measurements indicated that the antibiotics Ro21-6150 and A-130A are identical.

Chemical evidence has confirmed that angolosamine (2,3,6-trideoxy-3-dimethylamino-D-*arabino*-hexose) and *NN*-dimethylvancosamine (2,3,6-trideoxy-3-dimethylamino-3-*C*-methyl-L-*lyxo*-hexose) are linked as *C*-glycosides in the antibiotic kidamycin (347).[545]



(347)

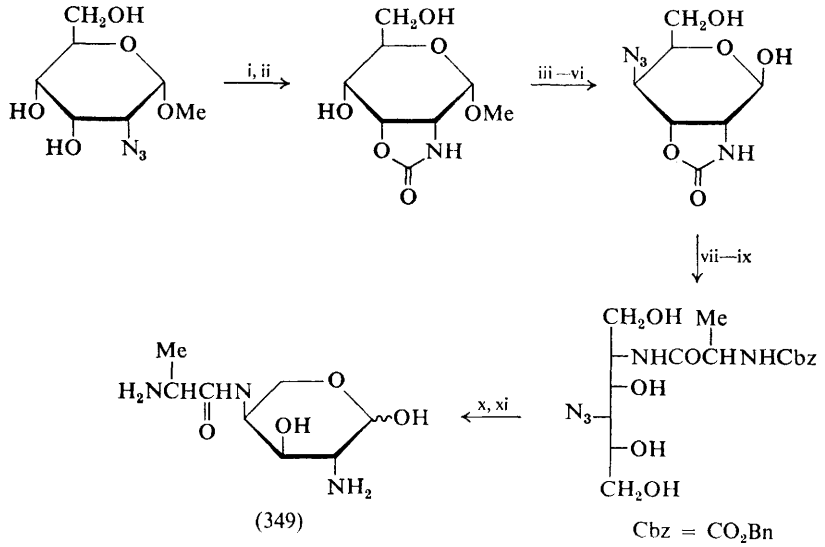


(348)

Umezawa's group has reported the total synthesis of streptomycin [546], following the recent synthesis of derivatives of dihydrostreptomycin [546a] (see Vol. 8, p. 134).

A new synthesis of gougerotin (348) from methyl 4-azido-4-deoxy-D-glucopyranoside has been outlined.[547] Analogues of gougerotin containing modifications to the sugar or the dipeptide or both have been described.[548] Prumycin (349) has been synthesized from methyl 2-azido-2-deoxy-α-D-allo-pyranoside by the reactions shown in Scheme 119.[549] 5′-*O*-Bromoacetyl- and 5′-*o*-chloroacetylpuromycin have been prepared for use in the affinity labelling of ribosomal

[543] M. Prystaš, L. Kalvoda, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1775.
[544] J. F. Blount, R. H. Evans, jun., Chao-Min Liu, T. Hermann, and J. W. Westley, *J.C.S. Chem. Comm.*, 1975, 853.
[545] M. Furukawa, I. Hayakawa, G. Ohta, and Y. Iitaka, *Tetrahedron*, 1975, **31**, 2989.
[546] S. Umezawa, Y. Takahashi, T. Usui, and T. Tsuchiya, *J. Antibiotics*, 1974, **27**, 997.
[546a] S. Umezawa, T. Yamasaki, Y. Kubota, and T. Tsuchiya, *Bull. Chem. Soc. Japan*, 1975, **48**, 563.
[547] F. W. Lichtenthaler, T. Morino, W. Winterfeldt, and Y. Sanemitsu, *Tetrahedron Letters*, 1975, 3527.
[548] C. Coutsogeorgopoulos, A. Bloch, K. A. Watanabe, and J. J. Fox, *J. Medicin. Chem.*, 1975, **18**, 771.
[549] H. Kuzuhara and S. Emoto, *Tetrahedron Letters*, 1975, 1853.



Reagents: i, H_2–Ni; ii, *p*-$NO_2C_6H_4OCOCl$; iii, TrCl–py; iv, MsCl–py; v, NaN_3–HMPT; vi, H_3O^+; vii, $NaBH_4$; viii, $Ba(OH)_2$; ix, *p*-$NO_2C_6H_4OCOCHMeNHCbz$; x, $NaIO_4$; xi, H_2–Pd

Scheme 119

proteins.[550] Syntheses of 3-methylshowdomycin and some related *C*-nucleosides are mentioned in Chapter 3.

The coupling of methyl 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside to tetracycline derivatives furnished products exhibiting antibacterial activity.[551] Gentamycin C_1 has been converted into 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] and 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl] derivatives; *S*-ethyl trifluorothioacetate [$CF_3C(O)SEt$] was used for selective protection of the amino-groups at C-3 and C-2′ in these transformations.[552] Lividomycin A has also been transformed into its 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative, which is active against bacteria resistant to the parent antibiotic,[553] and into the 6′-amino-1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-6′-deoxy derivative.[554]

Umezawa's group has reported the synthesis of 4′-deoxy-kanamycin *via* the coupling of an appropriately derivatized 6-amino-4,6-dideoxy-D-*xylo*-hexosyl chloride with a streptamine derivative.[555] The enzymic phosphorylation and antibacterial activity of the modified antibiotic were examined. Ribostamycin has been converted into butirosin B and its 3′,4′-dideoxy derivative, thus formally

[550] H. H. von Döhren and F. Eckstein, *Chem. Ber.*, 1975, **108**, 2959.
[551] W. Sobiczewski, *Acta polon. Pharm.*, 1975, **32**, 37 (*Chem. Abs.*, 1975, **83**, 164 498h).
[552] P. J. L. Daniels, J. Weinstein, and T. L. Nagabhushan, *J. Antibiotics*, 1974, **27**, 889.
[553] I. Watanabe, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 2124.
[554] I. Watanabe, A. Ejima, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 2303.
[555] S. Umezawa, Y. Nishimura, Y. Hata, T. Tsuchiya, M. Yagisawa, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 722.

completing the total synthesis of these antibiotics.[556] Syntheses of 4′-deoxy-kanamycin A [from 6′-(benzyloxycarbonyl)amino-kanamycin A][557] and 5″-amino-3′,4′,5″-trideoxy-butirosin A (from 5″-amino-5″-deoxy-butirosin A 3′,4′-dimethanesulphonate)[558, 559] have also been described.

Both α- and β-*epi*-4′-daunorubicin and α- and β-*epi*-4′-adriamycin have been synthesized and their biological activities assessed.[560]

The syntheses of amino-sugar components of several antibiotics and of analogues of streptozotoxins are noted in Chapter 8.

Isotopic labelling with D-[6-^{13}C]glucose has established that C-6 of streptidine is derived from C-6, rather than from C-2, of D-glucose in the biosynthesis of streptomycin by *Streptomyces griseus*.[561]

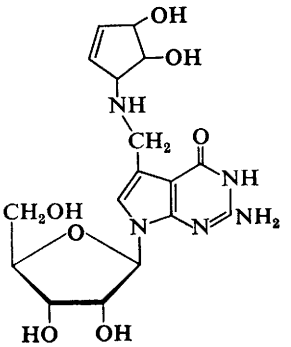
Cordycepin (3′-deoxyadenosine) has been shown to inhibit the synthesis of proteins in germinating cotton cotyledons, whereas 3′-deoxycytidine did not.[562]

The methanolysis of α-lipomycin to give 2,6-dideoxyhexose derivatives is mentioned in Chapter 3.

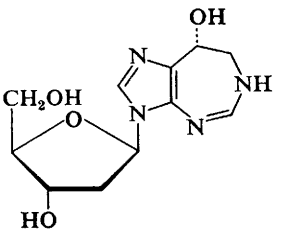
[556] D. Ikeda, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1974, **47**, 3136.
[557] T. Naito, S. Nakagawa, Y. Abe, K. Fujisawa, and H. Kawaguchi, *J. Antibiotics*, 1974, **27**, 838.
[558] P. W. K. Woo, *J. Antibiotics*, 1975, **28**, 522.
[559] H. Saeki, Y. Shimada, E. Ohki, and S. Sugawara, *J. Antibiotics*, 1975, **28**, 530.
[560] F. Arcamone, S. Penco, A. Vigevani, S. Redaelli, G. Franchi, A. Di Marco, A. M. Casazza, T. Dasdia, F. Formelli, A. Necco, and C. Soranzo, *J. Medicin. Chem.*, 1975, **18**, 703.
[561] M. H. G. Munro, M. Taniguchi, and K. L. Rinehart, *J. Amer. Chem. Soc.*, 1975, **97**, 4782.
[562] V. Walbot, A. Capdevila, and L. S. Dure, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 103.

21
Nucleosides

The first paper in a new journal devoted to carbohydrates, nucleosides, and nucleotides has reviewed the mechanisms of nucleoside synthesis by condensation reactions.[563] 5-Carboxymethyl-2′-*O*-methyluridine has been found as a component of yeast tRNA,[564] and the unknown nucleoside 'Q' present in some tRNAs has been identified as 7-(4,5-*cis*-dihydroxy-1-cyclopenten-3-ylaminomethyl)-7-deazaguanosine (350).[565] Syntheses of 9-β-D-glucopyranosyl and 7- and 9-β-D-glucofuranosyl derivatives of zeatin and 6-benzylaminopurine have been reported;



(350)



(351)

these nucleosides were compared with compounds produced as metabolites of the cytokinins, which were shown to have pyranoid structures.[566] A nucleoside isolated from the fermentation broth of a strain of *Streptomyces antibioticus* was shown to have the structure (351); it is a potent inhibitor of adenosine deaminase.[567] Cordycepin (3′-deoxyadenosine) inhibited protein synthesis in germinating cotton cotyledons, whereas 3′-deoxycytidine did not.[562]

Synthesis

A novel method for condensing appropriately substituted sugar derivatives having a free anomeric hydroxy-group with 6-chloropurine has used diethyl azodi-

[563] K. A. Watanabe, D. H. Hollenberg, and J. J. Fox, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 1.

[564] M. W. Gray, *Canad. J. Biochem.*, 1975, **53**, 735.

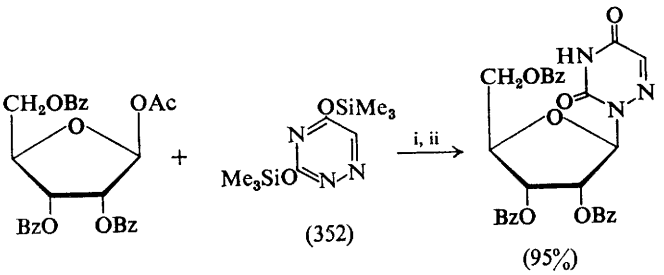
[565] H. Kasai, Z. Ohashi, F. Harada, S. Nishimura, N. J. Oppenheimer, P. F. Crain, J. G. Liehr, D. L. von Minden, and J. A. McCloskey, *Biochemistry*, 1975, **14**, 4198.

[566] D. E. Cowley, I. D. Jenkins, J. K. MacLeod, R. E. Summons, D. S. Letham, M. M. Wilson, and C. W. Parker, *Tetrahedron Letters*, 1975, 1015.

[567] P. W. K. Woo, H. W. Dion, S. M. Lange, L. F. Dahl, and L. J. Durham, *J. Heterocyclic Chem.*, 1974, **11**, 641.

carboxylate and methyldiphenylphosphine; nucleosides from 2,3,4,6-tetra-*O*-methyl-D-gluco- and -D-manno-pyranoses and 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, *etc.*, were prepared in this way.[568] In an unusual stereospecific synthesis of β-D-ribosyl-nucleosides, D-arabinose 2-toluene-*p*-sulphonate was treated with the sodium salt of either adenine or uracil in DMSO; the reaction apparently proceeds *via* the 1,2-oxiran to yield mainly the pyranosyl nucleoside and some furanosyl nucleoside.[569] The use of 3,4-di-*O*-benzoyl-D-arabinose 2-toluene-*p*-sulphonate gave only pyranosyl nucleosides, whereas 3,4-*O*-isopropylidene-2-*O*-toluene-*p*-sulphonyl-D-arabinose did not react. *N*-(Peracetylglycosyl)maleimides are sufficiently reactive to undergo transglycosylation reactions with N^6-benzoyladenine and N^4-benzoylcytosine; adenosine and cytidine have been prepared by this route.[570]

The use of trimethylsilyloxy derivatives, instead of alkoxy derivatives, of pyrimidines and other bases in the Hilbert–Johnson synthesis of nucleosides has resulted in better yields. Niedballa and Vorbrüggen have reported on the scope and limitations of this modified Hilbert–Johnson reaction. Thus, in the example shown in Scheme 120, stannic chloride in 1,2-dichloroethane was used to effect a



Reagents: i, $SnCl_4$–$ClCH_2CH_2Cl$; ii, aq. $NaHCO_3$

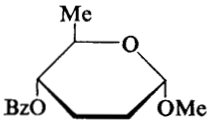
Scheme 120

condensation between 2,4-bis(trimethylsilyloxy)-6-azauracil (352) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose, although other Friedel–Crafts catalysts and acylated methyl glycofuranosides can also be used in the reaction.[571] The use of a trimethylsilylated 6-methyluracil derivative in the above reaction afforded mainly the N^3-riboside (68%), whereas in a more polar solvent (*e.g.* acetonitrile) the products were the N^1-riboside (41%), the N^3-riboside (52%), and the N^1N^3-bisriboside (3%); comparable results were obtained with trimethylsilylated 5,6-dimethyluracil.[571a] Peracetylated reducing disaccharides can also be used in the presence of stannic chloride to give the corresponding disaccharide nucleoside; for example, cellobiose octa-acetate reacted with the trimethylsilylated base (352) to give the N^1-cellobioside (80%); acetylated cello-triose and -tetraose also reacted.[572] Other nucleosides prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-

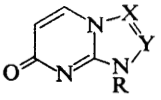
[568] W. A. Szarek, C. Depew, H. C. Jarrell, and J. K. N. Jones, *J.C.S. Chem. Comm.*, 1975, 648.
[569] A. M. Kritzyn and A. Holý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3211.
[570] A. L. Schwartz and L. M. Lerner, *J. Org. Chem.*, 1975, **40**, 24.
[571] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3654.
[571a] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3660.
[572] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3664.

ribofuranose by this procedure were 1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2-dihydropyridin-2-one,[573] 5-azacytidines,[574] and 5-methylaminomethyl-2-thiouridine (a rare nucleoside found in *E. coli* tRNA).[575] A later paper advocated the use of catalytic amounts of either of the strong Lewis acids trimethylsilyl perchlorate or trimethylsilyl trifluoromethanesulphonate in these reactions, particularly as the isolation of the product is simplified.[576]

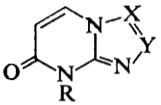
Lewis-acid activation of the glycosidic bond was used also to prepare cytosine derivatives from methyl 4-*O*-benzoyl-α-D-amicetoside (353), when inversion of the configuration at C-1 occurred to give the β-nucleosides.[577] Substitution at either N-7 or N-9 took place when D-glucofuranose penta-acetate was condensed with bis(trimethylsilyl)-N^6-benzoyladenine in dichloromethane in the presence of stannic chloride; lower yields of the nucleosides were obtained from D-xylo- and D-ribo-furanose tetra-acetates, and a D-glucuronamide nucleoside resulted following ammonolysis of the product obtained from D-glucurono-6,3-lactone triacetate.[578]



(353)



(354)



(355)

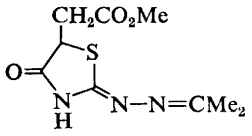
X = N; Y = CH or X = CH; Y = N
R = glycosyl

Nucleosides in the *s*-triazolo[2,3-*a*]-5-pyrimidinone and *s*-triazolo[4,3-*a*]-7-pyrimidinone series have been synthesized by way of silyl or fusion procedures.[579] The former procedure afforded the kinetically favoured product (354) substituted in the triazole ring, whereas the fusion procedure yielded the thermodynamically favoured product (355) substituted in the pyrimidine ring.

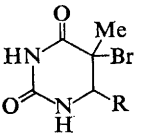
Fusion procedures have also been used to prepare nucleosides of 3-nitro-1,2,4-triazole[580] and ethyl 4-nitropyrazole-3-carboxylate.[581] Epimerization at C-2 occurred during the fusion of 3-acetamido-1,2,5-tri-*O*-acetyl-3-deoxy-β-D-ribofuranose with 2,6-dichloropurine, yielding some α- and β-D-arabinofuranosyl derivatives in addition to the expected products.[582]

Conventional procedures have been used to prepare ribonucleosides from a wide range of bases, including the thiazolidin-4-one (356),[583] the 5,6-dihydro-

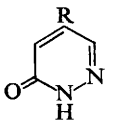
[573] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3668.
[574] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1974, **39**, 3672.
[575] H. Vorbrüggen and K. Krolikiewicz, *Angew. Chem. Internat. Edn.*, 1975, **14**, 255.
[576] H. Vorbrüggen and K. Krolikiewicz, *Angew. Chem. Internat. Edn.*, 1975, **14**, 421.
[577] T. Ogawa and M. Matsui, *J.C.S. Chem. Comm.*, 1975, 992.
[578] A. A. Akhrem, E. K. Adarich, L. N. Kulinkovich, I. A. Mikhailopulo, E. B. Posshchast'eva, and V. A. Timoshchuk, *Doklady Akad. Nauk S.S.S.R.*, 1974, **219**, 99 (*Chem. Abs.*, 1975, **82**, 86 532c).
[579] J. L. Barascut and J. L. Imbach, *Bull. Soc. chim. France*, 1975, 2561.
[580] J. L. Barascut, C. Tamby, and J. L. Imbach, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 77.
[581] I. A. Korbukh, N. G. Yakunina, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1975, **11**, 463 (*Chem. Abs.*, 1975, **83**, 28 500a).
[582] J. A. Montgomery and H. J. Thomas, *J. Carbohydrates, Nucleosides. Nucleotides*, 1975, **2**, 91.
[583] M. Hohnjec, J. Kobe, U. Valcavi, and M. Japelj, *J. Heterocyclic Chem.*, 1975, **12**, 909.



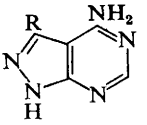
(356)



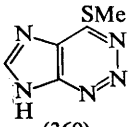
(357) R = OMe, OEt, OAc, *etc.*



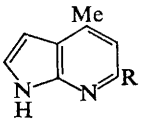
(358) R = OH or NH_2



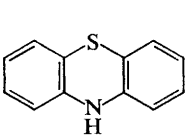
(359) R = H, C≡N, $CONH_2$, $CSNH_2$, *etc.*



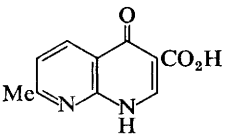
(360)



(361) R = H or Cl



(362)



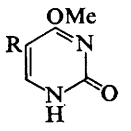
(363)

thymines (357),[584] the pyridazines (358),[585] 3-deaza-5-fluorocytosine,[586] 2-thiomethyl-2′-deoxyuracil,[587] 5-vinyluracil,[588] 1,4-dihydropyrazine-2,3-dione,[589] isoguanine,[590] several pyrazolo[3,4-*d*]pyrimidines (359),[591] thiazolo[5,4-*d*]pyrimidine-5,7-dione,[592] 4-methylthioimidazo[4,5-*d*]triazine (360),[593] indazole (substitution at either N-1 or N-2),[594] the 4-methyl-2,3-dihydropyrrolo[2,3-*b*]-pyridines (361),[595] the phenothiazine (362), and the 1,8-naphthyridine derivative (363).[596]

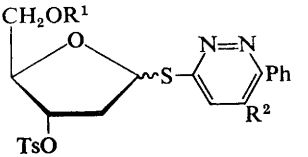
[584] R. Teoule, B. Fouque, and J. Cadet, *Nucleic Acid Res.*, 1975, **2**, 487.
[585] D. J. Katz, D. S. Wise, and L. B. Townsend, *J. Heterocyclic Chem.*, 1975, **12**, 609.
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[590] C. L. Schmidt and L. B. Townsend, *J.C.S. Perkin I*, 1975, 1257.
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[595] M. N. Preobrazhenskaya, T. D. Miniker, V. S. Martynov, L. N. Yakhontov, and D. M. Krasnokutskaya, *Zhur. org. Khim.*, 1974, **10**, 2449 (*Chem. Abs.*, 1975, **82**, 112 218b).
[596] C. Tapiero and J. L. Imbach, *J. Heterocyclic Chem.*, 1975, **12**, 439.

Syntheses of 1-deaza-[597] and 3-deaza-guanosine and its 5′-phosphate [598] have also been described.

Both N-1 and N-3 were substituted when the bis(trimethylsilyl) derivative of 5-fluoroimidazole-4-carboxamide reacted with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride; both the α- and β-glycosides were formed by substitution at N-1, and the β-nucleoside was shown to possess antiviral activity.[599] Both N-1 and N-2 of ethyl pyrazole-3-carboxylate reacted with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose, although the N^2-glycoside isomerized to the N^1-glycoside in the presence of iodine.[600] The N^1-glycoside only was obtained when 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-ribofuranose reacted with ethyl pyrazole-3-carboxylate.[601] In both cases, the ester groups were converted into the carboxamide by reaction with ammonia. In the synthesis of nucleosides from the 4-methoxy-2(1*H*)-pyrimidones (364) substitution took place initially at O-2 and then the glycosyl residue migrated to N-1.[602] An $S \rightarrow N$ migration of the glycosyl residue yielding the



(364) R = H or Me

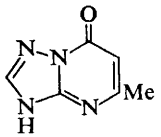


(365) R^1 = COC_6H_4Me-*p*; R^2 = H or Ph

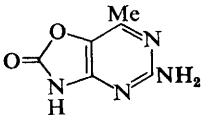
more stable α-glycosides was noted when the *S*-glycosides (365) were heated with mercuric bromide in benzene.[602a] A simple means of transforming uracil nucleosides into cytosine nucleosides has been reported,[603] and 2-thioadenosine has been obtained from adenosine 1-*N*-oxide by the action of carbon disulphide and sodium hydroxide.[604]

The discovery of naturally occurring nucleosides containing L-sugar components has prompted the synthesis of 1-α-L-arabinopyranosyl-uracil and -cytosine, 1-α-L-lyxopyranosyl-uracil and -cytosine, 9-α-L-arabino- and -lyxopyranosyladenines, and 1-α-L-xylopyranosyluracil (obtained by ring-opening of 2,2′-anhydro-1-α-L-xylopyranosyluracil).[605] Nucleosides of other sugars to be synthesized include those derived from the triazolo[1,5-*a*]pyrimidine (366) and D-glucose [606] and from the oxazolo[4,5-*d*]pyrimidine (367) and β-D-glucopyranose, β-D-arabinopyranose, β-D-xylopyranose, and β-D-ribofuranose,[607] the

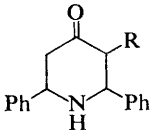
[597] B. L. Cline, R. P. Panzica, and L. B. Townsend, *J. Heterocyclic Chem.*, 1975, **12**, 603.
[598] P. D. Cook, R. J. Rousseau, A. M. Mian, R. B. Meyer, P. Dea, G. Ivanovics, D. G. Streeter, J. T. Witkowski, M. G. Stout, L. N. Simon, R. W. Sidwell, and R. K. Robins, *J. Amer. Chem. Soc.*, 1975, **97**, 2916.
[599] J. C. Reepmeyer, K. L. Kirk, and L. A. Cohen, *Tetrahedron Letters*, 1975, 4107.
[600] I. A. Korbukh, M. N. Preobrazhenskaya, and O. N. Yudina, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 363.
[601] I. A. Korbukh, L. N. Abramova, B. N. Stepanenko, and M. N. Preobrazhenskaya, *Doklady Akad. Nauk S.S.S.R.*, 1975, **220**, 240 (*Chem. Abs.*, 1975, **82**, 125 550d).
[602] M. Prystaš, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1786.
[602a] D. Heller and G. Wagner, *Pharmazie*, 1975, **30**, 207.
[603] H. Vorbrüggen, K. Krolikiewicz, and U. Niedballa, *Annalen*, 1975, 988.
[604] K. Kikugawa and H. Suehiro, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 159.
[605] M. Fuertes, J. T. Witkowski, and R. K. Robins, *J. Org. Chem.*, 1975, **40**, 2372.
[606] G. Wagner, G. Valz, B. Dietzsch, and G. Fischer, *Pharmazie*, 1975, **30**, 134.
[607] I. Ito, N. Oda, T. Kato, and K. Ota, *Chem. Pharm.* and *Bull.* (*Japan*), 1975, **23**, 2104.



(366)

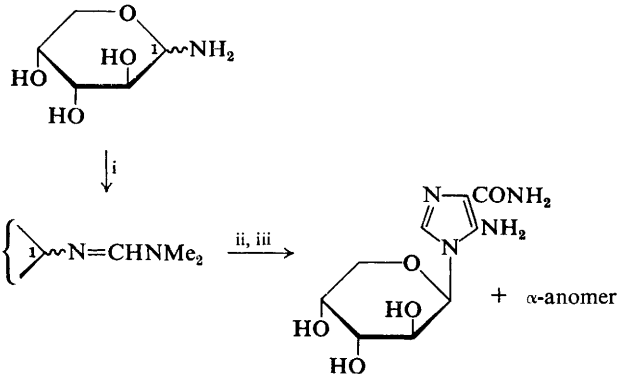


(367)



(368) R = H or Me

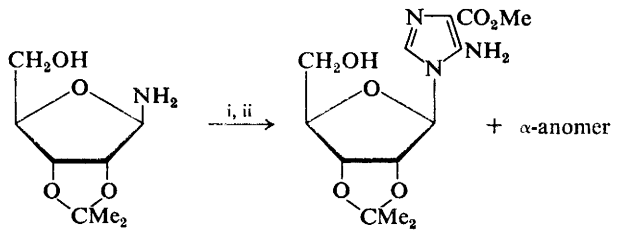
N-1-substituted glycosyl 4-nitrobenzotriazoles derived from β-D-glucopyranose, β-D-xylopyranose, and β-D-ribofuranose,[608] several pentosyl and deoxypentosyl nucleosides derived from 1,2,4-triazole-3-carboxamide (substitution occurred



Reagents: i, $Me_2NCH(OMe)_2$; ii, AcOH–MeOH; iii, $H_2NCH(CN)CONH_2$

Scheme 121

mainly at N-1),[609] the D-glucopyranosyl and D-xylopyranosyl derivatives of the bases (368),[610] a number of 5′-deoxy-ribonucleosides of imidazole,[611] and 9-α-D-idofuranosyladenine.[9] The β-D-arabinofuranosyl analogue of *S*-adenosylhomo-



Reagents: i, TsOH; ii, $EtOCH{=}NCH(CN)CO_2Me$

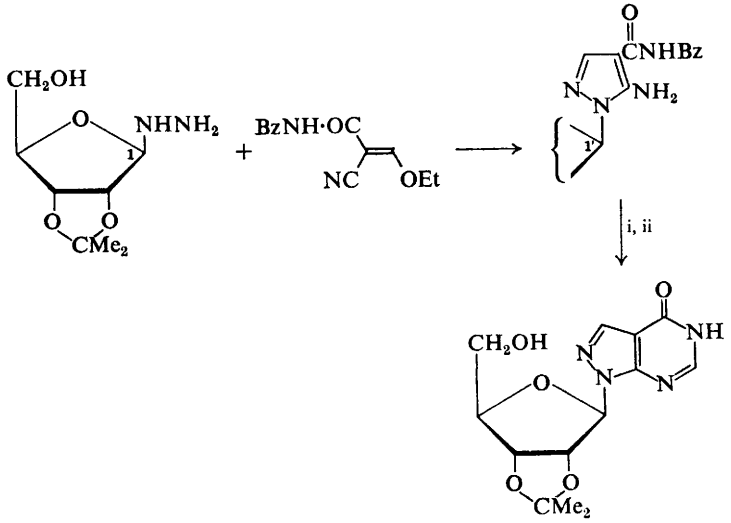
Scheme 122

[608] E. E. Rengevich, V. P. Chernetskii, and L. S. Usenko, *Ukrain khim. Zhur.*, 1975, **41**, 635 (*Chem. Abs.*, 1975, **83**, 193 617b).

[609] J. T. Witkowski, M. Fuertes, P. D. Cook, and R. K. Robins, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 1.

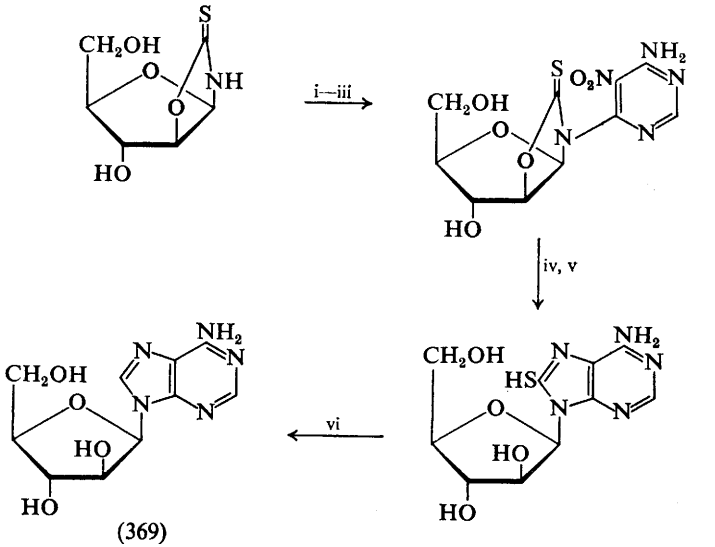
[610] I. N. Azerbaez, V. A. Afanas'ev, B. K. Kalimov, Zh. K. Dzhanakhmetova, and B. D. Abigorov, *Doklady Resp. Nauchno-Tech. Conf. Neftekhim., 3rd*, 1974, **1**, 61 (*Chem. Abs.*, 1975, **83**, 179 472u).

[611] P. C. Srivastava, A. R. Newman, T. R. Matthews, and R. K. Robins, *J. Medicin. Chem.*, 1975, **18**, 1237.



Reagents: i, MeONa–MeOH; ii, HCO_2Et

Scheme 123



(369)

Reagents: i, NaH; ii, $HgBr_2$; iii, 4-amino-6-chloro-5-nitropyrimidine; iv, H_2–Pd; v, Δ; vi, Ni–H_2

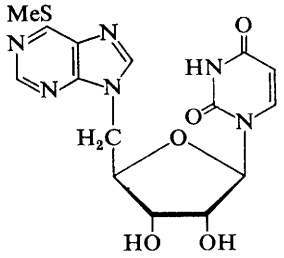
Scheme 124

cysteine[612] and 9-(3-deoxy-α-D-*threo*-pentofuranosyl)adenine[613] have been synthesized. Whistler and his co-workers have prepared several 5-halogeno-4′-thiouridines for biological testing.[614, 615] The reaction of 1-β-D-arabinofuranosyluracil with an excess of hydrogen selenide in pyridine–methyl cellosolve gave the 2- and 4-seleno derivatives.[616]

A number of nucleosides have been elaborated from glycosylamine derivatives: for example, imidazole nucleosides have been obtained by the routes outlined in Schemes 121[617] and 122,[618] and 1-β-D-ribopyranosylisatin was obtained by deacetylation of the product resulting from the action of oxalyl chloride on *N*-(2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl)aniline.[619] Glycosylhydrazines, which exist as equilibrium mixtures of acyclic and cyclic forms, have also been used to prepare ribonucleosides (Scheme 123).[620] A new synthesis of 9-β-D-arabinofuranosyladenine (369) is shown in Scheme 124.[621]

'Double-headed' Nucleosides and Homo- and Iso-nucleosides

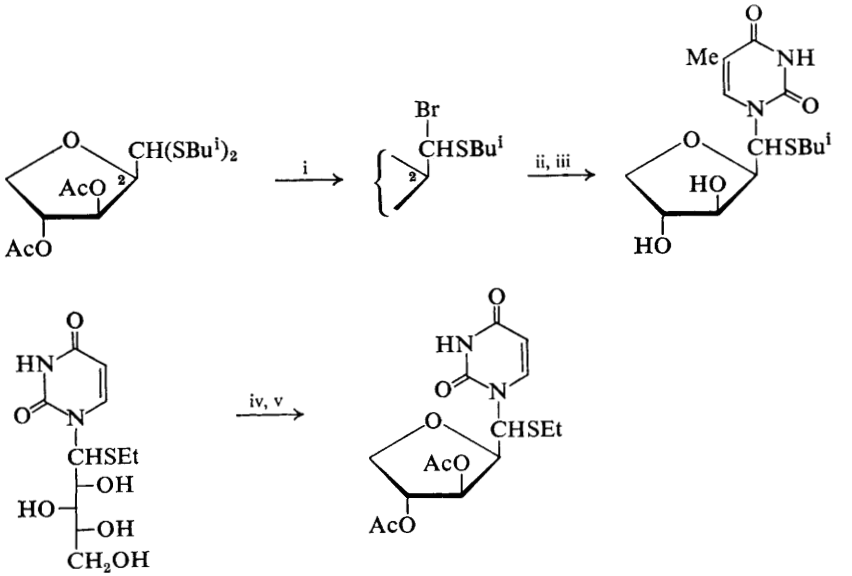
Several 'double-headed' nucleosides have been reported by Russian workers. Thus, treatment of 2′,3′-*O*-isopropylideneuridine 5′-toluene-*p*-sulphonate with the sodium salt of 6-methylthiopurine gave the purine–pyrimidine nucleoside (370) after removal of the protecting group; a related 'double-headed' nucleoside



(370)

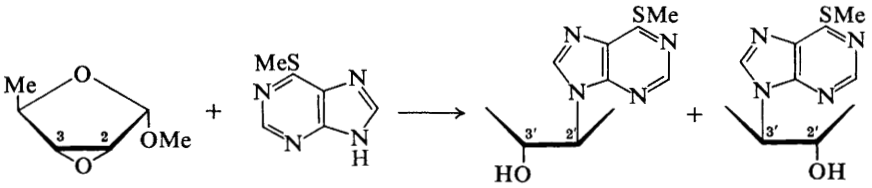
was also synthesized from 4-azauridine.[622] Other compounds to be prepared include the 5′-deoxy-5′-(indol-1-yl) and 5′-deoxy-5′-(6-methylthiopurin-9-yl) derivatives of adenosine.[623]

[612] R. T. Borchardt and Y. S. Wu, *J. Medicin. Chem.*, 1975, **18**, 300.
[613] H. S. El Khadem and T. D. Audichya, *J. Medicin. Chem.*, 1975, **18**, 438.
[614] M. Bobek, A. Block, R. Parthasarthy, and R. L. Whistler, *J. Medicin. Chem.*, 1975, **18**, 784.
[615] J. C. Kim, E.-S. Lee, C.-B. Kim, M. Bobek, and R. L. Whistler, *Tachan Hwahak Hoechi*, 1975, **19**, 130 (*Chem. Abs.*, 1975, **83**, 59 198n).
[616] C.-Y. Shiue and S.-H. Chu, *J. Org. Chem.*, 1975, **40**, 2971.
[617] G. Mackenzie and G. Shaw, *J.C.S. Chem. Comm.*, 1975, 47.
[618] V. V. Alenin and V. D. Domkin, *Zhur. obshchei Khim.*, 1975, **45**, 933 (*Chem. Abs.*, 1975, **83**, 43 662y).
[619] I. V. Yartseva, L. V. Ektova, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1975, **1**, 189 (*Chem. Abs.*, 1975, **83**, 131 857w).
[620] R. R. Schmidt, J. Karg, and W. Guilliard, *Angew. Chem. Internat. Edn.*, 1975, **14**, 64.
[621] R. Ranganathan, *Tetrahedron Letters*, 1975, 1185.
[622] E. A. Utkina, S. Ya. Mel'nik, M. N. Preobrazhenskaya, and N. N. Suvorov, *Zhur. org. Khim.*, 1975, **11**, 194 (*Chem. Abs.*, 1975, **82**, 140 420e).
[623] E. A. Utkina, S. Ya. Mel'nik, M. N. Preobrazhenskaya, and N. N. Suvorov, *Zhur. org. Khim.*, 1975, **11**, 910 (*Chem. Abs.*, 1975, **83**, 43 661x).

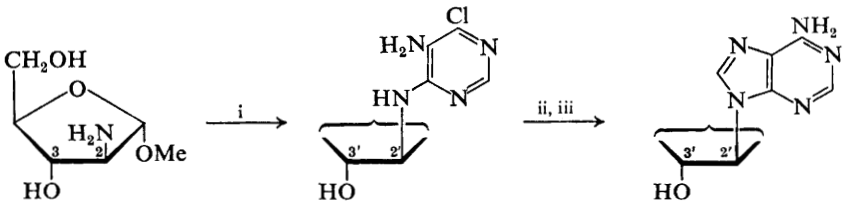


Reagents: i, Br_2; ii, 2,4-diethoxy-5-methylpyrimidine; iii, H^+–MeOH; iv, TsCl–py; v, Ac_2O–py

Scheme 125



Scheme 126



Reagents: i, 5-amino-4,6-dichloropyrimidine; ii, $(EtO)_3CH$–HCl; iii, NH_3–EtOH

Scheme 127

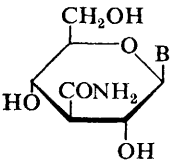
Homonucleosides have been obtained from 3,4-di-*O*-acetyl-2,5-anhydro-D-xylose di-isobutyl dithioacetal and from an acyclic-sugar nucleoside (Scheme 125).[624] The first of a series of papers on isonucleosides (*i.e.* nucleosides in which

[624] J. Defaye, D. Horton, S. S. Kokrady, and Z. Machon, *Carbohydrate Res.*, 1975, **43**, 265.

the base is not bonded to the anomeric carbon of the sugar residue) has described two synthetic approaches to purin-9-yl derivatives: either by ring-opening of an oxiran with the heterocyclic base (Scheme 126), or by elaboration from a pyrimidinyl compound (Scheme 127).[625]

Nucleosides with Branched-chain Components

The nucleosides (261)[432] and (371) (analogues of gougerotin)[429] were obtained from branched-chain amino-sugars described in Chapter 15. The assignment of configuration at the branch-point of nucleosides containing a saccharinic acid component is reported in a later section (see p. 156).

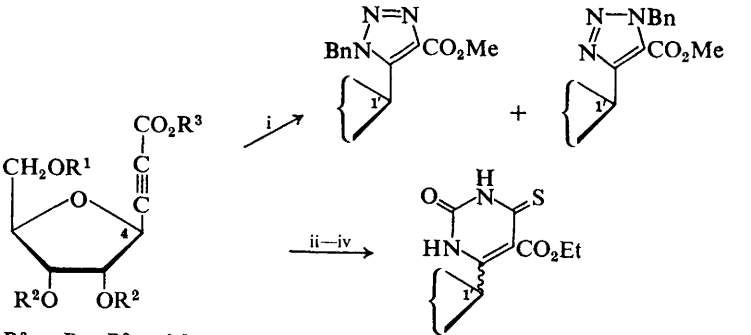


(371) B = cytosin-1-yl or thymin-1-yl

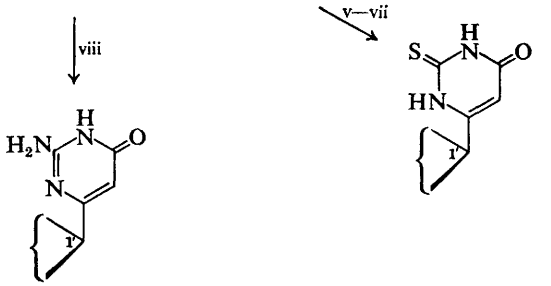
C-Nucleosides

All the syntheses of *C*-nucleosides reported during the past year have started from *C*-glycosides. Two groups have used the D-ribofuranosylethynes (372)[139a] and (373)[626] (see Scheme 128) to elaborate *C*-nucleosides, while a related sequence from a glycosylacetic acid ester used in the preparation of 5-β-D-ribofuranosylisocytosine (ψ-cytosine) is shown in Scheme 129.[627] Moffatt and his co-workers have used variously substituted derivatives of 2,5-anhydro-D-allose to prepare such *C*-nucleosides as (374),[135] (375), (376),[136] and (377)[137] (see Schemes 14—16). Another group has taken advantage of various D-ribofuranosyl *S*-benzylthioformimidates (RSCBn:NH), obtained from the corresponding nitrile, to prepare imidazole and purine *C*-nucleosides.[628—629a] A general synthetic route to 2-substituted, fused pyrimidones by the condensation of a glycosyl thioformimidate with a functionalized pyrazole has been noted already (see Scheme 17); the formycin B analogue (378) was also synthesized by this route.[140] In another approach, 2,5-anhydro-4-deoxy-D-*lyxo*-hexonic acid reacted with 4,5,6-triaminopyrimidine to give 8-(3-deoxy-α-D-*threo*-pentofuranosyl)adenine.[613] Other workers have prepared *C*-nucleosides from the 2,5-anhydro-DL-allose derivatives (379).[630] The *C*-glycoside 4-β-D-ribofuranosyl-

[625] J. A. Montgomery, S. D. Clayton, and H. J. Thomas, *J. Org. Chem.*, 1975, **40**, 1923.
[626] S. Y.-K. Tam, F. G. De Las Heras, R. S. Klein, and J. J. Fox, *Tetrahedron Letters*, 1975, 3271.
[627] C. K. Chu, K. A. Watanabe, and J. J. Fox, *J. Heterocyclic Chem.*, 1975, **12**, 817.
[628] A. Kolb, C. Gouyette, T. Huynh Dinh, and J. Igolen, *Tetrahedron*, 1975, **31**, 2914.
[629] A. Kolb, T. Huynh Dinh, and J. Igolen, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 37.
[629a] T. Huynh Dinh, A. Kolb, C. Gouyette, and J. Igolen, *J. Heterocyclic Chem.*, 1975, **12**, 111.
[630] G. Just and M. Ramjeesingh, *Tetrahedron Letters*, 1975, 985.



(372) $R^1 = R^2 = Bn$; $R^3 = Me$
(373) $R^1 = Tr$; $R^2, R^2 = CMe_2$; $R^3 = Et$

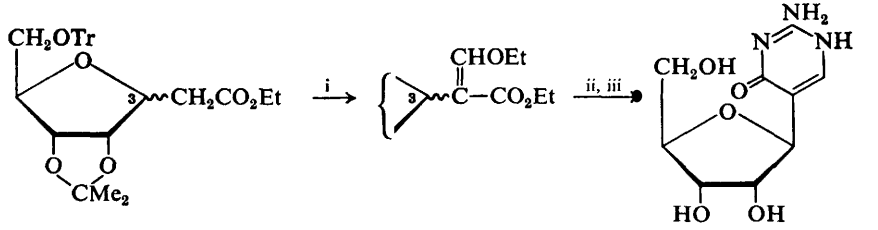


Reagents: i, BnN_3; ii, NH_3; iii, $CSNCO_2Et$; iv, NaOH; v, pyrrolidine; vi, H^+; vii, $(H_2N)_2C{=}S$; viii, $(H_2N)_2C{=}NH$

Scheme 128

1,3-dihydroxybenzene (21) (*i.e.* 1,3-dideazauridine)[128] has been mentioned in Chapter 3, which also contains details of the synthesis of the cyclopentyl analogue (29) of pseudouridine.[134]

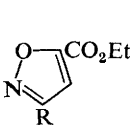
The direct reaction of lithiated pyridine, imidazole, and benzothiazole, *etc.*, with 2,3:5,6-di-*O*-isopropylidene-L-gulono-1,4-lactone and 2,3-*O*-isopropylidene-D-ribono-1,4-lactone has been used to obtain analogues of *C*-nucleosides.[631]



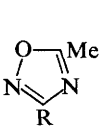
Reagents: i, HCO_2Et; ii, $(H_2N)_2C{=}NH$–MeONa; iii, MeOH–HCl

Scheme 129

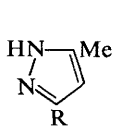
[631] H. Ogura and H. Takahashi, *J. Org. Chem.*, 1974, **39**, 1374.



(374)



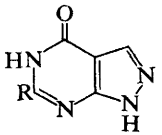
(375)



(376)

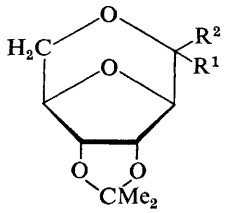


(377)



(378)

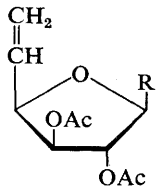
R = β-D-ribofuranosyl



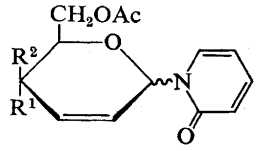
(379) R^1, R^2 = O or R^1 = H; R^2 = OH

Unsaturated Nucleosides

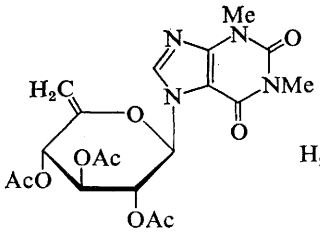
The synthesis of unsaturated nucleosides falls into two groups: first, where an unsaturated sugar is condensed with a base; second, where unsaturation is introduced into an existing nucleoside. Thus, the unsaturated hexofuranosyl derivative (380) was coupled to 6-benzamidochloromercuripurine by the titanium chloride method to give the adenine derivative (381).[632] In a somewhat different



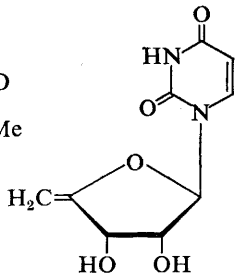
(380) R = OAc
(381) R = N^6-benzoyladenin-9-yl



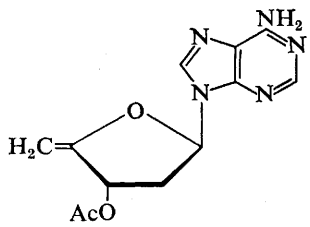
(382) R^1 = OAc; R^2 = H
or R^1 = H; R^2 = OAc



(383)



(384)

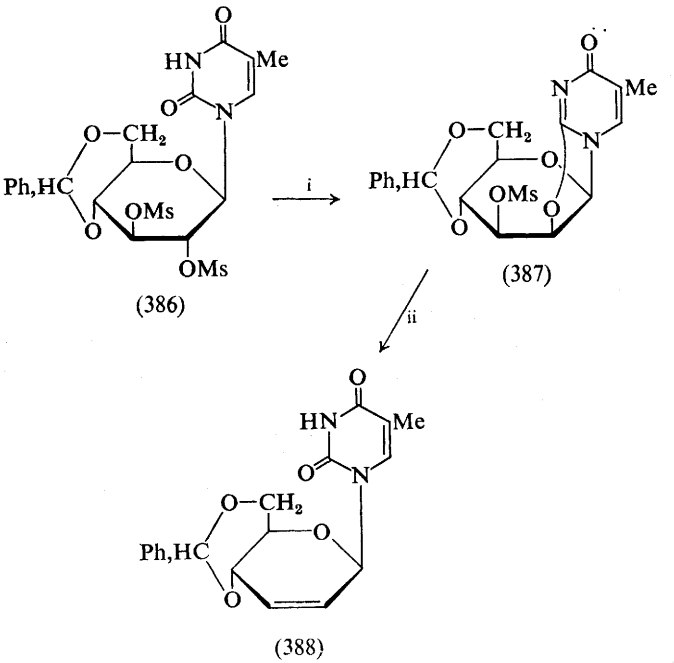


(385)

[632] L. M. Lerner, *Carbohydrate Res.*, 1975, **44**, 13.

approach, 2-pyridone reacted with 3,4,6-tri-*O*-acetyl-D-glucal or -D-galactal to furnish the 2′,3′-unsaturated nucleosides (382).[633]

Silver fluoride in pyridine is frequently used in the dehydrohalogenation of 5′-deoxy-5′-iodo-nucleosides; for example, the theophylline and uracil nucleosides (383) [634] and (384) [407], respectively, were prepared in this way (see Scheme 89), whereas 3′,5′-dideoxy-3′,5′-di-iodothymidine gave the diene (236) when treated with silver fluoride in pyridine. However, 9-(2,5-dideoxy-β-D-*glycero*-pent-4-enofuranosyl)adenine (385) was best prepared by the action of DBU on 3′-*O*-acetyl-5′-bromo-2′,5′-dideoxyadenosine.[635] In a synthesis of the 2′,3′-unsaturated



Reagents: i, NaOBz–DMF; ii, NaI–Zn–DMF

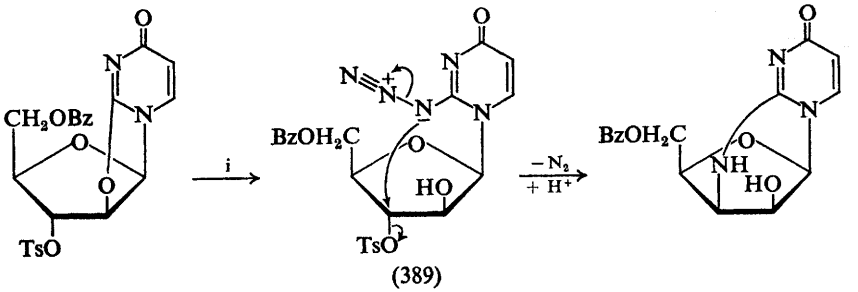
Scheme 130

hexopyranosyl-thymine (388), it was advantageous to convert the dimethanesulphonate (386) into the 2,2′-anhydronucleoside (387) before reaction with the Tipson–Cohen reagent (Scheme 130).[636] A 3′,4′-unsaturated inosine is mentioned in the section on halogeno-sugar nucleosides.

[633] G. Alonso, M. Fuertes, G. Garcia-Munoz, F. G. De Las Heras, and M. Stud, *Anales de Quim.*, 1974, **70**, 1077 (*Chem. Abs.*, 1975, **83**, 179 463s).
[634] A. J. Freestone, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1975, **43**, 239.
[635] N. Suciu and L. M. Lerner, *Carbohydrate Res.*, 1975, **44**, 112.
[636] T. Yamazaki, H. Shiraishi, K. Matsuda, H. Sugiyama, S. Seto, and N. Yamaoka, *J.C.S. Chem. Comm.*, 1975, 518.

Cyclonucleosides

A novel one-step synthesis of 2,3′-epimino-1-(3-deoxy-β-D-lyxofuranosyl)uracil is shown in Scheme 131; the azide (389) was suggested to be an intermediate.[637] A simple procedure for obtaining 2,2′-anhydropyrimidine nucleosides, and hence 1-β-D-arabinofuranosylribonucleosides, involved the reaction of cytidine or

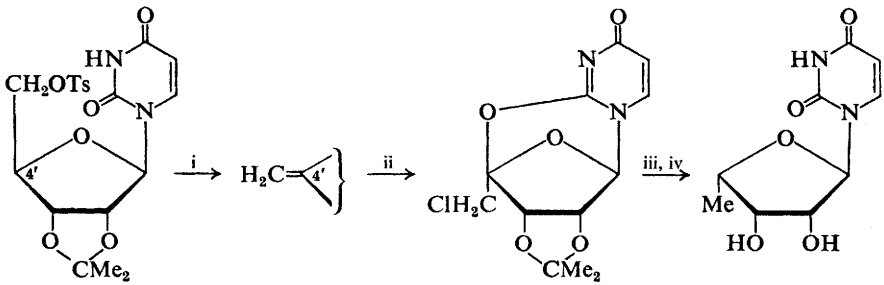


Reagent: i, $\overset{+}{N}H_4\ N_3^-$

Scheme 131

uridine with thionyl chloride in acetonitrile to give the corresponding 2′,3′-cyclic sulphite, which yielded the 2,2′-anhydro-nucleoside on heating with either dilute aqueous acid or sodium acetate in DMF.[638] The use of phosphoryl chloride to convert cytidine into 1-β-D-arabinofuranosylcytosine has also been reported.[639]

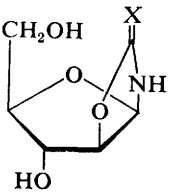
The non-nucleophilic base DBU has been used to prepare certain uracil and thymine anhydro-nucleosides from nucleosides having a leaving-group (*e.g.* methanesulphonyloxy) at C-2′, or C-3′, or C-5′; the ease of formation of the anhydro-nucleosides was established as 2,2′ > 2,3′ > 2,5′.[640] The preparation of 1-(5-deoxy-α-L-lyxofuranosyl)uracil *via* a 2,4′-anhydro-nucleoside is illustrated in Scheme 132.[641] The products obtained on reaction of 2,2′-anhydrouridine with



Reagents: i, Bu^tOK; ii, Bu^tOCl; iii, H_2–Pd–Et_3N; iv, H_3O^+

Scheme 132

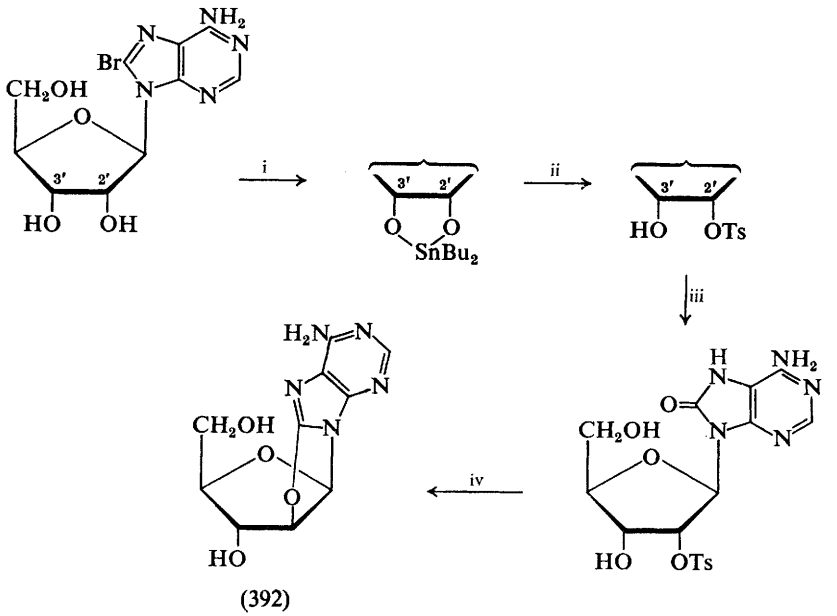
[637] T. Sasaki, K. Minamoto, and T. Sugiura, *J. Org. Chem.*, 1975, **40**, 3498.
[638] T. Sowa and K. Tsunoda, *Bull. Chem. Soc. Japan*, 1975, **48**, 505.
[639] Research Group for Utilization of Nucleic Acids of Worker–Peasant–Soldier Students (Class 1974), *Hua Hsueh Tung Pao*, 1974, 329 (*Chem. Abs.*, 1975, **82**, 156 617k).
[640] J. A. Secrist, tert., *Carbohydrate Res.*, 1975, **42**, 379.
[641] T. Sasaki, K. Minamoto, T. Asano, and M. Miyake, *J. Org. Chem.*, 1975, **40**, 106.



(390) X = NH
(391) X = O

bromine depend on the solvent used; the pyrimidine ring was cleaved in bromine water to give the imide (390) and then the urethane (391), whereas 5-bromo-1-(2-bromo-2-deoxy-β-D-ribofuranosyl)uracil was obtained when a solution of bromine in chloroform was used.[642] The 2′,5-dibromouridine was transformed into 2,2′-anhydro-1-β-D-arabinofuranosyl-5-bromouracil on treatment with sodium methoxide.

Ikehara's group has published several papers describing the synthesis of 2′,8-anhydroadenosine (392) (Scheme 133)[643] and its conversion into 9-β-D-arabino-furanosyl-5′,8-thioanhydroadenine (393) (see Scheme 134);[644] 9-β-D-xylofurano-

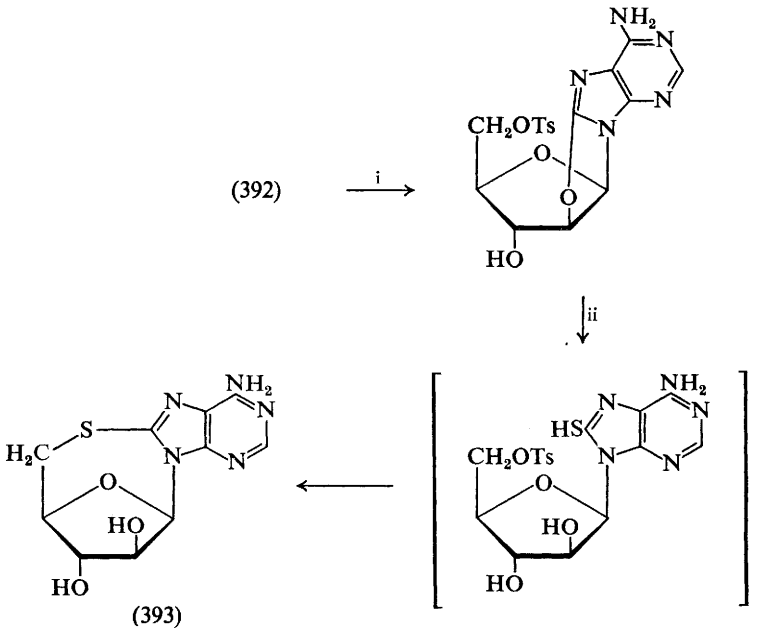


(392)

Reagents: i, Bu_2SnO; ii, TsCl–py; iii, AcOH–H_2O–AcONa; iv, NH_3

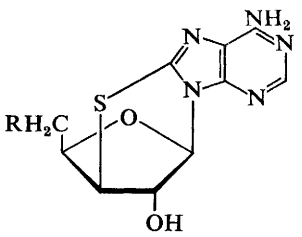
Scheme 133

[642] M. Merész, G. Y. Horvath, P. Sohar, and J. Kuszmann, *Tetrahedron*, 1975, **31**, 1873.
[643] M. Ikehara and T. Maruyama, *Tetrahedron*, 1975, **31**, 1369.
[644] M. Ikehara and Y. Ogiso, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1114.

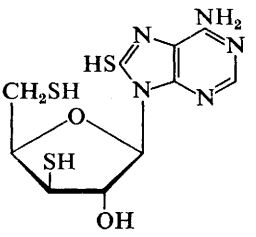


Reagents: i, TsCl–py; ii, NaSH–DMF

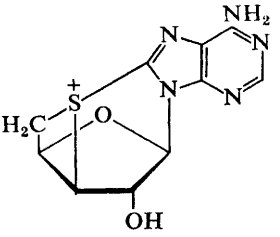
Scheme 134



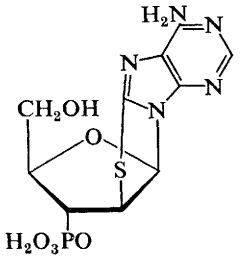
(394) R = OTs
(395) R = SH



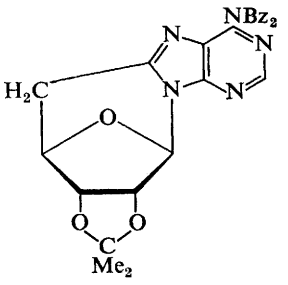
(396)



(397)

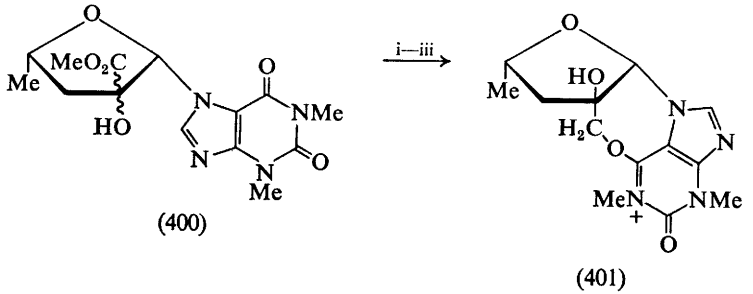


(398)



(399)

syl-5′,8-thioanhydroadenine was similarly obtained from 3′,8-anhydroadenosine. The 3′,8-thioanhydro-nucleoside 5′-toluene-*p*-sulphonate (394) reacted with sodium hydrogen sulphide to give the monothiol (395) and trithiol (396), possibly by way of the sulphonium ion (397).[645] Treatment of adenosine 2′- or 3′-phosphate with sodium hydrogen sulphide gave the 8-thiol derivative, which reacted



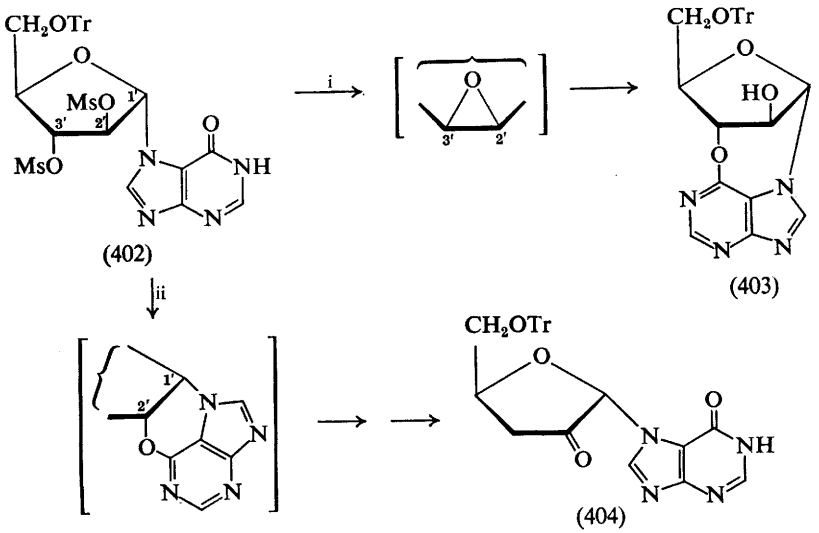
(400) (401)

Reagents: i, $NaBH_4$; ii, TsCl–py,; iii, Δ

Scheme 135

with DCC to give the 2′,3′-cyclic phosphate and then with trimethylsilyl chloride to give the 2′,8-thioanhydro-nucleoside 3′-phosphate (398).[646]

Reduction (tributyltin hydride) of N^6-dibenzoyl-5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneadenosine afforded the carbon-bonded cyclonucleoside (399) and its



(402) (403) (404)

Reagents: i, MeONa; ii, NaOBz–DMF

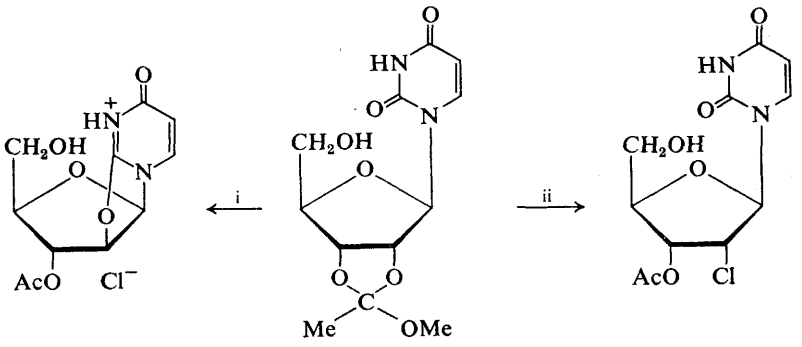
Scheme 136

[645] M. Ikehara and Y. Ogiso, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 121.
[646] M. Ikehara and T. Tezuka, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 67.

7,8-dihydro derivative in addition to the expected 5′-deoxy-nucleoside.[647] The configuration at the branch-point in the isomeric 'saccharinic acid' nucleosides (400) was determined by conversion of one of them into the cyclonucleoside (401) (Scheme 135).[648] The cyclonucleoside (403) was obtained when the nucleoside dimethanesulphonate (402) reacted with sodium methoxide, probably by ring-opening of an intermediate 2′,3′-oxiran; (402) also reacted with sodium benzoate to give the 3′-deoxy-2′-keto-nucleoside (404), possibly *via* an intermediate cyclonucleoside (Scheme 136).[649]

Halogeno-sugar Nucleosides

Trimethylsilyl chloride reacted with the nucleoside 2′,3′-orthoester (405) to give either 3′-*O*-acetyl-2,2′-anhydro-1-β-D-arabinofuranosyluracil or 3′-*O*-acetyl-2′-chloro-2′-deoxyuridine, depending on the solvent and conditions used (Scheme 137).[650] Two groups have reported the reaction of derivatives of 2,3-anhydro-β-



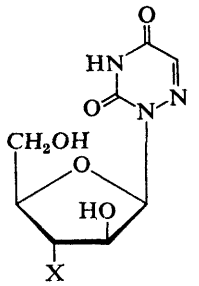
(405)

Reagents: i, Me_3SiCl–MeCN; ii, Me_3SiCl–$MeNO_2$

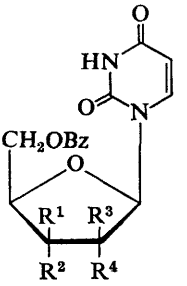
Scheme 137

D-lyxopyranosyl nucleosides with hydrogen halides to give the 3′-bromo- or 3′-chloro-3′-deoxy-nucleosides (406),[651] or a mixture of the 2′- and 3′-fluoro-deoxy-nucleosides (407).[651a] 9-(3-Deoxy-3-iodo-β-D-xylofuranosyl)hypoxanthine has been synthesized and converted into 3′-amino-3′-deoxy- and 3′,4′-unsaturated nucleosides as shown in Scheme 138.[652] Both 1-(2-deoxy-2-fluoro- and 2-chloro-2-deoxy-β-D-arabinofuranosyl)cytidine have been prepared from the corresponding halogeno-sugar derivatives by standard methods (see Schemes 43 and 46).[266, 270]

[647] K. N. V. Duong, A. Gaudemer, M. D. Johnson, R. Quillivic, and J. Zylber, *Tetrahedron Letters*, 1975, 2997.
[648] T. Halmos and K. Antonakis, *J.C.S. Perkin I*, 1975, 2138.
[649] Y. Mizuno, Y. Watanabe, K. Ikeda, and J. A. McCloskey, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1411.
[650] M. W. Logue, *Carbohydrate Res.*, 1975, **40**, C9.
[651] J. Brokeš and J. Beránek, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3071.
[651a] G. Kowollik and P. Langen, *Z. Chem.*, 1975, **15**, 147 (*Chem. Abs.*, 1975, **83**, 59 201h).
[652] F. W. Lichtenthaler, K. Kitahara, and K. Strobel, *Synthesis*, 1974, 860.

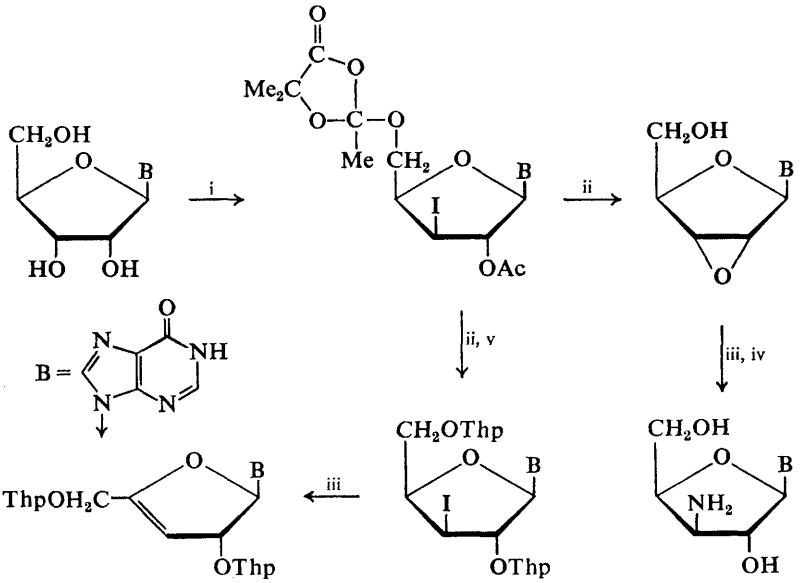


(406) X = Br or Cl



(407) $R^1 = R^4 = H$; $R^2 = F$; $R^3 = OH$
$R^1 = OH$; $R^2 = R^3 = H$; $R^4 = F$

Verheyden and Moffatt have prepared 5′-deoxy-5′-iodo-nucleosides by the reaction shown in Scheme 47; only 5′-deoxy-5′-iodo-4′-methoxy-nucleosides were obtained when the unprotected nucleoside or its 2′,3′-cyclic carbonate was used.[276]



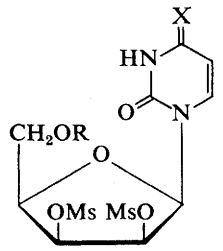
Reagents: i, $Me_2C(OAc)COI$; ii, MeONa–MeOH; iii, NaN_3; iv, H_2–Pd; v, dihydropyran–H^+

Scheme 138

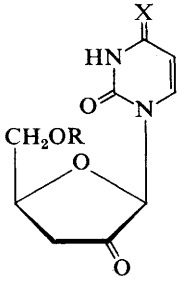
Keto-nucleosides and Nucleoside Carboxylic Acids

The reaction of a hypoxanthine nucleoside 2′,3′-dimethanesulphonate with sodium benzoate in DMF to give a 3′-deoxy-2′-keto-nucleoside has already been noted (Scheme 136), and analogous reactions with the cytidine and uridine derivatives (408) also gave the 3′-deoxy-2′-keto-nucleosides (409).[653] Attempted

[653] T. Sasaki, K. Minamoto, K. Hattori, and T. Sugiura, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 47.

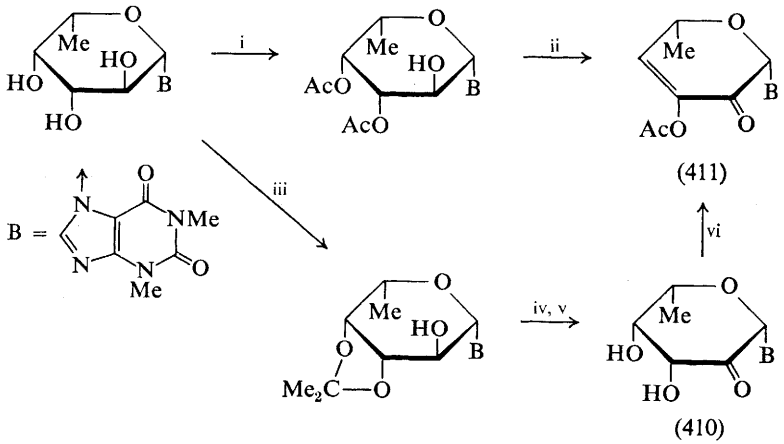


(408) X = NH; R = Tr
or X = O; R = Bn



(409) X = NH; R = Tr
or X = O; R = Bn

acetylation of the theophylline 2′-keto-nucleoside (410) with acetic anhydride in pyridine resulted in β-elimination to give the enone derivative (411), and this reaction has been used to determine the structures of partially acylated nucleosides (Scheme 139).[654] This and related work on keto-nucleosides by Antonakis's group have been summarized.[655]



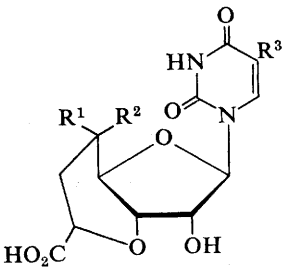
Reagents: i, Ac_2O–BF_3; ii, Ac_2O–DMSO–H^+; iii, Me_2CO–H^+; iv, DMSO–DCC–H^+; v, H_3O^+; vi, Ac_2O–py

Scheme 139

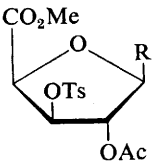
The unusual 3,7-anhydro-octofuranosyluronic acid nucleosides (412) have been isolated from *Streptomyces cacaoi* var. *asoensis*.[656] Oxidation of either guanosine or 7-β-D-ribofuranosyltheophylline afforded the corresponding 5′-carboxy derivatives,[657] and related uridine derivatives were obtained when

[654] M. Bessodes, M.-J. Arvor-Egron, A. Ollapally, and K. Antonakis, *Compt. rend.*, 1975, **280**, *C*, 1273.
[655] K. Antonakis, *Chimia* (*Switz.*), 1975, **29**, 59.
[656] K. Isono, P. F. Crain, and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1975, **97**, 943.
[657] K. E. Norris, O. Manscher, K. Brunfeldt, and J. B. Petersen, *Nucleic Acids Res.*, 1975, **2**, 1093.

bis(trimethylsilyl)uracil reacted with D-glucurono-6,3-lactone triacetate [658] and the D-xylofuranuronate (413)[659] in the presence of stannic chloride. 1-(Methyl 2-*O*-acetyl-3-*O*-toluene-*p*-sulphonyl-β-D-xylofuranosyluronate)uracil (414) underwent elimination of toluene-*p*-sulphonic acid on treatment with sodium azide in



(412) R^1, R^2 = O; R^3 = CO_2H
or R^1 = H; R^2 = OH; R^3 = CO_2H
or R^1 = H; R^2 = OH; R^3 = CH_2OH

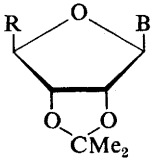


(413) R = OAc
(414) R = uracil-1-yl

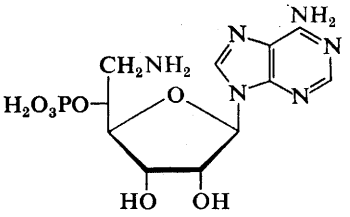
HMPT at room temperature to give the 3′,4′-unsaturated nucleoside. Details of the formation of 'saccharinic acid' nucleosides from 2′,3′-diketo-nucleosides have appeared [660] (*cf.* Vol. 8, p. 151).

Amino-sugar Nucleosides

A marked increase in the number of papers indicates renewed interest in amino-sugar nucleosides. The 5′-aldehydo-nucleosides (415) were used as starting materials in syntheses of the amino-acid derivative (416) [428] and the 5′-*C*-amino-methyl derivative (417) of adenosine.[661] The first synthesis was accomplished by



(415) R = CHO; B = uracil-1-yl or adenin-9-yl
(416) R = $CH(NH_2)CO_2H$; B = uracil-1-yl
(417) R = $CH(OH)CH_2NH_2$; B = adenin-9-yl



(418)

[658] A. A. Akhrem, V. A. Timoshchuk, and I. A. Mikhailopulo, *Zhur. obshchei Khim.*, 1975, **45**, 956 (*Chem. Abs.*, 1975, **83**, 43 663z).
[659] A. A. Akhrem, V. A. Timoshchuk, and I. A. Mikhailopulo, *Carbohydrate Res.*, 1975, **43**, 195.
[660] T. Halmos and K. Antonakis, *Carbohydrate Res.*, 1975, **43**, 208.
[661] F. Kappler and A. Hampton, *J. Org. Chem.*, 1975, **40**, 1378.

way of condensation of 5′-aldehydouridine with 2-phenyloxazolo-5-one in the well-known hydantoin or azolactone route, and the second synthesis by way of the reaction of 5′-aldehydoadenosine with nitromethane (the Fischer–Sowden reaction). The 5′-phosphate (418) and 5′-triphosphate (analogues of AMP and ATP, respectively) were prepared from (417).

Cytidine has been converted into 1-(5-amino-5-deoxy-β-D-arabinofuranosyl)-cytosine by a sequence of reactions involving *N*-benzoylation, sulphonation, acetylation, displacement with azide ion, *etc.*,[662] and 1-(3-amino-3-deoxy-β-D-arabinofuranosyl)-6-azauracil was derived from 2′,3′-di-*O*-methanesulphonyl-5′-*O*-trityl-6-azauridine *via* 2,2′- and 2′,3′-anhydro-nucleosides.[663] Other syntheses have been accomplished by the condensation of an appropriately derivatized amino-sugar with either a pyrimidine or purine derivative; for example, the Hilbert route was used to prepare 1-(2-amino-2-deoxy-α-D-arabino- and -β-D-xylo-furanosyl)cytosine.[664, 665] The reactivity of 1,3,4,6-tetra-*O*-acetyl-2-acylamido-2-deoxy-β-D-glucopyranoses in condensation reactions with 2,6-dichloropurine, theophylline, and 6-benzylaminopurine was shown to be in the order benzamido > acetamido > phthalimido.[666] 9-(3-Acetamido-2,5-di-*O*-acetyl-3-deoxy-β-D-ribofuranosyl)-2,6-dichloropurine has been synthesized and converted into the corresponding 2,6-diamino- and 6-amino-2-chloro(fluoro)-nucleosides.[667]

3′-Amino-3′,5′-dideoxyadenosine has been synthesized from 1,2-di-*O*-acetyl-3,5-dideoxy-3-trifluoroacetamido-D-ribofuranose in connection with studies on the toxicity of puromycin,[668] and the 5′-*O*-bromo(chloro)acetyl derivatives of puromycin have been prepared for use as affinity labels for ribosomal proteins.[550]

Phosphate and Related Esters of Nucleosides

The increase in the number of papers on nucleoside phosphates over the past year has been matched by an increase in the number of phosphorylating agents used; these reagents could be used with other carbohydrates as well as with nucleosides.

Adenosine reacted with such phosphorus acid amides as $P(NM_2)_3$, $Me_2NCOP(NMe_2)_2$, and $(Me_2NCO)_2PNMe_2$ in pyridine at room temperature to give adenosine 2′(3′)-phosphite, and the first reagent also furnished adenosine 2′(3′),5′-diphosphite at 100 °C.[669] Treatment of adenosine with triphenylphosphine and diethyl azodicarboxylate afforded the 3′,5′-cyclic ester (316) in 75% yield; unfortunately, (316) was of limited use in the synthesis of 2′-substituted adenosines, since most reagents cleaved the phosphorus-containing ring to give 5′-substituted products.[497]

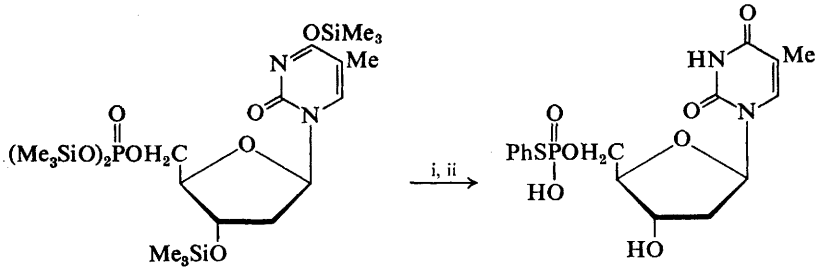
High yields (80—98%) of the corresponding 5′-phosphates were obtained when nucleosides reacted with a mixture of phosphoryl chloride, water, and pyridine (2 : 1 : 2) in acetonitrile; the active phosphorylating agent was identified as an

[662] T.-S. Lin and W. H. Prusoff, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 185.
[663] J. Brokeš and J. Beránek, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3061.
[664] M. L. Wolfrom and S. Inouye, *Carbohydrate Res.*, 1975, **42**, 305.
[665] M. L. Wolfrom, S. Inouye, and P. J. Conigliaro, *Carbohydrate Res.*, 1975, **42**, 317.
[666] N. Nakazaki, T. Takeda, T. Yoshino, M. Sekiya, and Y. Ishido, *Carbohydrate Res.*, 1975, **44**, 215.
[667] J. A. Montgomery, K. Hewson, and A. G. Laseter, *J. Medicin. Chem.*, 1975, **18**, 571.
[668] J. M. J. Tronchet, R. Graf, and J. Tronchet, *Helv. Chim. Acta*, 1975, **58**, 1497.
[669] I. P. Andrianova and E. E. Nifant'ev, *Zhur. obshchei Khim.*, 1975, **45**, 488 (*Chem. Abs.*, 1975, **82**, 171 335k).

adduct of tetrachloropyrophosphate and pyridinium chloride.[670] 2-*NN*-Dimethylamino-4-nitrophenyl phosphate in refluxing pyridine in the presence of acetic acid and triethylamine has been used to cause selective phosphorylation of primary hydroxy-groups.[671, 671a]

Phosphate diesters of nucleosides have been obtained by treating the mixed anhydride of a nucleotide and 2,4,6-trimethylbenzoic acid with alcohols,[672] and by the stepwise addition of suitably protected nucleosides and alcohols to the *N*-methylpyridinium salt of dichlorophosphoric acid.[673] Light-sensitive 2-nitrobenzyl esters of nucleotides have been prepared.[674] Aryl phosphoramidochloridates are effective phosphorylating agents.[675] Thus, treatment of 2′,3′-*O*-methoxymethyleneuridine with a two-fold excess of phenyl, 2-chlorophenyl, or 2,4-dichlorophenyl phosphorocyclohexylamidochloridate in the presence of 5-chloro-1-ethyl-2-methylimidazole gave the corresponding 5′-(aryl phosphoramidates) in good yield. The nucleoside 5′-phosphate could be obtained by treatment of the 5′-(aryl phosphoramidates) with aqueous alkali and then with dilute acid. Related 5′-phosphoramidates obtained with 2,2,2-tribromoethyl-phosphoromorpholinochloridate were used in the synthesis of nucleoside 5′-mono-, 5′-di-, and 5′-tri-phosphates.[676]

A synthesis of thymidine 5′-(*S*-phenyl phosphorothioate) (419) is shown in Scheme 140,[677] and related 3′-thioates have been used in polynucleotide syn-



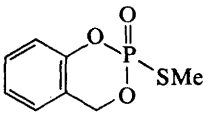
Reagents: i, PhSSPh; ii, H_2O

(419)

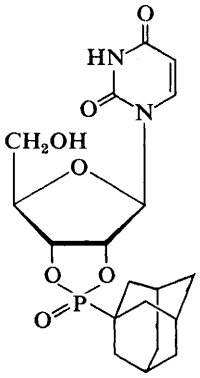
Scheme 140

theses.[678] Adenosine reacted with the cyclic phosphorothioate (420) to give a mixture of adenosine 5′-(*S*-methyl phosphorothioate) and adenosine 2′,3′-cyclic phosphate.[679]

[670] T. Sowa and S. Ouchi, *Bull. Chem. Soc. Japan*, 1975, **48**, 2084.
[671] Y. Taguchi and Y. Mushika, *Tetrahedron Letters*, 1975, 1913.
[671a] Y. Taguchi and Y. Mushika, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1586.
[672] N. I. Sokolova, V. V. Nosova, I. S. Vel'moga, and Z. A. Shabarova, *Zhur. obshchei Khim.*, 1975, **45**, 1197 (*Chem. Abs.*, 1975, **83**, 97 790g).
[673] M. Rubinstein and A. Patchornik, *Tetrahedron*, 1975, **31**, 2107.
[674] M. Rubinstein, B. Amit, and A. Patchornik, *Tetrahedron Letters*, 1975, 1445.
[675] J. H. van Boom, P. M. J. Burgers, R. Crea, W. C. M. M. Luyten, A. B. J. Vink, and C. B. Reese, *Tetrahedron*, 1975, **31**, 2953.
[676] J. H. van Boom, R. Crea, W. C. M. M. Luyten, and A. B. J. Vink, *Tetrahedron Letters*, 1975, 2779.
[677] T. Hata and M. Sekine, *J. Amer. Chem. Soc.*, 1974, **96**, 7363.
[678] M. Sekine and T. Hata, *Tetrahedron Letters*, 1975, 1711.
[679] M. Eto, M. Iio, Y. Kobayashi, H. Omura, and M. Eto, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2081.

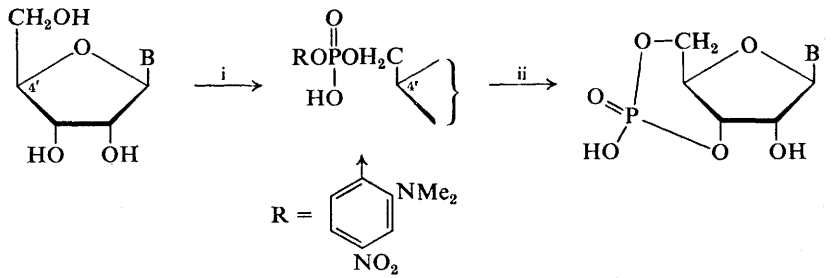


(420)



(421)

Diethyl pyrocarbonate has been used to convert uridine 2′- or 3′-phosphate into the 2′,3′-cyclic phosphate in high yield,[680] and an adamantyl 2′,3′-cyclic phosphonate (421) was obtained when uridine reacted with 1-adamantylphosphonyl chloride.[681] Phosphorylation of ribonucleosides with pyrophosphoryl chloride followed by hydrolysis in neutral solution afforded a simple synthesis of ribonucleoside 2′,3′-cyclic phosphate 5′-phosphates.[682] Two phosphorylating agents mentioned earlier in this section can also yield 3′,5′-cyclic phosphates: either by treatment of nucleoside 5′-(2-*NN*-dimethylamino-4-nitrophenyl phosphates) with acetic acid in pyridine (Scheme 141)[671, 683] or by treatment of nucleoside 5′-(*S*-methyl phosphorothioates) with iodine in pyridine.[679] Another route to 3′,5′-cyclic phosphates involved cyclization of the nucleoside 5′-trichloromethylphosphonates (422) (obtained by the action of trichloromethylphosphonyl

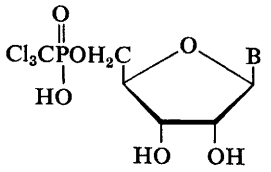


B = cytosin-1-yl, adenin-9-yl, guanin-9-yl, *etc.*

Reagents: i, $ROPO_3H_2$–DCC–DMF; ii, AcOH–py (high dilution)

Scheme 141

[680] F. Solymosy, L. Ehrenberg, and I. Fedorcsak, *Nucleic Acids Res.*, 1975, **2**, 985.
[681] M. N. Preobrazhenskaya, S. Ya. Mel'nik, D. M. Oleinik, E. S. Shepeleva, K. F. Turchin, and P. I. Sanin, *Bioorg. Khim.*, 1975, **1**, 277 (*Chem. Abs.*, 1975, **83**, 97 804q).
[682] A. Simoncsits and J. Tomasz, *Biochim. Biophys. Acta*, 1975, **395**, 74.
[683] Y. Taguchi and Y. Mushika, *Bull. Chem. Soc. Japan*, 1975, **48**, 1528.



(422)

dichloride and triethyl phosphate on the appropriate nucleoside) with potassium t-butoxide in DMSO.[684] Water-soluble carbodi-imides have been used to cyclize 3′-amino-3′-deoxyadenosine 5′-phosphate or its 3′-*N*-benzyl derivative in aqueous solution at pH 7.5 with the formation of a P—N bond.[685] A number of 2-substituted adenosine 3′,5′-cyclic phosphates have been prepared by the condensation of an appropriate aldehyde with 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 3′,5′-cyclic phosphate.[686] Diazoalkanes reacted with uridine 3′,5′-cyclic phosphate to give the corresponding triesters.[687]

Other Derivatives

Alkylation of 1-β-D-arabinofuranosylcytosine using either diethyl or dimethyl sulphate under basic conditions afforded all the possible *O′*-ethyl and *O′*-methyl ethers, which, after separation on an anion-exchange resin, were identified by ^{1}H n.m.r. spectroscopy; deamination of these alkylated derivatives gave the corresponding uracil nucleosides.[688] The distribution of products on mono-methylation of the *cis*-glycol system of adenosine with diazomethane can be influenced by inorganic catalysts (*e.g.* stannous chloride, stannic chloride, ferric chloride); for example, the ratio of 2′-*O*-methyl- to 3′-*O*-methyl-adenosine was 4 : 1 using cerium(III) chloride heptahydrate, whereas it was 1 : 3 using titanium-(IV) chloride.[689] In addition to ethylation, it was noted that methylation occurred when adenosine reacted with diazoethane in dimethoxyethane, the methyl group clearly originating from the solvent.[155] 3-Methyl-2-pyridyldiazomethane 1-oxide (45) has been recommended for the monoalkylation of the *cis*-glycol system of ribonucleosides since it reacts to give isomeric 2′- and 3′-*O*-(3-methyl-2-picolyl 1-oxide) derivatives, which are stable towards phosphorylation and which can be cleaved with methanolic ammonia after treatment with acetic anhydride.[161]

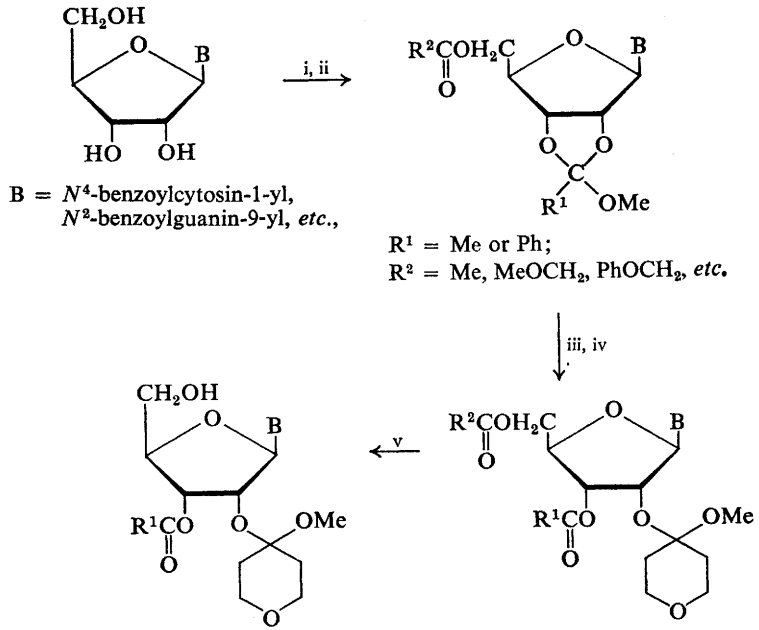
The 2′-*O*-(1-methoxyethyl) ethers of nucleoside 5′-phosphates have been prepared either by phosphorylation of the corresponding nucleoside 2′-ether or by methoxyethylation of the nucleoside 5′-phosphates.[690] Mixtures of the photo-labile 2′- and 3′-*O*-(2-nitrobenzyl)-ribonucleosides were obtained in good yields when adenosine, inosine, cytidine, and uridine were treated with 2-nitrophenyl-

[684] R. Marumoto, T. Nishimura, and M. Honjo, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2295.
[685] M. Morr, M.-R. Kula, and L. Ernst, *Tetrahedron*, 1975, **31**, 1619.
[686] R. B. Meyer, jun., H. Uno, R. K. Robins, L. N. Simon, and J. P. Miller, *Biochemistry*, 1975, **14**, 3315.
[687] J. Engels and W. Pfleiderer, *Tetrahedron Letters*, 1975, 1661.
[688] E. Darzynkiewicz and D. Shugar, *Acta Biochim. Polon.*, 1974, **21**, 305 (*Chem. Abs.*, 1975, **82**, 58 042z).
[689] M. J. Robins, A. S. K. Lee, and F. A. Norris, *Carbohydrate Res.*, 1975, **41**, 304.
[690] G. N. Bennett and P. T. Gilham, *Biochemistry*, 1975, **14**, 3152.

diazomethane in the presence of stannous chloride; the 2′-*O*-(2-nitrobenzyl)-ribonucleosides (separated by fractional crystallization or chromatography) underwent smooth cleavage to the parent nucleoside on photolysis in methanol–DMF.[691] Several 5′-*O*-carboxymethyl ethers of cytidine, 1-β-D-arabinofuranosylcytosine, and 2′-deoxyuridine have been prepared with a view to their attachment to proteins to form immunosuppressants.[692, 693]

The reaction of some 7-hexopyranosyltheophylline nucleosides with benzaldehyde in the presence of zinc chloride gave the expected benzylidene acetals with the α-D-mannopyranosyl (2′,3′:4′,6′-diacetal), β-D-galactopyranosyl (4′,6′-acetal), and β-D-glucopyranosyl (4′,6′-acetal) derivatives.[694]

A study has been made of the selective acylation of cytidine and 2′-deoxy-



Reagents: i, $R^1C(OMe)_3$–H^+; ii, R^2COCl or $(R^2CO)_2O$–py; iii, H_3O^+; iv, 5,6-dihydro-4-methoxy-2*H*-pyran–H^+; v, NH_3

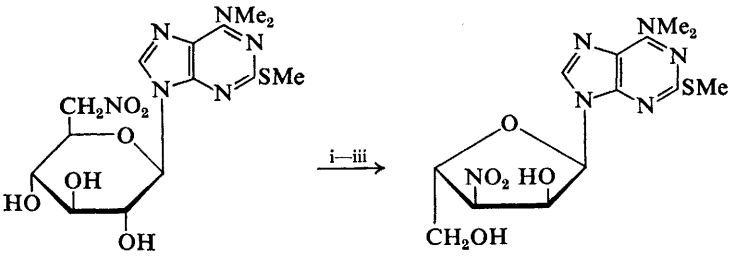
Scheme 142

cytidine derivatives.[695] A general procedure devised for the synthesis of 3′-*O*-acyl-2′-*O*-(methoxytetrahydropyranyl)-ribonucleosides (Scheme 142) is based on the rates of deacylation of a number of 5′-esters in aqueous or methanolic ammonia.[696] Purine ribonucleosides reacted with fuming nitric acid in the

[691] D. G. Bartholomew and A. D. Broom, *J.C.S. Chem. Comm.*, 1975, 38.
[692] A. Holý and H. Pischel, *Coll. Czech. Chem. Comm.*, 1974, **39**, 3863.
[693] H. Pischel, A. Holý, G. Wagner, and D. Cech, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2689.
[694] A. J. Freestone, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1975, **45**, 3.
[695] R. C. Bleaney, A. S. Jones, and R. T. Walker, *Tetrahedron*, 1975, **31**, 2423.
[696] C. B. Reese, J. C. M. Stewart, J. H. van Boom, H. P. M. de Leeuw, J. Nagel, and J. F. M. De Rooy, *J.C.S. Perkin I*, 1975, 934.

presence of urea and acetic anhydride to give nitrate esters (*e.g.* adenosine 5′-nitrate was obtained in 45% yield); these esters are used as coronary dilators.[697]

Treatment of nucleosides with a mixture of lithium azide, carbon tetrabromide, and triphenylphosphine in DMF at room temperature gave the 5′-azido-5′-deoxy-nucleosides in high yields.[698] Standard reactions were used to transform the (6-nitro-β-D-glucopyranosyl)-nucleoside (423) into the (3-nitro-α-L-ribofurano-syl)-nucleoside (424) (Scheme 143).[699]



(423) (424)

Reagents: i, $NaIO_4$; ii, MeONa–MeOH; iii, $NaBH_4$

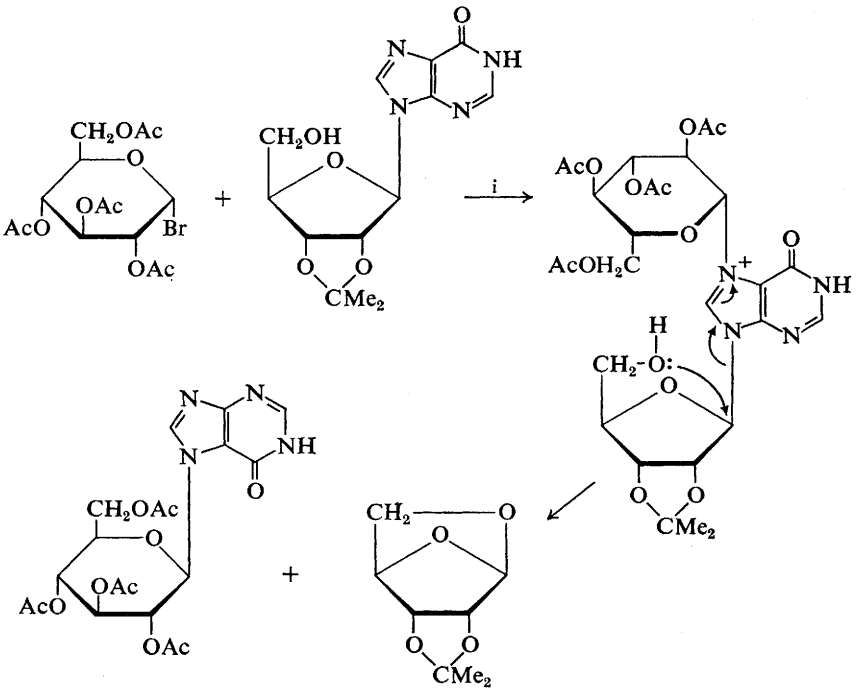
Scheme 143

Reactions

A novel transfer of a purin-9-yl residue occurred when 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide reacted with 2′,3′-*O*-isopropylideneinosine, yielding 7- and 9-β-D-glucopyranosylhypoxanthines and 1,5-anhydro-2,3-*O*-isopropylidene-β-D-ribofuranose (Scheme 144); the anhydro-sugar was not formed in the absence of the glycosyl halide.[700] A diglycosylhypoxanthine intermediate was invoked to explain this and analogous reactions of other ribonucleosides. The dialdehyde formed by oxidation of adenosine with periodate reacted with dimedone to give the structure (425).[701]

Treatment of 2′-deoxyadenosine with *N*-hydroxymethylglycine (prepared from formaldehyde and glycine) resulted in scission of the *N*-glycosyl bond, and it was proposed that the mutagenic and lethal effects of formaldehyde in cells may be due to this reaction.[702] 1-(2-Deoxy-β-D-*erythro*-pentofuranosyl)pyridone is much more labile than naturally occurring nucleosides, and its incorporation into dinucleotides has been investigated.[703] Adenosine and related compounds reacted with hydroxylamine *O*-esters to give the corresponding 1-amino derivatives.[704] Several 8-alkylated and 8-acylated derivatives of guanosine and inosine 3′,5′-cyclic phosphates have been obtained by the reaction of the parent nucleotides

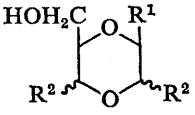
[697] R. Duschinsky, H. Walker, W. Wojnarowski, W. Arnold, G. Englert, and B. Pellmont, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 411.
[698] T. Hata, I. Yamamoto, and M. Sekine, *Chem. Letters*, 1975, 977.
[699] T. Lin and R. E. Harmon, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 357.
[700] F. W. Lichtenthaler and K. Kitahara, *Angew. Chem. Internat. Edn.*, 1975, **14**, 815.
[701] F. Hansske and F. Cramer, *Carbohydrate Res.*, 1975, **41**, 366.
[702] Yu. A. Semin, E. N. Kolomyitseva, and A. M. Poverennyi, *Bioorg. Khim.*, 1975, **1**, 317 (*Chem. Abs.*, 1975, **83**, 147 681v).
[703] I. Gregor, U. Séquin, and C. Tamm, *Helv. Chim. Acta*, 1975, **58**, 712.
[704] G.-F. Huang, M. Maeda, T. Okamoto, and Y. Kawazoe, *Tetrahedron*, 1975, **31**, 1363.



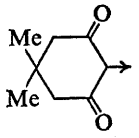
Reagent: i, $Hg(CN)_2$–$MeNO_2$

Scheme 144

with free radicals generated *in situ* either from hydroperoxides or by hydrogen-abstraction from the carbonyl group of aldehydes.[705] Platinum has been shown to catalyse hydrogen–deuterium exchange in nucleoside bases.[705a]



(425) R^1 = adenin-9-yl; R^2 =



Physical Measurements

^1H N.m.r. and Raman difference spectroscopy have shown that methylmercury(II) ions bind quantitatively to inosine at pH 8 by displacement of the proton at N-1.[706] The binding of Mn^{2+} ions to inosine 5′-triphosphate has been studied by

[705] L. F. Christensen, R. B. Meyer, jun., J. P. Miller, L. N. Simon, and R. K. Robins, *Biochemistry*, 1975, **14**, 1490.

[705a] M. Maeda and Y. Kawazoe, *Tetrahedron Letters*, 1975, 1643.

[706] S. Mansy and R. S. Tobias, *Biochemistry*, 1975, **14**, 2952.

^{1}H, ^{13}C, and ^{31}P n.m.r. spectroscopy; it was concluded that the complex in solution involves the interaction of a Mn^{2+} ion with one molecule of inosine 5′-triphosphate at one of two sites (N-7 or O-6).[707] The ^{1}H n.m.r. spectra of several ribonucleoside 3′,5′-cyclic phosphates have been recorded.[708] A pattern-recognition technique for determining the anomeric configuration of ribonucleosides has been developed; it uses the chemical shifts of H-1′, H-2′, and H-3′ and the corresponding ^{3}J coupling constants obtained from computer-simulated ^{1}H n.m.r. spectra of α- and β-D-ribofuranosides.[709] Although the α-configuration was inferred for 1-(2,3,5-tri-*O*-acetyl-D-ribofuranosyl)benzotriazole using a published ^{1}H n.m.r. spectroscopic method (J. A. Montgomery, *Carbohydrate Res.*, 1974, **33**, 184), the β-configuration was indicated on the basis of differences in the chemical shifts of the methyl signals of the corresponding 2′,3′-*O*-isopropylidene derivative.[710]

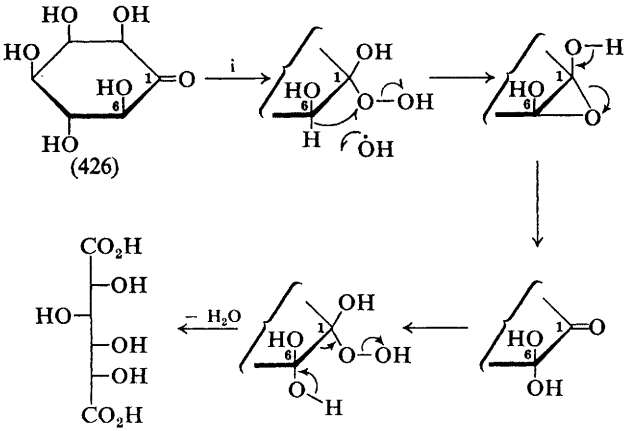
Extensive reference to the use of n.m.r. spectroscopy in studies of the conformations of nucleosides is made in Chapter 23, and the application of mass spectrometry and of polarimetry to nucleosides is referred to in Chapters 24 and 25, respectively.

[707] G. P. P. Kuntz and G. Kotowycz, *Biochemistry*, 1975, **14**, 4144.
[708] M. Kainosho and K. Ajisaka, *J. Amer. Chem. Soc.*, 1975, **97**, 6839.
[709] J. Zupan, J. Kobe, and D. Hadzi, *Analyt. Chem.*, 1975, **47**, 1702.
[710] J. L. Imbach and B. L. Kam, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 271.

22
Oxidation and Reduction

Oxidation

The oxidation of an aldose to the next lower aldose with ammonium vanadate in sulphuric acid or perchloric acid [26] has been mentioned in Chapter 2. Isbell and his co-workers have extended their studies of peroxide-cleavage reactions to include such carbocyclic compounds as 2,3,4,6/5-pentahydroxycyclohexanone (426) and *xylo*-trihydroxycyclohexane-1,2,3-trione, which gave DL-glucaric acid (see Scheme 145) and xylaric acid, respectively, on oxidation.[711] Mechanisms



Reagent: i, Na_2O_2

Scheme 145

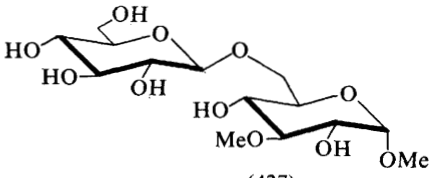
similar to those proposed earlier (see Vol. 8, p. 161) were invoked to account for the products obtained. Variations in the rates of degradation of reducing sugars and related compounds by alkaline hydroperoxide were attributed to the catalytic effects of ferrous or ferric salts, which increase the free-radical cleavage of the hydroperoxide adducts of reducing sugars at the expense of ionic cleavage.[712] The catalytic effects of iron salts can be counteracted by the addition of magnesium salts, due to the formation of a magnesium–iron complex.

The oxidation of alditols and methyl glycopyranosides, *etc.* with sodium metaperiodate in deuterium oxide has been followed by ^1H n.m.r. spectroscopy;

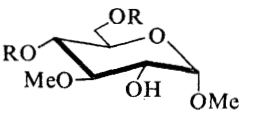
[711] H. S. Isbell, H. L. Frush, and Z. Orhanovic, *Carbohydrate Res.*, 1975, **43**, 93.
[712] H. S. Isbell, E. W. Parks, and R. G. Naves, *Carbohydrate Res.*, 1975, **45**, 197.

free aldehydo-groups were not present, but various hemiacetal and acetal forms were detected.[713] Procedures for determining the uptake of periodate, *etc.* in the oxidation of carbohydrates are mentioned in Chapter 26.

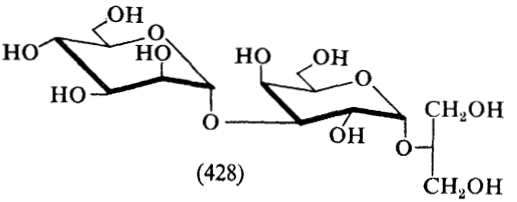
Acetal migration can lead to the formation of cyclic acetals that are resistant to mild hydrolysis with acid in the Smith degradation of polysaccharides. Lindberg and his co-workers have shown that the model disaccharides (427) and (428) are degraded to the expected products (429) and (430), respectively, without the formation of significant amounts of the cyclic acetals (431) and (432).[714]



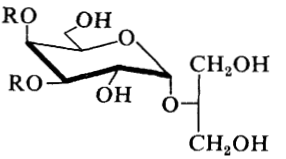
(427)



(429) R = H
(431) R, R = $CHCH_2OH$

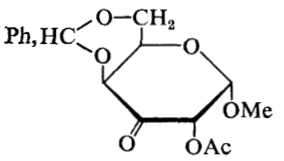


(428)

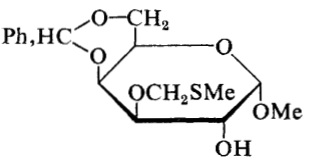


(430) R = H
(432) R, R = $CHCH_2OH$

Oxidation of 4,6:4′,6′-di-*O*-benzylidene-2,2′-di-*O*-toluene-*p*-sulphonyl-αα-trehalose with DMSO–phosphorus pentaoxide gave the symmetrical 3,3′-diulose, whereas the 3-ulose-3′-acetate was obtained when DMSO–acetic anhydride was used.[715] DMSO–acetic anhydride also converted methyl 4,6-*O*-benzylidene-α-D-galactopyranoside into a mixture of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-*xylo*-hexopyranosid-3-ulose (433) and the 3-*O*-(methylthio)methyl ether (434).[716]



(433)



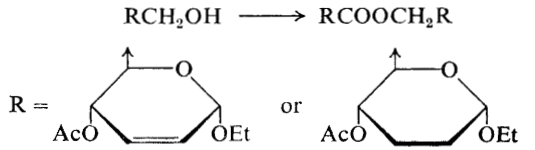
(434)

DMSO is also used in an improved two-stage procedure for the oxidation of primary and secondary alcohols to carbonyl compounds; the alcohol is first converted into the chloroformate, which affords the carbonyl compound on

[713] S. Hirano, T. Fukuda, and M. Sato, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 2539.
[714] B. Erbing, B. Lindberg, and S. Svensson, *Acta Chem. Scand.* (*B*), 1974, **28**, 1180.
[715] C.-K. Lee, *Carbohydrate Res.*, 1975, **42**, 354.
[716] J. Defaye and A. Gadelle, *Carbohydrate Res.*, 1975, **42**, 373.

treatment with DMSO and 1,2-epoxypropane (a non-basic acid scavenger) followed by triethylamine.[717]

The primary hydroxymethyl group of some carbohydrate derivatives was shown to undergo oxidative dimerization with the chromic oxide–bipyridyl complex, but an intermediate aldehyde does not appear to be formed (Scheme 146).[718] Other oxidizing agents failed to bring about this transformation. The



Reagent: CrO_3. 2py

Scheme 146

oxidation of methyl glycosides to methyl aldonates by ozone is discussed in a review on stereoelectronic control in the cleavage of tetrahedral intermediates.[719]

The platinum-catalysed oxidation of several partially protected ketoses has been examined by Heyns's group [438, 438a] (see Chapter 16).

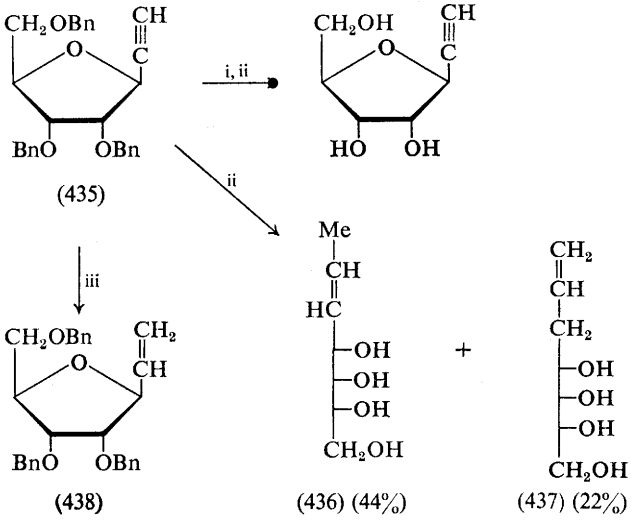
Reduction

Two new methods for the reduction of aldonolactones to aldoses have been developed for use in small-scale syntheses: either the lactone itself was reduced with diborane in THF, or an *O*-tetrahydropyranyl derivative was reduced with a 1 : 1 mixture of lithium aluminium hydride and aluminium chloride in ether.[720] The yield of aldose depends on a number of factors and may be low due to the ease of reduction of the aldose to the alditol. The catalytic hydrogenation of D-glucose in the temperature range 100—170 °C and at pressures of 20—80 atmospheres has been examined;[721] the effects of pH and promoters (*e.g.* magnesium, barium chloride, calcium sulphate) were also examined.[722] The rate of hydrogenation was enhanced at pH 8, and calcium sulphate was the most effective promoter at pH 6.8.

The selective reduction of an ω-iodo-group without affecting an azido-group can be achieved using sodium cyanoborohydride in HMPT; ω-toluene-*p*-sulphonates can also be reduced using a mixture of sodium iodide and sodium cyanoborohydride in the same solvent.[723] For example, reduction of methyl 2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-6-iodo-α-D-altropyranoside by this procedure gave methyl 2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-altropyranoside.

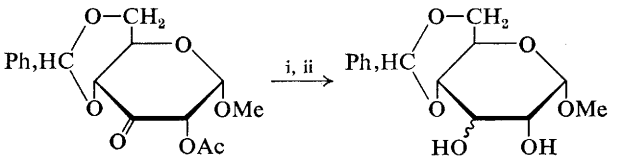
Prior conversion into the anion (with sodamide) enabled the benzyl groups in the D-ribofuranosylethyne derivative (435) to be cleaved, using sodium in liquid ammonia, without reduction of the alkynic group; otherwise the acyclic alkenes

[717] D. H. R. Barton and G. P. Forbes, *J.C.S. Perkin I*, 1975, 1614.
[718] M. B. Yunker and B. Fraser-Reid, *J.C.S. Chem. Comm.*, 1975, 61.
[719] P. Deslongchamps, *Tetrahedron*, 1975, **31**, 2463.
[720] S. S. Bhattacharjee, J. A. Schwarcz, and A. S. Perlin, *Carbohydrate Res.*, 1975, **42**, 259.
[721] M. Abdel-Akher, J. Ghali, M. S. Raouf, and M. Roushdi, *Stärke*, 1974, **26**, 352.
[722] M. Abdel-Akher, M. S. Raouf, J. Ghali, and M. Roushdi, *Stärke*, 1974, **26**, 383.
[723] H. Kuzuhara, K. Sato, and S. Emoto, *Carbohydrate Res.*, 1975, **43**, 293.



Reagents: i, $NaNH_2$; ii, Na–NH_3; iii, H_2–Lindlar catalyst

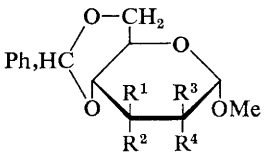
Scheme 147



solvent	*allo*	:	*gluco*
MeOH	100		0
Pr^iOH	50		50

Reagents: i, $NaBH_4$–ROH; ii, MeONa–MeOH

Scheme 148



(440) $R^1 = {}^2H$; $R^2 = R^4 = OAc$; $R^3 = {}^1H$
(441) $R^1 = R^4 = OAc$; $R^2 = {}^1H$; $R^3 = {}^2H$

(436) and (437) were obtained (Scheme 147).[724] Partial reduction to the D-ribo-furanosylethene (438) was accomplished using Lindlar catalyst in the presence of a small amount of quinoline.

The reduction of the glycosidulose derivative (439) with sodium borohydride proved to be particularly sensitive to the solvent used (see Scheme 148); exclusive formation of methyl 4,6-*O*-benzylidene-α-D-allopyranoside could be ensured by the addition of a small proportion of water to a solution of the reductant in isopropyl alcohol.[725] The reduction of (439) with sodium borodeuteride in dry isopropyl alcohol gave the 3- and 2-deuteriated compounds (440) and (441) after acetylation. The 2-deuteriated D-glucopyranoside derivative (441) presumably arises through attack of the reductant at C-2 of an intermediate 2,3-enolic species.

[724] J. G. Buchanan, A. R. Edgar, M. J. Power, and G. C. Williams, *Carbohydrate Res.*, 1975, **45**, 312.

[725] D. C. Baker, C. Boeder, J. Defaye, A. Gadelle, and D. Horton, *Carbohydrate Res.*, 1975, **40**, C6.

23

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

The use of n.m.r. spectroscopy in determining the conformations of pyranoid rings in mono-, di-, and poly-saccharides has been reviewed.[726] In comparing the relative merits of Fourier-transform and continuous-wave ^1H n.m.r. spectroscopy, it has been pointed out that the first technique offers the possibility for increasing both the sensitivity and the resolution, and for the selective removal of signals by taking advantage of differences in the relaxation times of different hydrogen nuclei; experiments with 1,2,3,4-tetra-*O*-acetyl-β-D-ribopyranose and methyl 2,3:4,5-di-*O*-isopropylidene-β-D-glucoseptanoside were used to illustrate these points.[727] The differences between the anomeric effects of chloro- and methoxy-groups have been discussed.[728] Both ^1H and ^{13}C n.m.r. spectroscopy were used to study the conformations of simple vicinal diesters; *gauche*-related oxygen functions seem to be preferred for electrostatic reasons, and this finding is relevant to the detailed conformational analysis of carbohydrate esters.[729]

Vorontsova and Bochkov have criticized the use of the Karplus equation and its modifications (with or without electronegativity corrections) for determining dihedral angles from measurements of vicinal coupling constants. Their criticism is based on the poor correlation obtained with such relatively rigid systems as α-D-xylopyranose 1,2,4-orthoesters[730] and 3-*O*-acetyl-β-L-arabinofuranose 1,2,5-orthobenzoate,[730a, 731] whose bond angles have been accurately determined from *X*-ray crystallographic data. The Russian workers propose the following equation:

$$^3J_{H,H'} = (4.37 + 4.39\cos\psi + 3.32\cos 2\psi) \times (1.28 - 0.04Y)$$

where ψ represents the smaller angle between the first C—H bond and the projection of the second C—H bond on to it and Y is the sum of the chemical shifts of the two hydrogen nuclei on the δ scale. The variation of the geminal coupling constant of the methylene signals of several derivatives of 1,2-*O*-isopropylidenetetrofuranoses bearing various substituents at C-3 has been examined.[732]

[726] V. S. R. Rao, *J. Indian Inst. Sci.*, 1974, **56**, 253.
[727] L. D. Hall, C. M. Preston, and J. D. Stevens, *Carbohydrate Res.*, 1975, **41**, 41.
[728] I. Tvaroška and T. Bleha, *Tetrahedron Letters*, 1975, 249.
[729] C. A. Kingsbury and C. R. Cowles, *J. Org. Chem.*, 1975, **40**, 1302.
[730] L. G. Vorontsova and A. F. Bochkov, *Org. Magn. Resonance*, 1974, **6**, 654.
[730a] L. G. Vorontsova and A. F. Bochkov, *Org. Magn. Resonance*, 1975, **7**, 313.
[731] A. F. Bochkov, V. M. Dashunin, A. S. Shashkov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1061.
[732] J. M. J. Tronchet, F. Barbalat-Rey, and J. Tronchet, *Carbohydrate Res.*, 1975, **41**, 1.

Grindley and Szarek have deduced the conformations of the seven-membered ring in 2,5-*O*-methylene- and 1,3:2,5-di-*O*-methylene-D-mannitol [202] and 1,6-di-*O*-benzyl-2,5-*O*-methylene-D-*arabino*-hex-3-ulose [203] in solution from the geminal and vicinal proton coupling constants.

Unambiguous assignment of the individual acetoxy-group resonances of methyl β-D-glucopyranoside tetra-acetate has been accomplished with the help of the 2-, 3-, 4-, and 6-*O*-trideuterioacetylated triacetates; the acetoxy-group resonances of partially acetylated and other derivatives of methyl β-D-glucopyranoside were similarly assigned.[733] The chemical shifts of H-6 for a number of pyrimidine nucleoside derivatives have been examined and related to a wide range of intramolecular interactions.[734] De Bruyn and Anteunis have warned against the use of H-1 chemical shifts to describe rotameric parameters about C-1—O-1 bonds when different non-reducing moieties are involved, since, as was shown with lactose and cellobiose, erroneous conclusions can be reached.[735] De Bruyn's group has also reported a 300 MHz ^{1}H n.m.r. spectroscopic study of β-D-allose, melibiose, lactose, and '3-ketolactose', which was shown to exist as the hydrated form in aqueous solution.[736]

The ^{29}Si n.m.r. spectra of trimethylsilylated methyl α-D-gluco-, α-D-manno-, α-D-galacto-, and α-L-rhamno-pyranosides have been measured; the trimethylsilyloxy-group at C-6 was identified by single-frequency proton decoupling at δ0.12 (*i.e.* the resonance of the Si—Me groups), when it appeared as a triplet because of residual coupling with H-6 and H-6′.[737] Stopped-flow ^{1}H n.m.r. methods have been used to study conformational changes induced in concanavalin A by Mn^{2+} and Ca^{2+} ions, and methyl α-D-mannopyranoside.[738]

Acyclic Systems

The conformations of several per-*O*-acetylaldononitriles and 5-(polyacetoxyalkyl)tetrazoles in [$^2H_5$]pyridine solution have been determined by ^{1}H n.m.r. spectroscopy; compounds having *arabino*, *galacto*, or *manno* configurations adopt an extended planar zig-zag conformation, whereas those having *xylo*, *ribo*, or *gluco* configurations favour a bent conformation.[739] A bent conformation for D-gluconononitrile penta-acetate was also reported by other workers.[740]

Pyranoid Systems

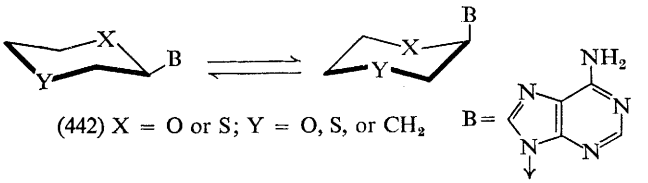
The internal energies of each of the sixteen possible $^4C_1$ conformations of the aldopentopyranoses (*i.e.* the α- and β-anomers of both the D- and L-forms) have been derived from Monte-Carlo calculations that take into account contributions made by van der Waals attractive and repulsive forces, polar interactions, and

[733] D. Horton and J. H. Lauterbach, *Carbohydrate Res.*, 1975, **43**, 9.
[734] J. Žemlička and J. P. Horwitz, *J. Amer. Chem. Soc.*, 1975, **97**, 4089.
[735] A. de Bruyn and M. Anteunis, *Bull. Soc. chim. belges*, 1975, **84**, 497 (*Chem. Abs.*, 1975, **83**, 114 813m).
[736] A. de Bruyn, M. Anteunis, J. van Beeumen, and G. Verhegge, *Bull. Soc. chim. belges*, 1975, **84**, 407 (*Chem. Abs.*, 1975, **83**, 114 772x).
[737] D. J. Gale, A. H. Haines, and R. K. Harris, *Org. Magn. Resonance*, 1975, **7**, 635.
[738] J. J. Grimaldi and B. D. Sykes, *J. Biol. Chem.*, 1975, **250**, 1618.
[739] A. M. Seldes, E. G. Gros, I. M. E. Thiel, and J. O. Deferrari, *Carbohydrate Res.*, 1975, **39**, 11.
[740] L. Maijs, O. K. Lukevits, V. A. Shcherbakov, and L. L. Shcherbakova, *Latv. P.S.R. Zinat. Akad. Vestis, Kim. Ser.*, 1975, 189 (*Chem. Abs.*, 1975, **83**, 97 743n).

bond torsion.[741] The calculated values are in good agreement with experimental values for the relative free energies of one group of sugars, namely α-D-xylo-, α-L-lyxo-, α- and β-D-lyxo-, β-L-arabino-, β-D-ribo-, and β-D-arabino-pyranose, indicating that the anomeric effect is included in the energy terms considered and that it can be attributed mainly to bond dipoles. Differences between the values for the observed and calculated free energies for α-L-arabino- and β-D-xylo-pyranose are considered to result from hydration by 'structured water', owing to the favourable stereochemistry of these sugars for hydration in the $^4C_1$ conformation. The agreement for the remaining sugars, which possess 1,3-interactions between diaxial oxygen atoms, is poor, for two reasons: the calculations are not able to treat high degrees of steric compression and the experimental data are less reliable. The authors conclude that this is a promising approach for calculating the conformational energies of carbohydrates (including polysaccharides) in aqueous solutions, if allowance is made for co-operative hydration of adjacent equatorial hydroxy-groups. The results of calculations of the conformational stabilities of α-D-arabino-, α-D-ribo-, and β-D-xylo-pyranose by the PCILO–CNDO/2 method were compatible with evidence obtained from n.m.r. spectroscopy and *X*-ray diffraction.[742] Further consideration has been given to the relation between the dihedral angle subtended by vicinal *gauche* protons in aldopyranoses and the observed coupling constants, paying particular attention to the electronegativities of the substituents and the orientation of the substituent relative to the coupled protons.[743]

In evaluating the stereochemical outcome of the free-radical addition of sodium hydrogen sulphite to several methyl hex-5-enopyranosides, the agreement between the experimental and calculated values was improved if an allowance of 0.45 kcal mol^{-1} was made for a 1,3-interaction between an axial hydroxy-group and the ring-oxygen atom (*e.g.* in the formation of methyl 6-deoxy-α-D-allo- and -β-L-talo-hexopyranoside 6-sulphonic acids).[361]

Three papers have reported the results of studies with simple tetrahydropyrans. 2-Hydroxytetrahydropyran has been shown to adopt a conformation placing the hydroxy-group in an axial orientation,[744] whereas the amino-group in related 2-aminotetrahydropyrans favours an equatorial orientation.[745] Both 3-chloro- and 3-bromo-tetrahydropyrans show a preference for the conformation in which the halogeno-group is equatorial.[746] The conformational equilibria of the nucleoside analogues (442) have also been studied; the proportion of the

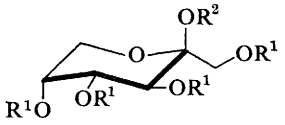


(442) X = O or S; Y = O, S, or CH_2 B=

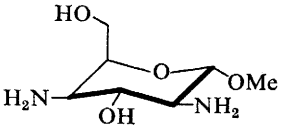
[741] D. A. Rees and P. J. C. Smith, *J.C.S. Perkin II*, 1975, 830.
[742] J. L. Cantara, J. Sygusch, and T. Cyr, *Chem. Phys. Aqueous and Gaseous Solutions, Proc. Symp.*, 1975, 59 (*Chem. Abs.*, 1975, **83**, 97 744v).
[743] T. P. Forrest, *Canad. J. Chem.*, 1974, **52**, 4095.
[744] A. El-Kafrawy and R. Perraud, *Compt. rend.*, 1975, **280**, *C*, 1219.
[745] J. Tesse, C. Glacet, and D. Couturier, *Compt. rend.*, 1975, **280**, *C*, 1525.
[746] C. B. Anderson and M. P. Geis, *Tetrahedron*, 1975, **31**, 1149.

conformer with the adenin-9-yl group located in an axial orientation increased from 5% with (442; X = S, Y = CH_2) to 90% with (442; X = S, Y = O).[747]

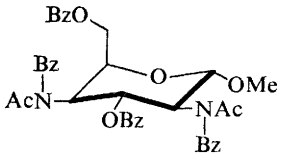
The ^{13}C (25.16 MHz) and ^1H n.m.r. (220, 300 MHz) spectra of permethylated gluco-, galacto-, and manno-pyranoses, and their 6-deoxy analogues, have been analysed with the aid of specific trideuteriomethylation, hetereonuclear spin-decoupling, and spectrum simulation.[748] Comparison of the spectral data showed that the ring conformation is not affected by the change from a methyl group to a methoxymethyl group; all compounds are present in the $^4C_1$(D) or $^1C_4$(L) conformation. Changes in orientation of the methoxy-groups had distinct effects on the chemical shifts of carbons and protons of the pyranose ring and of the methoxy-groups. The ^1H (300 MHz) n.m.r. spectra of D-gluco-, D-galacto-, and D-manno-pyranoses and their methyl glycosides yielded vicinal and geminal coupling constants corresponding to those reported for the peracetylated derivatives; the empirical rules proposed by Lemieux and Stevens (*Canad. J. Chem.*, 1966, **44**, 249) for calculating the chemical shifts of protons in aldohexopyranoses were refined and extended for the methyl glycosides.[749] Benzyl β-D-xylopyranoside and the corresponding 3-deoxy-3-fluoro derivative exist mainly in the $^4C_1$ conformation, whereas a considerable proportion (45%) of the $^1C_4$ form is present in benzyl 3-deoxy-β-D-*erythro*-pentopyranoside.[750] The 300 MHz ^1H n.m.r. spectrum of the β-pyranose form of D-fructose has been completely assigned.[751] A related study of the β-D-fructopyranose derivatives (443) showed that the $^2C_5$ conformation is favoured in solution.[752] The preferred conformations



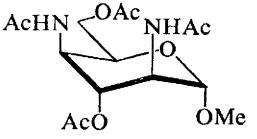
(443) R^1 = H; R^2 = Me
R^1 = Ac; R^2 = H, Ac, or Me



(444)



(445)

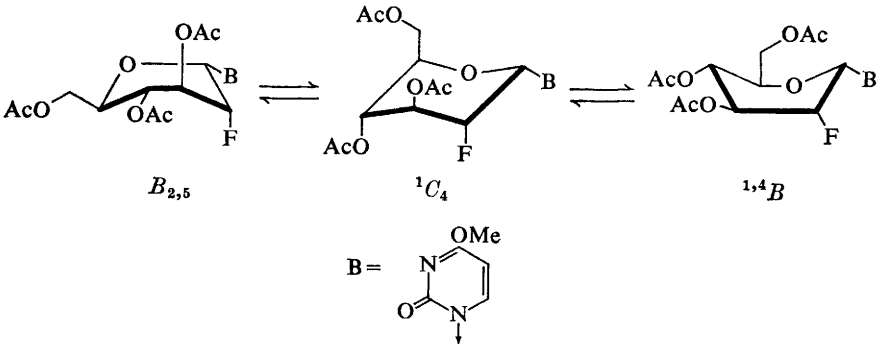


(446)

of the methyl 2,4-diamino-2,4-dideoxy-α-D-idopyranosides (444), (445), and (446) depend upon the extent of acylation of the amino-groups; thus, the $^1C_4$ conformation is preferred by the 2,4-diaminoglycoside (444) (76%) and its protonated

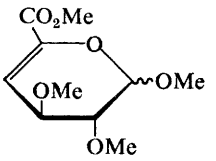
[747] W. A. Szarek, D. M. Vyas, and B. Achmatowicz, *Tetrahedron Letters*, 1975, 1553.
[748] J. Haverkamp, M. J. A. de Bie, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1975, **39**, 201.
[749] A. de Bruyn, M. Anteunis, and G. Verhegge, *Acta Cienc. Indica*, 1974, **1**, 83 (*Chem. Abs.*, 1975, **83**, 97 829b).
[750] A. de Bruyn, M. Anteunis, G. Aerts, and E. Saman, *Carbohydrate Res.*, 1975, **41**, 290.
[751] A. de Bruyn, M. Anteunis, and G. Verhegge, *Carbohydrate Res.*, 1975, **41**, 295.
[752] A. de Bruyn, M. Anteunis, and G. Verhegge, *Bull. Soc. chim. belges*, 1974, **83**, 476 (*Chem. Abs.*, 1975, **82**, 125 531y).

form (~ 84%) in deuterium oxide, and also by the 2,4-bis(di-*N*-acylamino)-glycoside (445) (93%) in [$^2H_4$]methanol or [^2H]chloroform, whereas the 2,4-diacetamidoglycoside (446) favours the $^4C_1$ conformation in proportions ranging from 92% in [^2H]chloroform to 56% in deuterium oxide.[753] An anomalous conformation has been observed for 4-methoxy-1-(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl)-2-(1*H*)-pyrimidone; although the corresponding β-anomer has the expected $^4C_1$ conformation, the α-anomer assumes a distorted conformation, with the range reflected by the equilibrium $B_{2,5} \rightleftharpoons {}^1C_4 \rightleftharpoons {}^{1,4}B$, the base retaining an equatorial or equatorial-like orientation (see Scheme 149).[754]



Scheme 149

^{1}H N.m.r. spectroscopy at 220 MHz has shown that the $^4C_1$ conformation is adopted by fully benzylated α- and β-D-glucopyranoses; inversion of the configuration at C-5 to give the corresponding L-idopyranose derivatives results in an inversion of conformation also.[755] Two groups have shown that derivatives of methyl or benzyl (methyl 4-deoxy-L-*threo*-hex-4-enopyranosid)uronate [*e.g.* (447)] adopt half-chair conformations that place the anomeric methoxy-group in



(447)

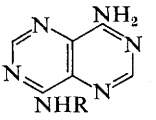
an axial orientation (*i.e.* α-anomer $^2H_1$; β-anomer $^1H_2$); however, the corresponding 1,2-*O*-isopropylidene derivatives adopt a half-boat conformation.[755, 756] ^{1}H N.m.r. spectroscopic studies on some per-*O*-acetyl-β-D-glucopyranosylamines have been reported.[757]

[753] H. Paulsen and H. Koebernick, *Carbohydrate Res.*, 1975, **45**, 205.
[754] J. Kiburis, A. B. Foster, and J. H. Westwood, *J.C.S. Chem. Comm.*, 1975, 44.
[755] J. Kiss and W. Arnold, *Helv. Chim. Acta*, 1975, **58**, 297.
[756] J. Alföldi, R. Palovčík, C. Peciar, J. Hirsch, and P. Kováč, *Carbohydrate Res.*, 1975, **44**, 133.
[757] S. H. Doss, S. Kondo, K. Tanaka, and Y. Tsuzuki, *Rev. Roumaine Chim.*, 1974, **19**, 1767 (*Chem. Abs.*, 1975, **83**, 28 518n).

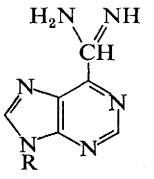
Furanoid Systems

220 MHz N.m.r. spectroscopy has shown that the conformation of the furanose ring of ribonucleoside 2′- and 3′-phosphates can be represented by the equilibrium $^3T_2 \rightleftharpoons {}^2T_3$; the relative proportions of these conformations are 1 : 1 for pyrimidine nucleosides and 3 : 2 for purine nucleotides.[758] Hence, the type of base present exerted more influence than the location of the phosphate group on the conformation adopted by the sugar ring. The conformations about the C-4′—C-5′ and C-2′(3′)—O-2′(3′) bonds were also studied. Analogous studies with adenosine, guanosine, and inosine indicated a slight preference for the $^2T_3$ conformation of the furanose ring.[759] A dynamic correlation was noted between the conformation of the D-ribofuranosyl ring and the glycosyl torsion angle of xanthosine in liquid ammonia. The $^3T_2$ conformation was present to the extent of 40% at room temperature, and the results of nuclear Overhauser and spin–lattice relaxation studies indicated that the $^3T_2$ and $^2T_3$ conformations correlate with *anti* and *syn* conformations, respectively, of the base.[760] Variable-temperature studies on nucleosides and their isopropylidene derivatives in liquid [$^3H_3$]ammonia were also reported.[761]

The D-ribofuranosyl ring in the glycosylamine (448) [previously thought to have the structure (449)] adopts the unusual ^0E conformation in the solid state.[762]



(448) R = β-D-ribofuranosyl



(449) R = β-D-ribofuranosyl

^{1}H N.m.r. spectroscopy has been used to investigate the conformations of 1- and 3-deaza-adenosines[763] and 6-substituted 2′-deoxyuridines.[764] Although the cyclonucleosides 8,2′-thioanhydroguanosine and 2,2′-anhydro-uridine, -3-aza-uridine, and -cytidine are quite rigid structures, the conformations in solution differ significantly from those determined in single crystals by *X*-ray diffraction: the D-ribofuranosyl ring of these compounds has the ^4E conformation in the crystal, whereas the E_1 conformation is adopted in DMSO and in aqueous solution.[765]

Semi-empirical potential-energy calculations have been carried out on purine and pyrimidine ribonucleoside 2′,3′-cyclic phosphates and the 5′-phosphates.[766]

[758] D. B. Davies and S. S. Danyluk, *Biochemistry*, 1975, **14**, 543.
[759] H. D. Luedemann, O. Roeder, E. Westhof, E. von Goldammer, and A. Mueller, *Biophys. Struct. Mech.*, 1975, **1**, 121 (*Chem. Abs.*, 1975, **83**, 179 469y).
[760] H. D. Luedemann, E. Westhof, and O. Roeder, *European J. Biochem.*, 1974, **49**, 143.
[761] E. Westhof, O. Roeder, I. Croneiss, and H. D. Luedemann, *Z. Naturforsch.*, 1975, **30c**, 131.
[762] P. Narayanan and H. M. Berman, *Carbohydrate Res.*, 1975, **44**, 169.
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[764] J. Cadet, R. Ducolomb, and C. Taieb, *Tetrahedron Letters*, 1975, 3455.
[765] W. Guschlbauer, Tran Dinh Son, M. Blandin, and J. C. Catlin, *Nucleic Acids Res.*, 1974, **1**, 855.
[766] N. Yathindra and M. Sundaralingam, *Biopolymers*, 1974, **13**, 2061.

The results indicated that the conformation of the furanose ring is related to that about the *N*-glycosidic bond: thus, *syn* and *anti* conformations about the *N*-glycosidic bond are preferred for ^0E and E_0 conformations of the furanose ring, respectively, irrespective of the type of base, whereas an unpuckered sugar ring favours the *syn* conformation for purine nucleosides and the *anti* conformation for pyrimidine nucleosides. The conformation about the C-4′—C-5′ bond also appears to depend on the conformation of the sugar ring: the ^0E conformation favouring the *gauche-trans* arrangement and the E_0 conformation favouring the *trans-gauche* arrangement. Semi-empirical calculations were also performed on 2′-*O*-methyladenosine and its 5′-mono- and 3′,5′-di-phosphates to assess the influence of the 2′-methoxy-group on the conformation adopted.[767] Calculations for the $^3T_2$ and $^2T_3$ conformations of the D-ribofuranosyl ring, both of which are observed in crystalline 2′-*O*-methyladenosine, indicated that the *anti* conformation is favoured in both cases, and that the C-4′—C-5′ bond shows a slight preference for the *gauche-gauche* arrangement with the $^3T_2$ conformation and for the *gauche-trans* arrangement with the $^2T_3$ conformation. Methylation at O-2′ had little effect on the conformations of the nucleoside and its 5′-phosphate, whereas it was more pronounced on that of the 3′,5′-diphosphate. The experimental results from ^{1}H and ^{31}P n.m.r. spectroscopy in conjunction with calculations on 8-bromo- and 8-methyl-thioadenosine 5′-phosphates revealed that the 8-substituents influence the conformations both about the C-1′—N-9 and C-4′—C-5′ bonds; these conformations were also related to the conformation of the sugar ring.[768] The methylene-proton (H-5′ and H-5″) resonances of 2′,3′-*O*-isopropylidene-N^3-methyluridine have been assigned by comparison with the δ values of the corresponding 2′- and 3′-phosphates, and by direct observation of the hydrogen-bonding between the 5′-hydroxy- and 2-carbonyl groups.[769] The relative populations of the C-4′—C-5′ rotamers were established as *gauche-gauche* > *gauche-trans* > *trans-gauche*, and are compatible with crystal-structure data. A number of derivatives of β-D-arabinofuranosyl-nucleosides have been examined by ^{1}H n.m.r. spectroscopy in neutral and in alkaline solutions.[769a] An appreciable change in the chemical shift of H-2′ signified that the 2′-hydroxy-group ionized at high pH values; 1-(3-*O*-methyl-β-D-arabinofuranosyl)uracil showed changes in its vicinal coupling constants compatible with a change to the $^2T_1$ conformation and to a predominantly *gauche-gauche* conformation about the C-4′—C-5′ bond. These observations are consistent with the formation of an intramolecular hydrogen-bond between O-2′ and O-5′, which is prevented by substitution at either of these positions. The conformation about the *N*-glycosidic bond remained *anti* both in neutral and alkaline solutions. Line-shape decomposition techniques have been used to determine the small $^5J_{5',1'}$ and $^4J_{6,1'}$ coupling constants in a ^{1}H n.m.r. spectroscopic investigation of the conformations about the *N*-glycosidic bond in uracil nucleosides and nucleotides.[770] The favoured *anti*

[767] P. Prusiner, N. Yathindra, and M. Sundaralingam, *Biochim. Biophys. Acta*, 1974, **366**, 115.
[768] R. H. Sarma, C.-H. Lee, F. E. Evans, N. Yathindra, and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1974, **96**, 7337.
[769] D. B. Davies and A. Rabczenko, *J.C.S. Perkin II*, 1975, 1703.
[769a] E. Darzynkiewicz, M. Remin, A. Dworak, and D. Shugar, *Cancer Biochem. Biophys.*, 1975, **1**, 85 (*Chem. Abs.*, 1975, **83**, 114 801f).
[770] T. Schleich, T. R. Lusebrink, B. P. Cross, and N. P. Johnson, *Nucleic Acids Res.*, 1975, **2**, 459.

conformation was unaffected by phosphorylation of the sugar residue, or changes in temperature, or transformation of the sugar residue into 2′-deoxy or D-arabinosyl analogues. The *syn* and *anti* conformations of nicotinamide mononucleotides were shown to be rapidly interconverting by means of the nuclear Overhauser effect.[771]

Di-, Oligo-, and Poly-saccharides

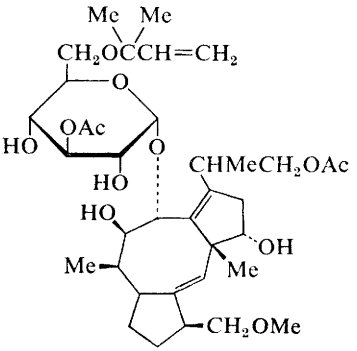
Monte-Carlo calculations previously carried out on monosaccharides have been applied to disaccharides, with inter-residue hydrogen-bonding also being taken into account.[772] Better agreement was obtained between the experimental and predicted free energies for maltose than by previous approaches, and the existence of seven crystal conformations and two solution conformations could be explained. The agreement for cellobiose is at least as good as that obtained by other approaches. One disadvantage of this approach is the extensive computation required. A theoretical study of the charge distributions and torsional potentials of six (1 → 4)-linked disaccharide units occurring in glycosaminoglycans has been undertaken with a view to simplifying the conformational analysis of these polysaccharides.[773]

High-resolution ^{1}H n.m.r. spectroscopy of D-glucobioses in deuterium oxide has shown that protons on, or vicinal to, the carbon atom involved in the glycosidic linkage are shifted downfield.[774] De Bruyn's group has also assigned the 300 MHz ^{1}H n.m.r. spectra of raffinose and melezitose (in D_2O)[775] and sucrose octa-acetate[776] (in [$^2H_6$]benzene and [^{2}H]chloroform, *etc.*) with the help of homo-INDOR experiments.

^{13}C N.M.R. Spectroscopy

Specifically deuteriated samples have been used in assigning the ^{13}C resonances in the ^{13}C n.m.r. spectra of D-glucose, D-xylose, 6-deoxy-D-glucose, D-mannose, L-rhamnose, D-galactose, L-arabinose, L-fucose, and their glycopyranosides; several related furanosides and alduronic acids were also examined.[777] Natural-abundance ^{13}C spectra of the anomeric forms of mycarose, mycaminose, chalcose, mycinose, and forosamine were used in assigning the ^{13}C resonances of the macrolide antibiotics leucomycin A_3 and A_5, spiramycin III, magnamycin A and B, chalcomycin, neutramycin, tylosin, and cirramycin A_1.[778] Lanthanide shift reagents and T_1 values assisted in the assignment of the ^{13}C resonances of the phytotoxic D-glucopyranoside fusicoccin (450) and some of its co-metabolites.[779] The structure proposed for the nucleoside antibiotic hikizimycin (see Chapter 20)

[771] W. Egan, S. Forsén, and J. Jacobus, *Biochemistry*, 1975, **14**, 735.
[772] D. A. Rees and P. J. C. Smith, *J.C.S. Perkin II*, 1975, 836.
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[774] A. de Bruyn, M. Anteunis, and G. Verhegge, *Bull. Soc. chim. belges*, 1975, **84**, 721 (*Chem. Abs.*, 1975, **83**, 193 603u).
[775] M. Anteunis, A. de Bruyn, and G. Verhegge, *Carbohydrate Res.*, 1975, **44**, 101.
[776] A. de Bruyn, M. Anteunis, and G. Verhegge, *Carbohydrate Res.*, 1975, **42**, 157.
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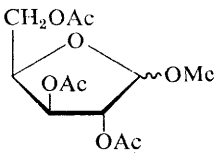
has been supported by comparison of its ^{13}C n.m.r. spectrum with those of 3(4)-amino-3(4)-deoxy-D-glucose and galactitol.[537] ^{13}C N.m.r. spectra have been reported for anomeric glycosides of *N*-acetylneuraminic acid,[780] glycosylamines, compounds formed by Amadori rearrangement, and related D-fructoses.[781] The tautomerism of several purines and of pyrrolo[2,3-*d*]pyrimidines, including nucleosides derived from them, has been examined by ^{13}C n.m.r. techniques.[782]

An examination of the proton-decoupled and coupled ^{13}C n.m.r. spectra of the D-pentopyranoses and some of their derivatives has shown that $^1J_{^{13}C\text{-}1,^1H\text{-}1}$ is 10 Hz lower when H-1 is axial than when it is equatorial.[783] The $^1J_{^{13}C\text{-}1,^1H\text{-}1}$ values can therefore be used for the determination of conformational equilibria. In agreement with this finding, tri-*O*-acetylamylose and tri-*O*-acetylcellulose have $^1J_{^{13}C\text{-}1,^1H\text{-}1}$ values of 176 and 163 Hz, respectively.[784] The ^{13}C–^{19}F coupling constants have been obtained from the proton-decoupled ^{13}C n.m.r. spectra of a number of glycosyl fluorides; $^1J_{^{13}C\text{-}1,F}$ values were found to be negative and to be larger in α-pyranosyl fluorides than in β-fluorides.[785] An axial substituent at C-2 in a pyranosyl fluoride increases both the $^1J_{^{13}C\text{-}1,F}$ and $^2J_{^{13}C\text{-}2,F}$ values. The $^3J_{^{13}C\text{-}3,F}$ values of β-pyranosyl fluorides are larger than those of α-pyranosyl fluorides, indicating a Karplus type of relationship. The absolute configuration of glycoses linked axially to O-4 and O-6 of deoxystreptamine can be determined from the ^{13}C chemical shifts of C-4, C-6, C-1′, and C-1″ at different pH values.[534] ^{13}C N.m.r. spectroscopy has demonstrated the presence of a β-linkage in C_{15}-iridoid D-glucopyranosides.[786]

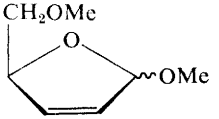
A thorough analysis of the ^{13}C n.m.r. spectra of methyl aldopento- and aldohexo-furanosides and several cyclopentanols has enabled the ^{13}C chemical shifts to be correlated with the configurations and other stereochemical features of vicinal diol groupings.[787] Values for both $^1J_{^{13}C\text{-}1,F}$ and $^2J_{^{13}C\text{-}2,F}$ in furanosyl

[780] V. Eschenfelder, R. Brossmer, and H. Friebolin, *Tetrahedron Letters*, 1975, 3069.
[781] W. Funcke and A. Klemer, *Annalen*, 1975, 1232.
[782] M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1975, **97**, 4627.
[783] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1975, **29**, 258.
[784] F. R. Taravel and P. J. A. Vottero, *Tetrahedron Letters*, 1975, 2341.
[785] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1975, **29**, 682.
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[787] R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, *Canad. J. Chem.*, 1975, **53**, 1424.

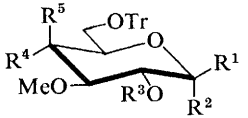
fluorides are smaller when the fluorine atom and the substituent at C-2 are in a *trans* arrangement than when they are in a *cis* arrangement.[785] The values of $^1J_{^{13}C\text{-}1,^1H\text{-}1}$ in the methyl furanosides (451) (α, 176.25 Hz; β, 172.50 Hz) and (452) (α, 176.00 Hz; β, 172.50 Hz) show little variation for each anomer and should be useful in determining the anomeric configurations of furanosides.[784]



(451)



(452)



(453) $R^1 = R^3 = H$; $R^2 = OMe$; $R^4 = Me$; $R^5 = OH$
$R^1 = H$; $R^2 = OMe$; $R^3 = Ms$; $R^4 = Me$; $R^5 = OH$
$R^1 = R^3 = H$; $R^2 = OMe$; $R^4 = OH$; $R^5 = Me$
$R^1 = OMe$; $R^2 = H$; $R^3 = R^4 = Me$; $R^5 = OH$

^{13}C N.m.r. spectroscopy has obvious advantages over ^1H n.m.r. spectroscopy in determining the configurations of branched-chain sugars. The ^{13}C n.m.r. spectra of several methyl hexopyranoside derivatives having a *C*-methyl group at either C-2 or C-4 [*e.g.* (453)] have been reported and discussed; axial *C*-methyl groups at C-4 are shifted upfield of the corresponding equatorial group by 5.3—6.4 p.p.m.[426] ^{13}C N.m.r. spectroscopy of 1,2-*O*-isopropylidene derivatives of apiose and dendroketose has permitted the configurations both at the branching point and at the anomeric centre to be established.[788]

The use of ^{13}C n.m.r. spectroscopy in the conformational analysis of various types of natural products (*e.g.* nucleoside phosphates and complex carbohydrates) has been reviewed.[789] The ^{13}C n.m.r. spectra of 6-chloro-6-deoxy-D-glucose, 6-*O*-methyl-D-glucose, ribitol, D-glucitol, 4-*O*-methyl-D-glucitol, maltose, maltitol, 6,6′-dichloro-6,6′-dideoxymaltose, 6′-chloro-6′-deoxymaltose, isomaltose, isomaltitol, and 6-chloro-6-deoxyisomaltose have been reported.[790] Replacement of a primary hydroxy-group with chlorine shifts the C-6 resonance downfield by 17 p.p.m., whereas it shifts upfield by 10 p.p.m. on methylation. The results were applied to investigations of microbial polysaccharides. Other oligo- and polysaccharides to be investigated by ^{13}C n.m.r. spectroscopy include six- and seven-membered cyclodextrins, (1 → 3)-, (1 → 4)-, and (1 → 6)-linked D-glucans, and D-glucans with mixed linkages; the ^{13}C resonances were assigned by comparison with the spectra of D-glucose and its *O*-methyl derivatives and those of differently linked D-gluco-bioses and -trioses.[791] This technique enabled the compositions,

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[789] I. C. P. Smith, H. J. Jennings, and R. Deslauriers, *Accounts Chem. Res.*, 1975, **8**, 306.
[790] P. Colson, K. N. Slessor, H. J. Jennings, and I. C. P. Smith, *Canad. J. Chem.*, 1975, **53**, 1030.
[791] P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1974, **96**, 8081.

structures, and principal sequences of several D-glucans to be determined. Conformational effects are also revealed by some of the spectra.

Lanthanide Shift Reagents

The ^{1}H n.m.r. spectra of peracetylated methyl D-hexopyranosides and methyl 2-acetamido-2-deoxy-D-hexopyranosides have been recorded in the presence of Eu(fod)$_3$.[792] Differences exist between the two sets of compounds; with the former compounds H-2, -3, -4, -6, and -6′ are most affected by the presence of the lanthanide shift reagent, whereas H-1, -2, -3, *N*-Ac, and *N*-H are most affected in the other series. The most marked shifts are shown by H-2 and *N*-Ac of the amino-sugar derivatives. Eu(fod)$_3$ has also been used in a simple method for determining the configurations of tertiary alcoholic centres in branched-chain sugars.[793] The displacement of the resonance frequency of specific protons produced by the addition of increasing amounts of Eu(fod)$_3$ was compared with that of the corresponding proton resonance of an alcohol of known stereochemistry. The configurations assigned were supported by statistical analysis of shift-gradient data for all the protons. The effects produced by Eu(fod)$_3$ and Pr(fod)$_3$ on the methyl signals of methyl 2,3,4,6-tetra-*O*-methyl-α- and -β-D-galactopyranosides have been recorded.[794] The following conformational assignments have been achieved by using Eu(fod)$_3$ to separate overlapping signals in the normal ^{1}H n.m.r. spectra: 4,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose ($^4H_5$), methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (intermediate between $^1C_4$ and $^5H_0$), and 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (intermediate between $^2C_5$ and $^2H_0$).[795]

In studies on nucleotides, Williams' group has employed edta complexes of lanthanide ions (La^{3+}, Pr^{3+}, Eu^{3+}, and Gd^{3+}) to probe the conformations of adenosine and cytidine 5′-phosphates at pH 7.5.[796] The conformation of cytidine 5′-phosphate at pH 2.0 was also investigated using lanthanide ions, and relaxation studies with Gd^{3+} cations confirmed the findings derived from the shift data.[797]

Spin–lattice Relaxation Times

Variations in the ^{13}C spin–lattice relaxation times (T_1 values) of carbohydrate derivatives have been demonstrated by Bock and Hall.[798] Thus C-1, -2, -3, -4, and -5 of methyl α-D-glucopyranoside (each bearing a single hydrogen substituent) have T_1 values in the range 0.9—1.2 s, whereas C-6 (bearing two hydrogen substituents) has a smaller T_1 value of 0.62 s that is not seriously influenced by rotation about the C-5—C-6 bond. By contrast, the glycosidic methyl carbon atom has a relatively large T_1 value (3.4 s), indicating that this carbon atom undergoes essentially free rotation in solution.

The replacement of hydrogen by deuterium at a specific position has been shown to reduce the spin–lattice relaxation times of other protons in a manner

[792] K. Izumi, *J. Biochem.* (*Japan*), 1974, **76**, 535.
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[796] C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J.C.S. Dalton*, 1974, 1762.
[797] C. D. Barry, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J.C.S. Dalton*, 1974, 1765.
[798] K. Bock and L. D. Hall, *Carbohydrate Res.*, 1975, **40**, C3.

that depends critically on the conformation of the molecule.[799] The data obtained can be used in conjunction with the ^{13}C spin–lattice relaxation times to furnish interproton distances. Average interproton distances were obtained for H-8—H-1′, —H-2′, and —H-3′ of 3,5′-anhydro-2′,3′-*O*-isopropylideneguanosine, which has a rigid conformation. The method was also used to study the distribution between the *syn* and *anti* conformations of nucleosides in solution.

Addition of very small proportions (~1 mmolar equivalent) of gadolinium nitrate to aqueous solutions of several mono- and di-saccharides has been found to cause appreciable changes in their proton spin–lattice relaxation times.[800] Useful information on the conformations of carbohydrate derivatives may be obtained if the binding of the gadolinium ion to the sugar is regiospecific. Gadolinium nitrate also induced striking changes in the ^{13}C n.m.r. spectrum of sodium α,β-D-galacturonate in deuterium oxide; notably, signals due to C-6 and C-1 of the α-anomer were reduced in apparent intensity, whereas there was no comparable effect on those due to C-6 and C-1 of the β-anomer.[801] The absence of comparable effects with the corresponding hexose implicates the carboxy-group in binding of the gadolinium ion. The effects produced on C-1 and C-6 (and also on H-1) make the gadolinium ion a sensitive diagnostic reagent for distinguishing between the anomeric forms of hexuronic acids.

The activation energy for pseudorotation of the furanose ring in adenosine, guanosine, inosine, and xanthosine in liquid [$^2H_3$]ammonia has been determined by analysis of the ^{13}C longitudinal relaxation rates of the carbon nuclei between +40 and −60 °C.[802] The average activation energy for pseudorotation in the purine nucleosides is 4.7 ± 0.5 kcal mol^{-1}; hydrogen-bonding between HO-5′ and the base in the pyrimidine nucleosides cytidine and uridine prevented pseudorotation of the D-ribofuranosyl ring.

[799] K. Akasaka, T. Imoto, S. Shibata, and H. Hatano, *J. Magn. Resonance*, 1975, **18**, 328.
[800] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1975, **41**, 53.
[801] B. Casu, G. Gatti, N. Cyr, and A. S. Perlin, *Carbohydrate Res.*, 1975, **41**, C6.
[802] O. Roeder, H. D. Luedemann, and E. von Goldammer, *European J. Biochem.*, 1975, **53**, 517.

24
Other Physical Methods

I.R. Spectroscopy

Comparison of the laser Raman spectra of α-D-glucopyranose, D-glucopyranosyluronic acid, 2-amino-2-deoxy-D-glucopyranose hydrochloride, and 2-acetamido-2-deoxy-D-glucopyranose has permitted the absorptions due to CO_2H, CH_2, $\overset{+}{N}H_3$, and NH groups to be assigned.[803] Raman difference spectroscopy, in conjunction with ^1H n.m.r. spectroscopy, has been used to show that methylmercury(II) ions bind quantitatively to inosine at pH 8 by displacement of the proton at N-1.[706]

The i.r. spectra of crystalline methyl α-D-gluco-, α-D-manno-, α-D-galacto-, and α-D-altro-pyranosides have been examined in detail using deuteriation and low-temperature techniques; in particular, bands due to the O—H stretching (~ 3000 cm^{-1}) and out-of-plane bending (400—800 cm^{-1}) were studied.[804] The ester, amide, and uronate contents of pectins and the uronate content of alginates have been determined, respectively, from the ester-carbonyl stretching band (1740 cm^{-1}), the amide I band (1650 cm^{-1}), and the carboxylate antisymmetric stretching band (1607 cm^{-1}) obtained from the spectra of solutions in deuterium oxide–phosphate buffer.[805] This method is accurate to within ± 2–4% and should be applicable to the determination of carboxylic acid derivatives in other polysaccharides. Analysis of the i.r. spectrum of erythromycin was assisted by examination of the i.r. spectra of deuteriated derivatives and some component sugars.[806]

U.V. Spectroscopy

A number of reversible changes were observed in the u.v. spectrum when frozen solutions of D-*erythro*-pentulose (D-ribulose) 5-phosphate were allowed to thaw.[807] Under the same conditions, the spectra of D-*threo*-pentulose(D-xylulose) 5-phosphate and D-*erythro*-pentulose 1,5-diphosphate were unchanged.

Mass Spectrometry

A review on field-desorption mass spectrometry has referred to studies on oligosaccharides, carbohydrate-containing antibiotics, plant glycosides, steroidal

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[804] A. J. Mitchell, *Austral. J. Chem.*, 1975, **28**, 335.
[805] S. M. Bociek and D. Welti, *Carbohydrate Res.*, 1975, **42**, 217.
[806] J. Dziegielewski, J. Hanuza, B. Jezowska-Trzebiatowska, and J. Kuduk-Jaworska, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1975, **23**, 513 (*Chem. Abs.*, 1975, **83**, 179 505g).
[807] T. Wood, *Carbohydrate Res.*, 1975, **40**, 407.

D-glucuronides, nucleosides, and nucleotides.[808] A very brief comment on the mass spectrometry of carbohydrate derivatives has also appeared.[809]

The mass spectra of some *O*-isopropylidene derivatives enriched in ^{13}C (*e.g.* 1,2:5,6-di-*O*-isopropylidene-α-D-[1-^{13}C]glucofuranose) have been recorded.[810] The use of ^{13}C-enrichment to aid in the analysis of mass-spectral fragmentation patterns has the advantage that the ^{13}C nucleus is less likely to be involved in intramolecular migration than other isotopic labels. Deuterium-labelling has also aided in the interpretation of the fragmentation patterns of 1,2:5,6-di-*O*-isopropylidene-α-D-gluco-, -D-allo-, and -D-gulo-furanoses.[811]

Several *O*-acetylated *N*-acylneuraminic acids (isolated from animal tissues) have been characterized by m.s.[812] They were identified as 4-*O*-acetyl-,9-*O*-acetyl-, 4,9-di-*O*-acetyl-, and 7,9-di-*O*-acetyl-*N*-acetylneuraminic acids, and 4-*O*-acetyl- and 9-*O*-acetyl-*N*-glycolylneuraminic acids on the basis of the typical fragmentation patterns of the TMS derivatives of their methyl esters. The fragmentations observed in the m.s. of amidosulphates of acetylated methyl α-D-galactopyranoside are characteristic of the position of the sulphate moiety.[813] The mass spectra of the pyridinium salts of acetylated D-glucose 2-, 3-, and 6-sulphates, and related derivatives of methyl α-D-glucopyranoside, have been compared with those of the corresponding sulphate methyl esters and D-glucose penta-acetate.[814] Three principal fragmentation patterns were detected, depending on the location of the sulphate group. Deuterium-labelling techniques have been used to elucidate the fragmentation patterns of butane- and methane-boronates of the dimethylphosphates of D-glucose, D-galactose, D-mannose, D-fructose, and methyl D-gluconate.[815] The advantages of these derivatives over those containing silicon were discussed.

The presence of free hydroxy-groups has been shown to change the fragmentation pathway of partially methylated alduronic acids by comparison with fully methylated derivatives.[816] Related studies have been carried out with partially and fully methylated derivatives of methyl (4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate.[817] The mass spectra of several (D-glucopyranosyluronic acid)-diglycerides have been reported.[818]

Electron-ionization and ion kinetic-energy spectra of the peracetates of a series of D-glucobioses have indicated that these techniques are useful in differentiating between the various types of glycosidic linkages.[819] Field-desorption m.s. has

[808] H. D. Beckey and H.-R. Schulten, *Angew. Chem. Internat. Edn.*, 1975, **14**, 403.
[809] J. F. G. Vliegenthart and J. P. Kamerling, *Biochem. Soc. Trans.*, 1975, **3**, 460.
[810] J. A. Schwarcz and A. S. Perlin, *Carbohydrate Res.*, 1975, **45**, 317.
[811] A. Buchs, A. Glangetas, and J. M. J. Tronchet, *Org. Mass Spectrometry*, 1975, **10**, 970.
[812] J. P. Kamerling, J. F. G. Vliegenthart, C. Versluis, and R. Schauer, *Carbohydrate Res.*, 1975, **41**, 7.
[813] A. I. Usov, V. V. Deryabin, O. S. Chizhov, V. I. Kadentsev, B. M. Zolotarev, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1084 (*Chem. Abs.*, 1975, **83**, 131 847t).
[814] A. F. Pavlenko, A. I. Kalinovskii, A. K. Dzizenko, N. I. Belogortseva, and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1973, 793 (*Chem. Abs.*, 1975, **82**, 4491b).
[815] J. Wiecko and W. R. Sherman, *Org. Mass Spectrometry*, 1975, **10**, 1007.
[816] V. Kováčik and P. Kováč, *Org. Mass Spectrometry*, 1975, **10**, 376.
[817] V. Kováčik and P. Kováč, *Analyt. Biochem.*, 1975, **64**, 45.
[818] S. G. Batrakov, E. F. Il'ina, B. V. Rozvnov, and L. D. Dergel'son, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 94 (*Chem. Abs.*, 1975, **82**, 125 561h).
[819] K. G. Das and B. Thayumanavan, *Org. Mass Spectrometry*, 1975, **10**, 455.

been used to examine permethylated di-, tri-, and tetra-saccharides; in addition to the appearance of intense peaks for the (M + 1) and molecular ions, intense peaks also resulted from inter-unit cleavage.[820] The mass spectra of the TMS derivatives of 2-*O*-β-D-glucopyranosyl-D-erythronic, 3-*O*-β-D-glucopyranosyl-D-arabinonic, and 4-*O*-β-D-glucopyranosyl-D-gluconic acids have been examined in connection with studies of the oxidative alkaline degradation of oligosaccharides.[821]

A detailed investigation of the chemical-ionization mass spectrometry of nucleosides has been reported,[822] and the electron-impact mass spectra of four *O*-methylinositols have been compared with those of the isomeric *C*-methyl derivatives.[823] Complex processes that involve the elimination of water have been observed in the mass spectra of cyclopentane-triols, -tetraols, and pentaols.[823a]

X-Ray Crystallography

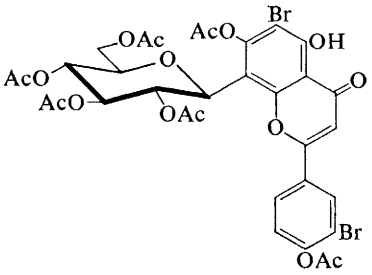
Bibliographies have appeared covering the results of determinations of the crystal structures of carbohydrates published during 1970—1972[824] and in 1973.[825] These bibliographies contain much more detail (*e.g.* the space group, calculated density, and brief comments concerning the conformation of the molecules) than aimed for in this section, where the names of the compounds examined are grouped under appropriate headings; solvent molecules of crystallization are not included.

Free Sugars and Simple Derivatives Thereof.—β-D-Galactopyranose and α-L-fucopyranose,[826] α-D-altropyranose penta-acetate,[827] and stachyose (the first tetrasaccharide to be so examined).[828]

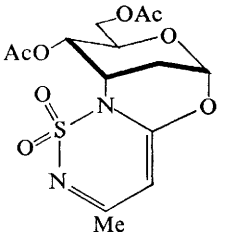
Glycosides.—Methyl α-D-altropyranoside (neutron diffraction was used to refine the crystal structure),[829] *O*-β-D-xylopyranosyl-L-serine and its copper(II) complex,[830] and a 'bromohexa-acetylvitexin' that was shown to be a 7 : 3-mixture of 2″,3″,4′,-4″,6″,7-hexa-*O*-acetyl-6-bromo-5-hydroxyvitexin and the 3′,6-dibromo-compound (454).[831]

Amino-sugar Derivatives.—2-Acetamido-2-deoxy-α-D-galactopyranose,[832] 2-acetamido-2-deoxy-α-D-glucopyranose,[833] and 2-acetamido-2-deoxy-β-D-mannopyranose.[834]

[820] J. Moor and E. S. Waight, *Org. Mass Spectrometry*, 1974, **9**, 903.
[821] R. Malinen and E. Sjöström, *Carbohydrate Res.*, 1975, **39**, 335.
[822] M. S. Wilson and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1975, **97**, 3436.
[823] A. Buchs and E. J. Charollais, *Compt. rend. Soc. Phys. Hist. Nat. Geneva*, 1974, **9**, 57 (*Chem. Abs.*, 1975, **83**, 179 444m).
[823a] A. Singy and A. Buchs, *Helv. Chim. Acta*, 1975, **58**, 2164.
[824] G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 445.
[825] G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 347.
[826] F. Longchambon, J. Ohannessian, D. Avenel, and A. Neuman, *Acta Cryst.*, 1975, **B31**, 2623.
[827] J. Ollis, V. J. James, and J. D. Stevens, *Cryst. Struct. Comm.*, 1975, **4**, 215 (*Chem. Abs.*, 1975, **83**, 51 320a).
[828] R. D. Gilardi and J. L. Flippen, *J. Amer. Chem. Soc.*, 1975, **97**, 6264.
[829] B. J. Poppleton, G. A. Jeffrey, and G. J. B. Williams, *Acta Cryst.*, 1975, **B31**, 2400.
[830] L. T. J. Delbaere, B. Kamenar, and K. Prout, *Acta Cryst.*, 1975, **B31**, 862.
[831] F. A. Jurnak and D. H. Templeton, *Acta Cryst.*, 1975, **B31**, 1304.
[832] A. Neuman, H. Gillier-Pandraud, F. Longchambon, and D. Rabinovich, *Acta Cryst.*, 1975, **B31**, 474.
[833] F. Mo and L. H. Jensen, *Acta Cryst.*, 1975, **B31**, 2867.
[834] A. Neuman, H. Gillier-Pandraud, and F. Longchambon, *Acta Cryst.*, 1975, **B31**, 2628.



(454)



(455)

Acid Derivatives.—*N*-(2-Chloroethyl)-D-gluconamide,[835] α-D-glucuronate,-$CaBr_2$,[836] calcium sodium D-galacturonate and strontium sodium D-galacturonate,[837] and L-*xylo*-hexulosonic acid.[838]

Anhydro-sugar Derivatives.—1,6-Anhydro-β-D-glucopyranose,[839] 1,6-anhydro-L-iditol,[840] and 1,4:2,5:3,6-trianhydro-D-mannitol.[841]

Nucleosides, Nucleotides, and their Derivatives.—Uridine,[842] uridine 5′-(methyl oxyacetate),[843] 6-chloro-9-β-D-ribofuranosylpurine,[844] 6-methyl-9-β-D-ribofuranosylpurine,[844a] 5-acetyl-1-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)uracil,[845] 2′-deoxycytidine,[846] the 1 : 1-complex of cytidine and *N*-benzyloxycarbonylglutamic acid,[847] 1-(3-*O*-methyl-β-D-arabinofuranosyl)cytosine,[848] 2′-deoxyadenosine 5′-(sodium phosphate),[849] 9-β-D-arabinofuranosyladenine hydrochloride,[850] 8-β-D-ribofuranosyladenine,[851] 8-(2-deoxy-α-D-*erythro*-pentofuranosyl)-6-hydroxypurine,[852] 1-methyl-4-β-D-erythrofuranosyl-4-imidazoline-2-thione,[853] and 4,6-di-*O*-acetyl-1,3′-anhydro-2,3-dideoxy-3-[3-hydroxy-5-methyl-(2*H*)-1,2,6-thiadiazin-2-yl 1,1-dioxide]-α-D-*ribo*-hexopyranose (455).[854]

[835] L. O. G. Satzke and M. F. Mackay, *Acta Cryst.*, 1975, **B31**, 1128.
[836] L. DeLucas, C. E. Bugg, A. Terzis, and R. Rivest, *Carbohydrate Res.*, 1975, **41**, 19.
[837] S. E. B. Gould, R. O. Gould, D. A. Rees, and W. E. Scott, *J.C.S. Perkin II*, 1975, 237.
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[839] K. B. Lindberg, *Acta Chem. Scand.* (*A*), 1974, **28**, 1181.
[840] P. Luger, R. Reinhardt, and H. Paulsen, *Chem. Ber.*, 1975, **108**, 3201.
[841] F. W. B. Einstein and K. N. Slessor, *Acta Cryst.*, 1975, **B31**, 552.
[842] E. A. Green, R. D. Rosenstein, R. Shiono, D. J. Abraham, B. L. Trus, and R. E. Marsh, *Acta Cryst.*, 1975, **B31**, 102.
[843] K. Morikawa, K. Torii, Y. Iitaka, and M. Tsuboi, *Acta Cryst.*, 1975, **B31**, 1004.
[844] H. Sternglanz and C. E. Bugg, *Acta Cryst.*, 1975, **B31**, 2888.
[844a] T. Takeda, Y. Ohashi, Y. Sasada, and M. Kakudo, *Acta Cryst.*, 1975, **B31**, 1202.
[845] D. W. Jones, P. W. Rugg, G. Shaw, and J. M. Sowden, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 165.
[846] D. W. Young and H. R. Wilson, *Acta Cryst.*, 1975, **B31**, 961.
[847] T. Hata, M. Yoshikawa, S. Sato, and C. Tamura, *Acta Cryst.*, 1975, **B31**, 312.
[848] G. I. Birnbaum, E. Darzynkiewicz, and D. Shugar, *J. Amer. Chem. Soc.*, 1975, **97**, 5904.
[849] B. S. Reddy and M. A. Viswamitra, *Acta Cryst.*, 1975, **B31**, 19.
[850] T. Hata, S. Sato, M. Kaneko, B. Shimizu, and C. Tamura, *Bull. Chem. Soc. Japan.* 1974, **47**, 2758.
[851] O. Lefebvre-Soubeyran, J. P. Declercq, G. Germain, T. Huynh-Dinh, and J. Igolen, *Compt. rend.*, 1975, **281**, *C*, 655.
[852] A. Ducruix and C. Pascard-Billy, *Acta Cryst.*, 1975, **B31**, 1987.
[853] A. Conde, E. Moreno, and R. Márquez, *Acta Cryst.*, 1975, **B31**, 648.
[854] C. Foces-Foces, P. Smith-Verdier, F. Florencio-Sabate, and S. Garćia-Blanco, *Acta Cryst.*, 1975, **B31**, 140.

Antibiotics and Components Thereof.—Pillaromycin [the component sugar was shown to be 2,3,6-trideoxy-4-*C*-(hydroxymethylcarbonyl)-L-*threo*-hexose] (246),[413] 5-deoxy-3-*C*-formyl-β-L-lyxofuranose trimethylene dithioacetal,[855] and the corresponding 1,2-*O*-isopropylidene derivative.[856]

Other Derivatives.—5-Chloro-5-deoxy-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-4-thio-β-L-arabinofuranose,[857] 7-azido-8-deoxy-1,2:3,4-di-*O*-isopropylidene-6,7-*S*-trimethylene-6,7-dithio-α-D-*erythro*-D-*galacto*-octopyranose,[858] 1(*R*)-2,3,4,5-tetra-*O*-acetyl-1-*S*-ethyl-1-[purin-9-yl-6(1*H*)-thione]-1-thio-D-arabinitol,[859] and *myo*-inositol hexaphosphate (dodeca-sodium salt).[860]

E.S.R. Spectroscopy

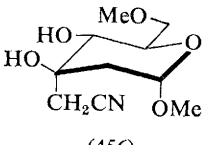
Ferric chloride-photosensitized oxidations of several alcohols (*e.g.* ethanol, cyclohexanol, and 2-methylpropan-2-ol) have been conducted in the cavity of an e.s.r. spectrometer at temperatures between −150 and −196 °C.[861] Except for rigid glasses of 2-methylpropan-2-ol, all the photolysed alcohols investigated yielded free-radicals detectable by the spectrometer; alcohols in rigid glasses should provide better models for cellulose in the solid state than alcohols in solution. Free radicals formed on γ-irradiation of single crystals of trehalose and sucrose,[862] on oxidation of some carbohydrate imidazolines (see Scheme 60),[350] and on treatment of L-ascorbic acid with hydrazine in oxygenated alkaline solution [483] have been examined by e.s.r. spectroscopy.

[855] W. Depmeier and O. H. Jarchow, *Acta Cryst.*, 1975, **B31**, 945.
[856] W. Depmeier and O. H. Jarchow, *Acta Cryst.*, 1975, **B31**, 939.
[857] W. Clegg, *Acta Cryst.*, 1975, **B31**, 2722.
[858] C. Riche and C. Pascard-Billy, *Acta Cryst.*, 1975, **B31**, 2565.
[859] A. Ducruix and C. Pascard-Billy, *Acta Cryst.*, 1975, **B31**, 2250.
[860] G. E. Blank, J. Pletcher, and M. Sax, *Acta Cryst.*, 1975, **B31**, 2584.
[861] O. Hinojosa, J. A. Harris, and J. C. Arthur, jun., *Carbohydrate Res.*, 1975, **41**, 31.
[862] A. Graslund and G. Lofroth, *Acta Chem. Scand.* (*B*), 1975, **29**, 475.

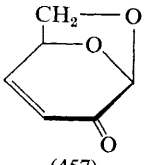
25
Polarimetry

Nakanishi's group continues to develop new methods for determining the absolute configuration of cyclic and acyclic vicinal glycols and amino-alcohols. The addition of an underivatized glycol (or amino-alcohol) to a solution of nickel bis(acetylacetonate) yields mainly a bidentate complex and induces Cotton effects at both ~ 300 nm and ~ 630 nm, which can be correlated with the absolute configuration of the glycol (or amino-alcohol).[863] A complementary procedure uses praseodymium tris(dipivalomethanate) [$Pr(dpm)_3$] in a dry, non-polar solvent; for example, the absolute structure of the branched-chain sugar (456) was corroborated by the appearance of a negative peak at 313 nm on complexation with $Pr(dpm)_3$.[864]

The variations in the optical rotations of aqueous solutions of D-glucose, sucrose, and maltose, *etc.*, on addition of calcium chloride have been investigated.[865] Measurement of the Cotton effects induced by the addition of germanic acid to aqueous solutions of D-fructose in the pH range 6—10 indicated that a bidentate complex is formed.[866]



(456)



(457)

The optical rotations of α-lactose, β-lactose, and equilibrated solutions thereof have been measured at 546 and 589 nm between 10 and 35 °C.[867] Although the measured optical rotation of an equilibrated solution of lactose showed good agreement with literature values, revised values were reported for the pure α- and β-forms.

In a study of the chiroptical properties of some substituted-phenyl glycosides, it was noted that steric and electronic effects associated with *ortho*-substituents may change the sign of some of the c.d. bands of the aromatic chromophore.[868]

[863] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5409.
[864] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5417.
[865] M. Boruch, *Zesz. Probl. Postepow Nauk Roln.*, 1974, **159**, 205 (*Chem. Abs.*, 1975, **83**, 10 644r).
[866] P. J. Antikainen, *Finn. Chem. Letters*, 1974, 159 (*Chem. Abs.*, 1975, **82**, 43 669k).
[867] T. J. Buma and H. K. C. van der Veen, *Ned. Melk-Zuiveltijdschr.*, 1974, **28**, 175 (*Chem. Abs.*, 1975, **83**, 79 504n).
[868] A. Levai, A. Liptak, I. Pinter, and G. Snatzke, *Acta Chim. Akad. Sci. Hung.*, 1975, **84**, 99 (*Chem. Abs.*, 1975, **82**, 98 327c).

Investigations of the o.r.d. and c.d. spectra of laevoglucosenone (457) have attributed the unusually large rotation {$[\alpha]_D$ − 524°(EtOH)} to the Cotton band at 357 nm.[869]

Rees and his co-workers have examined the c.d. spectra of several methyl α- and β-glycopyranosyluronic acids.[870] The sign of the c.d. curve of the free acid is determined by the configuration at C-5, and an anomalous long-wavelength band of opposite sign to the main $n \rightarrow \pi^*$ band is present when HO-4 has an equatorial orientation. The same generalizations apply to salts of the acids, although the shape and the position of the bands are different. These variations can be used to investigate the structures of uronic acid residues in such complex polysaccharides as alginates. C.d. studies on the α- and β-ketosides of *N*-acetylneuraminic acid and some of its derivatives have also been undertaken with a view to interpreting the c.d. data of complex biological molecules.[871] C.d. and o.r.d. spectra for several aryl D-glucuronides have been reported.[104]

The conformational mobilities of pyrimidine nucleosides in aqueous solution have been investigated by c.d. measurements in the temperature range −100 to +40 °C; changes in the main c.d. band were ascribed to oscillation of the base about the glycosidic bond and to pseudorotation of the sugar ring.[872] The c.d. spectra of uridine, thymidine, 2′-deoxyadenosine, adenosine, and 9-β-D-arabinofuranosyladenine in aqueous solution have been measured at pH 7 and 2,[873] and the c.d. spectra of benzoyl, 4-nitrobenzoyl, and anisoyl derivatives of adenosine, uridine, and 1-β-D-ribofuranosylbenzimidazole have also been recorded.[874]

The c.d. spectra of formycin and formycin B have been compared with those of 2,5′- and 4,5′-anhydroformycin, which are derived from the *anti* and *syn* conformation, respectively, of formycin.[141] The spectra indicated that formycin and formycin B adopt mainly the *anti* conformation in aqueous solution, although there is a higher proportion of formycin in the *syn* conformation in acid solution.

[869] A. Ohnishi, E. Takagi, and K. Kato, *Bull. Chem. Soc. Japan*, 1975, **48**, 1959.
[870] E. R. Morris, D. A. Rees, G. R. Sanderson, and D. Thom, *J.C.S. Perkin II*, 1975, 1418.
[871] G. Keilich, R. Brossmer, V. Eschenfelder, and L. Holmquist, *Carbohydrate Res.*, 1975, **40**, 255.
[872] A. Andre and W. Guschlbauer, *Nucleic Acids Res.*, 1974, **1**, 803.
[873] W. C. Brunner and M. R. Maestre, *Biopolymers*, 1975, **14**, 555.
[874] A. F. Usatyi, W. N. Nesawibatko, and M. N. Semjenowa, *Tetrahedron*, 1975, **31**, 1071.

26
Separatory and Analytical Methods

Chromatographic Methods

Gas–Liquid Chromatography.—A two-part review by Dutton has discussed the g.l.c. of sugars and their derivatives.[875]

Mixtures of *O*-methyl ethers obtained by partial methylation of a number of methyl glycopyranosides have been acetylated and the products identified by g.l.c.–m.s.[876] The use of partially ethylated alditol acetates for the analysis by g.l.c. of the components of polysaccharides has been described.[877] The ethyl analogues are eluted before the corresponding methyl derivatives, and this often allows the separation of many polysaccharide components by g.l.c. that are not separable as their partially methylated alditol acetates. The use of molar flame-responses calculated on the basis of effective carbon responses has been advocated for the accurate determination of partially methylated and partially ethylated alditol acetates by g.l.c.[878]

Peracetylated aldononitriles of tetra-, tri-, and di-*O*-methyl ethers of D-manno-pyranose have been separated by g.l.c. and then analysed by m.s.[879] Various fragment ions comprising the mass spectra were identified (with the aid of deuteriomethylation) and related to the structure of the parent methylated sugar. A method for determining the degree of polymerization of oligosaccharides and polysaccharides (*e.g.* D-xylan) is based on measurement of the ratio of acetylated alditol to acetylated aldononitrile after reduction of the poly(oligo)-saccharide with sodium borohydride, hydrolysis with acid, and treatment of the components in the hydrolysate with hydroxylamine, acetic anhydride, *etc.*[880]

Mixtures of common aldoses have been analysed by g.l.c.–m.s. of their *O*-iso-propylidene derivatives, which, in most cases, give rise to clearly different mass spectra.[881] Gas–liquid chromatographic data for the butaneboronic esters and TMS derivatives of some monosaccharides and alditols have been reported; butaneboronic esters are useful for the qualitative identification of certain mono-saccharides, and are of particular value in the quantitative analysis of mixtures of D-glucose and D-fructose.[882]

[875] G. G. S. Dutton, *Adv. Carbohydrate Chem. Biochem.*, 1973, **28**, 11; *ibid.*, 1974, **30**, 10.
[876] Yu. N. El'kin, N. I. Shul'ga, T. I. Vakorina, and A. K. Dzizenko, *Analyt. Biochem.*, 1975, **68**, 9.
[877] D. P. Sweet, P. Albersheim, and R. H. Shapiro, *Carbohydrate Res.*, 1975, **40**, 199.
[878] D. P. Sweet, R. H. Shapiro, and P. Albersheim, *Carbohydrate Res.*, 1975, **40**, 217.
[879] F. R. Seymour, R. D. Plattner, and M. E. Slodki, *Carbohydrate Res.*, 1975, **44**, 181.
[880] I. M. Morrison, *J. Chromatog.*, 1975, **108**, 361.
[881] S. Morgenlie, *Carbohydrate Res.*, 1975, **41**, 285.
[882] P. J. Wood, I. R. Siddiqui, and J. Weisz, *Carbohydrate Res.*, 1975, **42**, 1.

TMS derivatives of methyl (methyl 4-deoxy-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronates [883] and of the monosaccharides obtained on methanolysis of glycoproteins and glycopeptides [884] have been examined by g.l.c.

Column and Ion-exchange Chromatography.—Dextran-based, boronarylated polymers having dipolar 2-{[(4-boronophenyl)methyl]-ethylammonio}ethyl and -diethylammonio}ethyl groups have been used for absorption of compounds containing a *cis*-diol grouping (*e.g.*, adenosine, D-ribose, α-D-glucose, D-gluconic acid, *etc.*).[885] The chromatographic behaviour of carbohydrates is defined by their structure and conformation, which are responsible for different stabilities of the boronic complexes formed.[885, 886]

The effectiveness of aluminate ion for separating carbohydrates by column chromatography with a strongly basic anion-exchange resin has been evaluated.[887] With water as eluant, the retention volumes for carbohydrates on an aluminate-resin column generally decrease in the order: ketoses > aldoses > alditols > methyl glycosides; retention increases with molecular size. Chromatography of reducing sugars must be carried out both rapidly and at low temperature to avoid alkaline degradation and interconversion of the sugars. The chromatographic separation of aldoses, alditols, and other polyhydroxy-compounds on anion-exchange resins in the borate form has been investigated.[499]

Variation of the counter-ion attached to a polystyrene-based, strong cation-exchange resin has been shown to affect the chromatographic behaviour of sugars and other polyhydroxy-compounds.[888] The separation of mixtures of sugars can often be significantly improved by choosing a suitable counter-ion, and, in certain cases, α- and β-anomers of monosaccharides can be separated.

Anomeric 2-amino-2-deoxy-D-glucosides, -galactosides, and -mannosides have been separated by chromatography on an Amberlite cation-exchange resin,[889, 889a] and ion-exchange chromatography has also been used to separate 2-amino-2,6-dideoxyhexoses (components of bacterial polysaccharides) and some of their methyl ethers.[890] A rapid, automated method for the analysis of mixtures of aminodeoxyalditols and neutral alditols using an ion-exchange resin in the borate form has been reported.[891]

Paper Chromatography.—A new solvent system (nitromethane–acetic acid–ethanol–water saturated with boric acid, 8 : 1 : 1 : 1) that separates the common monosaccharides from each other, and that also separates alditols from each other and from their parent monosaccharides, has been reported.[892] Paper-

[883] D. Anderle, P. Kováč, and J. Hirsch, *J. Chromatog.*, 1975, **105**, 206.
[884] J. P. Kamerling, G. J. Gerwig, J. F. G. Vliegenthart, and J. R. Clamp, *Biochem. J.*, 1975, **151**, 491.
[885] A. M. Yurkevich, I. I. Kolodkina, E. A. Ivanova, and E. I. Pichuzhkina, *Carbohydrate Res.*, 1975, **43**, 215.
[886] E. A. Ivanova, S. I. Panchenko, I. I. Kolodkina, and A. M. Yurkevich, *Zhur. obshchei Khim.*, 1975, **45**, 208 (*Chem. Abs.*, 1975, **82**, 156 599f).
[887] J. A. Rendleman, jun., and J. E. Hodge, *Carbohydrate Res.*, 1975, **44**, 155.
[888] R. W. Goulding, *J. Chromatog.*, 1975, **103**, 229.
[889] S. Hirano, Y. Akimoto, M. Ishigami, and Y. Koga, *Analyt. Biochem.*, 1975, **66**, 627.
[889a] S. Hirano, M. Ishigami, and Y. Ohe, *J. Biochem.* (*Japan*), 1975, **78**, 421.
[890] M. Yaguchi, V. Daoust, and M. B. Perry, *Carbohydrate Res.*, 1975, **39**, 131.
[891] L. Hough, A. M. Y. Ko, and P. Wusteman, *Carbohydrate Res.*, 1975, **44**, 97.
[892] J. F. Robyt, *Carbohydrate Res.*, 1975, **40**, 373.

chromatographic separations of steroidal β-D-glucuronides and their esters have been achieved in non-polar solvent systems.[893, 894]

Thin-layer Chromatography.—Improved or more-rapid separations by t.l.c. have been reported for mono-, di-, tri- and oligo-saccharides,[895–897] naturally occurring pentitols and hexitols,[898] iridoid D-glucopyranosides,[899] methylated nucleosides,[900] and D-arabinosyl-, D-ribosyl-, and 2-deoxy-D-*erythro*-pentosyl-nucleotides.[901]

A simple system for the computerized storage of information obtained by t.l.c. and paper chromatography has been devised.[902]

High-pressure Liquid Chromatography.—Mixtures of D-galactose, D-fructose, D-ribose, D-xylose, and L-rhamnose have been separated by high-pressure liquid chromatography on a cation-exchange resin eluted with a 3 : 1-mixture of acetone and isopropyl alcohol.[903] Conditions have also been defined for the separation of starch hydrolysates, of mixtures of D-fructose, D-glucose, sucrose, melibiose, raffinose, *etc.*,[904, 905] and of oestrogen D-glucuronides (urinary steroid hormones).[906]

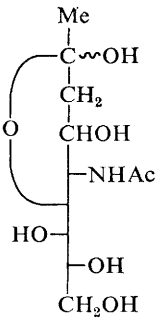
Other Analytical Methods

Titanium compounds have been shown to affect the measurement of D-glucose oxidase (and therefore D-glucose) by the D-glucose–D-glucose oxidase–peroxidase–2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) assay system.[907] A new automated assay specific for D-fructose has been devised, based on the chromophore produced by dehydration of D-fructose in hydrochloric acid.[908] New manual and automated methods have been reported for the determination of ω-deoxy-sugars (*e.g.* L-fucose and saccharinic acids), based on measurement of the acetaldehyde released by periodate oxidation;[909] for the specific determination of aldonic acids by consecutive reaction with periodate and 2,3,4-trihydroxybenzoic acid;[910] and for the determination of glyoxylic acid from aldonic acids and of pyruvic acid from 2-*C*-methylaldonic acids, which allow their determination in admixture.[911] Measurement of the glyoxylic acid released by oxidation with periodate has also been used to establish the substitution pattern of *O*-glycosylaldonic acids in eluates from anion-exchange resins; 3-*O*-substituted aldonic acids do not give glyoxylic acid on oxidation with periodate, in contrast to 4-

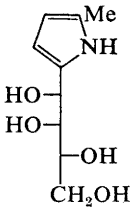
[893] V. R. Mattox, J. E. Goodrich, and R. D. Litwiller, *J. Chromatog.*, 1975, **108**, 23.
[894] V. R. Mattox, R. D. Litwiller, and J. E. Goodrich, *J. Chromatog.*, 1975, **109**, 129.
[895] R. A. de Zeeuw and G. G. Dull, *J. Chromatog.*, 1975, **110**, 279.
[896] S. A. Hansen, *J. Chromatog.*, 1975, **105**, 388.
[897] S. A. Hansen, *J. Chromatog.*, 1975, **107**, 224.
[898] B. P. Kremer, *J. Chromatog.*, 1975, **110**, 171.
[899] T. J. Danielson, E. M. Hawes, and C. A. Bliss, *J. Chromatog.*, 1975, **103**, 216.
[900] T. W. Munns and H. F. Sims, *J. Chromatog.*, 1975, **111**, 403.
[901] P. M. Schwartz and J. C. Drach, *J. Chromatog.*, 1975, **106**, 200.
[902] I. M. Jakovljevic and R. H. Bishara, *J. Chromatog.*, 1975, **110**, 398.
[903] W. Funasaka, T. Hanai, and K. Fujimura, *J. Chromatog. Sci.*, 1974, **12**, 517.
[904] J. C. Linden and C. L. Lawhead, *J. Chromatog.*, 1975, **105**, 125.
[905] H. Hyakutake and T. Hanai, *J. Chromatog.*, 1975, **108**, 385.
[906] Sj. van der Wal and J. F. K. Huber, *J. Chromatog.*, 1974, **102**, 353.
[907] J. F. Kennedy and I. M. Kay, *Carbohydrate Res.*, 1975, **44**, 291.
[908] J. F. Kennedy and M. F. Chaplin, *Carbohydrate Res.*, 1975, **40**, 227.
[909] R. G. Jones and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 162.
[910] R. G. Jones, A. R. Law, and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 209.
[911] R. G. Jones, A. R. Law, and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 15.

and 6-*O*-substituted aldonic acids.[910] A gravimetric version of a published procedure for the determination of uronic acids by decarboxylation has been reported.[912]

A specific, colorimetric determination of 2-acetamido-2-deoxy-D-galactose is based on its reaction with pentane-2,4-dione under anhydrous basic conditions to give a product identified as (458).[913] Mild alkaline hydrolysis of (458) yields 2-methylpyrrole, which can be determined by the chromophore (λ_{max} 548 nm)



(458)



(459)

produced with acidified 4-(dimethylamino)benzaldehyde (Ehrlich reagent); alternatively, under the acidic conditions of the last reaction (458) is hydrolysed to (459), which then produces a chromophore (λ_{max} 525 nm) on reaction with 4-(dimethylamino)benzaldehyde. 2-Acetamido-2-deoxy-D-glucose did not produce a chromophore when similarly treated. Methods for the assay of 2-amino-2-deoxy-D-glucose in the presence of primary amines have been evaluated.[914]

Trimethylated methyl D-glucopyranosides obtained by methanolysis of methylated D-glucans have been identified by oxidation of the unsubstituted hydroxy-groups with DMSO–phosphorus pentoxide and conversion of the resulting glycopyranosiduloses into their 2,4-dinitrophenylhydrazones, which could be measured spectrophotometrically after chromatographic separation.[915]

Periodate ion has been determined by the formation of a chromophore with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)[916] and by means of an iodide-selective electrode.[917] The 2,4-dinitrophenylhydrazones derived from aldehydic fragments produced on periodate oxidation of carbohydrates have been separated by t.l.c., after which the individual hydrazones could be determined spectrophotometrically.[918]

New reagents have been recommended for the detection on chromatograms of non-reducing disaccharides (sodium periodate–silver nitrate in aqueous

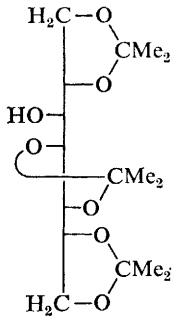
[912] A. E. Castagne and I. R. Siddiqui, *Carbohydrate Res.*, 1975, **42**, 382.
[913] F. Serafini-Cessi, *Biochem. J.*, 1975, **149**, 513.
[914] R. L. Benson, *Carbohydrate Res.*, 1975, **42**, 192.
[915] N. Kashimura, K. Yoshida, and K. Onodera, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1725.
[916] G. Mahuzier, B. S. Kirkacharian, and C. Harfouche-Obeika, *Analyt. Chim. Acta*, 1975, **76**, 79.
[917] S. Honda, K. Sudo, K. Kakehi, and K. Takiura, *Analyt. Chim. Acta*, 1975, **77**, 274.
[918] S. Honda, K. Kakehi, and K. Takiura, *Analyt. Chim. Acta*, 1975, **77**, 125.

acetone followed by a solution of sodium hydroxide),[919] trehalose (by incubation with trehalase),[920] and glycosides of mandelonitrile (2,3,5-triphenyltetrazolium hydroxide [921] and hydroxylamine–ferric chloride [922]).

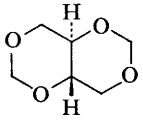
[919] T. Yamada, M. Hisamatsu, and M. Taki, *J. Chromatog.*, 1975, **103**, 390.
[920] G. Caldes and B. Prescott, *J. Chromatog.*, 1975, **111**, 466.
[921] U. Schwarzmaier, *J. Chromatog.*, 1975, **114**, 235.
[922] U. Schwarzmaier, *J. Chromatog.*, 1975, **111**, 430.

27
Alditols

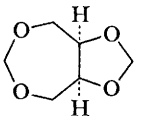
One of the two products formed on acetonation of D-*glycero*-D-*galacto*-heptitol (perseitol) has been identified as the 1,2:4,5:6,7-tri-*O*-isopropylidene derivative (460) [12] (see also Scheme 2). Acid-catalysed monobutylidenation of 1-deoxy-D-galactitol (L-fucitol) yielded the diastereoisomeric 2,3-acetals under kinetic control, whereas the 4,6-acetal is favoured under conditions of thermodynamic control; a small proportion of the 4,5-acetals was also isolated.[923] Acid-catalysed



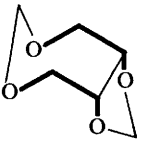
(460)



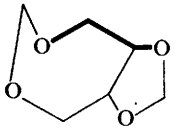
(461)



(462)



(463)



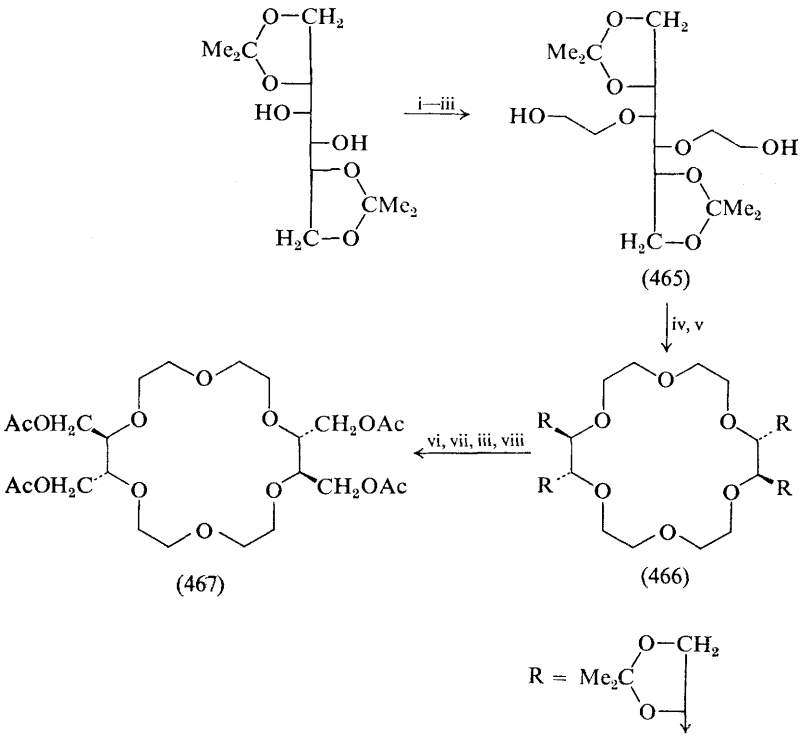
(464)

methylenation of erythritol furnished 1,3:2,4- and 1,4:2,3-di-*O*-methylene-erythritol [(461) and (462), respectively] and a small proportion of 1,4-anhydro-2,3-*O*-methylene-erythritol; constitutional assignments made to the diacetals were based on their ^{1}H n.m.r. and mass spectra.[200] Acid-catalysed equilibration of the diacetals (461) and (462) indicated that there is a small free-energy difference of 1.37 kcal mol^{-1} in favour of the former at room temperature; thus, it appears that the *gauche* oxygen–oxygen interactions in the *gauche, gauche* (463) and *anti, gauche* (464) conformations of the 1,4:2,3-diacetal (462) do not result in any appreciable stabilization of this isomer under the equilibrium conditions used (*cf.* Vol. 7, p. 198).

Configurationally chiral cryptands have been synthesized from carbohydrate precursors; *e.g.* 1,2:5,6-di-*O*-isopropylidene-D-mannitol was transformed into

[923] T. G. Bonner, E. J. Bourne, D. Lewis, and L. Yüceer, *J.C.S. Perkin I*, 1975, 1323.

the DD-cryptand (467) by the sequence of reactions outlined in Scheme 150.[924] The chiral host (466) showed differences in its complexation of enantiomeric guests [*e.g.* (±)-(*RS*)-α-phenylethylammonium hexafluorophosphate].



Reagents: i, CH_2=$CHCH_2Br$; ii, O_3; iii, $NaBH_4$; iv, TsCl–py; v, (465)–NaH–DMSO; vi, H^+; vii, $NaIO_4$; viii, Ac_2O–py

Scheme 150

Erythritol, L-arabinitol, and D-mannitol reacted with 2,4,6-tri-isopropylbenzenesulphonyl chloride (*i.e.* a bulky sulphonylating reagent) to give the corresponding αω-disulphonates, whereas ribitol, xylitol, allitol, and D-glucitol afforded 1,4-anhydro-ω-sulphonates.[251] The reaction of galactitol with 2,4,6-tri-isopropylbenzenesulphonyl chloride gave 1,6-di-*O*-(2,4,6-tri-isopropylbenzenesulphonyl)galactitol and 1,4-anhydro-6-*O*-(2,4,6-tri-isopropylbenzenesulphonyl)-DL-galactitol.

The synthesis of 5-(tetra-*O*-acetyl-L-*arabino*-tetritol-1-yl)tetrazole, *etc.*, by reaction of acetylated aldononitriles with ammonium azide has been noted in Chapter 10.[344] Related pentitol-1-yl heterocyclic compounds have been prepared

[924] W. D. Curtis, D. A. Laidler, J. F. Stoddart, and G. H. Jones, *J.C.S. Chem. Comm.*, 1975, 833, 835.

from 2,3:4,5-di-*O*-isopropylidene-L-arabinose [925] and 2,3:5,6-di-*O*-cyclohexylidene-D-mannono-1,4-lactone.[926]

The properties, synthesis, stability, and metabolism of 1,4:3,6-dianhydro-D-glucitol dinitrate have been discussed.[927] The i.r. and Raman spectra of powdered 1,5-anhydro-xylitol, -ribitol, and -L-arabinitol have been reported; Raman spectra were also recorded for solutions of the anhydro-sugars in water and in [$^2H_6$]DMSO.[928]

Conductometric measurements have indicated that neither D-glucitol nor D-mannitol forms a complex with the hydroxides of alkali and alkaline-earth metals;[490] this is in contrast to recent reports (see Vol. 8, pp. 123 and 188) of the binding of alditols with alkaline-earth metal cations. The extent of complexation of alditols with the Pr^{3+} cation in aqueous solution has been assessed by ^1H n.m.r. spectroscopy.[929] The following configurational sequence of tridentate ligands with the Pr^{3+} cation was established: *xylo* > *threo* > *arabino*(*lyxo*) > *glycero* > *ribo*. Secondary hydroxy-groups form complexes more readily than primary hydroxy-groups; and *gauche*- or 1,3-interactions introduced on complexation decrease the stability of the complex.

[925] Yu. A. Zhdanov, V. G. Alekseeva, V. N. Fomina, and V. N. Mirnyi, *Doklady Akad. Nauk S.S.S.R.*, 1975, **221**, 100 (*Chem. Abs.*, 1975, **83**, 43 646w).

[926] Yu. A. Zhadanov, V. G. Alekseeva, and V. N. Fomina, *Doklady Akad. Nauk S.S.S.R.*, 1974, **219**, 867 (*Chem. Abs.*, 1975, **82**, 125 542c).

[927] L. A. Silvieri and N. J. Deangelis, *Analyt. Profiles Drug Subst.*, 1975, **4**, 225 (*Chem. Abs.*, 1975, **83**, 136 765z).

[928] L. J. Pitzner and R. H. Atalla, *Spectrochim. Acta*, 1975, **31A**, 911.

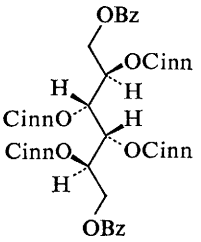
[929] A. P. G. Kieboom, T. Spoormaker, A. Sinnema, J. M. van der Toorn, and H. van Bekkum, *Rec. Trav. chim.*, 1975, **94**, 53.

28

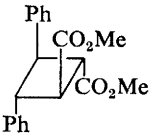
The Synthesis of Optically Active Non-carbohydrate Compounds

The use of derivatives of L-ascorbic acid in attempts to synthesize the naturally occurring spirodilactones leucodrin and conocarpin is noted in Chapter 17.[485]

Efficient asymmetric induction has been accomplished through the photoaddition reactions of residues linked to D-mannitol as the chiral agent; thus, the tetracinnamate derivative (468) underwent smooth intramolecular ($2\pi + 2\pi$) photocycloaddition to give cyclobutanes having the truxinic acid (head-to-head)



(468) Cinn = PhCH $\overset{t}{=}$CHCO



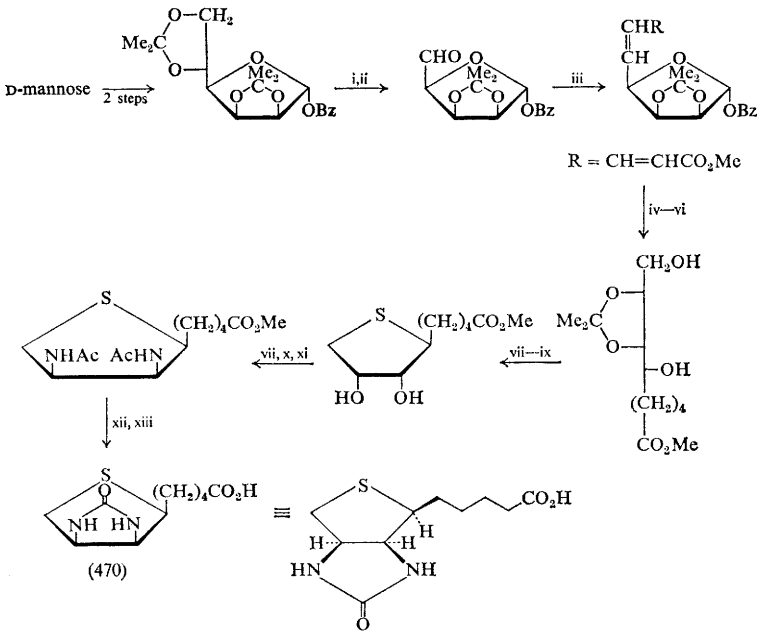
(469)

constitution.[930] The chiral reagent used to induce asymmetry was readily removed by ester exchange with methanol to yield, *inter alia*, optically active dimethyl δ-truxinate (+)-(469).

The stereospecific total synthesis of (+)-biotin (470) has been achieved from D-mannose by the route shown in Scheme 151.[931] Canadian[932] and Japanese[933] groups have independently established the absolute configuration of the naturally occurring antifungal compound avenaciolide (471) isolated from *Asperigillus avenaceus*. The route used by Anderson and Fraser-Reid[932] is illustrated in Scheme 152, and that used by the Japanese workers[933] follows closely similar lines.

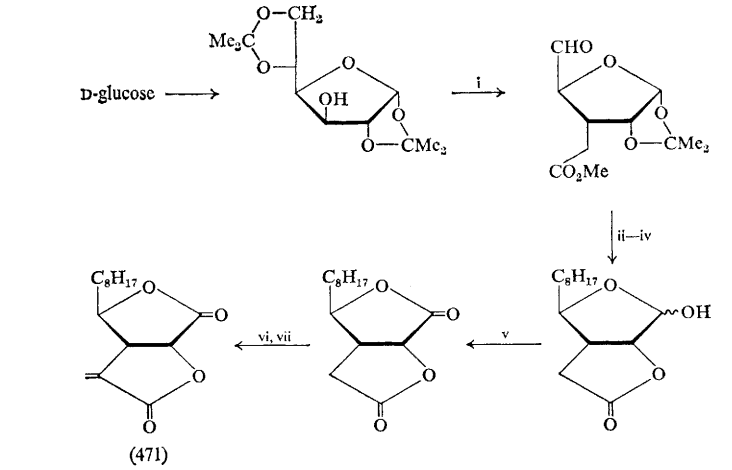
Furanoid derivatives have provided convenient starting materials for the synthesis of 11-oxaprostaglandins (see also Vol. 8, p. 36); one[934] of the two[935] routes to 11-oxaprostaglandins recently reported by Lourens and Koekemoer is outlined in Scheme 153.

[930] B. S. Green, Y. Rabinsohn, and M. Rejtö, *Carbohydrate Res.*, 1975, **45**, 115.
[931] H. Ohrui and S. Emoto, *Tetrahedron Letters*, 1975, 2765.
[932] R. C. Anderson and B. Fraser-Reid, *J. Amer. Chem. Soc.*, 1975, **97**, 3870.
[933] H. Ohrui and S. Emoto, *Tetrahedron Letters*, 1975, 3657.
[934] G. J. Lourens and J. M. Koekemoer, *Tetrahedron Letters*, 1975, 3715.
[935] G. J. Lourens and J. M. Koekemoer, *Tetrahedron Letters*, 1975, 3719.



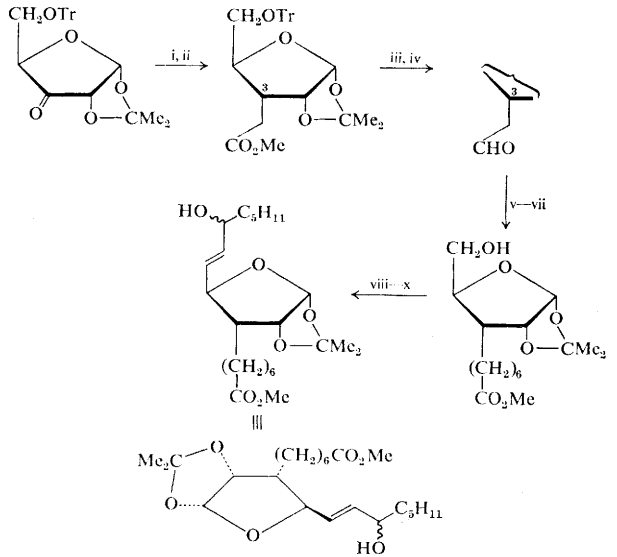
Reagents: i, H_3O^+; ii, $NaIO_4$; iii, $Ph_3P{=}CHR$; iv, H_2–Pd; v, MeONa–MeOH; vi, $NaBH_4$; vii, MsCl–py; viii, Na_2S–HMPT; ix, aq. HCO_2H; x, NaN_3–HMPT; xi, H_2–Pt–MeOH–Ac_2O; xii, $Ba(OH)_2$; xiii, $COCl_2$

Scheme 151



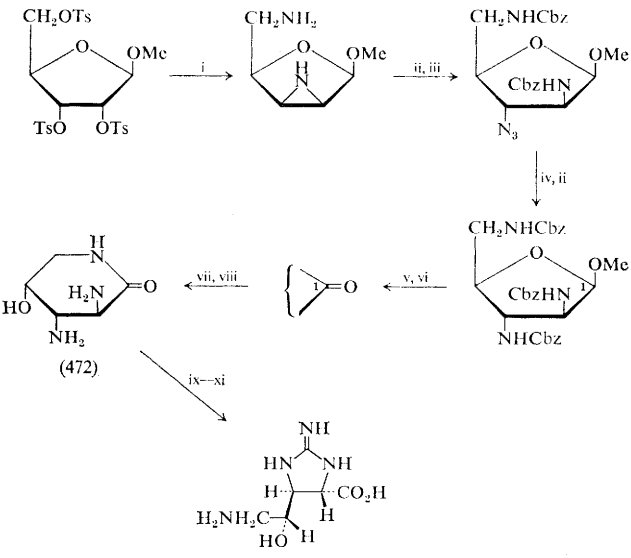
Reagents: i, Vol. 3, p. 125; ii, $C_7H_{15}P^+Ph_3Br^-$–THF–BuLi; iii, H_2–Pd–C; iv, H^+; v, CrO_3–Me_2CO; vi, $MeO_2COMgOMe$; vii, HCHO–Et_2NH–AcOH

Scheme 152



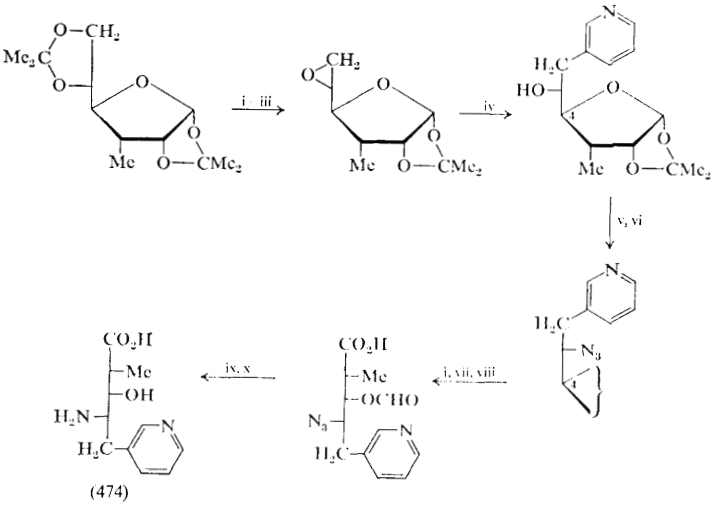
Reagents: i, $(MeO)_2P(O)CH_2CO_2Me$; ii, H_2–Ni; iii, $LiAlH_4$–THF; iv, CrO_3–py; v, $Ph_3P{=}CH(CH_2)_3CO_2Na$; vi, H_2–Pt–AcOH; vii, CH_2N_2–Et_2O; viii, DMSO–DCC–CF_3CO_2H; ix, $(MeO)_2P(O)\bar{C}HCOC_5H_{11}Na^+$; x, $Zn(BH_4)_2$

Scheme 153



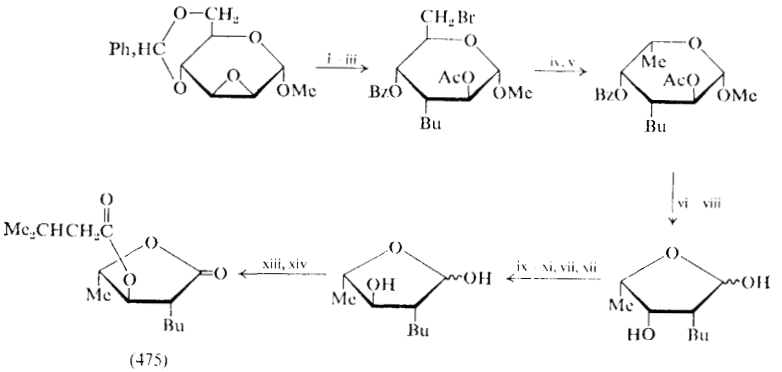
Reagents: i, Vol. 3, p. 87; ii, BnOCOCl(CbzCl); iii, NaN_3–DMF; iv, H_2–Pd; v, TsOH–H_2O–dioxan; vi, CrO_3–AcOH; vii, HBr–AcOH; viii, NaOH; ix, BrCN; x, HCl; xi, Dowex-50(NH_4^+) resin

Scheme 154



Reagents: i, AcOH; ii, TsCl–py; iii, base; iv, C_5H_4NLi–Et_2O; v, MsCl–py; vi, NaN_3; vii, $NaIO_4$; viii, Br_2–H_2O; ix, H_3O^+; x, Pd–H_2

Scheme 155



Reagents: i, BuMgCl; ii, Ac_2O–py; iii, NBS–CCl_4; iv, AgF–py; v, H_2–Pd; vi, Ac_2O–AcOH–H_2SO_4; vii, MeONa; viii, $NaIO_4$; ix, MeOH–HCl; x, MsCl–py; xi, NaOBz–DMF; xii, H_3O^+; xiii, Br_2–H_2O; xiv, $(Me_2CHCH_2CO)_2OCl$–py

Scheme 156

Streptolidine (473), a guanidinoamino-acid that occurs as a component of the streptothricin group of antibiotics, has been synthesized by way of the lactam form (472) of (2*S*, 3*S*, 4*R*)-2,3,5-triamino-4-hydroxypentanoic acid (Scheme 154).[936] (2*R*, 3*S*, 4*S*)-4-Amino-3-hydroxy-2-methyl-5-(pyrid-3-yl)pentanoic acid (474), a component of the antibiotic pyridomycin, has been derived from 3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-allofuranose by the sequence of reactions outlined in Scheme 155.[937]

[936] S. Kusumoto, S. Tsuji, and T. Shiba, *Bull. Chem. Soc. Japan*, 1974, **47**, 2690.
[937] M. Kinoshita and S. Mariyama, *Bull. Chem. Soc. Japan*, 1975, **48**, 2081.

A stereospecific synthesis of naturally occurring (+)-blastmycinone (475) is shown in Scheme 156; (2*R*)-diastereoisomers of the natural product were also prepared from the same starting material.[938]

[938] S. Aburaki, N. Konishi, and M. Kinoshita, *Bull. Chem. Soc. Japan*, 1975, **48**, 1254.

Part II

MACROMOLECULES

1
Introduction

The format of Part II differs little from that used in previous Reports.

A few changes have been made in Chapter 5 (Glycoproteins, *etc.*), where the biosynthesis of glycosaminoglycans is considered separately from that of other glycoproteins. Cell-surface glycoproteins are also dealt with under a separate heading. A notable trend over the past year has been the interpretation of biological phenomena at a molecular level. For example, the three-dimensional structure of concanavalin A has been determined, so that rationalizations of the binding mechanisms of lectins at a molecular level are in prospect; and more insight has been gained into the phosphorylation mechanisms that control the turnover of glycogen *in vivo*.

Increasing use is being made of affinity chromatography in the isolation and purification of enzymes (Chapter 6), and many of the matrices used are based on polysaccharides (Chapter 8). Several new enzymes have been reported over the past year; these include sulphatases and enzymes (*e.g.* phosphohexosidases) that cleave units in derivatized form. As in previous Reports, the authors' designation of the enzyme is given, although the full specificity of the enzyme may not be known. It is also pertinent to note that the E.C. numbering system assigns only one number of each enzyme, although it is becoming increasingly apparent that there may be two or more variants of a particular enzyme. An index of the enzymes reported appears at the end of Chapter 6.

The term 'glycanase' has been used instead of the term 'glycosidase' to describe enzymes that are specific for polysaccharides, irrespective of whether they act by an *endo* or *exo* process. The classification of enzymes described as *exo*-β-D-acetamidodeoxyhexosidases is difficult, since there is often no information to indicate whether or not the enzyme hydrolyses polysaccharides, *etc.* References to such enzymes are included in the section on glycoside hydrolases. In previous Reports, enzymes effecting the hydrolysis of D-fructosidic linkages have been classified under the E.C. number 3.2.1.26, whereas they may now be found under the E.C. numbers 3.2.1.26 and 3.2.1.80. The unspecific term 'sialidase' has been replaced by the term 'neuraminidase' in this Report. The classification of *exo*- and *endo*-D-glucanases also presents a problem, since these enzymes often exhibit a wide range of specificities and action patterns. The E.C. numbering system has been used to classify these enzymes whenever possible, but the section on miscellaneous glucanases should also be consulted.

Many novel derivatives of polysaccharides and glycoproteins, *etc.*, prepared over the past year are reported in Chapter 8. Theoretical aspects of immobilized enzymes are also considered in some detail in this chapter. It is encouraging to note that industrial applications of immobilized enzymes have gained impetus during the past year or so.

2
General Methods

BY R. J. STURGEON

Analysis

The composition of polysaccharides has been determined quantitatively by acid hydrolysis and paper chromatography of the reduced (sodium borotritide) sugars.[1] Values for uronic acids were corrected for the amount of aldobiouronic acid measured. The quantitative analysis of sugars by the phenol–sulphuric acid method is affected by such impurities as nitrite, nitrate, and lactate ions.[2] D-Glucose liberated by hydrolysis of glucans containing α-(1 → 4)- and α-(1 → 6)-linkages with an acid amyloglucosidase has been estimated by the D-glucose oxidase procedure.[3] Pre-coated t.l.c. plates impregnated with sodium tetraborate have given good separations of monoglucosyl- and monogalactosyl-ceramides.[4] Solvent systems giving improved separations of gangliosides have been devised.[5] Quantitative analyses of the ester, amide, and uronate contents of pectins and alginates have been achieved by measurement of the corresponding absorption bands obtained from the i.r. spectra of solutions of the polymers in D_2O–phosphate buffers.[6] A continuous nephelometric procedure for the monitoring of glycosaminoglycans eluted from chromatographic columns has been reported.[7] Estimates of the molecular weights of mucopolysaccharides have been obtained by measurement of their electrophoretic mobilities on thin layers of Sephadex and cellulose.[8] Polysaccharide and glycoprotein fractions have been examined in the ultracentrifuge at equilibrium under conditions of meniscus-depletion; the data obtained have been used to evaluate qualitatively and semi-quantitatively the molecular-weight distribution functions.[9] A simple method has been reported for the determination of ^{35}S-sulphated polysaccharides.[10] After precipitation onto paper, sulphated glycosaminoglycans were determined separately by treatment with chondroitinase A, B, or C. A conductimetric method has been used for the determination of carboxy-groups and sulphate groups in heparin and other mucopolysaccharides.[11] A method for labelling acidic polysaccharides (*e.g.* the major cell-wall antigenic components) with ^{125}I has been reported; it has possible

[1] C. A. Knutson, *Carbohydrate Res.*, 1975, **43**, 225.
[2] T. Fujikawa, M. Shiomi, S. Mukai, and M. Wada, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 483.
[3] J. C. Murat and A. Serafty, *Clinical Chem.*, 1974, **20**, 1576.
[4] J. Robert and G. Rebel, *J. Chromatog.*, 1975, **110**, 393.
[5] K. Eberlein and G. Gercken, *J. Chromatog.*, 1975, **106**, 425.
[6] S. M. Bociek and D. Welti, *Carbohydrate Res.*, 1975, **42**, 217.
[7] K. Blau and C. S. Dodge, *Analyt. Biochem.*, 1974, **58**, 650.
[8] G. Tortolani and E. Romagnoli, *Experientia*, 1975, **31**, 389.
[9] R. A. Gibbons, S. N. Dixon, and D. H. Pocock, *Biochem. J.*, 1973, **135**, 649.
[10] A. Wasteson, K. Uthne, and B. Westmark, *Biochem. J.*, 1973, **136**, 1069.
[11] B. Casu and U. Gennaro, *Carbohydrate Res.*, 1975, **39**, 168.

applications in measuring the primary binding to antibodies.[12] A new reagent of the Schiff-type has been used to demonstrate the presence of DNA and polysaccharides.[13] An improved separation of di-, tri-, and oligo-saccharides present in starch hydrolysates by t.l.c. is obtained when lactic acid is included in the solvent system.[14, 15] Oligosaccharides containing D-glucose units linked in various ways have been separated by partition chromatography on ion-exchange resins.[16] A series of D-xylose-containing oligosaccharides (up to D-xylohexaose) were separated by ion-exchange chromatography in borate buffers after oxidation to the corresponding oligomeric aldonic acids with hypoiodite.[17] Strongly basic resins having a styrene-divinylbenzene matrix have been shown to be ideal for the chromatography of oligomeric aldonic acids.[18] Oligomers containing up to sixteen residues were separated on resins containing a low proportion of divinylbenzene. Satisfactory separation of silylated derivatives of disaccharides by g.l.c. has been achieved on open, tubular, glass capillary columns coated with liquid phases of low polarity.[19] Glutaraldehyde has been used to attach glucose oxidase, amyloglucosidase, invertase, and β-galactosidase, either separately or in combination, to the inside surface of nylon tubes; the enzyme systems were then used to estimate sucrose, lactose, and maltose.[20] The fractionation and identification of fatty acids and monosaccharides in cellular structures have been achieved by selective depolymerization by methanolysis, followed by separation of the derived trifluoroacetates by g.l.c.[21] G.l.c. data for butaneboronic esters and TMS derivatives of some monosaccharides and alditols have been described; mass spectroscopic data for butaneboronic esters of D-fructose, D-glucose, D-galactose, and D-mannose were also reported.[22]

Analyses of mixtures of common aldoses by g.l.c.–m.s. of their *O*-isopropylidene derivatives,[23] and of aldoses and polyols by g.l.c. of their polyacetates and aldononitrile acetates [24] have been reported. Variation of the counter-ion attached to a polystyrene-based, strong cation-exchanger has been shown to affect the chromatographic behaviour of sugars and polyols on the resin.[25] The separation of the components of several mixtures was improved appreciably by the choice of a suitable counter-ion. The automated determination of simple saccharides by ion-exchange chromatography of their borate complexes has been reported.[26] Conditions have been recorded for the separation of D-glucose, D-fructose, sucrose, melibiose, raffinose, and three isomeric ketoses by liquid chromato-

[12] J. Dawes and W. H. McBride, *Immunochemistry*, 1975, **12**, 855.
[13] R. Cogliati and A. Gautier, *Compt. rend.*, 1973, **277**, *D*, 387.
[14] S. A. Hansen, *J. Chromatog.*, 1974, **105**, 388.
[15] S. A. Hansen, *J. Chromatog.*, 1975, **107**, 224.
[16] J. Havlicek and O. Samuelson, *Analyt. Chem.*, 1975, **47**, 1854.
[17] Y. Sakai, K. Okawa, and Y. Kamiyama, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 545.
[18] J. Havlicek and O. Samuelson, *J. Chromatog.*, 1975, **114**, 383.
[19] S. Adam and W. G. Jennings, *J. Chromatog.*, 1975, **115**, 218.
[20] D. J. Inman and W. E. Hornby, *Biochem. J.*, 1974, **137**, 25.
[21] E. Jantzen, K. Bryn, and K. Bøvre, *Acta Path. Microbiol. Scand.* (*B*), 1974, **82**, 753.
[22] P. J. Wood, I. R. Siddiqui, and J. Weisz, *Carbohydrate Res.*, 1975, **42**, 1.
[23] S. Morgenlie, *Carbohydrate Res.*, 1975, **41**, 285.
[24] C. D. Pfaffenberger, J. Szafranek, M. G. Horning, and E. C. Horning, *Analyt. Biochem.*, 1975, **63**, 501.
[25] R. W. Goulding, *J. Chromatog.*, 1975, **103**, 229.
[26] E. F. Walborg, L. E. Kondo, and J. M. Robinson, in 'Methods in Enzymology', Vol. 41B, ed. W. A. Wood, Academic Press, New York, 1975, p. 10.

graphy.[27] The simultaneous assay of neutral sugars and amino-sugars has been reported by an automated procedure involving the use of ion-exchange chromatography.[28] A practical, high-speed liquid chromatographic procedure has been devised to separate components of the tricarboxylic acid cycle from monosaccharides.[29] Sulphate has been used as the counter-ion in a rapid separation of reducing sugars on ion-exchangers with neutral borate buffers; the sugars were determined by a new method using 4-hydroxybenzoic acid hydrazide as the colorimetric reagent.[30] The chromatography of carbohydrates on ion-exchange resins in the aluminate form has been investigated.[31] With water as the sole eluant, the retention volumes for carbohydrates on an aluminate-resin column were found to decrease in the order: ketoses > aldoses > alditols > methyl glycosides. Resins having a low hydroxide content were preferred for chromatographic separations, since the extent of alkaline degradation and interconversion of reducing sugars was reduced. Mono- and di-saccharides have been separated from each other using vapour-programmed t.l.c.; quantitation was obtained by *in situ* reflectance spectrometry.[32] A paper-chromatographic system that separates the commonly occurring monosaccharides from each other, and that also separates alditols from their parent monosaccharides, has been reported.[33] In a new fluorometric procedure, reducing sugars (4—400 nanomole) were determined by reaction with ethylenediamine sulphate without interference from either glycosides or neutral polysaccharides.[34] Fluorometric determinations of pentoses (with anthrone) and hexoses (with 5-hydroxy-1-tetralone) have been used in the analysis of the carbohydrates present in sea water.[35] Since the concentration of carbohydrates in sea water is low, it is necessary to separate them from other materials before they are assayed. Microdetermination of the individual neutral sugars, amino-sugars, and *N*-acetylneuraminic acid of glycoproteins and glycolipids has been achieved by reduction (sodium borotritide) of the monosaccharides present in acid hydrolysates and separation of the alditols as their borate complexes by paper electrophoresis.[36] The release and determination of neutral sugars and amino-sugars of glycoproteins have been studied, and colorimetric and g.l.c. methods of analysis have been compared.[37]

A kinetic determination of 2-ketohexoses has been based on their reactions with the cysteine–sulphuric acid reagent; sorbose gave a distinct absorption spectrum, whereas D-fructose, psicose, and tagatose were distinguished from each other by differences in the rate of increase of the absorbance at 412 nm.[38] Procedures used in colorimetric ultramicroassays of reducing sugars,[39] and

[27] J. C. Linden and C. L. Lawhead, *J. Chromatog.*, 1975, **105**, 125.
[28] G. Keilich and D. Ziegler, *Z. physiol. Chem.*, 1975, **356**, 417.
[29] H. Hyakutake and T. Hanai, *J. Chromatog.*, 1975, **108**, 385.
[30] A. M. C. Davies, D. S. Robinson, and R. Couchman, *J. Chromatog.*, 1974, **101**, 307.
[31] J. A. Rendleman and J. E. Hodge, *Carbohydrate Res.*, 1975, **44**, 155.
[32] R. A. de Zeeuw and G. G. Dull, *J. Chromatog.*, 1975, **110**, 279.
[33] J. F. Robyt, *Carbohydrate Res.*, 1975, **40**, 373.
[34] S. Honda, K. Kakimoto, K. Sudo, K. Kakehi, and K. Takiura, *Analyt. Chim. Acta*, 1974, **70**, 133.
[35] H. Hirayama, *Analyt. Chim. Acta*, 1974, **70**, 141.
[36] S. Takasaki and A. Kobata, *J. Biochem. (Japan)*, 1974, **76**, 783.
[37] A. E. Clarke, V. M. Maritz, J. Presser, and M. A. Denborough, *Biochem. Med.*, 1974, **9**, 342.
[38] D. J. Bissett, T. E. Hanson, and R. L. Anderson, *Microchem. J.*, 1974, **19**, 71.
[39] G. Avigad, in ref. 26, p. 24.

enzymic methods available for the estimation of D-glucose, D-galactose, D-fructose, D-mannose, L-fucose, and 2-amino-2-deoxy-D-glucose have been reported.[40] The chromophore produced by dehydration of D-fructose in hydrochloric acid has been used in the direct analysis of the ketose in the presence of D-glucose.[41] D-Fructose can be reduced quantitatively with D-mannitol dehydrogenase in the presence of high concentrations of D-glucose and sucrose.[42] The colour yield obtained when D-glucose reacts with the anthrone–sulphuric acid reagent is more than doubled in the presence of hydrochloric and formic acids; approximately equal colour yields were obtained from D-glucose and D-fructose by using suitable reaction times.[43] D-Glucose has been separated from the other components present in physiological fluids on thin layers of mixed anion- and cation-exchange resins.[44] A reflectance method for the determination of the levels of D-glucose in blood samples has been compared with the hexokinase method.[45] The sensitivity of the direct *o*-toluidine procedure for the measurement of D-glucose was increased by the exclusion of water and by the use of high concentrations of boric acid.[46] Good agreement has been achieved between the hexokinase method and Trinder's D-glucose oxidase method for the measurement of D-glucose in sera and urine.[47] A study of the latter method by the simplex technique demonstrated that the activity of D-glucose oxidase is the most crucial variable of the reagent. The direct analysis of D-glucose in whole blood samples has been achieved by a method that uses a mixed-bed reactor containing immobilized D-glucose oxidase.[48] Levels of D-glucose in blood samples have also been measured by means of the enzymic oxidation of D-glucose on a column of immobilized D-glucose oxidase; hydrogen peroxide formed in the reaction was determined with a mixed luminol–ferricyanide reagent, which produced chemiluminescence proportional to the sugar concentration.[49] This technique is suitable for the analysis of D-glucose in urine if uric acid is first removed. Titanium compounds have been found to affect the measurement of D-glucose by the D-glucose–D-glucose oxidase–peroxidase–2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) assay system.[50] The validity of measurement of the activity of D-glucose oxidase immobilized on supports based on titanium oxides was affected by complexation of the intermediate hydrogen peroxide with the support, perhaps resulting in the formation of titanic peroxides. D-Glucose has been estimated with the D-glucose oxidase–peroxidase system using 4-aminodimethylaniline to produce the chromogen.[51] An enzyme-based fluorometric method for the analysis of D-glucose in blood samples has been developed; the hexokinase–D-glucose 6-phosphate dehydrogenase reagents were lyophilized on silicone rubber pads, and existing spectrophotometric procedures were adapted

[40] H. Schachter, in ref. 26, p. 3.
[41] M. F. Chaplin and J. F. Kennedy, *Carbohydrate Res.*, 1975, **40**, 227.
[42] K. Yamanaka, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 371.
[43] M. A. Jermyn, *Analyt. Biochem.*, 1975, **68**, 332.
[44] D. L. Bloxam, *Biochem. Med.*, 1974, **10**, 61.
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[47] J. A. Lott and K. Turner, *Clinical Chem.*, 1975, **21**, 1754.
[48] W. Dritschilo and M. K. Weibel, *Biochem. Med.*, 1974, **9**, 32.
[49] D. T. Bostick and D. M. Hercules, *Analyt. Chem.*, 1975, **47**, 447.
[50] J. F. Kennedy and I. M. Kay, *Carbohydrate Res.*, 1975, **44**, 291.
[51] F. Ionescu-Stoian, *Rev. Roumaine Biochim.*, 1974, **11**, 25.

for the subsequent analysis of D-glucose.[52] Direct injection enthalpimetry has been applied to the determination of D-glucose in the hexokinase-catalysed reaction with ATP.[53] Microcalorimetric measurements on this reaction demonstrated a linear relationship between the measured heat and the concentration of D-glucose, enabling the method to be used in determining the concentration of D-glucose in human sera.[54] Methods of analysis for blood sugar have been compared.[55] ω-Deoxy-sugars have been determined by manual and automated procedures[56] that involve spectrophotometric measurement of the acetaldehyde released on periodate oxidation.

Conditions have been established for the quantitative recovery of 2-amino-2-deoxy-D-glucose and -D-galactose after the hydrolysis of glycoproteins with toluene-*p*-sulphonic acid.[57] In a procedure for the simultaneous determination of the neutral sugars and amino-sugars of glycoproteins by g.l.c., the amino-sugars were deaminated with nitrous acid and converted into the 2,5-anhydro-alditol acetates.[58] Methyl glycosides of amino-sugars have been converted into the corresponding *N*-acetylated derivatives prior to analysis by g.l.c.[59] A combination of g.l.c. and colorimetric procedures has been used to identify the amino-sugars present in connective tissues; after the amino-sugars had been converted into TMS derivatives, a two-phase extraction system was used to remove any amino-acids.[60] A modified procedure has been developed to enable 2-amino-2-deoxy-D-glucose to be converted into 2-acetamido-2-deoxy-D-glucose in the presence of other primary amines.[61] Ligand-exchange chromatography has been used in the separation of 2-amino-2-deoxy-D-glucose, -D-galactose, and -D-mannose on ion-exchange resins in either copper or nickel forms.[62] The u.v. absorption of the copper complexes eluted from the resin permitted the amino-sugars to be detected and measured.

Muramic acid has been determined quantitatively by a procedure that involves its degradation to acetaldehyde *via* lactic acid.[63] Acetaldehyde was measured spectrophotometrically, and other components present in hydrolysates of bacterial cell walls did not interfere with the determination. The analysis of natural and synthetic *N*- and *O*-acylated sialic acids by g.l.c. of the TMS ethers has been described.[64] The *O*-acyl groups were not lost when *N*-trimethylsilylimidazole was used as the silylating reagent. A quantitative determination of neuraminic acid by g.l.c.–m.s. (using multiple ion-detection) has been reported.[65] A modified periodate–resorcinol procedure, which avoids extraction of the

[52] S. W. Kiang, J. W. Kuan, and G. G. Guilbault, *Clinical Chem.*, 1975, **21**, 1799.
[53] C. D. McGlothlin and J. Jordan, *Analyt. Chem.*, 1975, **47**, 786.
[54] R. N. Goldberg, E. J. Prosen, B. R. Staples, R. N. Boyd, G. T. Armstrong, R. L. Berger, and D. S. Young, *Analyt. Biochem.*, 1975, **64**, 68.
[55] Y. Goto, T. Toyota, and Y. Ishitoya, *J. Exp. Med. (Japan)*, 1974, **112**, 279.
[56] R. G. Jones and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 162.
[57] A. K. Allen and A. Neuberger, *F.E.B.S. Letters*, 1975, **60**, 76.
[58] W. H. Porter, *Analyt. Biochem.*, 1975, **63**, 27.
[59] J. R. Etchison and J. J. Holland, *Analyt. Biochem.*, 1975, **66**, 87.
[60] K. G. Braund, P. Ghosh, T. K. F. Taylor, and L. H. Larsen, *Analyt. Biochem.*, 1975, **64**, 10.
[61] R. L. Benson, *Carbohydrate Res.*, 1975, **42**, 192.
[62] J. D. Navratil, E. Murgia, and H. F. Walton, *Analyt. Chem.*, 1975, **47**, 122.
[63] O. Hadžija, *Analyt. Biochem.*, 1974, **60**, 512.
[64] J. Casals-Stenzel, H. P. Buscher, and R. Schauer, *Analyt. Biochem.*, 1975, **65**, 507.
[65] I. Mononen and J. Kärkkäinen, *F.E.B.S. Letters*, 1975, **59**, 190.

chromogen with an organic solvent, has been applied to the automated analysis of bound *N*-acetylneuraminic acid in sera.[66] An automated assay for sialic acid has been devised by combining the resolving power of ion-exchange chromatography with the specificity and sensitivity of the thiobarbituric acid system.[67] D-Glucose has been shown to suppress the reaction of 2-deoxy-D-*erythro*-pentose in the determination of sialic acid with thiobarbituric acid; D-glucose apparently competes with the deoxypentose for periodate ions.[68] A manual procedure for measuring sialic acid in blood and tissues and an automated procedure for measuring sialic acid in blood have been combined and modified in order that the concentration of sialic acid in both blood and tissues can be determined by continuous flow analysis.[69] The claim that an *o*-phenanthroline reagent can be used in the determination of sialic acid has been invalidated; changes in the absorbance at 307 nm are caused by variation of the pH of the medium rather than by the formation of complexes.[70] A method for the quantitative analysis of 2-keto-3-deoxyoctonate (KDO) and 3,6-dideoxyhexoses is based on assays by periodate–thiobarbiturate before and after reduction with sodium borohydride.[71]

Detailed procedures for the colorimetric determination of hexuronic acids have been reported,[72] and a micromethod for the estimation of non-conjugated uronic acids relies on their fluorescence in the presence of ethylenediamine sulphate;[73] glycosidically linked uronic acids were not hydrolysed under the neutral conditions of the reaction. A decarboxylation procedure for the determination of uronic acids has been modified to allow the carbon dioxide evolved to be measured gravimetrically.[74] Several mono-, di-, and tri-*O*-methyl ethers of methyl (methyl α-D-glucopyranosid)uronates have been separated by g.l.c., and separations of the corresponding TMS, trifluoroacetyl, and acetyl derivatives were also achieved.[75] Uronic acids have been identified by g.l.c. after conversion into the corresponding aldonic acid butaneboronates; aldoses normally found with uronic acids in polysaccharide hydrolysates can also be separated and measured by this procedure.[76] Manual and automated determinations have been described for aldonic acids and *O*-glycosyl derivatives thereof; the acid is oxidized with periodate ion and the resulting glyoxylic acid is determined with 2,3,4-trihydroxybenzoic acid.[77] Glyoxylic acid was shown to interfere with the determination of pyruvic acid by the lactate dehydrogenase procedure, but it did not interfere in a procedure using salicylaldehyde.[78]

Non-reducing mono- and oligo-saccharides have been detected on paper chromatograms by a periodate–silver nitrate reagent.[79] A number of solvent

[66] E. Rey, L. Gerbaut, and C. Lombart, *Clinical Chem.*, 1975, **21**, 412.
[67] M. J. Krantz and Y. C. Lee, *Analyt. Biochem.*, 1975, **63**, 464.
[68] C. H. Smith, T. M. Donohue, and R. Depper, *Analyt. Biochem.*, 1975, **67**, 290.
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[70] S. L. Snyder, N. S. Mathewson, and P. Z. Sobocinski, *Clinical Chem.*, 1974, **20**, 387.
[71] T. I. Burtseva, L. I. Glebko, and Y. S. Ovodov, *Analyt. Biochem.*, 1975, **64**, 1.
[72] G. Avigad, in ref. 26, p. 29.
[73] S. Honda, K. Sudo, K. Kakehi, H. Yuki, and K. Takiura, *Analyt. Chim. Acta*, 1975, **77**, 199.
[74] A. E. Castagne and I. R. Siddiqui, *Carbohydrate Res.*, 1975, **42**, 382.
[75] D. Anderle and P. Kováč, *J. Chromatog.*, 1974, **91**, 463.
[76] F. Eisenberg, *Analyt. Biochem.*, 1974, **60**, 181.
[77] R. G. Jones, A. R. Law, and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 209.
[78] R. G. Jones, A. R. Law, and P. J. Somers, *Carbohydrate Res.*, 1975, **42**, 15.
[79] T. Yamada, M. Hisamatsu, and M. Taki, *J. Chromatog.*, 1975, **103**, 390.

systems have been reported for use in the efficient separation of polyols.[80, 81] D-Glucitol and D-mannitol in pharmaceuticals have been separated and determined by g.l.c. of their tri-n-butylbisboronic esters.[82] The accuracy of an enzyme-based fluorometric method for the estimation of D-glucitol has been assessed; the results underline the difficulties and limitations of determining this hexitol in nanomolar quantities.[83] A rapid, automated method has been reported for the analysis of mixtures of neutral and aminodeoxy-alditols on an ion-exchange resin in the borate form.[84]

New qualitative tests have been reported for the detection of iodate and periodate ions based on their reactions with *o*-phenylenediamine, thiourea, and sodium sulphite.[85] A potentiometric method for the determination of sodium metaperiodate has used an iodide-selective electrode.[86] A ^{1}H n.m.r. spectroscopic method for the direct determination of formic acid liberated on oxidation of carbohydrates with periodate was shown to be accurate for 10—100 μmol of acid.[87] Glyoxal and hydroxyaldehydes formed on periodate oxidation of carbohydrates have been separated by t.l.c.; the separated components were then determined by spectrophotometric measurement of their 2,4-dinitrophenylhydrazones.[88] A convenient method for the analysis of such fragments by liquid chromatography on small open columns was also developed. Free glycolaldehyde has been determined spectrophotometrically by reaction with 3-methyl-2-benzthiazolehydrazine and conversion of the resulting hydrazone into an osazone with 2,4-dinitrophenylhydrazine.[89] A sensitive colorimetric method for the determination of formaldehyde is based on its reaction with tryptophan in the presence of sulphuric acid and iron, nickel, or cobalt salts.[90] An automated spectrofluorometric determination of formaldehyde as 3,5-diacetyl-1,4-dihydro-2,6-dimethylpyridine has been described.[91] The method was used in determinations of serine and cysteine, and for monitoring the fractions derived from glycoproteins and peptides.

The relative rates of reaction of various carbohydrases have been measured using an oscillating capillary-tube recording viscometer.[92] A regression analysis of the curves obtained from complete reactions gave the equation for each curve and allowed the rate of reaction to be defined. A new method for monitoring the activity of certain carbohydrases, which are normally assayed by means of reducing sugars, has been used to locate carbohydrase activity in eluates from chromatographic columns.[93]

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Structural Methods

The application of enzymic methods to the structural analysis of polysaccharides has been reviewed.[94] The second part of a review on the application of g.l.c. to carbohydrates includes details of methods of methylation, and has collected m.s. data for TMS, acetyl, trifluoroacetyl, and methyl derivatives of sugars.[95] The high reactivity of methyl trifluoromethanesulphonate (methyl triflate) makes it a suitable reagent for methylation of carbohydrates under mild conditions.[96] Since the high reactivity of this triflate limits the choice of solvent for the methylation, hydrophilic carbohydrates cannot normally be methylated by this procedure.

A new strategy for the modification of polysaccharides containing uronic acid residues has been applied to *Klebsiella* type 47 capsular polysaccharide, which contains side-chains of 4-*O*-α-L-rhamnopyranosyl-D-glucopyranosyluronic acid attached to the main chain.[97] Hydroxy- and carboxy-groups were first substituted by treatment with methyl vinyl ether, after which the substituent at C-4 of the uronic acid residues was eliminated with base. Removal of the modified uronic acid residues and protecting groups with acid furnished an essentially linear polysaccharide. 1,2-Cyclopentanedione has been identified as a decomposition product of the alkaline degradation of reducing carbohydrates substituted at C-3 or C-4.[98] A series of deoxyaldaric acids corresponding in structure to the 3-deoxy-2-*C*-(hydroxymethyl)aldonic (isosaccharinic) acids was formed when hydrocellulose and xylan were treated with alkali or oxygenated alkali.[99] Thus, degradation of hydrocellulose produced quantities of 2-methyltartronic, 2-(2-hydroxyethyl)tartronic, 2-deoxy-3-*C*-(hydroxymethyl)tetraric, 2-(2,3-dihydroxypropyl)tetronic, 3-deoxy-2-*C*-hydroxymethyl-*erythro*-pentaric, and 3-deoxy-2-*C*-hydroxymethyl-*threo*-pentaric acids. A 5-deoxy-5-nitromaltitol derivative (synthesized from maltose) has been used as a model compound in a study of the selective cleavage of a (1 → 4)-glycosidic bond at the reducing-end residue of methylated oligosaccharides.[100] Treatment of this compound with alkali resulted in cleavage of 87% of the glycosidic bonds. The m.s. of the TMS derivatives of 2-*O*-β-D-glucopyranosyl-D-erythronic, 3-*O*-β-D-glucopyranosyl-D-arabinonic, and 4-*O*-β-D-glucopyranosyl-D-gluconic acids have been recorded for use in studies of the oxidative, alkaline degradation of oligosaccharides.[101]

Mass spectral data for the primary fragmentations of partially ethylated alditol acetates have been tabulated so as to allow easy reference in the identification of these polysaccharide derivatives.[102] A newly developed, highly polar, capillary column gave improved separations of many ethylated alditol acetates when compared with the corresponding methylated derivatives.[103] Results have been presented which demonstrate that the molar flame-responses of partially methylated and partially ethylated alditol acetates should be calculated on the basis of

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their effective carbon responses.[104] The m.s. of a number of permethylated oligosaccharides containing aldohexose units only or at least one D-fructose unit have been determined.[105] It was concluded that the m.s. of these derivatives can be used to detect D-fructose units and to provide information on the ratio of pyranose to furanose units and the positions of the glycosidic linkages. A scheme for isolating the individual methyl ethers obtained by partial methylation of methyl glycopyranosides has been reported.[106] Useful information on the methylation–fragmentation analysis of mannans has been recorded in a study of the g.l.c.–m.s. patterns of methylated and deuteriomethylated *O*-acetylaldononitriles of D-mannose.[107]

The analysis of 2-acetamido-2-deoxyaldoses by g.l.c.–m.s. of the TMS-*O*-methyloximes, and conditions for the separation of these derivatives by g.l.c. have been reported.[108] The TMS derivatives of 2-acetamido-2-deoxy-D-aldoses have been identified by application of a pattern-recognition procedure to their mass spectra.[109] 2-Amino-2,6-dideoxy-D-glucose(quinovosamine) and -D-galactose(fucosamine) and methyl ethers thereof have been separated on a microscale by ion-exchange chromatography in order to assist in the identification of 2-amino-2,6-dideoxyhexoses present in lipopolysaccharides.[110] A number of methyl (methyl 4-deoxy-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronates (identified by g.l.c.–m.s. of the TMS derivatives) have been found among the products of methylation of conjugates containing D-glucuronic and D-galacturonic acids.[111] The main fragmentation mechanism for methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate has been deduced using trideuteriomethylated derivatives and by the measurement of metastable transitions.[112] Lithium in ethylamine has been used for the demethylation of methylated sugars without cleavage of the glycosidic bond.[113]

A review on the use of ^{13}C n.m.r. spectroscopy in deducing the conformations of biological molecules includes applications of the technique to polysaccharides.[114] A structural analysis of polysaccharides has been reported in which the methylated sugars are identified by ^{1}H n.m.r. spectroscopy; the method was demonstrated with an α-(1 → 3)-linked D-glucan and glycogen.[115] The ^{1}H and ^{13}C n.m.r. spectra of permethylated D-gluco-, D-galacto-, and D-manno-pyranosides and their 6-deoxy analogues have been analysed with the aid of specific trideuteriomethylation, heteronuclear spin-decoupling, and spectrum simulation.[116] Comparison of spectral data for the aldohexose derivatives and their 6-deoxy

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analogues showed that the ring conformation is not significantly affected by the presence or absence of a methoxy-group at C-6. Changes in the orientation of the methoxy-groups have perceptible effects on the chemical shifts of carbons and protons of the pyranoid rings and of the methoxy-groups. An assessment has been made of various techniques used in assigning signals in the ^{13}C n.m.r. spectra of polysaccharides, and of their possible interpretation in terms of chemical structure.[117] Gadolinium (Gd^{3+}) ions have been shown to interact selectively with aldohexuronic acids and poly(glycosiduronic acids), thereby modifying the relaxation pathways of the ^{1}H and ^{13}C nuclei.[118] Lanthanide shift reagents have also been used to increase the dispersion of the ^{1}H n.m.r. spectra of permethyl ethers of D-galactose.[119] ^{31}P N.m.r. spectra for a number of purified yeast *O*-phosphonohexoglycans have been recorded.[120] The data were correlated with established aspects of individual and collective polymer structures, permitting conclusions to be drawn regarding the chemical environment of the phosphate groups and the assignment of anomeric configurations to the hexosyl phosphate residues.

A g.l.c. procedure that establishes the DP of oligo- and poly-saccharides has been developed.[121] The oligo(poly)saccharide was reduced prior to hydrolysis and conversion of the reducing sugars liberated into their oxime derivatives; the DP was determined from the ratio of acetylated aldononitrile to acetylated alditol. A similar technique, which determined the ratio of aldose acetate to alditol acetate, was used to measure the DP of amylose.[122]

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3
Plant and Algal Polysaccharides

BY R. J. STURGEON

Starch

Percolation with perchloric acid has been used instead of maceration for extracting starch from small amounts of plant material.[1] A method using a modified analytical system based on the Paloheimo–Vainio method has been reported for the determination of starch.[2] The kinetics of complex formation and factors influencing the molar extinction coefficients were examined. Amylopectin has been determined spectrophotometrically by measuring the iodine complex at two wavelengths; the method gave more accurate results than the conventional, single-wavelength procedure.[3] Amylose and amylopectin have been determined by g.l.c. using a procedure based on end-group analysis.[4] Inclusion complexes formed between unsaturated fatty acids and amylose have been found to stabilize the fatty acids against autoxidation.[5]

The proportions of polysaccharide-chain segments in an ordered state have been compared in spherical and lenticular starch granules.[6] The spherical type of particle was shown to possess a more ordered structure, and it was suggested that variations in the arrangement of the polysaccharide could account for some of the differences between the biochemical properties of the two forms of granule. The conformation and packing in the crystal of anhydrous V-amylose have been investigated by a combination of linked-atom model building and *X*-ray diffraction analysis.[7] A new model for the packing of the chains in the unit cell and the presence of water inside the helix were proposed. A combined *X*-ray and stereochemical approach has provided evidence supporting a helical arrangement of the chains and antiparallel packing of the chains in the unit cell.[8] I.r. and Raman spectroscopic studies of the changes involved in the conversion of V-amylose into the more extended B-form are consistent with a mechanism involving extension of the helix and changes in hydrogen bonding at the hydroxymethyl groups.[9] The spin–spin relaxation times of the protons of starch suspensions in water have been observed by pulsed ^{1}H n.m.r. spectroscopy.[10] The marked changes in the τ-values on gelatinization were attributed to increases in the mobility and hydration of the

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polymer chains. The ability of amylose to form complexes with dyes has been studied by c.d. and absorbance spectroscopy.[11] A number of dyes formed chiral complexes with the polysaccharide, and the ability to form complexes depended on the nature and position of the substituents on the dye. In a study of the decomposition of maltose and amylose by aqueous alkali under anaerobic conditions, a chromophore appears to be formed *via* 4-deoxy-D-*glycero*-hexo-2,3-diulose, which arises by β-elimination in the propagative, stepwise degradation of amylose chains.[12]

The physicochemical properties of banana-fruit starch and starch components have been reported; the β-amylolysis values of the amylose components range from 76 to 98%.[13] Amylose and dextran exhibited identical calibration curves on gel-permeation chromatography using DMSO–water or 4M-guanidine hydrochloride as solvents, thereby allowing commercially available dextrans to be used as markers in the calibration of amylose.[14] The molecular-weight distributions of corn, potato, and rice starches have been estimated by gel-permeation chromatography.[15] Amylose from starch has been further fractionated by gel filtration on agarose beads.[16] Differences in the viscosities of amylopectin from waxy rice starches have been ascribed to differences in molecular weight, since the samples studied had similar average chain lengths.[17] The gel consistency of non-waxy rice starch has been attributed mainly to the amylopectin fraction.[18] Rice starch has been modified by γ-irradiation to produce a soluble starch of low viscosity; an increase in reducing power, together with some molecular breakdown, was also observed.[19] Malondialdehyde has been identified among the products of γ-irradiated corn starch by conversion into the thiobarbiturate derivative.[20] The amounts of deoxy-compounds needed for the production of malondialdehyde on oxidation with periodate have been determined in irradiated potato starch.[21] Isotope dilution analysis has been used to determine a number of low-molecular-weight carbonyl compounds formed on γ-irradiation of [U-^{14}C]maize starch.[22]

Chromatographic and enzymic studies of the insoluble residues obtained by hydrolysis of various cereal starches with acid have suggested a new model for the structure of waxy maize amylopectin.[23] A review dealing with the mechanism of action of α-amylases has discussed the compositions of the residual oligosaccharides.[24] The results of a study of the catalytic activity of poly(acrylic acid–styrenesulphonic acid) towards amylose in aqueous solution at low pH indicated that interactions between the carboxy-groups of the copolymer and the hydroxy-groups of the substrate make no contribution to the rate of reaction.[25]

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Beer has been reported to contain four distinct groups of dextrins having DPs 5—10, 11—16, 17—21, and 22—27.[26] The first group of dextrins was found to be either linear or singly branched, whereas the others contained two, three, and four α-(1 → 6)-D-glucosidic linkages, respectively. Thus, the majority of the α-(1 → 6)-linkages of amylopectin appear to survive the brewing process, with 25% of them appearing as singly branched oligosaccharides and 35% as multiply branched oligosaccharides. The influence of the DP of a series of malto-oligosaccharides on the adsorption of water vapour and its hysteresis has been reported.[27] Significant proportions of D-glucose 6-phosphate have been detected in acid hydrolysates of waxy maize and waxy rice starches; a phosphorylated oligosaccharide (average DP 6) containing one mole of phosphate was isolated after extensive treatment of the polysaccharides with α-amylase.[28] Enzymic debranching of starches from maize of various genotypes has been achieved in the presence of high concentrations of DMSO.[29] It has been suggested that the substrate for Q-enzyme consists of two chains of amylose that have become associated through hydrogen-bonding and that, possibly, form a double helix.[30] The secondary, tertiary, and quaternary structures of amylose and amylopectin are also implicated in the action of this branching enzyme.

The soluble and starch-grain-bound forms of ADP-D-glucose:α-1,4-glucan-α-4-glucosyltransferase (starch synthase) from maize, avocado, and sweet potato have been found to exhibit a discontinuity in their Arrhenius plots at about 12 °C.[31] Discontinuity in the Arrhenius plots of cold-sensitive plants may be due to a change of phase in the lipid portion of the enzyme–lipid–amylose complex at a critical temperature. Two enzymes having D-glucosyl- and D-glucosyl-glucan-transferase activities have been isolated from *Spinacia oleracea*; the physiological function of these enzymes may be to produce oligosaccharide primers for starch synthase or phosphorylase.[32] A particulate preparation from potato composed mainly of proplastids was able to transfer D-glucose from UDP-D-[^{14}C]glucose to an endogenous protein to form a labelled glycoprotein having short glucan chains which is considered to act as an acceptor for the synthesis of larger glucan chains in the biosynthesis of starch.[33] On the basis of data obtained on the properties of the enzyme responsible for this reaction, a simple scheme of reactions was proposed to explain how the enzyme and D-glucose-1-phosphate-dependent activities are involved in the synthesis of α-(1 → 4)-glucan chains of the starch type. The isolation of a non-sedimentable fraction that catalysed the transfer of D-[^{14}C]glucose from D-[^{14}C]glucosyl-1-phosphate to the endogenous protein acceptor in the absence of added primer furnished additional evidence that a glycoprotein intermediate initiates the synthesis of α-(1 → 4)-glucan.[34] The labelled glucosylated product was suscep-

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tible to amylolytic and proteolytic enzymes, and both the glycoprotein and the α-(1 → 4)-glucan derived from it could be used as primers in transglucosylating systems with either ADP- or UDP- or GDP-D-[^{14}C]glucose as D-glucosyl donors.

Cellulose

Aspects of the structure of cotton cellulose have been reviewed.[35] It has been suggested that the helical structure proposed for cellulose I on the basis of morphological and structural studies could be extended to cellulose II and to the examination of other types of helix. The crystallite structure of cellulose has been investigated by analysis of the degradation products of model compounds by gel-permeation chromatography.[36] A detailed folding-chain model for regenerated cellulose was constructed, and various aspects of this structure were discussed. The hydrolysis of cellulose was suggested to take place in three ways; *viz*, a first-order, random scission at the folds; a zero-order, peeling reaction at the ends of the crystallites; and a first-order, random scission on the lateral surfaces. Changes in the accessibility of the hydroxy-groups and in the extent of lateral crystalline order in passing from fibrous cotton cellulose II to hydrocelluloses during hydrolysis have been examined.[37] The behaviour of cellulose II on hydrolysis with acid was generally similar to that of fibrous cotton cellulose I. Annealation and an increased order in cellulose II have been observed on heating cellulose at temperatures far removed from the estimated melting point of the polysaccharide.[38]

Differences in the low-frequency region of the Raman spectra of celluloses I and II have been interpreted in terms of conformations involving different orientations about the β-(1 → 4)-D-glucosidic linkages.[39] Diffraction studies with low-angle neutrons have provided information on the structure of cellulose I that cannot be obtained from *X*-ray studies.[40] The results were generally in agreement with those based on electron-diffraction studies, except that the *a* and *c* axes were found to be about twice as long as those determined by *X*-ray studies; a new value for the *b* axis was also proposed. A folded-chain model for cellulose II has been developed from experimental work and theoretical considerations.[41]

The proposal that transverse sectioning of natural cellulose fibres can be used to test theories on the structure of microfibrils has been examined theoretically.[42] It was concluded that fibre-sectioning experiments described in the literature do not disprove the folded-chain model, and it was suggested that clearly divided sections exist along the axis of the microfibril at intervals of 200 Å. This cannot be explained in terms of a fringed micelle model. However, it is possible that cellulose II has a folded-chain conformation, since a single molecule of cellulose can be folded back and forth in the (101) plane to form a sheet-like structure that fits into the unit cell. A cellulose molecule can achieve a sharp U-turn in the

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[42] C. Holt and D. B. Sellen, *J. Polymer Sci., Polymer Phys.*, 1975, **13**, 1.

(101) plane by placing one D-glucose unit only in a half-boat conformation. A crystallite probably consists of a number of sheets held together by secondary forces in the (101) plane. Thus, the crystallites are closely packed at the surface of each fold at the longitudinal edges to make up the cellulose microfibril. According to this model, the oxygen atom of the D-glucosidic linkage in the fold, where hydrolysis with acid would have to take place, partly protrudes from the surface of the crystallite; a pair of oxygen atoms forming the glycoside linkages at the folds are then facing each other and are accessible for hydrolysis. This model explains why the chains of cellulose II are cleaved at these sites on hydrolysis with acid.

Low-frequency i.r. spectra of some cellulose polymorphs, decrystallized cellulose, and cellopentaose have been recorded.[43] Correlations between the absorption bands in these spectra were in accord with those previously established for bands at higher frequency. The crystal structure of cellulose from *Valonia ventricosa* has been investigated using diffraction data obtained from oriented specimens and employing a rigid-body, least-squares refinement procedure; a parallel-chain model was proposed.[44] An increase in the adsorption of iodine by cellulose has been observed on raising the temperature, with a second-order transition at 25 °C.[45] This appears to result from progressive penetration of iodine molecules into the pores of the cellulose structure as the temperature is raised. The solubilities of cellulose and cellulosic pulps in aqueous solutions of zinc chloride and sodium zincate have been shown to depend on the purity and DP of the samples, but depolymerization of the samples also occurred.[46] The non-Newtonian behaviour of solutions of cellulose in cadoxen has been observed, and a flow equation has been derived.[47] Alkali cellulose with an acceptable DP has been prepared by oxidation of cellulose in dilute solutions of periodate ion, followed by mercerization.[48] 1,6-Anhydro-3-deoxy-β-D-*ribo*-hexopyranose has been isolated from the pyrolysis products of cellulose; it was identified by i.r., u.v., and n.m.r. spectroscopy, and is considered to be a product of thermolysis intermediate between levoglucosan and levoglucosenone (1,6-anhydro-3,4-dideoxy-β-D-*glycero*-hex-3-enopyranos-2-ulose).[49] There is no evidence to indicate that levoglucosan is a primary product of the acid-catalysed pyrolysis of cellulose to give levoglucosenone, so that the principal mechanism of its formation must involve an unidentified precursor.[50]

The hydrolysis of dextrin (as a model for cellulose) has been studied in the presence of poly(vinyl alcohol–vinylsulphonic acid).[51] Although the rate of hydrolysis increased with decreasing molecular weight of the substrate, the rates in the presence of copolymer were always greater than those with sulphuric acid on substrates of the same molecular weight. Both the enthalpy and the entropy of activation in the presence of the copolymer were smaller than those with

[43] A. J. Michell, *Cellulose Chem. Technol.*, 1974, **8**, 439.
[44] K. H. Gardner and J. Blackwell, *Biochim. Biophys. Acta*, 1974, **343**, 232.
[45] K. Aziz and M. A. Abou-State, *Cellulose Chem. Technol.*, 1974, **8**, 443.
[46] K. Garves, *Holzforschung*, 1974, **28**, 168.
[47] J. M. Perena, E. Riande, and G. M. Guzman, *Cellulose Chem. Technol.*, 1974, **8**, 413.
[48] V. Mašura, *Cellulose Chem. Technol.*, 1974, **8**, 21.
[49] A. Ohnishi, E. Takagi, and K. Kato, *Chem. Letters*, 1974, 1361.
[50] A. Broido, M. Evett, and C. C. Hodges, *Carbohydrate Res.*, 1975, **44**, 267.
[51] K. Arai and N. Ise, *Makromol. Chem.*, 1975, **176**, 37.

sulphuric acid. The effects of inorganic salts on the rate of hydrolysis of dextrin by the copolymer were also reported; the rate was enhanced when sodium chloride and potassium nitrate were added, but it was retarded when sodium and potassium sulphates were added.[52] When inorganic salts were replaced by organic solvents in the same system, the rate of hydrolysis decreased to a minimum and then increased at higher concentrations of organic solvent.[53] Factors besides the dielectric permittivity of the mixed solvent system appear to be important.

The scission of glycosidic linkages during the degradation of cellulose by aqueous, oxygenated alkali has been shown to proceed in two steps (rate constants k_1 and k_2), although initially the process is complicated by the formation of intramolecular cross-linkages.[54] Relationships were derived giving the values of k_1 and k_2, and the number of cross-linkages was calculated from the weight-average and number-average DPs of the cellulose. A relationship between the degree of delignification and the extent of degradation of cellulose has been used to predict the outcome of oxygen-bleaching of wood pulp.[55] Delignification and the extent of cleavage of cellulose chains during oxygen-bleaching were retarded in different ways by additives and by pre-treatment of the pulp. Degradation of cellulose depended on the catalytic effect of transition-metal ions on the decomposition of peroxides. The effects of iron and magnesium ions on hydrogen peroxide and on the dialkyl peroxides formed on reaction of methyl β-D-glucopyranoside with oxygenated alkali have been examined.[56] The reaction is considered to be radical autoxidative in which initiation, propagation, and termination processes are influenced by the presence of catalysts. The nature of the degradation products of cellulose treated with oxygen–alkali has been shown to depend on the presence of iron and cobalt compounds.[57] The proportion of D-gluconic acid to D-mannonic acid end-groups was greater in the presence of these catalysts, whereas the reverse was true in their absence. Pre-treatment of birch meal with sodium hydrogen carbonate at high temperatures prior to digestion in the presence of oxygen resulted in an increased rate of delignification.[58] An increased pressure of oxygen resulted in a decreased rate of depolymerization of cellulose. Metal compounds that catalyse the delignification and retard the depolymerization of cellulose during oxygen-cooking were removed when wood meal was soaked in acids.[59] Added aluminium salts had a similar effect, due to removal of the catalytically active metal compounds with the precipitated aluminium hydroxide. Copper salts exerted a strong catalytic effect on the degradation of cellulose during oxygen–hydrogen carbonate treatment, but the effects were masked by H_4edta.[60] The ability of oxidants to convert the terminal aldehydic groups of hydrocellulose into aldonic acids has been studied.[61] Erythronic acid end-groups were formed principally by oxidation

[52] K. Arai and Y. Ogiwara, *Makromol. Chem.*, 1975, **176**, 2491.
[53] K. Arai and Y. Ogiwara, *Makromol. Chem.*, 1975, **176**, 2501.
[54] S. Jovanović, A. Palma, and G. V. Schulz, *Makromol. Chem.*, 1974, **175**, 3503.
[55] R. Gustavsson and B. Swan, *Tappi*, 1975, **58**, 120.
[56] J. T. McCloskey, J. D. Sinkey, and N. S. Thompson, *Tappi*, 1975, **58**, 56.
[57] L. Löwendahl and O. Samuelson, *Svensk Papperstidn.*, 1974, **77**, 593.
[58] K. Abrahamsson and O. Samuelson, *Svensk Papperstidn.*, 1974, **77**, 693.
[59] K. Abrahamsson and O. Samuelson, *Svensk Papperstidn.*, 1975, **78**, 135.
[60] M. Manoucheri and O. Samuelson, *Svensk Papperstidn.*, 1975, **78**, 211.
[61] H. Heikkilä and E. Sjöström, *Cellulose Chem. Technol.*, 1975, **9**, 3.

under alkaline conditions (*e.g.* anthraquinone 2-sulphonate, nitrobenzoate, and oxygen–alkali), whereas D-gluconic acid end-groups were formed under acidic conditions (*e.g.* chlorite and hydrogen sulphite); appreciable amounts of D-mannonic acid end-groups were also detected after treatment with hydrogen sulphite.

Cell-surface and intracellular β-(1 → 4)-D-glucan synthase activities have been measured in connection with studies of the stage and direction of cell growth in *Pisum sativum*.[62] An examination of membrane preparations of hormone-treated epicotyls of *P. sativum* has indicated that cellulose synthases in the Golgi and endoplasmic reticulum are not themselves loci for cellulose synthesis *in vivo*, but represent enzymes in transit to sites of action at the wall–protoplast interface, where they appear to operate only if the integrity of the cellular organization is maintained.[63] The initial rate of transfer of D-glucose from UDP-D-[^{14}C]glucose approached the observed rate of deposition *in vivo* of cellulose in intact tissues.

Gums and Mucilages

Acacia gum from exudates of species of a subseries Juli florae has been shown to contain more acidic groups and to have a higher viscosity, methoxy-content, and molecular weight, but a lower proportion of L-rhamnosyl and L-arabinosyl residues, than the majority of other *Acacia* gums.[64] Graded hydrolysis of bael (*Aegle marmelos*) gum with acid has furnished 3-*O*-β-D-galactopyranosyl-L-arabinose, 5-*O*-β-D-galactopyranosyl-L-arabinose, and 3-*O*-β-D-galactopyranosyl-D-galactose, and the acidic oligosaccharides 3-*O*-(β-D-galactopyranosyluronic acid)-D-galactose and 3-*O*-(β-D-galactopyranosyluronic acid)-3-*O*-β-D-galactopyranosyl-D-galactose.[65] Although the results give some insight into the polysaccharides present in the degraded gum, they do not permit a plausible structure of the gum polysaccharide to be proposed. A tentative structure (1) has been assigned to the repeating unit of mangle (*Rhizophora mangle*) gum based on the results of graded hydrolysis with acid, methylation, oxidation with periodate, and Smith and Barry degradations.[66]

The neutral and acidic sugar components of the acidic gums from *Prunus* species have been identified by a combination of methods that included a titrimetric procedure.[67] A partially degraded gum derived from *P. persica* containing residues of D-galactose, D-mannose, D-xylose, L-arabinose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid has been used as a source of carbon for the growth of *Aspergillus flavus* and for the production of *exo*- and *endo*-glycanohydrolases that might be used in structural studies of polysaccharides.[68] The seed mucilage of *Ocimium basilicum* has been shown to contain a heterogeneous collection of polysaccharides composed of residues of D-glucose, D-galactose, D-mannose, L-arabinose, D-xylose, L-rhamnose, D-galacturonic acid, and

[62] G. Shore, Y. Raymond, and G. A. MacLachlan, *Plant Physiol.*, 1975, **56**, 34.
[63] G. Shore and G. A. MacLachlan, *J. Cell Biol.*, 1975, **64**, 557.
[64] D. M. W. Anderson and M. C. L. Gill, *Phytochemistry*, 1975, **14**, 739.
[65] A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydrate Res.*, 1975, **41**, 219.
[66] M. Sarkar and C. V. N. Rao, *Carbohydrate Res.*, 1975, **41**, 163.
[67] J. Rosík and K. C. B. Wilkie, *Phytochemistry*, 1975, **14**, 1019.
[68] A. Kardošová, J. Rosík, and J. Kubala, *Phytochemistry*, 1975, **14**, 341.

→ 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 → 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 → 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 → 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 → 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 → 4)-GalUA*p*-(1 → 2)-Rha*p*-(1 →

(1)

D-mannuronic acid.[69] Graded hydrolysis with acid yielded an acid-stable core polysaccharide containing residues of D-glucose and D-mannose (10 : 2). A mucilage isolated from the tubers of *Bletilla striata* has been identified as a glucomannan containing *O*-acetyl groups (4%); methylation analysis indicated that the polysaccharide is composed mainly of β-(1 → 4)-linked aldohexopyranosyl residues that are branched at C-2 of some of the D-mannosyl residues.[70] Root-cap slimes from five varieties of maize have been shown to consist of a mixture of neutral, weakly acidic, and acidic polysaccharides.[71]

Pectins

Apple-fruit pectin has been visualized in electron microscopy by staining with Ruthenium Red.[72] Pectins have been separated from methylated RNA and RNA-precursors by virtue of their solubility in ethanolic solutions of lithium chloride.[73] The pK_0 values of pectic and alginic acids have been shown to be virtually independent of the nature of the polyelectrolyte and the charge density; the pK_0 values were close to that of the monomer unit and were unaffected by the position of the carboxy-group on the hexose ring.[74] The presence of Fe^{2+} and Fe^{3+} ions has been reported to make the pectins less susceptible to the action of pectolytic enzymes.[75] Gel filtration and gel electrophoresis have demonstrated the heterogeneous nature, both in molecular size and charge, of pectic substances from tomatoes.[76] I.r. spectroscopy of the cell walls of *Nitella axilliformis* has indicated that, in addition to reorientation of the cellulose microfibrils, changes in the ultrastructure of the matrix polysaccharides take place; these are revealed by changes in the orientation of pectic carboxy-groups during extension growth.[77] The products of the hydrolytic action of several polygalacturonases on pectin have been analysed by gel-filtration chromatography.[78] In addition to the identification of normal *exo*- and *endo*-enzymes, one enzyme preparation yielded quantities of mono-, di-, and penta-saccharides from the polysaccharide. Optimum conditions for the isolation of pectin from potato pulps have been reported.[79] An acidic polysaccharide complex isolated from willow-bark pectin has been subjected to partial hydrolysis with acid.[80] The structures of the acidic and neutral oligosaccharides were established using chromatographic methods, enzymic hydrolysis, and m.s. of the products of methylation analysis. The following acidic oligomers were characterized: 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, 4-*O*-(β-D-glucopyranosyluronic acid)-L-fucose, 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose, 6-*O*-(β-D-galactopyranosyluronic acid)-D-galactose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-galactose, 4-*O*-(α-D-galactopyranosyluronic acid)-D-

[69] R. N. Tharanathan and Y. V. Anjaneyalu, *Indian J. Chem.*, 1974, **12**, 1164.
[70] M. Tomoda, S. Nakatsuka, and N. Satoh, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2710.
[71] K. Wright, *Phytochemistry*, 1975, **14**, 759.
[72] D. E. Hanke and D. H. Northcote, *Biopolymers*, 1975, **14**, 1.
[73] K. M. Jakob, J. Gressel, and I. Tal, *Analyt. Biochem.*, 1975, **63**, 457.
[74] M. Rinaudo and M. Milas, *J. Polymer Sci., Polymer Chem.*, 1974, **12**, 2073.
[75] Z. I. Protsenko and E. N. Kharyanov, *Priklad. Biokhim. i Mikrobiol.*, 1975, **11**, 237.
[76] E. R. Stein and H. E. Brown, *J. Agric. Food Chem.*, 1975, **23**, 526.
[77] H. Morikawa and M. Senda, *Plant Cell Physiol.*, 1974, **15**, 1139.
[78] J. H. McLendon, *Phytochemistry*, 1975, **14**, 377.
[79] O. Abousteit and W. Kempf, *Stärke*, 1974, **26**, 417.
[80] R. Toman, S. Karácsonyi, and M. Kubačková, *Carbohydrate Res.*, 1975, **43**, 111.

galacturonic acid, and the homologous tri-, tetra-, and penta-saccharides. Among the neutral oligosaccharides isolated were 4-*O*-β-D-galactopyranosyl-D-galactose and the homologous tri- and tetra-saccharides, and 6-*O*-β-D-galactopyranosyl-D-galactose and the homologous β-(1 → 6)-linked trisaccharide.

Hemicelluloses

The relationship of cellulose and hemicelluloses in growing plant cells has been the subject of a review.[81] A method affording minimal contamination with cytoplasmic constituents has been developed for the preparation of 'cell-wall material' from plant tissues.[82] The roles of cell-walls and wall-degrading enzymes in infective processes in plants have been reviewed.[83] Chromatography on DEAE–cellulose and paper electrophoresis have been used to study soluble pectins, hemicelluloses, and cellulose.[84] The four main stages in the analysis of foodstuffs have been outlined; examples of methods applicable to cellulosic and non-cellulosic cell-wall polysaccharides of plants were given.[85] The results of experiments on *in vitro* cell-binding with barley aleurone cells, which contain arabinoxylan and cellulose, have supported the suggestion that non-covalent bonds between the pentosan and the cellulose fibres play a part in maintaining the structure of the cell wall.[86] It was also suggested that bonding among the pentosan chains is utilized in forming the cell wall. I.r. spectroscopy has been used in the analysis of hemicelluloses, particularly in investigations of inter-chain bonding.[87] In a study of the photodegradation of the polysaccharides of wood pulp, the cleavage of glycosidic bonds was found to be of primary importance, whereas oxidation had only a secondary role.[88] Differential alkali-extraction of hemicellulosic polysaccharides and glycoproteins from non-delignified, primary cell walls of lupin hypocotyls has indicated that nearly all the hemicellulose and glycoproteins are not attached to other cell-wall components.[89] A derivative of taurine–Sapogen T has been reported to inhibit the depolymerization of hemicelluloses during hypochlorite bleaching, producing better yields of materials exhibiting high viscosities.[90] A holocellulose from the bark of loblolly pine (*Pinus taeda*) yielded basic, neutral, and acidic fractions having a broad range of molecular weights (<100—>900) when degraded with boiling piperidine.[91] The formation of these polymers could involve an initial nucleophilic displacement by piperidine at the glycosidic linkages of the polysaccharide; the initially formed glycosylamine would be degraded further by rearrangement, dehydration, enolization, and polymerization.

The cell walls of barley endosperm have been shown to contain a microfibrillar phase embedded in an amorphous matrix.[92] The microfibrillar components are

[81] P. Albersheim, *Sci. American*, 1975, **232** (4), 80.
[82] R. R. Selvendran, *Phytochemistry*, 1975, **14**, 1011.
[83] P. Albersheim and A. J. Anderson-Prouty, *Ann. Rev. Plant Physiol.*, 1975, **26**, 31.
[84] V. N. Ezhov and E. I. Datunashvili, *Priklad. Biokhim. i Mikrobiol.*, 1974, **10**, 908.
[85] D. A. T. Southgate, *J. Assoc. Public Analysts*, 1974, **12**, 114.
[86] M. McNiel, P. Albersheim, L. Taiz, and R. L. Jones, *Plant Physiol.*, 1975, **55**, 64.
[87] E. Gruber, J. Mohammed, and J. Schurz, *Cellulose Chem. Technol.*, 1974, **8**, 423.
[88] S. Hernádi, *Cellulose Chem. Technol.*, 1975, **9**, 31.
[89] J. A. Monro, R. W. Bailey, and D. Penny, *Carbohydrate Res.*, 1975, **41**, 153.
[90] J. Rutkowski, *Cellulose Chem. Technol.*, 1975, **9**, 167.
[91] E. J. Parish, G. D. McGinnis, G. P. Belue, and P. Fang, *Carbohydrate Res.*, 1975, **39**, 384.
[92] G. B. Fincher, *J. Inst. Brewing*, 1975, **81**, 116.

thought to include cellulose, tightly bound arabinoxylan, and a polysaccharide rich in D-mannosyl residues, whereas the matrix is composed of arabinoxylan (25%) and β-glucan (75%).

The digestion of cell-wall polysaccharides of spear grass and of cotton cellulose in bovine rumen has been examined by viscometry and *X*-ray diffraction.[93] The hemicelluloses of grass partly digested in the rumen and of faeces fibres have been isolated and shown by viscometry to have molecular weights similar to those of the material isolated from the original grass. This finding substantiates earlier conclusions that the digestion-resistant hemicelluloses are chemically identical with the digestible hemicelluloses and that the resistance to digestion is due to protection by lignin. The molecular weight of cotton cellulose decreased slowly during digestion in bovine rumen, and the polysaccharide retained its fibrous form. Since the crystallinity decreased at the same time, it was concluded that amorphous and crystalline regions of cellulose are attacked at roughly the same rate. The cell-free rumen liquor of a steer fed with spear grass has been shown to contain macromolecular substances in which carbohydrates and lignin-derived compounds are covalently bound to each other.[94] Some of the lignin–carbohydrate linkages were labile to alkali, whereas treatment with dilute sulphuric acid converted part of the complex into an acetone-soluble material containing both carbohydrate and lignin-derived compounds. It was concluded that soluble lignin–carbohydrate complexes formed from grasses by the actions of micro-organisms may account for dissolution and, hence, the apparent digestion of about half of the total intake of lignin. Alkaline extracts of the holocellulose from spruce wood have been subjected to ion-exchange chromatography, yielding fractions representing different lignin–carbohydrate complexes.[95] Three lignin–carbohydrate complexes (13—30% carbohydrate) isolated from ripe cotton capsules by extraction with *NN*-diethylformamide contained D-xylosyl, L-arabinosyl, D-glucosyl, and D-galactosyl residues.[96]

The thermal stabilities of model compounds containing glycosidic and acetal-type linkages analogous to those found between lignin and carbohydrates, and those of lignin–carbohydrate complexes isolated from birch and spruce woods, have been investigated.[97] The acetal-type linkages were generally more resistant to thermal degradation than were glycosidic linkages, and complete acetylation increased the thermal stability of both glycosidic and acetal linkages. A survey has been made of the solubility in alkali of hemicelluloses from a number of plants in connection with delignification processes.[98] It appears that the wall hemicelluloses extracted are attached to either lignin or other cell-wall constituents by alkali-labile linkages.

The rates of delignification of the cell walls of *Lolium perenne* and *Trifolium pratense* at different stages of maturity have been determined after treatment with sodium chlorite.[99] If the lignin content was less than 10%, delignification

[93] R. J. Beveridge and G. N. Richards, *Carbohydrate Res.*, 1975, **43**, 163.
[94] B. D. E. Gaillard and G. N. Richards, *Carbohydrate Res.*, 1975, **42**, 135.
[95] D. Fengel and M. Przylenk, *Svensk Papperstidn.*, 1975, **78**, 17.
[96] L. S. Smirnova and K. A. Abduazimov, *Khim. prirod. Soedinenii*, 1974, 397.
[97] B. Košiková, D. Joniak, and M. Košik, *Cellulose Chem. Technol.*, 1975, **9**, 61.
[98] R. W. Bailey and S. E. Pickmere, *Phytochemistry*, 1975, **19**, 501.
[99] I. M. Morrison, *Phytochemistry*, 1975, **14**, 505.

was completed in 30 min. The yield and composition of hemicelluloses extracted with alkali were found to depend on the duration of the delignification reaction. A non-lignin loss of about 10% has been reported during kraft pulping of wood; this is partly accounted for by loss of cellulose and hemicelluloses.[100] The loss of hemicelluloses is related directly to the removal of lignin, due to either chemical or physical linkages between them. Analysis of the spent liquor from oxygen–hydrogen carbonate cooking of birch wood has revealed that it contains about half the dissolved lignin in the form of low-molecular-weight compounds, as well as xylan and organic acids derived from degradation of the hemicelluloses.[101] Identification of the acids indicated that degradation of the carbohydrates occurs by the same reactions as in the bleaching of wood pulp by oxygen. The rates of delignification, of dissolution of hemicelluloses, and of depolymerization of carbohydrates during oxygen–hydrogen carbonate cooking have been shown to increase with increasing concentrations of sodium hydrogen carbonate and with decreasing partial pressures of carbon dioxide.[102] A high concentration of carbon dioxide retarded delignification and resulted in the formation of more reducing hexose moieties.[103] *O*-Acetyl groups in cell-wall preparations from grasses and other higher plants have been detected by i.r. spectroscopy, and their presence has been confirmed chemically.[104] The proportion of *O*-acetyl groups present is considered to influence both the physical state, and the degradation by enzymes, of the cell-wall polysaccharides.

Seven oligosaccharides have been isolated from the tuberous roots of *Asparagus cochinchinensis*.[105] Oxidation with periodate and methylation analysis were used to identify *neo*-kestose and six oligosaccharides possessing non-reducing, linear structures composed of (2 → 1)-linked β-D-fructofuranosyl residues having a *neo*-kestosyl residue at one end. A fructosan (mol. wt. 5×10^3—1.8×10^4) containing (2 → 6)-glycosidic linkages has been isolated from different parts of timothy grass (*Phleum pratense*).[106]

The chemical composition and characterization of the β-glucan in malt have been reported; the actions of β-glucan hydrolases on this polysaccharide were also investigated.[107] Improved purification procedures suggested that the β-glucan content of malt is lower than that normally reported. The pollen-tube wall of *Lilium longiflorum* has been shown to contain alkali-resistant fibrils composed of β-(1 → 3)-(1 → 4)-linked D-glucans.[108] A determination of non-starchy polysaccharides based on viscometric measurement of extractable β-glucans has been developed to aid in the breeding of barley.[109] Treatment of coleoptile segments of *Avena* with auxin has been shown to result in a decrease in the non-cellulosic D-glucose component of the cell walls, owing to the production of an *exo*-glucanase.[110] Heat-shock on β-glucan synthases in the leaves of

[100] C. H. Mathews, *Svensk Papperstidn.*, 1974, **77**, 629.
[101] O. Samuelson and L. A. Sjöberg, *Cellulose Chem. Technol.*, 1974, **8**, 39.
[102] K. Abrahamsson and O. Samuelson, *Svensk Papperstidn.*, 1975, **78**, 417.
[103] M. Manouchehri and O. Samuelson, *Svensk Papperstidn.*, 1975, **78**, 451.
[104] J. S. D. Bacon, A. H. Gordon, E. J. Morris, and V. C. Farmer, *Biochem. J.*, 1975, **149**, 485.
[105] M. Tomoda and N. Satoh, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2306.
[106] W. Kühbauch, *Z. Pflanzenphysiol.*, 1974, **74**, 121.
[107] G. Barwald, *Monatsh.*, 1974, **27**, 25.
[108] W. Herth, W. W. Franke, H. Bittinger, A. Kuppel, and G. Keilich, *Cytobiologie*, 1974, **9**, 344.
[109] V. M. Bendelow, *J. Inst. Brewing*, 1975, **81**, 127.
[110] D. J. Nevins, *Plant Cell Physiol.*, 1975, **16**, 495.

Phaseolus vulgaris and *Vigna sinensis* severely inhibited the synthesis of β-(1 → 4)-D-glucosyl linkages, but stimulated the synthesis of β-(1 → 3)-D-glucosyl linkages.[111] A particulate fraction from pollen tubes and ungerminated pollen from *Lilium longiforum* incorporated D-[^{14}C]glucose from UDP-D-[^{14}C]glucose into a lipid fraction and a β-(1 → 3)-linked glucan that liberated laminaribiose as the only radioactive disaccharide on partial hydrolysis with acid.[112] The enzyme isolated from the growing pollen tubes synthesized β-(1 → 3)-glucan more rapidly, and produced a higher proportion of alkali-insoluble glucan, than did the enzyme from ungerminated pollen.

A glucomannan isolated from the tubers of *Arum maculatum* has been purified by ion-exchange chromatography.[113] Enzymic studies, oxidation with periodate, and i.r. spectroscopy indicated the presence of only β-(1 → 4)-glycosidic linkages in this polysaccharide. A number of oligosaccharides, including cellobiose, a mannopentaose, and several oligosaccharides containing both D-glucosyl and D-mannosyl residues, have been isolated and characterized after digestion of konjac (*Amorphophallus konjac*) glucomannan with a β-mannanase.[114] These and earlier investigations indicated a structure (2) for this β-(1 → 4)-linked glucomannan.[115]

Glc → Glc → Man → Man → Glc → Man → Man → Man → Man → Man →
Glc → Glc → Man

the hexoses are β-D-(1 → 4)-linked

(2)

The distribution of *O*-acetyl groups in pine glucomannan has been determined.[116] The method used involved acetalation of the polysaccharide with methyl vinyl ether, removal of the *O*-acetyl groups by treatment with alkali, and cleavage of the glycosidic linkages of the D-mannosyl residues carrying the original *O*-acetyl groups by a technique based on oxidation, β-elimination, and mild hydrolysis with acid. After removal of the acetal functions, the composition and molecular-weight distribution of the resulting mixture of oligosaccharides indicated that the *O*-acetyl groups are irregularly distributed in the polysaccharide. Acid hydrolysis of the galactomannans in germinating seeds of lucerne, carob, honey locust, guar, and soybean proceeded rapidly to the monomeric units once scission of the main chain was initiated, rather than by partial depolymerization of all the chains.[117] An arabinogalactan present in wheat flour has been characterized.[118] Approximately 60% of the D-galactosyl residues are linked β-(1 → 6) and have L-arabinosyl residues attached at C-3; part of the remaining D-galactosyl residues appear to be substituted by branches having β-(1 → 3)- and β-(1 → 6)-linkages.

[111] C. Musolan, L. Ordin, and J. I. Kindinger, *Plant Physiol.*, 1975, **55**, 328.
[112] D. Southworth and D. B. Dickinson, *Plant Physiol.*, 1975, **56**, 83.
[113] M. Koleva and C. Achtardjieff, *Pharmazie*, 1975, **30**, 111.
[114] H. Shimahara, H. Suzuki, N. Sugiyama, and K. Nisizawa, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 293.
[115] H. Shimahara, H. Suzuki, N. Sugiyama, and K. Nisizawa, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 301.
[116] L. Kenne, K. G. Rosell, and S. Svensson, *Carbohydrate Res.*, 1975, **44**, 69.
[117] B. V. McCleary and N. K. Matheson, *Phytochemistry*, 1975, **14**, 1187.
[118] H. Neukom and H. Markwalder, *Carbohydrate Res.*, 1975, **39**, 387.

An arabinan from the bark of white willow (*Salix alba*) has been reported to contain approximately 90 α-L-arabinofuranosyl residues that are either (1 → 5)- or (1 → 3)- or (1 → 2)-linked, with some of these residues branched through positions 2 or 3 or 5.[119] Degradation of spear-grass hemicellulose B with an *endo*-hemicellulase produced a series of β-(1 → 4)-linked D-xylopyranosyl oligomers, the arabinosylxylobiose (3), and the arabinosylxylotriose (4).[120] The structures (3) and (4) were elucidated by methylation and enzymic analyses.

α-L-Ara*f*-(1 → 3)-β-D-Xyl*p*-(1 → 4)-D-Xyl

(3)

α-L-Ara*f*-(1 → 3)-β-D-Xyl*p*-(1 → 4)-β-D-Xyl*p*-(1 → 4)-D-Xyl

(4)

A hardwood xylan has been partially hydrolysed by a xylanase obtained from a *Streptomyces* sp., and the resulting xylo-oligosaccharides were used as the source of carbon for growing *Candida guilliermondii*.[121] Only xylo-biose and -triose were selectively metabolized, leaving the corresponding tetra- to hexa-saccharides in the culture broth. Further evidence has shown that the release of soluble xyloglucan from the cell walls of peas results from enzymic action on the cell wall and coincides with wall-extension.[122] Treatment of pea (*Pisum sativum*) tissues with either auxin or acid produced a similar effect on the cell wall, in agreement with Cleland's suggestion that the action of auxin is brought about by the secretion of hydrogen ions. A linear galactan containing only β-(1 → 3)-linkages has been isolated from tissue-cultured rose cells.[123] The principal polysaccharide extracted with alkali from the rose cells contains a backbone of (1 → 4)-linked D-xylosyl residues and non-reducing D-glucopyranosyl, L-arabinofuranosyl, and L-fucopyranosyl end-groups.[124]

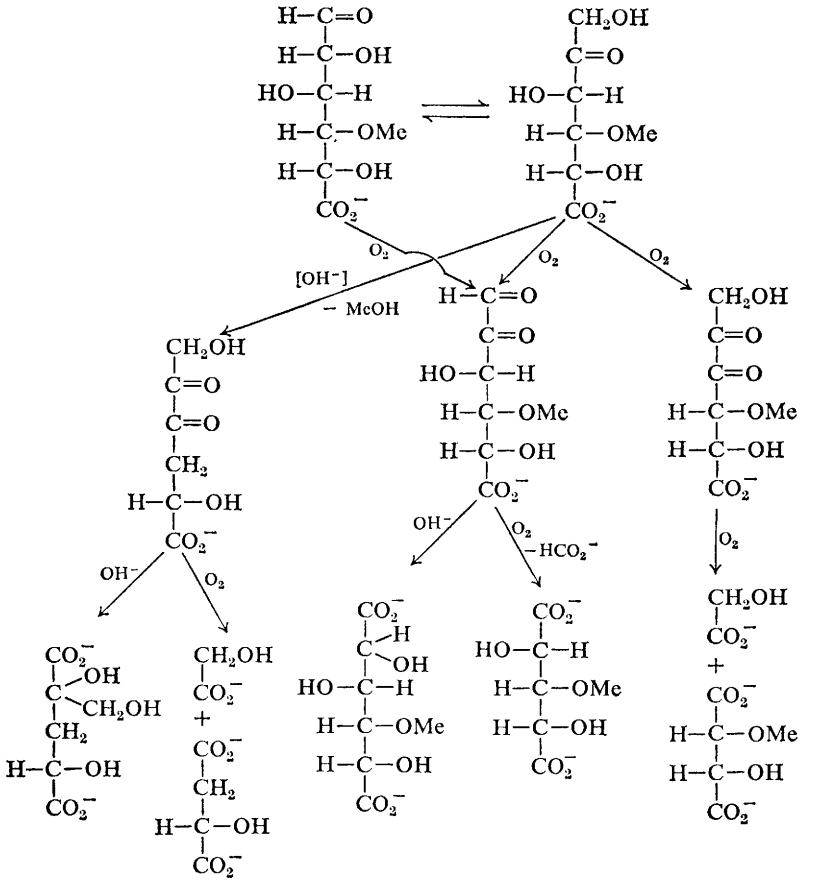
Two polysaccharides isolated from the seeds of *Phaseolus mungo* have been purified by precipitation in ethanol; they were electrophoretically homogeneous, and contained D-glucosyl, D-galactosyl, and L-arabinosyl residues.[125] A hetero-polysaccharide exhibiting blood-group H-like activity has been isolated from the seeds of *Euonymus sieboldiana*; ion-exchange and gel-permeation chromatography were used in its purification.[126] The heteropolysaccharide, which inhibited the haemagglutination of human group O red-blood cells and eel anti-serum, is composed of D-glucose and L-rhamnose (3 : 1) and small proportions of L-arabinose, D-mannose, D-galactose, and L-fucose. Each of three galacto-xyloglucans isolated from the seeds of tamarind, nasturtium, and balsamine was found to possess a core of β-(1 → 4)-linked D-glucosyl units, some of which are substituted at C-6 with either β-D-xylosyl or 2-*O*-β-D-galactosyl-D-xylosyl residues.[127]

[119] Š. Karácsony, R. Toman, F. Janaček, and M. Kubačková, *Carbohydrate Res.*, 1975, **44**, 285,
[120] R. F. H. Dekker and G. N. Richards, *Carbohydrate Res.*, 1975, **43**, 335.
[121] I. Kusakabe, T. Yasui, and T. Kobayashi, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 383.
[122] M. Jacobs and P. M. Ray, *Plant Physiol.*, 1975, **56**, 373.
[123] A. Mollard and F. Barnoud, *Carbohydrate Res.*, 1975, **39**, C16.
[124] A. Mollard and F. Barnoud, *Carbohydrate Res.*, 1975, **39**, C13.
[125] A. K. Chakraborty, *J. Indian Chem. Soc.*, 1975, **52**, 172.
[126] S. Yamamoto and T. Shinohara, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1863.
[127] J. E. Courtois and P. Le Dizet, *Anales de Quim.*, 1974, **70**, 1067.

D-Galactose, D-glucose, L-arabinose, D-xylose, D-galacturonic acid, and D-glucuronic acid are constituents of an acidic, water-soluble polysaccharide isolated from the bark and pith of stems of *Alcea rugosa*.[128] Two electrophoretically homogeneous polysaccharides have been isolated from the sapwood holocellulose of mature lateral shoots of sugar maple (*Acer saccharum*).[129] The results of methylation, oxidation with periodate, and selective degradation of these polysaccharides indicated that each consists of a chain of (1 → 4)-linked D-glucopyranosyl and D-xylopyranosyl residues with branching at C-2 of some D-xylosyl residues; the branches are terminated with residues of either D-xylopyranose or 4-*O*-methyl-D-glucopyranosyluronic acid. The polysaccharides differ in both the degree of branching and the molar ratio of D-glucose, D-xylose, and 4-*O*-methyl-D-glucuronic acid.

Elimination of the residues of 4-*O*-methyl-D-glucuronic acid from methylated birch xylan has been followed by specific cleavage of the xylan backbone at the original branched D-xylosyl residues using a technique involving sequential oxidation, β-elimination, and mild hydrolysis with acid.[130] The molecular-weight distribution of the resulting methylated oligosaccharides suggested that the 4-*O*-methyl-D-glucuronic acid residues are irregularly distributed in the xylan. Three previously unknown acids isolated from acid hydrolysates of birch glucuronoxylan have been identified as 4-*O*-methyl-α-D-glucopyranosyluronic acid α-D-xylopyranose 1,2′:2,1′-dianhydride, 4-*O*-methyl-α-D-glucopyranosyluronic acid β-D-xylopyranose 1,2′:2,1′-dianhydride, and 4-*O*-methyl-α-D-glucopyranosyluronic acid α-D-xylofuranose 1,2′:2,1′-dianhydride.[131] An equilibrium exists between the dianhydrides and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose in acid media. 4-*O*-Methyl-D-glucuronic acid residues were cleaved from hardwood xylan during both one-stage oxygen pulping and two-stage oxygen–alkali processes.[132] An investigation of the reactions of 4-*O*-methyl-D-glucuronic acid in alkaline solution has led to the isolation and identification (as TMS derivatives) of a number of deoxypentaric, deoxytetraric, 4-*O*-methylmannaric, 3-*O*-methylarabinaric, *O*-methylerythraric, and *O*-methylthrearic acids.[133] Reaction pathways that parallel those resulting in the degradation of cellulose were proposed to account for the formation of these dicarboxylic acids (Scheme 1). The thermal stabilities of polysaccharide fractions extracted from hornbeam wood were found to increase in the order: 4-*O*-methyl-D-glucuronoxylan < pectic substances < glucomannan < cellulose.[134] Thermolysis of 4-*O*-methyl-D-glucuronoxylan resulted in the formation of shorter chains with a higher content of D-glucuronic acid, owing to preferential cleavage of (1 → 4)-linked β-D-xylosyl residues.[135]

[128] I. S. Kozhina and G. Z. Mamatov, *Khim. prirod. Soedinenii*, 1974, 146.
[129] P. C. Bardalaye and G. W. Hay, *Carbohydrate Res.*, 1974, **37**, 339.
[130] K. G. Rosell and S. Svensson, *Carbohydrate Res.*, 1975, **42**, 297.
[131] K. Larsson, *Carbohydrate Res.*, 1975, **44**, 199.
[132] L. A. Lindström and O. Samuelson, *Tappi*, 1975, **58**, 147.
[133] L. Löwendahl, G. Petersson, and O. Samuelson, *Carbohydrate Res.*, 1975, **43**, 355.
[134] A. Ebringerová, J. Hirsch, A. Kramár, and R. Domanský, *Cellulose Chem. Technol.*, 1974, **8**, 49.
[135] A. Ebringerová, J. Hirsch, A. Kramár, and R. Domanský, *Cellulose Chem. Technol.*, 1974, **8**, 61.



Scheme 1

Algal Polysaccharides

Agarose and derivatives thereof carrying *O*-methyl, *O*-sulphate, *O*-hydroxyethyl, or *O*-carboxyethylidene groups in various positions have been studied by measurement of the optical rotations, *X*-ray diffraction, and computerized molecular-model building.[136] All the samples showed essentially the same order–disorder transition during gel–sol interconversion. A double-helix model was proposed for the basic structure, with each chain forming a left-handed, three-fold helix that is translated axially relative to its partner by exactly half the distance of the pitch. The model accounts for the sign and magnitude of the shift in optical rotation that accompanies sol–gel transitions. Digestion of agarose with an agarase from *Pseudomonas elongata* cleaved the β-(1 → 4)-linkages of the polysaccharide with the release of neoagaro-biose and -tetraose.[137] Investigation

[136] S. Arnott, A. Fulmer, W. E. Scott, I. C. M. Dea, R. Moorhouse, and D. A. Rees, *J. Mol. Biol.*, 1975, **90**, 269.

[137] M. A. Vattuone, E. A. De Flores, and A. R. Sampietro, *Carbohydrate Res.*, 1975, **39**, 164.

of the acetolysis products of the sulphated polysaccharide of the seaweed *Aeodes ulvoidea* has resulted in the isolation and characterization of a number of oligosaccharides.[138] The isolation of a tetrasaccharide possessing alternate D- and L-galactosyl residues demonstrated the hitherto unexpected presence of L-galactose in the polysaccharide. It was not possible to propose a structure for the repeating unit owing to the complexity of the acetolysis products.

The structure of an extracellular polysaccharide elaborated by the marine diatom *Chaetoceros affinis* has been examined.[139] Most of the L-rhamnosyl residues are located on the outer regions of the molecule, whereas L-fucosyl and D-galactosyl residues are present in both the inner and outer regions. Structural investigations have been reported on the extracellular, sulphated polysaccharide extracted from *C. curvisetus*; the component sugars found were D-galactose, L-rhamnose, and L-fucose (in furanose and pyranose forms).[140] Methylation analysis and partial hydrolysis with acid revealed that the L-fucofuranosyl residues are present both as end-groups in (1 → 2)-linkages and as branch points in the polysaccharide. The L-fucopyranosyl residues occur mainly as branch points, some being (1 → 3)-linked, most of the L-rhamnosyl residues are (1 → 2)-linked, and the D-galactosyl residues are (1 → 3)-linked. Spheroplasts have been prepared from the cells of actively growing *Chlorella* species after extensive degradation of the cell walls with a mixture of polysaccharide-degrading enzymes.[141] A polysaccharide having structural features similar to those of the alginic acid of *Laminaria digitata* has been isolated from the brown seaweed *Cystoseira barbata*.[142] Three chemically distinct segments were demonstrated to coexist in the macromolecule; each segment contains different proportions of D-mannuronic and L-guluronic acids. One segment has a structure in which the residues of D-mannuronic and L-guluronic acids alternate in a definite order, another segment appears to be a homopolymer of D-mannuronic acid, and the third segment is composed of blocks of L-guluronic acid residues separated by a single residue of D-mannuronic acid.[143]

The diffraction patterns of magnesium ι-carrageenate have provided direct evidence that this polysaccharide exists as a double-helix.[144] A molecular model for ι-carrageenate derived from these measurements consists of two identical right-handed, three-fold helical chains, with one of the chains translated axially relative to the other. The helix-to-coil transition of ι-carrageenan has been studied by optical measurements and differential scanning calorimetry, and is described by a two-state 'all-or-none' mechanism.[145] A mucilagenous sulphated galactan with properties similar to those of κ-carrageenan has been isolated from the red alga *Hypnea charoides*.[146] The polysaccharide is composed of D-galactose, 3,6-anhydro-D-galactose, and sulphate ester (3 : 1 : 2); the sulphate groups are

[138] A. J. R. Allsobrook, J. R. Nunn, and H. Parolis, *Carbohydrate Res.*, 1975, **40**, 337.
[139] B. Smestad, A. Haug, and S. Myklestad, *Acta Chem. Scand.* (*B*), 1974, **28**, 662.
[140] B. Smestad, A. Haug, and S. Myklestad, *Acta Chem. Scand.* (*B*), 1975, **29**, 337.
[141] E. Braun and H. G. Aach, *Planta*, 1975, **126**, 181.
[142] C. I. Simionescu, V. I. Popa, V. Rusan, and A. Liga, *Cellulose Chem. Technol.*, 1975, **9**, 213.
[143] C. I. Simionescu, V. I. Popa, A. Liga, and V. Rusan, *Cellulose Chem. Technol.*, 1975, **9**, 547.
[144] S. Arnott, W. E. Scott, D. A. Rees, and C. G. A. McNab, *J. Mol. Biol.*, 1974, **90**, 253.
[145] D. S. Reid, T. A. Bryce, A. H. Clark, and D. A. Rees, *Faraday Discuss. Chem. Soc.*, 1974, **57**, 230.
[146] H. Shimahara and N. Sugiyama, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 2569.

probably located on O-4 of the D-galactosyl residues. The types and proportions of carrageenans in the red algae *Iridaea cordata*, *I. heterocarpa*, *I. lineare*, *Gigartina exasperata*, and *G. papillata* have been examined.[147] The gametophytes contain 93% κ-carrageenan, whereas the tetrasporophytes contain 95% λ-carrageenan.

Changes in the structure and composition of the cell walls of *Fucus gardneri* have been related to the development of the embryo.[148] Alginic acid, fucoidin (present only at the rhizoid end of cell-walls), and cellulose were identified as the principal structural components of the embryo wall at 24 h after fertilization; a sulphated xylogalactofucoglucuronan and a β-(1 → 3)-glucan were also found in the embryo cell wall. During early embryogenesis in *F. distichus*, exogenous ^{35}S-sulphate was incorporated into acid-soluble polymers composed of L-fucose, which appeared only in the rhizoid cells of the two-celled embryo.[149] The lack of incorporation of sulphate into the fucan during the first few hours after fertilization does not appear to result either from permeability barriers preventing sulphate from entering into the zygotes or from changes in the intracellular sulphate pool. Indirect evidence suggested that enzymic sulphation may be responsible for retaining the fucan sulphate in the rhizoid cell. In order to understand the occurrence of fucoidin and its analogues in Phaeophyta, sulphated polysaccharides have been isolated from a number of brown seaweeds so that their sugar compositions could be examined.[150] The sulphated polysaccharides from *Chordariales*, *Laminaria*, and *Kjellmaniella* in the Laminariales appear to contain simple fucan sulphates, whereas those from *Eisenia*, *Undaria*, and *Alaria* in Laminarales and Fucales are heterogeneous in composition. Efforts have been made to establish the most convenient and efficient method of extraction of the polysaccharides from *Phyllophora nervosa*.[151]

A combination of methylation, treatment with alkali, and partial methanolysis of the alkali-treated polysaccharide to give derivatives of agarobiose was used to establish the structure of the galactan sulphate of *Polysiphonia lanosa*.[152] The polysaccharide belongs to the agar class, in which 3-linked derivatives of β-D-galactose alternate with 4-linked derivatives of α-L-galactose in a repeating sequence. However, in addition to D-galactose, the 3-linked units of the galactan sulphate include 6-*O*-methyl-D-galactose, D-galactose 6-sulphate, and 6-*O*-methyl-D-galactose 4-sulphate, and the 4-linked units also include L-galactose 6-sulphate, 2-*O*-methyl-L-galactose 6-sulphate, and 3,6-anhydro-L-galactose. Thus, the galactan sulphate belongs to the general family of agar (-A-B-A-B-) types having a masked repeating structure. An extracellular, sulphated polysaccharide composed of D-xylose and L-galactose (1 : 1) and D-glucuronic acid (9%) has been isolated from cultures of *Porphyridium cruentum*.[153] On the basis of data from methylation analysis, partial hydrolysis with acid, and Smith degradation, the polymer was found to have mainly (1 → 4)-linkages with branches at position 3 of D-xylose and positions 2 or 6 of D-galactose.

[147] J. R. Waaland, *Phytochemistry*, 1975, **14**, 1359.
[148] A. M. Novotny and M. Forman, *Planta*, 1975, **122**, 67.
[149] W. E. Hogsett and R. S. Quatrano, *Plant Physiol.*, 1975, **55**, 25.
[150] T. Fujikawa and K. Nakashima, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 455.
[151] C. I. Simionescu and V. I. Popa, *Cellulose Chem. Technol.*, 1975, **9**, 119.
[152] J. F. Batey and J. R. Turvey, *Carbohydrate Res.*, 1975, **43**, 133.
[153] D. G. Medcalf, J. R. Scott, J. H. Brannon, G. A. Hemerick, R. L. Cunningham, J. H. Chessen, and J. Shah, *Carbohydrate Res.*, 1975, **44**, 87.

An α-(1 → 3)-linked mannan, a sulphated glucuronoxylorhamnan, and amylose have been separated from aqueous extracts of *Urospora penicilliformis*.[154] The sulphated polysaccharide was shown to contain a (1 → 3)-linked backbone of L-rhamnosyl residues, some of which are sulphated at O-2 and O-4, D-glucuronic acid and D-xylose as non-reducing end-groups, and 1,4-, 1,3-, and 1,2,4-linked D-xylosyl residues. Further structural studies on zosterine (a complex polysaccharide isolated from *Zostera marina*) have shown that it comprises a block structure composed of galacturonan, apiogalacturonan, and heteroglycanglycuronan interlinked by residues of D-galacturonic acid and L-rhamnose.[155]

Miscellaneous Polysaccharides

Various water-soluble polysaccharides have been isolated from lichens of *Lasallia*, *Usnea*, and *Cladonia* species; the partially *O*-acetylated D-glucan from *L. pensylvanica* contains β-(1 → 6)-linkages and possesses antitumour activity, while the (1 → 3)-— (1 → 4)-linked β-glucan of *U. rubescens* was identified as lichenin.[156] A polysaccharide (DP 150) isolated from *Umbillicaria mammulata* has been shown to be a β-(1 → 6)-linked D-glucan having 9% of the residues acetylated at O-3.[157] A water-soluble glucan and an alkali-soluble glucan have been isolated from the oak lichen *Evernia prunastri*.[158] On the basis of i.r. spectroscopy, methylation analysis, periodate oxidation, and hydrolyses with acid and enzymes, the polysaccharides were shown to be composed principally of (1 → 3)- and (1 → 4)-linked α-D-glucopyranosyl residues in different proportions: the extent of branching in the polysaccharides also differs. The presence of some β-linkages cannot be excluded in view of slight hydrolysis with β-D-glucosidase.

[154] E. J. Bourne, M. L. Megarry, and E. E. Percival, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 235.
[155] Y. S. Ovodov, R. G. Ovodova, V. I. Shibaeva, and L. V. Mikheyskaya, *Carbohydrate Res.*, 1975, **42**, 197.
[156] Y. Nishikawa, K. Ohki, K. Takahashi, G. Kurono, F. Fukuoka, and M. Emori, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2692.
[157] A. H. Heckendorf and Y. Shimizu, *Phytochemistry*, 1974, **13**, 2181.
[158] M. Hranisavljević-Jakovljevic, J. Miljković-Stojanović, R. Dimitrijević, and V. Mićović, *Carbohydrate Res.*, 1975, **39**, 115.

4
Microbial Polysaccharides

Teichoic Acids

A study of morphological mutants of *Bacillus subtilis* and *B. licheniformis* has shown that a reduction in the proportion of negatively charged polymers in the cell wall is commonly encountered, owing either to a failure to form them or to an increase in the synthesis of mucopeptides at the expense of that of teichoic acids.[1] Alterations in the localization or production of lytic enzymes were also implicated. The interaction of concanavalin A with the poly(D-glucosylglycerol phosphate) present in cell walls of *B. subtilis* has been used to study the organization of surface teichoic acids in the ultrastructure.[2] A model was proposed having the surface teichoic acid oriented perpendicular to the long axis of the cell. Hydrolysis of cell walls of *B. subtilis* by either autolysins or lysozyme exposed glycosylated teichoic acids, as judged by the increased precipitation with [^{14}C]-concanavalin A.[3] The number of reactive sites for concanavalin A increased significantly after only limited digestion of the walls. Quantitative analysis of either [^{14}C]-concanavalin A-treated or hydrolysed cell walls indicated that approximately half of the teichoic acid exposed is on the cell surface, whereas the remainder is probably embedded within the peptidoglycan matrix. Cell-wall and membrane teichoic acids from several bacteria have been shown to form soluble complexes with polysaccharides and bovine plasma albumin in alcohols.[4] Polysaccharides (*e.g.* dextran, amylose, D-galactan, levan, and lichenin) containing different monomeric units and anomeric configurations complexed with the teichoic acids, indicating that the interaction is relatively non-specific. The ability of teichoic acids to bind polysaccharides and proteins in solvents of moderate dielectric permittivity (*e.g.* ethanol) suggests that these polymers may serve as complexing agents for hydrophilic molecules found in membranes.

The teichoic acid in the wall of *B. stearothermophilus* possesses a repeating structure of 1-*O*-α-D-glucopyranosylglycerol phosphate units joined by phosphodiester linkages between adjacent glycerol and D-glucopyranosyl units.[5] The walls did not agglutinate with concanavalin A, nor did the isolated teichoic acid precipitate with the lectin. No evidence was obtained to support the presence of a teichoic acid composed of units of D-glucosyl-(1 → 2)-poly(glycerol phosphate), as previously reported. An antigenic determinant isolated from *Butyrivibrio*

[1] H. J. Rogers, H. M. Pooley, P. F. Thurman, and C. Taylor, *Ann. Microbiol.*, 1974, **125B**, 135.
[2] D. C. Birdsell, R. J. Doyle, and M. Morgenstern, *J. Bacteriol.*, 1975, **121**, 726.
[3] R. J. Doyle, M. L. McDaniel, J. R. Helman, and U. N. Streips, *J. Bacteriol.*, 1975, **122**, 152.
[4] R. J. Doyle, A. N. Chatterjee, U. N. Streips, and F. E. Young, *J. Bacteriol.*, 1975, **124**, 341.
[5] A. J. Anderson and A. R. Archibald, *Biochem. J.*, 1975, **151**, 115.

fibrisolvens reacted with antisera specific to the poly(glycerol phosphate) backbone of membrane teichoic acids and glycerol-lipoteichoic acids of lactobacilli.[6] Inhibition studies on the antigen–antibody reaction and chemical studies on the antigen suggested that a poly(glycerol phosphate) backbone acts as the antigenic determinant. Pneumococcal teichoic acids have been shown to inhibit the induction of competence in incompetent cells of *Diplococcus pneumoniae*; the inhibitory effect was similar to that observed with 2-amino-2-deoxy-D-glucose and -D-galactose.[7] Pneumococci grown on a medium containing choline are known to incorporate choline into the wall teichoic acid with the formation of autolysin-sensitive cell walls. The autolysin-catalysed hydrolysis of amide linkages in the peptidoglycan of *D. pneumoniae* required an additional interaction between the enzyme and the choline residues in the teichoic acid portion of the cell wall.[8] The binding of Mg^{2+} ions to the wall teichoic acid of *Lactobacillus buchneri* has been measured in the presence of different concentrations of sodium chloride.[9] This teichoic acid bound fewer Mg^{2+} ions when the concentration of sodium chloride was increased, and, at the same time, Mg^{2+} ions were bound less tightly to the polymer. Changes have been reported in the optical properties of Acridine Orange after binding with the ribitol teichoic acid of *Staphylococcus aureus*.[10] Extraction of *Staph. epidermidis* with dilute phosphate buffers solubilized a teichoic acid containing units of 2-acetamido-2-deoxy-D-glucose and glycerol.[11] The antigenicity of this teichoic acid agreed with the findings that it contains 1,3-phosphodiester linkages, and that the amino-sugar, which is present in the β-configuration, is the principal antigenic determinant.[12] Purified cell walls of *Streptococcus mutans* have been prepared by disruption of cell suspensions without the use of proteolytic enzymes; this procedure ensures that all cell-wall constituents are retained for chemical analysis.[13] The cell wall was reported to be composed of glycerol teichoic acid (9%), non-peptidoglycan polysaccharide (34%), and peptidoglycan (50%). Antisera to whole cells of *S. mutans* cross-reacted with antigens extracted from other Gram-positive species and from other oral Streptococci.[14] A purified glycerol teichoic acid showed that the cross-reactions are specific for a determinant on the teichoic acid backbone. Examination of the culture media and cells of *S. mutans* and *S. faecalis*, after exponential- and stationary-phase growth, revealed the presence of a glycerol teichoic acid.[15] The presence of large amounts of extracellular glycerol teichoic acid appears to result from excretion rather than from cell lysis.

The cell walls of *B. subtilis* were able to synthesize teichuronic acid when grown under growth-limiting conditions of phosphate at a low dilution rate, whereas teichoic acid was synthesized at a high dilution rate.[16] The purified teichuronic

[6] M. E. Sharpe, J. H. Brock, and B. A. Phillips, *J. Gen. Microbiol.*, 1975, **88**, 355.
[7] M. Rejholcová, M. Ticha, M. Kohoutova, and J. Kocourek, *J. Bacteriol.*, 1974, **120**, 1478.
[8] J. V. Höltje and A. Tomasz, *J. Biol. Chem.*, 1975, **250**, 6072.
[9] P. A. Lambert, I. C. Hancock, and J. Baddiley, *Biochem. J.*, 1975, **149**, 519.
[10] V. V. Ignatov, A. A. Shcherbakov, and V. Y. Krestianov, *Mikrobiologia*, 1975, **44**, 224.
[11] G. S. Johnsen, C. Endersen, A. Grov, and P. Oeding, *Acta Pathol. Microbiol. Scand.* (*B*), 1975, **83**, 226.
[12] G. S. Johnsen, A. Grov, and P. Oeding, *Acta Pathol. Microbiol. Scand.* (*B*), 1975, **83**, 235.
[13] H. R. Cooper, F. W. Chorpenning, and S. Rosen, *Infection and Immunity*, 1975, **11**, 823.
[14] F. W. Chorpenning, H. R. Cooper, and S. Rosen, *Infection and Immunity*, 1975, **12**, 586.
[15] R. Joseph and G. D. Shockman, *Infection and Immunity*, 1975, **12**, 333.
[16] J. Wright and J. E. Heckels, *Biochem. J.*, 1975, **147**, 187.

acid was shown to be composed of alternating residues of D-glucopyranosyluronic acid substituted at C-4 and 2-acetamido-2-deoxy-D-galactopyranose substituted at C-3; it has an average chain-length of 20 disaccharide units. Mutant strains of *Micrococcus lysodeikticus* were unable to synthesize cell-wall teichuronic acid under the phosphate-limiting conditions required by the parent strain to synthesize the polysaccharide.[17] No increase in the synthesis of teichoic acid was noted, nor were other anionic polysaccharides detected in cell-wall preparations of the mutants. The absence of other anionic polymers in the mutants was compatible with the development of a requirement for a higher concentration of Mg^{2+} ions, supporting the view that teichuronic acid in *M. lysodeikticus* participates in the binding of bivalent cations.

When the growth temperature for a *rod* mutant of *B. subtilis* is changed from 30 to 45 °C and the organism becomes round, the synthesis of mucopeptides increases, whereas that of teichoic acid decreases.[18] Membrane preparations from certain L-phase variants isolated from *B. licheniformis* and *B. subtilis* were unable to synthesize peptidoglycan when grown in osmotically stabilized media, but they were able to synthesize poly(glycerol phosphate), teichuronic acid, and a polymer of 2-acetamido-2-deoxy-D-glucose.[19] The biosynthesis of a teichoic acid linked covalently to peptidoglycan has been studied in membrane and cell-wall preparations of *B. licheniformis*.[20] Attachment of most ($\sim 80\%$) of the teichoic acid requires the synthesis of cross-linked peptidoglycan, whereas some teichoic acid is attached in the absence of peptidoglycan. Evidence was also obtained for the participation of a membrane-bound precursor in the synthesis of covalently-linked teichoic acid. The biosynthesis of peptidoglycan, teichoic acid, and teichuronic acid by reverting protoplasts of *B. licheniformis* has been studied.[21] At complete reversion, the synthesized cell-wall material possessed characteristics similar to those of the parent bacilli in that it contained three polymers in roughly the same proportions. Teichoic acid and nascent peptidoglycan synthesized at the same time by *Diplococcus pneumoniae* cells are attached covalently to one another.[22] Both peptidoglycan and teichoic acid are incorporated into the cell surface in a growth zone located at the cell's equator. Once physically linked in this zone, the peptidoglycan–teichoic acid complex is conserved during growth and division of the bacteria. In contrast to the high rates of turnover of bacillary walls, the cell walls of Pneumococci undergo little, if any, turnover. In addition to poly(ribitol phosphate), the cell walls of *Staphylococcus aureus* contain glycerol phosphate residues that are not removed either on digestion with proteolytic enzymes or by extraction with phenol.[23] Three or four glycerol phosphate residues are present in a chain which is attached covalently to the peptidoglycan by way of a phosphodiester linkage to *N*-acetylmuramic acid. Degradative studies indicated that the poly(ribitol phosphate) chains of the teichoic acid may be attached to the cell wall by linkage to this oligomeric glycerol phosphate.

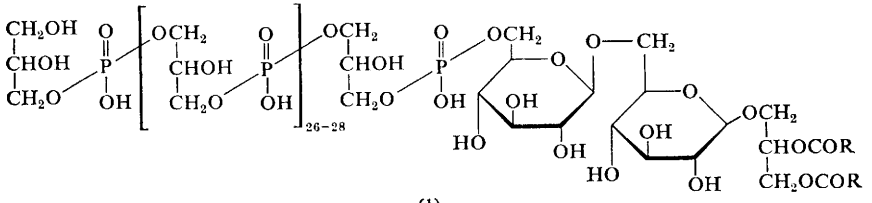
[17] M. Yamada, A. Hirose, and M. Matsuhashi, *J. Bacteriol.*, 1975, **123**, 678.
[18] H. J. Rogers, P. F. Thurman, C. Taylor, and J. N. Reeve, *J. Gen. Microbiol.*, 1974, **85** 335.
[19] J. B. Ward, *J. Bacteriol.*, 1975, **124**, 668.
[20] A. W. Wyke and J. B. Ward, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 877.
[21] T. S. J. Elliot, J. B. Ward, and H. J. Rogers, *J. Bacteriol.*, 1975, **124**, 623.
[22] A. Tomasz, M. McDonnell, M. Westphal, and E. Zanati, *J. Biol. Chem.*,1975, **250**, 337.
[23] J. E. Heckels, A. R. Archibald, and J. Baddiley, *Biochem. J.*, 1975, **149**, 637.

Evidence has been presented to show that peptidoglycan can act as a donor of glycerol and phosphate residues in the synthesis of teichoic and lipoteichoic acids.[24] Thus, a particulate fraction from *Streptococcus sanguis* catalysed the synthesis of polymers containing glycerol 3-phosphate units from peptidoglycan.

The antigenic properties of lipoteichoic acids have been reviewed.[25]

Pulse-chase experiments have demonstrated that the distribution of labelled teichoic acid between the cell wall and the membrane of *Diplococcus pneumoniae* is constant over the chase period, suggesting that the wall teichoic acid and the membrane lipoteichoic acid are synthesized simultaneously and independently.[26] It has been suggested that the physiological role *in vivo* of the choline-containing lipoteichoic acid (Forssman antigen) of *D. pneumoniae* is to control the autolysin activity.[27] A new view of penicillin-induced lysis of bacteria has been based on treatment of an autolysin-deficient mutant of *Pneumococcus* with penicillin.[28] Inhibition of cell-wall synthesis by any means triggered the release of bacterial autolytic enzymes by destabilizing an endogenous complex composed of autolysin inhibitor (lipoteichoic acid) and autolytic enzyme. Chloramphenicol and other inhibitors of protein synthesis are considered to protect bacteria against penicillin-induced lysis by interfering with the activities of the autolytic enzymes, rather than by depleting the concentration of these enzymes at the cell surface. That the principal physiological function of lipoteichoic acids is to control bacterial autolytic enzymes is supported by the findings that the addition of pneumococcal lipoteichoic acid to culture media of growing *Pneumococcus* caused chain formation, prevented lysis during the stationary phase of growth, and inhibited any lysis by penicillin and by the pneumococcal bacteriophage Dp-1.[29]

A lipoteichoic acid (1) extracted from *Staphylococcus aureus* with phenol consists of chains composed of 28 glycerol phosphate units attached to an acylated



(1) R = fatty acid

diglucosylglycerol.[30] The D-glucosyl residues of the lipoteichoic acid are present as diacylated 1-*O*-β-gentiobiosylglycerol, and the teichoic acid chain is attached by way of a phosphodiester linkage involving O-6 of the non-reducing, terminal D-glucosyl residue. These conclusions conflict with previous reports on the lipoteichoic acid carrier extracted from membranes of *Staphylococcus aureus*

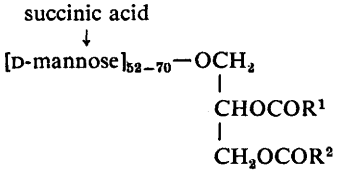
[24] L. Edmur and T. H. Chiu, *F.E.B.S. Letters*, 1975, **55**, 216.
[25] A. J. Wicken and K. W. Knox, *Science*, 1975, **187**, 1161.
[26] E. B. Briles and A. Tomasz, *J. Bacteriol.*, 1975, **122**, 335.
[27] J. V. Höltje and A. Tomasz, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1690.
[28] A. Tomasz and S. Waks, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4162.
[29] J. V. Höltje and A. Tomasz, *J. Bacteriol.*, 1975, **124**, 1023.
[30] M. Duckworth, A. R. Archibald, and J. Baddiley, *F.E.B.S. Letters*, 1975, **53**, 176.

with phenol [31] The lipoteichoic acid carrier is considered to be identical with the membrane lipoteichoic acid, and is claimed to consist of chains of 12—14 residues of glycerol phosphate, with one mole each of D-glucose and fatty acid present in the chains.[32] Culture fluids from a number of strains of oral Streptococci and of Lactobacilli contain an erythrocyte-sensitizing antigen possessing the properties of a lipoteichoic acid; this was shown by means of antibodies specific for the poly(glycerol phosphate) backbone of the lipoteichoic acid.[33]

The teichoic acid of the lipoteichoic acid isolated from *Streptococcus lactis* has been shown to contain 16 or 17 units of glycerol phosphate, approximately half of which are substituted with α-D-galactopyranosyl residues; the glycolipid portion contains α-D-glucopyranosyl-(1 → 2)-α-D-glucopyranosyl-(1 → 1)-glycerol.[34] α-D-Galactosyl residues are the only sugar constituents of the teichoic acid. Thus, this lipoteichoic acid differs from any previously described, insofar as the component sugar of the glycolipid differs from that of the teichoic acid.

The binding of Group A *Streptococci* to cell membranes has indicated that ester-linked fatty acids are involved in the spontaneous adsorption of lipoteichoic acids onto erythrocytes.[35] Both lipoteichoic acid and mesosomal vesicles were released from *S. faecalis* by autolysis with *N*-acetylmuramidase.[36] Evidence that the lipoteichoic acid is not associated with the isolated mesosomal residues was obtained. Partial hydrolysis with acid has shown that the poly(glycerol phosphate) of the membrane lipoteichoic acid of *S. faecalis* is covalently linked, by way of a phosphodiester bond, to phosphatidylkojibiosyldiglyceride.[37]

Lipomannans have been isolated from membranes of *Micrococcus lysodeikticus*, *M. flavus*, and *M. sodonensis*, whereas lipoteichoic acids could not be detected.[38] The suggestion that lipoteichoic acids are replaced by lipomannans was strengthened by chemical and physical similarities between the two polymers: the lipomannans contain glycerol, ester-linked fatty acids, and monoesterified succinic acid, conferring on them both hydrophobic properties and charge. The lipomannan (2) isolated from *M. lysodeikticus* contains a chain of about 60



(2) $R^1 = R^2$ = fatty acids

D-mannosyl residues with two branch points, and is joined at the reducing end to a glycerol residue bearing two fatty acid residues. Succinic acid residues on the lipomannan enable it to bind Mg^{2+} ions. This lipomannan was shown to be

[31] F. Fiedler and L. Glaser, *J. Biol. Chem.*, 1974, **249**, 2684.
[32] F. Fiedler and L. Glaser, *Carbohydrate Res.*, 1974, **37**, 37.
[33] J. L. Markham, K. W. Knox, A. J. Wicken, and M. J. Hewett, *Infection and Immunity*, 1975, **12**, 378.
[34] A. J. Wicken and K. W. Knox, *Infection and Immunity*, 1975, **11**, 973.
[35] I. Ofek, E. H. Beachey, W. Jefferson, and G. L. Campbell, *J. Exp. Med.*, 1975, **141**, 990.
[36] R. Joseph and G. D. Shockman, *J. Bacteriol.*, 1975, **122**, 1375.
[37] M. C. W. Ganfield and R. A. Pieringer, *J. Biol. Chem.*, 1975, **250**, 702.
[38] D. A. Powell, M. Duckworth, and J. Baddiley, *Biochem. J.*, 1975, **151**, 387.

associated with the cytoplasmic membrane, probably by intercalation of the fatty acid residues with those of membrane lipids. In another study of the membrane of *M. lysodeikticus*, the isolated lipomannan was shown to have a different fatty acid composition and a different ratio of hexose to acyl groups.[39] These differences could be a consequence of the different conditions used to grow the micro-organism. *In vitro* experiments indicated that the deacylated lipomannan does not bind to membranes, supporting the view that the acyl groups are responsible for binding.

The pattern of cleavage of the cell wall of *B. subtilis* by purified *N*-acetylmuramic acid:L-alanine amidase has been studied in the absence, and in the presence, of a modifier protein that combines stoicheiometrically with the enzyme to stimulate its activity.[40] The modifier protein did not affect the rate of the enzymic reaction, but changed the mode of cleavage of the cell wall from a random pattern to a sequential pattern. Binding of the enzyme to the cell wall and functional interaction with the modifier protein occurred only when the cell wall contained teichoic acids. It appears that cell-wall teichoic acids act as specific 'allosteric' ligands for bacterial lytic enzymes.

Peptidoglycans

From an examination of molecular models, two configurations have been proposed for the glycan strands and the peptide side-chains of peptidoglycans; each configuration will accommodate any chemical modification that has been observed in bacteria without the need to change the configuration of the peptide backbone.[41] In one model, the glycan strands are parallel, with their lengths and widths predominantly in the plane of the cell wall; the cross-bridging portions of the peptide side-chains are in a separate parallel plane at right angles to the glycan strands, and the peptide chains are adpressed to the glycan strands and stabilized by three hydrogen-bonds for each disaccharide–peptide subunit. In the other model, the peptide side-chains are raised away from the glycan strands in an extended configuration. The compact and extended conformations are thought to be interconvertible.

A radioimmunoassay using a synthetic pentapeptide hapten has been developed for measurement of the pentapeptide determinant of peptidoglycans.[42]

Analysis of the component peptidoglycans in the cell walls has been used in the identification of the genus *Actinomyces*.[43] The peptidoglycan of *Actinomyces meyeri* is of the lysine type, whereas that of *Corynebacterium granulosum* contains diaminopimelic acid. The principal components of the peptidoglycan present in cell walls of *A. viscosus* have been identified as 2-acetamido-2-deoxy-D-glucose, *N*-acetylmuramic acid, alanine, glutamic acid, and lysine (1 : 1 : 4 : 4 : 4).[44] Electrophoretic separation of the products obtained by partial hydrolysis of the peptidoglycan with acid indicated a structure similar to that of other lysine-

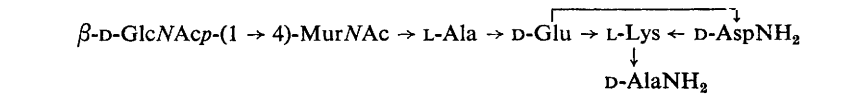
[39] D. D. Pless, A. S. Schmit, and W. J. Lennerz, *J. Biol. Chem.*, 1975, **250**, 1319.
[40] D. R. Herbold and L. Glaser, *J. Biol. Chem.*, 1975, **250**, 7231.
[41] E. H. Oldmixon, S. Glauser, and M. L. Higgins, *Biopolymers*, 1974, **13**, 2037.
[42] B. Heymer, D. Bernstein, K. H. Schleiffer, and R. M. Krause, *J. Immunol.*, 1975, **114**, 1191.
[43] M. Závadova, J. Chaloupka, P. Křečková, J. Adensámová, and J. Pokorný, *Folia Microbiol.*, 1974, **19**, 177.
[44] M. J. Reed and R. T. Evans, *Arch. Oral Biol.*, 1974, **19**, 1083.

containing peptidoglycans, although there are also highly complex cross-bridges. The incorporation of newly synthesized peptidoglycan into pre-existing cell walls of *B. licheniformis* was inhibited by β-lactam antibiotics.[45] Cells of *B. megaterium* were unable to synthesize peptidoglycan from externally added precursors (*e.g.* UDP-2-acetamido-2-deoxy-D-glucose and UDP-*N*-acetylmuramyl-L-alanyl-D-glutamyl-*meso*-diaminopimelyl-D-alanyl-D-alanine) unless they were first treated with toluene.[46] The newly synthesized peptidoglycan is attached to the existing cell wall by peptide cross-bridges. A study of the enzymes involved in the synthesis of peptidoglycan precursors has shown that the specificity for addition of the third amino-acid residue to the peptide side-chain is absolute; thus, extracts of *Corynebacterium poinsettiae* are capable of adding L-homoserine only to UDP-*N*-acetylmuramylglycyl-D-glutamine.[47] A phage endolysin, identified as an *N*-acetylmuramyl-L-alanine amidase, hydrolysed the cell-wall peptidoglycan of *Clostridium saccharoperbutylacetonicum* with the release of a polysaccharide and a peptide containing D-glutamic acid, DL-alanine, and diaminopimelic acid (1 : 2 : 1).[48] The peptide unit contains the sequence L-Ala-D-Glu-D-diaminopimelyl-D-Ala and appears to link *N*-acetylmuramic acid residues on different polysaccharide chains. Cell-wall peptidoglycans from *E. coli* and *Salmonella typhimurium* were hydrolysed to dialysable non-reducing fragments by partially purified preparations of the bacteriolytic activities in lysates of phages λ or Vi II.[49, 50] The absence of reducing properties in the structures of the two principal fragments was ascribed to the presence of a 1,6-anhydro-*N*-acetylmuramic acid residue at the 'reducing-end' of each chain. Both sources are considered to contain at least two types of hydrolytic activities: an *endo*-peptidase, which is responsible for cleavage of peptide cross-linkages, and a new type of *N*-acetylmuramidase, which cleaves not only β-(1 → 4)-linkages between *N*-acetylmuramic acid and 2-acetamido-2-deoxy-D-glucose in the glycan moiety, but also catalyses dehydration to form residues of 1,6-anhydro-*N*-acetylmuramic acid. The mode of incorporation of diaminopimelic acid into the peptidoglycan and that of insertion of phage-λ receptor into the external membrane of *E. coli* have been compared.[51] Resistance to digestion by trypsin was conferred on the principal envelope glycoprotein from *E. coli* on association with peptidoglycan.[52]

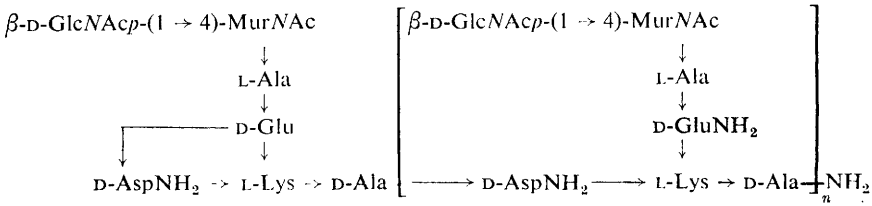
A mutant of *E. coli* has been described in which the cells showed an irregular morphology associated with the leakage of β-galactosidase and other intracellular proteins.[53] An investigation of the relative rates of peptidoglycan synthesis in the mutant and parent strains did not reveal any impairment to the synthesis of UDP-*N*-acetylmuramylpentapeptide. It appears that either the mutant cells are defective in the peptidoglycan layer or the peptidoglycan does not interact with other components of the cell envelope. Muramic acid was not detected in the hydrolysates of cell-wall preparations from a number of strains

[45] Z. Tynecka and J. B. Ward, *Biochem. J.*, 1975, **146**, 253.
[46] W. P. Schrader and D. P. Fan, *J. Biol. Chem.*, 1974, **249**, 4815.
[47] A. W. Wyke and H. R. Perkins, *J. Gen. Microbiol.*, 1975, **88**, 159.
[48] S. Ogata, Y. Tahara, and M. Hongo, *J. Gen. Appl. Microbiol.*, 1975, **21**, 65.
[49] J. Van Heijenoort and A. Taylor, *Ann. Microbiol.*, 1974, **125B**, 183.
[50] A. Taylor, B. C. Das, and J. Van Heijenoort, *European J. Biochem.*, 1975, **53**, 47.
[51] A. Ryter, *Ann. Microbiol.*, 1974, **125B**, 167.
[52] J. P. Rosenbusch, *J. Biol. Chem.*, 1974, **249**, 8019.
[53] K. Olden, S. I. Ito, and T. H. Wilson, *J. Bacteriol.*, 1975, **122**, 1310.

of halophilic cocci, supporting the view that the absence of this sugar is characteristic of all of these bacteria, since the cell walls do not contain peptidoglycans commonly found in bacteria.[54] Glycine, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and 2-amino-2-deoxyguluronic acid are the principal cell-wall components, but account for only 7—15% of the dry weight of cell walls. Since this is much less than the amount of peptidoglycan present in the cell walls of common micrococci, it is likely that components other than amino-acids and amino-sugars are responsible for the structure, shape, and rigidity of the cell walls. A fraction (20—30%) of the peptidoglycan isolated from *Lactobacillus acidophilus* has been shown to be immune to turnover in growing cells.[55] This immunity may be restricted either to a specific, chemically different portion, or to a topographical portion (*e.g.* the cell poles), of the cell wall. Three neutral glycopeptides possessing antitumour properties were released from the cell walls of *L. bulgaricus* by digestion with lysozyme and pepsin.[56] The glycopeptides were shown to contain muramic, aspartic, and glutamic acids, 2-amino-2-deoxy-D-glucose, alanine, and lysine (1 : 1 : 1 : 1 : 2 : 1), and to possess molecular weights of 1×10^3, 2×10^3, and 1×10^4; the results of sequence studies indicated the structures (3)—(5), respectively, for these glycopeptides.



(3)



(4) $n = 1$
(5) $n = 8$—10

Both N- and O-substitution in the cell-wall peptidoglycans of *L. fermentum* have been studied in relation to the growth phase; the lytic activities and the effect of trypsin on the peptidoglycans were also examined.[57] Extensive *O*-acetylation occurs as the cells enter the stationary growth phase, and the sensitivity of the cells to lysozyme decreases at the same time. Unsubstituted amino-sugars, principally 2-amino-2-deoxy-D-glucose, occur in the cell walls during both exponential and stationary phases, but not in the corresponding peptidoglycans. The sensitivity to lysozyme of isolated cell walls that had been *N*-acetylated chemically

[54] R. Reistad, *Arch. Mikrobiol.*, 1975, **102**, 71.
[55] L. Daneo-Moore, J. Coyette, M. Sayare, D. Boothby, and G. D. Shockman, *J. Biol. Chem.*, 1975, **250**, 1348.
[56] I. G. Bogdanov, P. G. Dalev, A. I. Gurevich, M. M. Kolosov, V. P. Mal'kova, and L. A. Plemyannikova, *F.E.B.S. Letters*, 1975, **57**, 259.
[57] I. M. Logardt and H. Y. Neujahr, *J. Bacteriol.*, 1975, **124**, 73.

decreases, and autolysis of the modified cell walls is inhibited. Trypsin was shown to enhance the sensitivity of native cell walls to lysozyme, but had no effect on cell walls that had been *O*-deacetylated and *N*-acetylated, suggesting that it acts as an esterase in removing *O*-acetyl groups from lysozyme-resistant walls.

The association of membrane peptidoglycans has been established in the synthesis *in vitro* of the peptidoglycans of *Micrococcus luteus*.[58] Incubation of *M. luteus* cells in the presence of penicillin G led to the accumulation in the culture medium of a linear peptidoglycan (mol. wt. 3.8×10^4) containing *ca.* 50 disaccharide (β-D-Glc*N*Ac-(1 → 4)-Mur*N*Ac) units, half of which have *N*-acetylmuramic acid residues substituted by the hexapeptide L-Ala-D-Glu(Gly)-L-Lys-D-Ala-D-Ala.[59] Penicillin G also promoted the secretion of a hexapeptide in amounts equal to that of the unsubstituted *N*-acetylmuramic acid residues on the secreted peptidoglycan. Secretion of the peptidoglycan was attributed to inhibition by penicillin of the attachment of newly synthesized peptidoglycans to pre-existing wall by transpeptidation, whereas secretion of the hexapeptide results from the action of a penicillin-insensitive amidase on newly synthesized peptidoglycan. Hydrolysis of the amino-sugar backbone of the peptidoglycan present in a *Myxobacter* sp. by an *endo-N*-acetylmuramidase released monomeric, dimeric, and trimeric peptides.[60] The monomeric peptide, which is attached covalently by way of *N*-acetylmuramic acid residues of the backbone, contains the sequence L-Ala-D-Glu-L-*meso*-diaminopimelyl-D-Ala. Several antigens associated with the cell walls of the genus *Pseudomonas* have been described.[61] A highly antigenic, strain-specific antigen was identified as a high-molecular-weight protein or lipoprotein in the form of an envelope surrounding the cells. Polysaccharides or lipopolysaccharides situated below this antigen are less antigenic than the strain-specific antigen. The mucopeptide component of the cell also exhibited antigenic activity that depends both on its carbohydrate and polypeptide components.

The chemical and immunological properties of cell walls of the anaerobic coryneforms *Propionibacterium acnes* and *Corynebacterium parvum* have been shown to differ from those of the cell wall of *C. diphtheriae*.[62] Although each organism appears to contain the same type of peptidoglycan, differences in the associated polysaccharide fractions are apparent. No differences were detected in the peptidoglycans of a number of novobiocin-resistant, pleiotropic mutants of Staphylococci.[63] The pleiotropy of these strains appeared to result from a genetic change that interferes with the control of the biosynthesis of teichoic acids. Cells of *E. coli* and *Salmonella typhimurium* defective in peptidoglycans have been shown to be similar to intact plasmolysed cells insofar as they allow partial penetration of low-molecular-weight oligosaccharides but exclude saccharides of molecular weights above 1×10^3.[64] However, the outer membrane, rather than the peptidoglycan, appears to determine the size of uncharged hydrophilic molecules that can penetrate the cell wall.

[58] G. Pellon, C. Bordet, and G. Michel, *Ann. Microbiol.*, 1974, **125B**, 149.
[59] D. Mirelman, R. Bracha, and N. Sharon, *Biochemistry*, 1974, **13**, 5045.
[60] E. Harcke, F. Masson, and H. Kühlwein, *Arch. Mikrobiol.*, 1975, **103**, 251.
[61] A. M. Tunstall, G. Gowland, and G. Hobbs, *J. Appl. Bacteriol.*, 1975, **38**, 159.
[62] I. Azuma, K. Sugimura, T. Taniyama, A. A. Aladin, and Y. Yamamura, *Jap. J. Microbiol.*, 1975, **19**, 265.
[63] R. Z. Korman, *J. Bacteriol.*, 1975, **124**, 724.
[64] T. Nakae and H. Nikaido, *J. Biol. Chem.*, 1975, **250**, 7359.

The biosynthesis and release of relatively non-cross-linked, soluble fragments of peptidoglycans by protoplasts of *Streptococcus faecalis* have been related to the absence of suitable acceptors in pre-existing walls.[65] The amounts of soluble fragments of peptidoglycan synthesized by the protoplasts were of the same order of magnitude as those of insoluble peptidoglycan expected to be produced by an equivalent amount of intact Streptococci.[66] When the autolysis of glycan chains by *N*-acetylmuramidase was suppressed in growing *S. faecalis* protoplasts, relatively large glycan chains with infrequent peptide cross-linkages were synthesized.[67] During rod-to-sphere transformation in the growth of *Vibrio* spp., the peptidoglycans are not attached to other cell-wall components, and are fragmented by autolysis by an *endo*-acetamidodeoxyhexosidase and an *endo*-peptidase.[68] Since the peptidoglycan layer is no longer intact, the cells become spherical, and convoluted layers of the cell wall straighten and expand away from the cytoplasmic membrane taking with them any attached peptidoglycan. Polysaccharide antigens and peptidoglycans have been detected in cell walls of the spirochaetes *Leptospira biflexa* and *Treponema pallidum*; L-ornithine, a component of the peptidoglycan of *T. pallidum*, was replaced by diaminopimelic acid in the peptidoglycan occurring in *L. biflexa*.[69]

Lipopolysaccharides

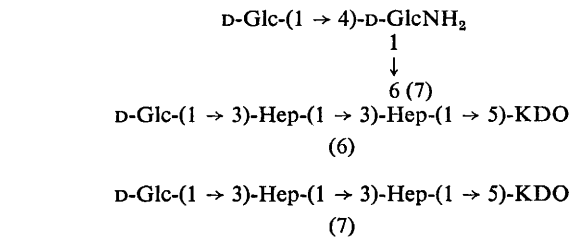
The chemistry and immunochemistry of bacterial lipopolysaccharides have been reviewed.[70, 71]

Electrodialysis has been used to remove a large proportion of the metal ions and amines that frequently contaminate preparations of lipopolysaccharides.[72]

Six different O-antigenic specificities have been detected in serological studies on the lipopolysaccharide of *Bacteroides fragilis*.[73] Serological activity was destroyed by periodate oxidation of the lipopolysaccharide. The polysaccharide moiety of a lipopolysaccharide occurring in a rough strain of *E.coli* is composed of D-galactosyl, D-glucosyl, L-*glycero*-D-*manno*-heptosyl, some D-mannosyl, and an unusually high proportion of 3-deoxy-D-*manno*-octulosonic acid residues; it represents the complete *E. coli* R2 core.[74] Treatment of *E. coli* with hot phenol–water resulted in partial degradation of the lipopolysaccharide, with the release of relatively long-chain fatty acids into the phenolic phase.[75] The traditional method using phenol for the extraction of lipopolysaccharides has been compared with a new method using aqueous butan-1-ol; both methods solubilized closely similar

[65] R. S. Rosenthal, D. Jungkind, L. Daneo-Moore, and G. D. Shockman, *J. Bacteriol.*, 1975, **124**, 398.
[66] R. S. Rosenthal and G. D. Shockman, *J. Bacteriol.*, 1975, **124**, 410.
[67] R. S. Rosenthal and G. D. Shockman, *J. Bacteriol.*, 1975, **124**, 419.
[68] D. A. Baker and R. W. A. Park, *J. Gen. Microbiol.*, 1975, **86**, 12.
[69] I. Azuma, T. Taniyama, Y. Yamamura, Y. Yanagihara, Y. Hattori, S. Yasuda, and I. Mifuchi, *Jap. J. Microbiol.*, 1975, **19**, 45.
[70] K. Jann and O. Westphal, in 'The Antigens', ed. M. Sela, Academic Press, London and New York, 1975, Vol. 3, p. 1.
[71] O. Westphal, *Internat. Arch. Allergy Appl. Immunol.*, 1975, **49**, 1.
[72] C. Galanos and O. Lüderitz, *European J. Biochem.*, 1975, **54**, 603.
[73] T. Hofstad, *Acta Pathol. Microbiol. Scand.* (*B*), 1975, **83**, 477.
[74] P. N. Taylor, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 148.
[75] S. Okuda, M. Sato, H. Uchiyama, and H. Takahashi, *J. Gen. Appl. Microbiol.*, 1975, **21**, 169.

lipopolysaccharides from *E. coli*.[76] Each preparation contains two physically and chemically distinct lipopolysaccharides: one (mol. wt. 1.5×10^6) has long antigenic side-chains and is not dissociated by detergents, whereas the other has short antigenic side-chains and is dissociated into units of molecular weight $\sim 9 \times 10^4$ by detergents. The hexose–heptose region of core oligosaccharides derived from lipopolysaccharides isolated from *E. coli* BB and from a number of R-phage-selected mutants with defective cell walls has been identified as heptose-(1 → 5)-3-deoxy-2-octulosonate.[77] Chemical analyses of the carbohydrate compositions of lipopolysaccharides from a number of mutants have been used to propose a schematic composition for a lipopolysaccharide from *E. coli* K-12.[78] The lipopolysaccharides are considered to be composed of four regions that reflect well-defined steps in their biosynthesis: the regions contain (i) lipid A, deoxyoctulosonic acid, and a phosphorus component, (ii) only heptose, (iii) only D-glucose, or (iv) D-glucose, D-galactose, and L-rhamnose. A series of lipopolysaccharide-specific bacteriophages were used in comparing three mutants of *Salmonella* with a number of mutants of *E. coli* K-12. The results revealed basic similarities in both the first and second regions of the structures of these lipopolysaccharides, and supported the proposal that there are four regions in the lipopolysaccharides' structures. Phage blocking with intact and with hydrolysed lipopolysaccharides has suggested that lipid A is part of the receptor site for phage $\phi 5$.[79] Studies with a bacteriophage infective for a mutant of *E. coli* deficient in UDP-D-galactose 4-epimerase showed that no adsorption occurs when the complete lipopolysaccharide is present in the cell membrane.[80] The core portion of the cell-wall lipopolysaccharide of *E. coli* J-5 (a mutant deficient in UDP-D-galactose epimerase) has been subjected to detailed structural analysis.[81] A mixture of dephosphorylated core oligosaccharides was resolved into three components by a combination of paper chromatography and electrophoresis, and ion-exchange chromatography. Smith degradation and specific enzymic and chemical procedures were used to identify two of the oligosaccharides as (6) and (7). By using particulate fractions of the cell envelope as a source of D-galactosyltransferase, it was shown that D-[^{14}C]galactose is transferred from



KDO = 3-deoxy-2-octulosonic acid; Hep = heptose

[76] D. C. Morrison and L. Leive, *J. Biol. Chem.*, 1975, **250**, 2911.
[77] P. Prehm, S. Stirm, B. Jann, and K. Jann, *European J. Biochem.*, 1975, **56**, 41.
[78] H. G. Boman and D. A. Monner, *J. Bacteriol.*, 1975, **121**, 455.
[79] H. G. Boman and D. A. Monner, *J. Bacteriol.*, 1975, **121**, 465.
[80] C. J. Pazoles and C. F. Kulpa, *Canad. J. Microbiol.*, 1975, **21**, 1217.
[81] N. A. Fuller, M. C. Wu, R. G. Wilkinson, and E. C. Heath, *J. Biol. Chem.*, 1973, **248**, 7938.

UDP-D-galactose to the terminal, non-reducing D-glucosyl residues of these oligosaccharides. The tetrasaccharide repeating unit (8) of the O-specific side-chains of the cell-wall lipopolysaccharide from *E. coli* 075 has been established by methylation analysis and Smith degradation.[82]

$$\rightarrow 3)\text{-}\alpha\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow$$
$$\begin{array}{c} 4 \\ \uparrow \\ 1 \end{array}$$
$$\beta\text{-D-Man}p$$

(8)

A polymer containing mainly *O*-acetylated D-*glycero*-D-*galacto*-heptosyl residues and an unidentified aldose has been located in the outer part of the cell wall of *Eubacterium saburreum*.[83] An endotoxin isolated from *Fusobacterium necrophorum* has been shown to be a lipopolysaccharide composed of amino-sugars (7%), reducing sugars (50%), heptose (6%), 3-deoxy-2-octulosonic acid (0.8%), lipid A (21%), and phosphorus (1.7%).[84] The lipopolysaccharide appears mainly as a ribbon-like, trilaminar structure under the electron microscope. Envelopes from competent cells of *Haemophilus influenzae* have been found to contain elevated levels of a lipopolysaccharide having a composition different from that present in log-phase cells.[85] The lipopolysaccharide synthesized by competent cells is less extensively phosphorylated and, therefore, less ionic than that present in log-phase cells. Both heptose and 3-deoxy-2-octulosonate have been detected in different strains of *Fusobacterium* and *Leptotrichia buccalis*, but not in *Bacteriodes* sp.[86]

Immunological analysis of glycolipids and lipopolysaccharides of various mycoplasmas has shown that the antigenic specificity is directly related to the structures of the saccharide chains in these polymers.[87] A purified mannan isolated (by extraction with phenol) from *Micrococcus lysodeikticus* cells has been found to bind to membranes *in vitro*, to have an apparent molecular weight $5—10 \times 10^4$, and to be composed of D-mannosyl, succinyl, fatty acid, and glycerol residues.[88] Structural investigations indicated that the mannan chain is linked to a 1,2-di-*O*-acylglycerol. A lipopolysaccharide consisting of lipid A, phosphate, D-galactose, L-arabinose, D-glucose, 2-amino-2-deoxy-D-glucose, and a dideoxy-sugar has been isolated from a thermophilic actinomycete (*Micropolyspora faeni*).[89] Animals produced mainly IgM antibodies to the lipopolysaccharide, although humans also produced IgG antibodies. All preparations of lipopolysaccharides isolated from several strains of *Neisseria gonorrhoeae* lack O-antigenic side-chains, but contain residues of D-glucose, D-galactose, 2-amino-2-deoxy-D-glucose, heptose, 3-deoxy-2-octulosonate, phosphate, and identical

[82] C. Erbing, S. Svensson, and S. Hammarström, *Carbohydrate Res.*, 1975, **44**, 259.
[83] T. Hofstad, *Acta Pathol., Microbiol. Scand. (B)*, 1975, **83**, 471.
[84] M. M. Garcia, K. M. Charlton, and K. A. McKay, *Infection and Immunity*, 1975, **11**, 371.
[85] K. C. Zoon and J. J. Scocca, *J. Bacteriol.*, 1975, **123**, 666.
[86] T. Hofstad, *J. Gen. Microbiol.*, 1974, **85**, 314.
[87] T. Sugiyama, P. F. Smith, T. A. Langworthy, and W. R. Mayberry, *Infection and Immunity*, 1974, **10**, 1273.
[88] D. D. Pless, A. S. Schmit, and W. J. Lennarz, *J. Biol. Chem.*, 1975, **250**, 1319.
[89] M. R. Hollingdale, *J. Gen. Microbiol.*, 1975, **86**, 250.

fatty acids.[90] No significant differences were found between the lipopolysaccharides of virulent and avirulent gonococci or between penicillin-sensitive and -resistant strains.

The cell-wall constituents of *Proteus mirabilis* have been systematically isolated in an attempt to localize an antitumour principle present only in the lipopolysaccharide layer of the cell wall.[91] The principal constituents of the purified lipopolysaccharide are D-galactose, amino-sugar, 3-deoxy-2-octulosonate, and myristic, β-hydroxymyristic, and diaminopimelic acids. A heptose-deficient mutant of *P. mirabilis* synthesized a lipopolysaccharide composed mainly of 3-deoxy-2-octulosonate, 2-amino-2-deoxy-D-glucose, and fatty acids, but containing no neutral sugars.[92] The lipopolysaccharide exhibited much the same antitumour activity as that of the wild type, excepting that the toxicity and pyrogenicity were lower.

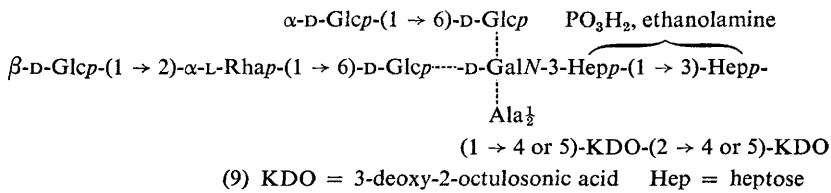
The compositions of lipopolysaccharides derived from 31 sero-groups of *P. mirabilis* have been reinvestigated; a new scheme incorporating 11 chemotypes was proposed.[93] Four distinct strains of *P. mirabilis* have been shown to contain two lipopolysaccharide fractions.[94] One of the lipopolysaccharides in each of the four strains appears to be similar and contains only small proportions of strain-specific constituents; the other, a water-soluble lipopolysaccharide, was purified by ion-exchange chromatography and found to contain such strain-specific constituents as uronic acid, amino-acids, amino-sugars, neutral sugars, ethanolamine, and phosphate, depending on the particular strain.

Cells of *Pseudomonas aeruginosa*, grown in media deficient in Mg^{2+} ions, were resistant to lysis by H_4edta, and the ultrastructure of the outermost layer of the outer cell-wall membrane was altered.[95] Envelopes from these cells contained less phosphorus, but more total carbohydrate and 3-deoxy-2-octulosonate, than those from H_4edta-sensitive cells. The lipopolysaccharides of *P. aeruginosa* and a phage-resistant mutant thereof are heterogeneous.[96] Growth experiments suggested that every strain of *P. aeruginosa* produces several lipopolysaccharides, each containing a common polysaccharide, but the relative proportions of the lipopolysaccharides can vary with the conditions of growth. Lipopolysaccharides from a number of strains of *P. aeruginosa*, representing seven serotypes on the Habs scheme, have been analysed.[97] Two polysaccharides, one of which appeared to be the common core polysaccharide, were obtained from most strains. The principal components identified in the core polysaccharides were D-glucose, L-rhamnose, 2-amino-2-deoxy-D-galactose, alanine, phosphate, and heptose. High-molecular-weight polysaccharides also obtained from most of the strains appeared to be heterogeneous and to consist mainly of amino-sugars, although L-rhamnose is one of the principal components of four of these polysaccharides

[90] A. Stead, J. S. Main, M. E. Ward, and P. J. Watt, *J. Gen. Microbiol.*, 1975, **88**, 123.
[91] M. Arai, M. Nakahara, K. Hamano, and H. Okazaki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1813.
[92] M. Nakahara, N. Kitahara, K. Hamano, M. Arai, and H. Okazaki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1821.
[93] K. Kotelko, I. Fromme, and Z. Sidorczyk, *Bull. Acad. polon. Sci., Ser. Sci. biol.*, 1975, **23**, 249.
[94] J. Gmetner, *European J. Biochem.*, 1975, **58**, 621.
[95] H. E. Gilleland, J. D. Stinnett, and R. G. Eagon, *J. Bacteriol.*, 1974, **117**, 302.
[96] I. R. Chester and P. M. Meadow, *European J. Biochem.*, 1975, **58**, 273.
[97] S. G. Wilkinson and L. Galbraith, *European J. Biochem.*, 1975, **52**, 331.

and L-arabinose is present in another. 2-Amino-2,6-dideoxy-L-galactose was most commonly found, but quinovosamine (2-amino-2,6-dideoxy-D-glucose), 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and an aminohexuronic acid (see ref. 99) were also detected. The results were discussed in terms of the serological classification of these bacteria and of their sensitivity of H_4edta.

Two water-soluble polymers were isolated after mild hydrolysis of the lipopolysaccharide of *P. aeruginosa* with acid.[98] The polymer of higher molecular weight retained O-antigenic specificity and contained 2-amino-2,6-dideoxy-L-galactose, whereas that of lower molecular weight corresponded to the core material and contained residues of D-glucose, L-rhamnose, heptose, 3-deoxy-2-octulosonate, 2-amino-2-deoxy-D-galactose, alanine, and phosphate. Structural studies on the neutral oligosaccharides and other results suggested the structure (9) for the core.



(9) KDO = 3-deoxy-2-octulosonic acid Hep = heptose

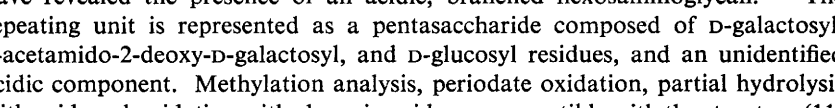
2-Amino-2-deoxy-D-galacturonic acid has been identified as a component of the lipopolysaccharide of *P. aeruginosa*; it probably occurs in the region of the polysaccharide responsible for O-antigenic specificity.[99] In an attempt to establish a relationship between the amino-sugars present in the lipopolysaccharides, the serotype and sensitivity to pyocin of different strains of *P. aeruginosa*, it was noted that only those strains sensitive to pyocin contain quinovosamine.[100]

The growth phase has been shown to influence the level of synthesis of lipopolysaccharides in a number of S-forms of *Salmonella* and in a rough mutant.[101] A decline in the proportion of lipopolysaccharides in the S-forms was found during the exponential phase of growth, with a discontinuity in the synthesis of lipopolysaccharides in the stationary phase. The level of synthesis of lipopolysaccharides increased substantially during the exponential phase of growth of a rough mutant. A correlation has been reported between the immunofluorescence of the 'core glycolipid' antigen of *S. minnesota* and the results obtained (by gel diffusion) on measuring antibodies directed against the antigen in sera.[102] Lipopolysaccharides have been extracted from the parent and two strains of *S. typhimurium* that are resistant to Felix O (FO) phage, which has its receptor located on the core lipopolysaccharide.[103] The amounts of lipopolysaccharide and O-specific sugars in the cores, and the average length of the O-chains, are almost identical in the parent and mutant strains. Neither passive haemagglutination nor methylation analysis revealed the presence of incomplete cores in the mutant strains. The phage-resistant strains appear to result from a

[98] D. T. Drewry, K. C. Symes, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1975, **149**, 93.
[99] S. G. Wilkinson and A. P. Welbourn, *Biochem. J.*, 1975, **149**, 783.
[100] S. F. Koval and P. M. Meadow, *J. Gen. Microbiol.*, 1975, **91**, 437.
[101] S. Schlecht, *Z. Bakt. Parasit. Infekt. Hygiene*, 1975, **232A**, 61.
[102] L. S. Young, K. R. Hoffman, and P. Stevens, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 389.
[103] A. A. Lindberg and S. Svensson, *J. Gen. Microbiol.*, 1975, **87**, 11.

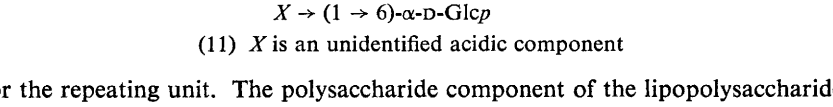
mutation in the synthesis of the core, which leaves few, if any, completed cores accessible to the FO phage. The labelling of cell walls or outer membranes of *S. typhimurium* with ferritin-conjugated antibodies directed against the polysaccharide moiety of the lipopolysaccharide has been examined.[104] The results suggested that transmembrane movement of lipopolysaccharides takes place, but only when the temperature is high enough to allow such movement. Extraction of the O-specific polysaccharide from *S. zuerich* afforded three fractions, which were examined in some detail.[105] The antigenic lipopolysaccharide serratigen from *Serratia marcescens* has been shown to contain D-galacturonic acid and D-galactosyl residues, which occur partly as a (1 → 3)-linked α-D-galactan.[106] The immunological heterogeneity of these polysaccharides results from the presence of two types of O-specific polysaccharide in the cells: one of them contains O-factor 1, which is associated with the presence of an α-D-glucopyranosyl residue in the side-chain, whereas the other does not.

Chemical evidence has indicated that the O-specific polysaccharide isolated from *Shigella boydii* has a pentasaccharide repeating unit containing D-mannosyl, D-galactosyl, 2-amino-2-deoxy-D-galactosyl, and D-glucuronic acid residues (2 : 1 : 1 : 1).[107] The structure (10) was proposed for the polysaccharide on the evidence of methylation, periodate oxidation, and Smith degradation.[108]



(10)

Structural studies on the O-specific polysaccharide of *Sh. dysenteriae* type 3 have revealed the presence of an acidic, branched hexosaminoglycan.[109] The repeating unit is represented as a pentasaccharide composed of D-galactosyl, 2-acetamido-2-deoxy-D-galactosyl, and D-glucosyl residues, and an unidentified acidic component. Methylation analysis, periodate oxidation, partial hydrolysis with acid, and oxidation with chromic oxide are compatible with the structure (11)



(11) *X* is an unidentified acidic component

for the repeating unit. The polysaccharide component of the lipopolysaccharide present in *Sh. dysenteriae* type 6 has been subjected to mild hydrolysis with acid

[104] P. F. Mühlradt and J. R. Golecki, *European J. Biochem.*, 1975, **51**, 343.
[105] H. O. Nghiêm and A. M. Stauub, *Carbohydrate Res.*, 1975, **40**, 153.
[106] T. Ikekawa and Y. Ikeda, *Chem. and Pharm. Bull. Japan*, 1975, **23**, 163.
[107] B. A. Dmitriev, L. V. Backinowsky, Y. A. Knirel, V. L. L'vov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2335.
[108] B. A. Dmitriev, L. V. Backinowsky, V. L. L'vov, Y. A. Knirel, N. K. Kochetkov, and N. A. Khomenko, *Carbohydrate Res.*, 1975, **41**, 329.
[109] B. A. Dmitriev, L. V. Backinowsky, V. L. L'vov, N. K. Kochetkov, and I. L. Hofman, *European J. Biochem.*, 1975, **50**, 539.

to furnish an acidic hexosaminoglycan and a core oligosaccharide fraction.[110] The hexosaminoglycan, which contains equal proportions of D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-galactose, and also an unidentified acidic component (*X*), was *N*-deacetylated and then hydrolysed and deaminated to give 3-*O*-(2-amino-2-deoxy-β-D-galactopyranosyl)-D-galactose and 2,5-anhydro-3-*O*-(6-*O*-α-D-galactopyranosyl-α-D-glucopyranosyl)-talitol. Based on the identification of these fragments, methylation analysis of the polysaccharide, together with determinations of the linkage configurations by chromic oxide oxidation, the hexosaminoglycan can be considered to have the repeating structure (12).

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow 6)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow$$
$$\begin{matrix} 4 \\ \uparrow \\ 1 \\ X \end{matrix}$$

(12) *X* is an unidentified acidic component

Elevated levels of *O*-acetyl residues in a phage-converted strain of *Sh. flexneri* have indicated that the phage determines a specific acetylase, which is responsible for the acetylation of L-rhamnosyl residues in the 3,4-group antigen complex, and expression of the group-6 antigen.[111] The presence of undecaprenyl phosphate has been demonstrated in *Sh. flexneri*; in addition, a UDP-D-glucose:polyprenol monophosphate glucosyltransferase, which transfers D-glucose from UDP-D-glucose to undecaprenyl phosphate, was solubilized.[112] Immunoelectrophoresis of antigens from *Sh. newcastle* has revealed that the K antigen is complexed with the high-molecular-weight component of the O antigen.[113] Methods for separating the components of the K,O-antigen complex were devised, and the chemical composition of the individual antigens was determined. The polysaccharide of K antigen is composed of residues of D-galacturonic acid, D-galactose, D-glucose, and L-rhamnose (1 : 1 : 1 : 2), whereas heptose residues replace the uronic acid residues in the O antigen. A method involving ultracentrifugation has yielded serologically individual K and O antigens from *Sh. newcastle*.[114] Perosamine (4-amino-4,6-dideoxy-D-mannose) has been identified as a constituent of the lipopolysaccharide of *Vibrio cholerae*.[115] A number of fatty acids are attached by way of ester or amide linkages to 2-amino-2-deoxy-D-glucosyl residues in the lipid-A component of the lipopolysaccharide from *Xanthomonas sinensis*.[116]

The polysaccharide component of the serologically active lipopolysaccharide from *Yersinia pseudotuberculosis* is composed of residues of 3-deoxy-2-octulosonate, L- and D-*glycero*-D-*manno*-heptose, D-mannose, D-glucose, paratose, L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose.[117] The type-specific,

[110] B. A. Dmitriev, Y. A. Knirel, and N. K. Kochetkov, *Carbohydrate Res.*, 1975, **44**, 77.
[111] P. Gemski, D. E. Koeltzow, and S. B. Formal, *Infection and Immunity*, 1975, **11**, 685.
[112] W. Jankowski, T. Mankowski, and T. Chojnacki, *Acta Biochem. Polon.*, 1975, **22**, 67.
[113] V. G. Petrukhin, Y. A. Belaya, and K. K. Ivanov, *Zhur. Mikrobiol. Epidemiol. Immunobiol.*, 1973, **50**, 91.
[114] V. G. Petrukhin, Y. A. Belaya, and K. K. Ivanov, *Zhur. Mikrobiol. Epidemiol. Immunobiol.*, 1975, **52**, 78.
[115] J. W. Redmond, *F.E.B.S. Letters*, 1975, **50**, 147.
[116] E. T. Rietschel, O. Lüderitz, and W. A. Volk, *J. Bacteriol.*, 1975, **122**, 1180.
[117] Y. S. Ovodov, R. S. Gorshkova, and S. V. Tomshich, *Immunochemistry*, 1974, **11**, 777.

antigenic lipopolysaccharide of the spirochaete *Leptospira canicola* contains L-rhamnosyl, D-xylosyl, L-arabinosyl, D-galactosyl, and 2-amino-2-deoxy-D-glucosyl residues, in addition to lipids and 16 amino-acids.[118] A sharp drop in the antigenic activity was observed when the lipopolysaccharide was oxidized with sodium periodate, indicating that the carbohydrate moiety is responsible for the antigenicity.

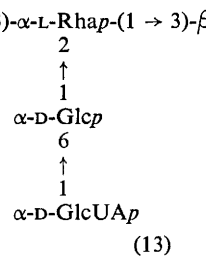
Capsular Polysaccharides

A water-insoluble, capsular polysaccharide has been found to accumulate on the cells of *Azotobacter vinelandii* before a water-soluble polysaccharide appears in the culture medium; both polysaccharides contain residues of D-glucose, L-rhamnose, L-guluronic acid, and D-mannuronic acid.[119] The presence of GDP-D-mannose dehydrogenase in crude, cell-free extracts containing these polysaccharides implied a close similarity between the biosynthesis of alginates in marine Phaeophyceae and that of polysaccharides of the soil micro-organisms. An association between calcium ions and uronic acids has been reported in the encystment of *A. vinelandii*.[120] An analytical g.l.c. procedure has been used to identify the monosaccharides (as TMS derivatives) present in the capsular polysaccharides of *Clostridium perfringens*.[121] Quantitative differences between the compositions of the principal polysaccharides enabled a number of strains to be differentiated.

The D-glucuronic acid residues of pneumococcal type III polysaccharide, which is composed of a repeating sequence of cellobiouronic acid residues joined through β-(1 → 3)-linkages, have been reduced to give a neutral β-D-glucan with alternate (1 → 3)- and (1 → 4)-linkages.[122] *Pneumococcus* type II capsular polysaccharide is composed of singly-branched hexasaccharide repeating units, for which three structures have so far been proposed.[123] The correct structure (13) has been determined by consecutive eliminations of the sugar residues in the side-chain.[124] Thus, the terminal D-glucuronic acid residue was eliminated by treating the fully methylated and esterified polysaccharide first with base and then with weak acid. The hydroxy-group at C-6 in the penultimate D-glucosyl residue released by this elimination was transformed into the corresponding 6-*O*-toluene-*p*-sulphonate, and the residue thereafter eliminated by treatment with base. As the side-chains were completely removed by these reactions, they must contain only two sugar residues. Further confirmation of the structure (13) was obtained when a Smith degradation of the polysaccharide yielded the tetrasaccharide L-Rha*p*-(1 → 3)-L-Rha*p*-(1 → 3)-L-Rha*p*-(1 → 2)-erythritol, which was characterized by methylation analysis.

The capsular type-specific antigen of *Haemophilus influenzae* type b contains equal proportions of D-ribose, ribitol, and phosphate.[125] The results of periodate

[118] N. Kasai and R. Yanagawa, *Z. Bakteriol. Parasiten Kd. Infect. Hyg. I. Abt. A*, 1974, **228**, 533.
[119] I. Couperwhite and M. F. McCallum, *J. Microbiol. Serol.*, 1975, **41**, 25.
[120] W. J. Page and H. L. Sadoff, *J. Bacteriol.*, 1975, **122**, 145.
[121] C. M. Paine and R. Cherniak, *Canad. J. Microbiol.*, 1975, **21**, 181.
[122] M. A. Anderson and B. A. Stone, *F.E.B.S. Letters*, 1975, **52**, 202.
[123] O. Larm, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1973, **31**, 120.
[124] L. Kenne, B. Lindberg, and S. Svensson, *Carbohydrate Res.*, 1975, **40**, 69.
[125] R. M. Crisel, R. S. Baker, and D. E. Dorman, *J. Biol. Chem.*, 1975, **250**, 4926.

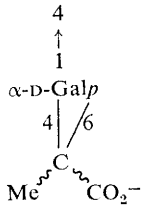


(13)

oxidation, hydrolyses with acid and alkali, and ^{1}H n.m.r. spectroscopy indicated that the antigen is a poly(ribosylribitol phosphate).

The capsular polysaccharide of *Klebsiella* type 47 contains side-chains attached to the main chain by way of D-glucuronic acid residues.[126] The side-chains were removed to give a linear polysaccharide by the following sequence of reactions: (i) substitution of hydroxy- and carboxy-groups with methyl vinyl ether, (ii) β-elimination by treatment with base, and (iii) removal of modified uronic acid residues and protecting groups by mild hydrolysis with acid.

Investigations using periodate oxidation, methylation analysis, and ^{1}H n.m.r. spectroscopy have revealed the structure (14) for alkali-treated *Klebsiella* type 11

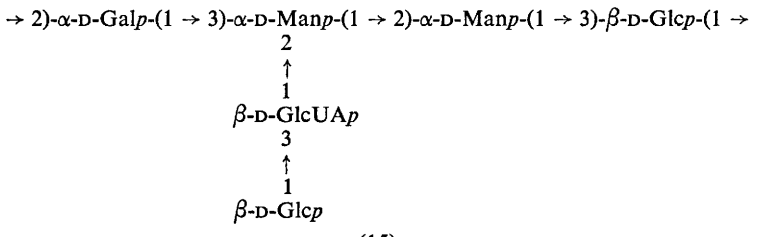


(14)

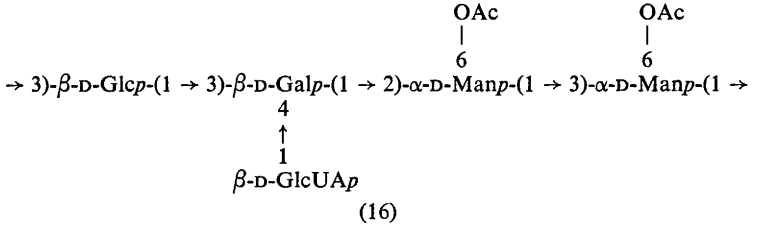
capsular polysaccharide.[127] The structural basis for some serological cross-reactions of the *Klebsiella* K11 antigen was considered, and it was shown that rabbit antisera against the *Klebsiella* K11 test-strain predominantly contain K agglutinins specific for branch-terminal 4,6-*O*-(1-carboxyethylidene)-D-galactose. The capsular polysaccharides from *Klebsiella* types 28, 59, and 81 have been studied, principally by a combination of methylation analysis, Smith degradation, and uronic acid degradation, followed by oxidation and elimination of the substituents of the oxidized residues; the polysaccharides consist of the repeating units (15)—(17), respectively.[128–130]

The *z*-average diffusion coefficient (D_z) of a meningococcal polysaccharide and its second moment (D_z^2) have been determined from the time-dependent

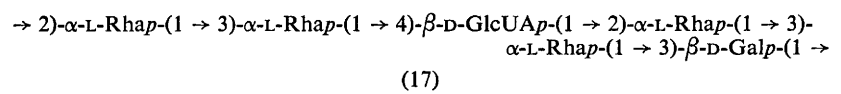
[126] M. Curvall, B. Lindberg, and J. Lönngren, *Carbohydrate Res.*, 1975, **41**, 235.
[127] H. Thurow, Y. M. Choy, N. Frank, H. Niemann, and S. Stirm, *Carbohydrate Res.*, 1975, **41**, 241.
[128] M. Curvall, B. Lindberg, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1975, **42**, 95.
[129] B. Lindberg, J. Lönngren, U. Rudén, and W. Nimmich, *Carbohydrate Res.*, 1975, **42**, 83.
[130] M. Curvall, B. Lindberg, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1975, **42**, 73.



(15)



(16)



(17)

correlation function.[131] The very low values observed for (D_z) and the large width of the polydispersity index, which represents the *z*-average normalized variance, suggested a higher degree of aggregation of the polysaccharide at finite concentrations than a monomer–dimer type of self-association. A lectin present in the haemolymph of a clam (*Tridacna maxima*) precipitated with pneumococcal type XIV polysaccharide; the combining sites of the lectin appear to be most complementary to 2-acetamido-2-deoxy-β-D-galactopyranosyl residues in the polysaccharide.[132]

Extracellular and Intracellular Polysaccharides

An improved method has been reported for the manufacture of Schardinger β-dextrin using a cyclodextrin:glucosyltransferase occurring in a *Bacillus* sp.[133] *X*-Ray analysis has shown that the structure of an α-cyclodextrin–methanol pentahydrate adduct is similar to that of the corresponding adduct with propan-1-ol.[134] The crystal structure of an α-cyclodextrin–*p*-iodoaniline complex has been investigated using the heavy-atom technique.[135] Large differences have been observed in the c.d. spectra of complexes of 1- and 2-substituted naphthalenes with β-cyclodextrin, indicating that steric effects influence the structures of the complexes.[136] Calculation of the rotational strength by the method of Kirkwood and Tinoco revealed that 2-substituted naphthalenes are included axially. The

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[132] B. A. Baldo and G. Uhlenbruck, *Carbohydrate Res.*, 1975, **40**, 143.
[133] M. Matzuzawa, M. Kawano, N. Nakamura, and K. Horikoshi, *Stärke*, 1975, **27**, 410.
[134] B. Hingerty and W. Saenger, *Nature*, 1975, **255**, 396.
[135] K. Harata and H. Uedaira, *Nature*, 1975, **253**, 190.
[136] K. Harata and H. Uedaira, *Bull. Chem. Soc. Japan*, 1975, **48**, 375.

following derivatives have been synthesized in order to enhance the hydrophobic character of cyclodextrins: heptakis-(2,6-di-*O*-propyl)-, heptakis-(2,6-di-*O*-methyl)-, pentakis-(6-*O*-methyl)-, and permethyl-β-cyclodextrin.[137] These derivatives stimulated the synthesis of fatty acids catalysed by a fatty acid synthase from *Mycobacterium smegmatis*, the efficacy of the derivatives decreasing in the order given. Hydrophobic forces are assumed to operate in the formation of complexes between cyclodextrins and long-chain ligands of acyl-coenzyme A.

A review on dextrans has dealt with their structures and biosynthesis, their role in dental caries, and their uses in industry.[138]

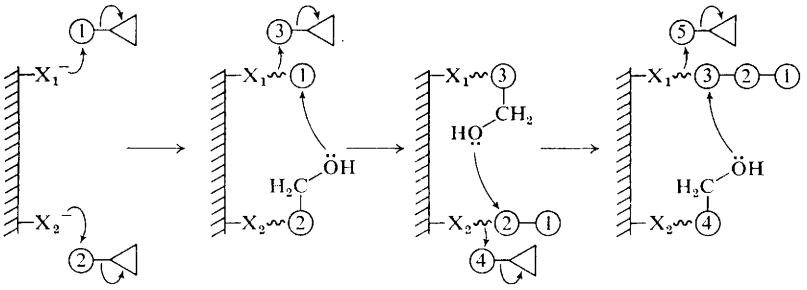
The introduction of carbonyl and carboxylic acid groups into, and the depolymerization of, dextran with Fenton's reagent have been studied.[139]

The degree of hydration of dextrans of various molecular weights in dilute aqueous solution has been determined by a ^1H n.m.r. relaxation method.[140] In the molecular-weight range 1.7×10^4—1.1×10^5, only a fraction of the D-glucosyl residues was found to be hydrated, the degree of hydration increasing with the molecular weight. The data obtained are considered to reflect intersegmentary interactions in a dextran molecule, which interfere with its complete hydration. The state of adsorbed water in a dextran gel has been investigated by near-i.r. spectroscopy and gravimetric-adsorption techniques; for example, the water combination ($\nu + \delta$) band at 5184 cm^{-1} was examined as a function of the relative humidity.[141] The lineshapes of this band were analysed by a Fourier-inversion technique, and information on the microdynamics of adsorbed water molecules was resolved on the picosecond time-scale. A comparison of the microdynamic and thermodynamic data indicated that the hydration mechanism is highly co-operative at all relative humidities. The tritium–proton exchange constant of a dextran has been measured.[142] A high-molecular-weight dextran containing 96% of its α-D-glucopyranosyl residues in (1 → 6)-linkages and the remainder as (1 → 3)-branch-points has been shown to give erroneous results on periodate oxidation.[143] Resistance to periodate attack of some 'hindered' C-3 hydroxy-groups may be due to bonding with aldehydo-groups formed by periodate oxidation. However, it seems likely that resistance to complete oxidation with periodate is related to the abnormal resistance of the polysaccharide to complete methylation through some form of temporary protection (*e.g.* non-covalent) of the hydroxy-groups at C-3. These effects may also be related to the variable macromolecular associations indicated in studies of the dextran by gel-permeation chromatography. Glycoproteins and polysaccharides that dissolve in saturated solutions of ammonium sulphate bind norleucine and valine; hence, dextrans have been fractionated on the basis of their molecular weights on columns of Agarose substituted with valine.[144] A sample of clinical dextran has been fractionated using aqueous ethanol.[145] The molecular-weight distribution was in

[137] R. Bergeron, Y. Machida, and K. Bloch, *J. Biol. Chem.*, 1975, **250**, 1223.
[138] R. L. Sidebotham, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 371.
[139] L. Ahrgren and A. N. De Belder, *Stärke*, 1975, **27**, 121.
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[141] J. A. Texter, R. Kellerman, and K. Klier, *Carbohydrate Res.*, 1975, **41**, 191.
[142] N. V. B. Marsden, *Acta Chem. Scand.* (*B*), 1974, **28**, 1100.
[143] G. J. Leonard and G. N. Richards, *Carbohydrate Res.*, 1975, **41**, 143.
[144] H. Bussey, R. A. Rimerman, and G. Hatfield, *Analyt. Biochem.*, 1975, **64**, 380.
[145] J. M. Barrales-Rienda, A. Horta, and E. Salz, *J. Macromol. Sci. Chem.*, 1974, **A8**, 715.

good agreement with the exponential functions of Tung and Schulz, but the agreement was improved by using a linear combination of the exponential, logarithmic, and normal functions.

The mechanism of biosynthesis of dextran has been studied by incubation of *Leuconostoc mesenteroides* dextransucrase, either attached to the cells or insolubilized on polyacrylamide beads, with [^{14}C]sucrose.[146] It is proposed that the enzyme has two catalytic sites, which both attack sucrose to give D-glucosyl complexes (see Scheme 1). The hydroxy-group at C-6 of one of the D-glucosyl



X_1 and X_2 represent nucleophilic groups at the active sites;
○ = D-glucose; △ = D-fructose; ○—◁ = sucrose; and
○—○ = (1→6)-linked α-D-glucopyranosyl residues

Scheme 1

residues attacks C-1 of the other bound D-glucosyl residue (and oligomers and polymers in later stages of the biosynthesis) to form an α-(1 → 6)-linkage; this releases one of the catalytic groups, which then attacks another molecule of sucrose to form a new enzyme–D-glucosyl complex, and so on. The dextran chain grows by extrusion from the enzyme when D-glucosyl units are transferred from sucrose to the active site and are inserted between the enzyme and the reducing-end of the dextran chain.

The influence of the molecular structure on the tolerogenicity of a dextran from *L. mesenteroides* B512 has been reported.[147] This dextran (mol. wt. 8 × 10^7) is composed of (1 → 6)-linked α-D-glucosyl residues (96%) with side-chains containing one or two α-D-glucosyl residues linked to the main chain through (1 → 3)-linkages; the side-chains do not contribute to the immunological specificity, which is wholly contained in the α-(1 → 6)-linkages. However, the tolerogenic response of *L. mesenteroides* B1355 was shown to depend on the presence of α-(1 → 3)-linkages (35%) in a dextran of molecular weight 4 × 10^7.[148] A distinction between the tolerogenicity and immunity of the α-(1 → 6)- and α-(1 → 3)-linked epitopes of *L. mesenteroides* B1355 dextran has been reported.[149] The effects of dextranases from *Actinomyces israelii* and *Bacteroides ochraceus* on the synthesis

[146] J. F. Robyt, B. K. Kimble, and T. F. Walseth, *Arch. Biochem. Biophys.*, 1974, **165**, 634.
[147] J. G. Howard, G. Vicari, and B. M. Courtenay, *Immunology*, 1975, **29**, 585.
[148] J. G. Howard and B. M. Courtenay, *Immunology*, 1975, **29**, 599.
[149] J. G. Howard, B. M. Courtenay, and G. Vicari, *Immunology*, 1975, **29**, 611.

of a water-insoluble glucan by a dextransucrase from *Streptococcus mutans* have been examined.[150] Very low levels of the dextranases inhibited the synthesis of this glucan by 60% and reduced the adherence of *S. mutans* to smooth surfaces by 80%. Two extracellular fractions exhibiting D-glucosyltransferase activity have been isolated from *S. mutans*.[151] One of the fractions is involved in the synthesis of both soluble and insoluble α-D-glucans containing (1 → 3)- and (1 → 6)-linkages, whereas the other fraction catalysed the formation of a soluble (1 → 6)-linked α-D-glucan only. Some strains of *S. mutans* representative of group IIId were found to synthesize and to degrade less of the intracellular polysaccharides than a large number of strains representative of other groups of *S. mutans*.[152] The cariogenicity in group IIId is considered to be independent of the synthesis of intracellular polysaccharides. Dextran-induced agglutination of *S. mutans* cells has been shown to be independent of cell-bound dextransucrase activity.[153] The cells possess a greater affinity for branched dextrans of low molecular weight than for linear dextrans of the same molecular weight, indicating that size alone does not determine the effectiveness of dextran to induce agglutination. A soluble dextransucrase produced by a mutant of *S. mutans* converted sucrose into alcohol- and water-insoluble glucans some three to six times faster than did the parent strain.[154] Structural studies on a native dextran produced by *S. sanguis* indicated that it contains D-glucosyl residues substituted at C-6 or C-3, or both, in addition to unsubstituted D-glucosyl residues.[155] The proportion of D-glucosyl residues substituted at C-3 was diminished in a dextran synthesized from sucrose by a partially purified dextransucrase, implying that several enzymes are involved in the synthesis of dextrans.

The intracellular polysaccharide of *Clostridium butyricum* was completely hydrolysed with amyloglucosidase, indicating the presence of (1 → 4)- and (1 → 6)-linked α-D-glucosyl residues.[156] Debranching of the β-limit dextrin derived from the polysaccharide by digestion with pullulanase revealed the presence of a glycogen-like structure having some external chains longer than those of normal glycogen. The reserve glucan from *C. pasteurianum* has been assumed hitherto to be a single polysaccharide; however, it has now been fractionated (by gel permeation chromatography) into two polysaccharides that resemble amylopectin and dextran.[157] Methylation analysis of the dextran-like material confirmed the presence of (1 → 6)-D-glucosyl residues in the polysaccharide, which has a weight-average molecular weight of 7.5×10^4 and a number-average molecular weight of 2.7×10^4. An enzyme complex isolated from *E. coli* was able to synthesize a glycoprotein containing (1 → 4)-linked α-D-glucosyl residues from ADP-D-glucose, which is considered to be the specific donor in the synthesis of bacterial glycogen, and from UDP-D-glucose.[158]

[150] C. F. Schachtele, R. H. Staat, and S. K. Harlander, *Infection and Immunity*, 1975, **12**, 309.
[151] H. K. Kuramitsu, *Infection and Immunity*, 1975, **12**, 738.
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[155] A. T. Arnett and R. M. Mayer, *Carbohydrate Res.*, 1975, **42**, 339.
[156] J. L. Bergère, M. Rousseau, and C. Mercier, *Ann. Microbiol.*, 1975, **126A**, 295.
[157] R. G. Brown, B. Lindberg, and E. J. Laishley, *Canad. J. Microbiol.*, 1975, **21**, 1136.
[158] R. Barengo, M. Flawiá, and C. R. Krisman, *F.E.B.S. Letters*, 1975, **53**, 274.

Close agreement has been obtained between values for the parameters in the Hill equation for the *in vivo* synthesis of glycogen by *E. coli* and values obtained for the synthesis of ADP-D-glucose *in vitro*, suggesting that this metabolite is the allosteric regulator of the synthesis of bacterial glycogen *in vivo*.[159] Co-ordination of the synthesis of glycogen, the utilization of D-glucose, and glycolysis has been demonstrated in *E. coli*.[160] Quantitative evidence has been provided for a mechanism in which ATP acts as a primary signal in regulating the rate of glycolysis.[161] The resultant change in the activity of phosphofructokinase alters the level of either D-fructose 1,6-diphosphate or D-glucose 6-phosphate, which serves to regulate the activity of glycogen synthase and the utilization of D-glucose, thus allowing a co-ordinated control of all three processes. No differences in the α- or β-amylolysis, chain lengths, and iodine values were detected in the glycogen synthesized by a mutant of *E. coli* at a rate 28% higher than that of the parent strain.[162] Higher concentrations of D-fructose 1,6-diphosphate and pyridoxal phosphate were required to produce half of the maximal allosteric activation of ADP-D-glucose pyrophosphorylase, when compared to those in the parent organism. From these and other results it was concluded that D-fructose 1,6-diphosphate is the physiological allosteric activator of ADP-D-glucose pyrophosphorylase in *E. coli*.

The branched glucan synthesized from sucrose by amylosucrase (EC 2.4.1.4) from *Neisseria perflava* cells was partially hydrolysed by β-amylase.[163] Synthesis of the polysaccharide required a primer, which could be replaced in *in vitro* experiments by either amylopectin or glycogen. The biosynthesis of granulose by *C. pasteurianum* has been shown to require the presence of ADP-D-glucose pyrophosphorylase and granulose synthase (ADP-D-glucose:α-1,4-glucan glucosyltransferase) and to depend on the availability of a suitable polyglucan primer.[164] The breakdown of granulose in the cells was effected by a constitutive α-1,4-polyglucan phosphorylase.[165]

The results of sucrose density-gradient centrifugation of a cell-free, particulate enzyme system from *Acetobacter xylinum* indicated that a polymeric intermediate is involved in the synthesis of cellulose from UDP-D-glucose by the system.[166] The intermediate exhibits the properties of an oligomer of D-glucose and is normally attached to the heaviest particle of the suspension; it was preferentially adsorbed onto fragments of preformed cellulose when released by hydrolysis. The synthesis of cellulose by *A. xylinum* has also been investigated by using D-[6-^{2}H]glucose. ^{1}H N.m.r. spectroscopy and m.s. of the derived D-glucitol hexa-acetate indicated that *ca.* 70% of D-glucose was incorporated without modification of the label at C-6.[167] However, other processes are involved, since

[159] D. N. Dietzler, M. P. Leckie, C. J. Lais, and J. L. Magnani, *J. Biol. Chem.*, 1975, **250**, 2383.
[160] D. N. Dietzler, M. P. Leckie, P. E. Bergstein, and M. J. Sughrue, *J. Biol. Chem.*, 1975, **250**, 7188.
[161] D. N. Dietzler, M. P. Leckie, J. L. Magnani, M. J. Sughrue, and P. E. Bergstein, *J. Biol. Chem.*, 1975, **250**, 7194.
[162] J. Preiss, E. Greenberg, and A. Sabraw, *J. Biol. Chem.*, 1975, **250**, 7631.
[163] G. Okada and E. J. Hehre, *J. Biol. Chem.*, 1974, **249**, 126.
[164] R. L. Robson, R. M. Robson, and J. G. Morris, *Biochem. J.*, 1974, **144**, 503.
[165] R. L. Robson and J. G. Morris, *Biochem. J.*, 1974, **144**, 513.
[166] J. Kjosbakken and J. R. Colvin, *Canad. J. Microbiol.*, 1975, **21**, 111.
[167] D. Gagnaire and F. R. Taravel, *F.E.B.S. Letters*, 1975, **60**, 317.

deuterium was also incorporated at C-1 and C-2 of the cellulose, arising from the *pro-S* and *pro-R* deuterium atoms at C-6 of the labelled hexose, respectively. A nascent form of cellulose microfibril in *A. xylinum* has been proposed, consistent with previous views that polymeric intermediate(s) are involved in the biosynthesis of cellulose, and that the chains associate to form the microfibril.[168] The relation between the yield of cellulose, weight-average DP, and the times of synthesis and growth of bacterial populations of *A. xylinum* has been described.[169] The bacterium exerts a strict control over the synthetic processes by initiating the synthesis of a defined number of polysaccharide chains during either cell division or another event related to it. Low-molecular-weight compounds, such as α-D-glucose 1-phosphate, UDP-D-glucose, and nucleotides, involved in the synthesis of cellulose by *A. xylinum* are localized in the cell-envelope region and, possibly, in the periplasm.[170] Other evidence indicated that the organism possesses a second permeability barrier outside the cytoplasmic membrane. Cell-envelope fractions of *A. xylinum* were able to synthesize cellulose from UDP-D-glucose, D-glucose 1- and 6-phosphates, and D-glucose.[171] The essential enzymes are distributed throughout the cell-envelope region, with both inner (cytoplasmic) and outer (cell-wall) membranes contributing to the synthesis. A comparison of the properties of the cell-envelope fraction with those prepared by other methods indicated that the method used to disrupt the cells may influence the substrate specificity. Cellodextrins of increasing molecular weight have been found to stimulate the synthesis of cellulose from UDP-D-glucose.[172] These and previous studies suggested that the cell envelope is necessary for the synthesis of a more immediate precursor of cellulose than UDP-D-glucose, probably by way of a lipid intermediate. A model outlining the sequence and sites of reactions leading to the synthesis of precursors of cellulose was proposed. Regenerated sheets of cellulose with a selective uniplanar and biaxial orientation were obtained by mercerization of the cellulose from *A. xylinum*; *X*-ray analysis showed that the orientation mechanism probably involves movement of the crystallites as a unit.[173] The polar hydroxy-groups may also be partly responsible for the orientation observed.

Curdlan, a glucan isolated from *Alcaligenes faecalis*, has been used to adsorb the tannins from fruit and vegetables.[174] A method for labelling acidic polysaccharides has been described; for example, the principal cell-wall antigens were labelled with ^{125}I and were then used to measure the primary binding to antibodies.[175] The binding properties and inhibition studies indicated that the *Corynebacterium parvum* antigen is heterogeneous, and that 2-acetamido-2-deoxy-D-glucosyl residues and either acetate or pyruvate contribute to the antigenic determinants. A polysaccharide containing *O*-acetylated D-*glycero*-D-*galacto*-heptose as the main constituent has been located in the outer part of the

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[169] M. Marx-Figini and B. G. Pion, *Biochim. Biophys. Acta*, 1974, **338**, 382.
[170] D. Cooper and R. J. Manley, *Biochim. Biophys. Acta*, 1975, **381**, 78.
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[173] M. Takai, Y. Tsuta, J. Hayashi, and S. Watanabe, *J. Polymer Sci., Polymer Chem.*, 1974, **12**, 221.
[174] T. Nakabayashi, *J. Food Sci. Technol.*, 1974, **21**, 341.
[175] J. Dawes and W. H. McBride, *Immunochemistry*, 1975, **12**, 855.

cell wall of *Eubacterium saburreum*.[176] Two antigenic specificities – one of which was abolished by deacetylation – were determined from precipitin and complement-fixation studies. The principal cell-wall polymer of *Halococcus morrhuae* has been identified as a complex sulphated heteropolysaccharide composed of D-glucosyl, D-galactosyl, D-galactosyluronic acid, D-glucosyluronic acid, 2-acetamido-2-deoxy-D-glucosyl, 2-acetamido-2-deoxy-D-galactosyl, 2-amino-2-deoxy-L-gulosyluronic acid, acetate, and sulphate residues.[177] Attempts to fractionate the cell-wall into chemically distinct polymers were unsuccessful.

The chemistry and immunostimulant properties of the cell walls of mycobacteria and related organisms have been reviewed.[178] Concanavalin A has been shown to react with three antigenic polysaccharides present in culture filtrates of *Mycobacterium tuberculosis*; these polysaccharides were isolated by direct precipitation and affinity chromatography.[179] A mannan esterified with succinic acid has been isolated from extracts of mesosomal and plasma membranes of *Micrococcus lysodeikticus*.[180] The polysaccharide was precipitated by concanavalin A, but it was attacked only slightly by jack-bean α-mannosidase.[181] I.r. spectroscopy indicated the presence of both esterified and free carboxy-groups in the mannan. Mesosomal membranes isolated from *M. lysodeikticus*, unlike preparations of plasma membranes, were unable to catalyse the incorporation of D-[^{14}C]mannose from GDP-D-[^{14}C]mannose into the mannan; it appears that the membrane system is unable to synthesize the carrier lipid undecaprenyl D-mannosyl phosphate.[182] It was suggested that the juxtaposition of the mesosomal vesicles and the enveloping plasma membrane *in vivo* allows the mannan located on the mesosomal vesicles to accept D-mannosyl units from the carrier lipid located in the plasma membrane.

^{13}C N.m.r. spectroscopy has been used to analyse a number of meningococcal polysaccharide antigens containing sialic acid.[183] Complete assignments of the spectra for both serogroup B and de-*O*-acetylated serogroup C polysaccharides were based on the spectroscopic data obtained for related monomers and on supporting chemical evidence. The serogroup B polysaccharide was shown to be an α-(2 → 8)-linked homopolymer of sialic acid, identical in structure to colominic acid from *E. coli*, whereas the serogroup C polysaccharide contains α-(2 → 9)-linked sialic acid residues. The *O*-acetyl substituents were shown to be located at C-7 and C-8 of the sialic acid residues in the unmodified serogroup C polysaccharide. An attempted taxonomic classification of the *Rhizobium* genus has been based on the compositions of the *exo*-polysaccharides.[184] Fragmentation analysis of extracellular acidic polysaccharides from a number of *Rhizobium* strains has yielded several neutral oligosaccharides and the oligosaccharides (18)—(20) containing residues of D-glucuronic acid.[185]

[176] T. Hofstad, *Acta Pathol. Microbiol. Scand.* (*B*), 1975, **83**, 471.
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[179] T. M. Daniel, *Amer. Rev. Respiratory Diseases*, 1974, **110**, 634.
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β-D-GlcUA*p*-(1 → 4)-D-GlcUA*p* (18)

β-D-GlcUA*p*-(1 → 4)-D-Glc*p* (19)

β-D-GlcUA*p*-(1 → 4)-β-D-GlcUA*p*-(1 → 4)-D-Glc*p* (20)

2-Amino-2-deoxy-D-galactose has been identified as a constituent of the cell walls of one of the bacteriophage-typable revertants isolated from pleiotropic mutants of *Staphylococcus aureus*.[186] An anti-phagocytic surface antigen isolated from *S. aureus* has been shown to contain 2-amino-2-deoxy-D-mannuronic acid and 2-amino-2,6-dideoxy-L-galactose.[187] Immunochemical studies suggested that this antigen, although consisting of the same components, is immunologically distinct from staphylococcal T-antigen.[188] The sticky, insoluble glucan present in *Streptococcus mutans* is thought to be important in the pathogenesis of periodontal diseases and dental caries, since it promotes the formation of dental plaque; hydrolysis of this glucan by an *endo*-α-(1 → 3)-glucanase from a *Flavobacterium* sp. liberated isomaltose, nigerose, and nigertriose.[189] Purified preparations of soluble and insoluble polysaccharides synthesized by *S. mutans* and *S. sanguis* have been found to contain phosphate, so that the polysaccharides could act as ion-exchange materials in dental plaque, thereby influencing the flow of ions to and from the tooth's surface.[190] Immunochemical analysis has revealed that the group d antigen of *S. mutans* contains polysaccharide material, composed of D-galactosyl and D-glucosyl residues, and ~10% protein.[191] Inhibition studies with haptens indicated that the immunodominant region of the antigen contains D-galactosyl residues. Polysaccharide antigens prepared from serotype a and d strains of *S. mutans* have been shown to contain both the serotype-specific determinant and a common a–d determinant.[192] The a and d antigenic polysaccharides recovered from specific antibody–antigen complexes had identical sugar compositions. Structural features of the polysaccharides of groups A, C, and A-variant (AV) Streptococci have been investigated by means of periodate oxidation, methylation analysis, and immunochemical comparison of the intact and periodate-oxidized polysaccharides.[193] The data indicated that similar (1 → 2)- and (1 → 3)-linked chains of L-rhamnosyl residues are present in both group A and AV polysaccharides; the group A polysaccharide also contains 2-acetamido-2-deoxy-D-glucosyl residues at the non-reducing termini, whereas the group AV polysaccharide is a homopolymer of L-rhamnose. Group C polysaccharide contains the same type of L-rhamnose chain, with some 2-acetamido-2-deoxy-D-galactosyl residues occupying terminal positions and with the remainder (1 → 3)-linked. The group antigens of streptococcal groups P and U have been identified as polysaccharides, and are located in the cell walls.[194] Both

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[194] G. E. Wessman, *Infection and Immunity*, 1975, **12**, 156.

polysaccharides are composed of L-rhamnosyl, D-glucosyl, D-galactosyl, and 2-amino-2-deoxy-D-glucosyl residues, and the P-antigen also contains 2-amino-2-deoxy-D-galactosyl residues. The polysaccharide antigens of streptococcal group B types Ia and Ib contain D-galactosyl and 2-acetamido-2-deoxy-D-glucosyl residues, but in different proportions.[195] The results of hapten inhibition and of chemical and biochemical studies suggested that the specificity of the type Ia polysaccharide depends on 2-acetamido-2-deoxy-D-glucosyl residues and that of type Ib on unacetylated amino-sugar residues. A method involving the use of lytic enzymes has been reported for the extraction of polysaccharides from *S. fradiae*.[196]

Fungal Polysaccharides

Glucans.—The development of cleistothecia during the growth of *Aspergillus nidulans* has been shown to require the presence of Mn^{2+} ions.[197] Analysis of mycelia grown under a deficiency of Mn^{2+} ions revealed that a (1 → 3)-α-D-glucan, which is the main source of carbon and energy for fructification, is virtually absent from the cell walls. When the levels of several enzymes involved in the metabolism of carbohydrates were measured in an attempt to explain the absence of (1 → 3)-α-D-glucan, phosphoglucomutase showed only 60% of the activity present under normal growth conditions in the presence of Mn^{2+} ions; it was argued that reduction in the activity of this enzyme is connected with the low amounts of glucan synthesized.[198] Samples of hydrated, crystalline biopolymers for electron diffraction have been obtained using a method involving quick freezing of hydrated crystals; the technique was illustrated with single crystals of the linear nigeran from *A. niger*.[199]

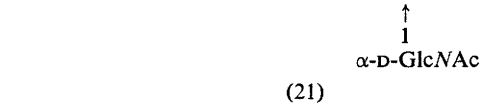
The effects of biotin on the composition and ultrastructure of the cell wall of *Candida albicans* have been studied in relation to mycelial morphogenesis of the organism.[200] Cell walls synthesized under a deficiency of biotin contained greater proportions of alkali-insoluble glucan, but smaller proportions of mannan and alkali-insoluble glucomannan, than did those synthesized under a sufficiency of biotin. Observations relating to the biosynthesis of the cell wall of *Cryptococcus diffluens* grown at non-permissive temperatures have been reported.[201] UDP-D-glucose, a precursor of glucan synthesis, that accumulated was excreted at non-permissive, but not at permissive, growth temperatures. Homogeneous, laminarin-like polysaccharides have been isolated from the zoospores and the mycelia of *Phytophthora palmivora*.[202] Methylation analysis and enzymic degradation indicated the presence of (1 → 3)-linked β-D-glucosyl residues, with one or two branches joined by (1 → 6)-linked β-D-glucosyl linkages. These polysaccharides may be distinctive storage materials of the oomycetous fungi. A combination of methylation, Smith degradation, and enzymic techniques has

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[202] M. C. Wang and S. Bartnicki-Garcia, *Carbohydrate Res.*, 1974, **37**, 331.

shown that the hyphal-wall polysaccharide of *Pythium acanthicum* consists of a main chain of (1 → 3)-linked β-D-glucan that is substituted by a short chain at C-6 of every third D-glucosyl residue.[203] Electron microscopy indicated that the inner surface of the cell wall is microfibrillar, whereas the outer surface is not. The microfibrillar layer is covered by an amorphous matrix consisting of a branched (1 → 3 and 6)-linked β-D-glucan that could be removed by an *exo*-β-(1 → 3)-glucanase. The microfibrils remaining after treatment with this enzyme are composed of glucans containing (1 → 3)- and (1 → 4)-linked β-D-glucosyl residues that are occasionally substituted at C-6.

Polysaccharides have been used to bind sterols, making them soluble in water.[204] The principal sterol-binding polysaccharides of *Penicillium roquefortii* and *Rhizopus arrhizus* were identified as glucans, and that of *Saccharomyces carlsbergenisis* as a mannan. The extents of inhibition of the syntheses of mannan and glucan in *S. cerevisiae* by 2-deoxy-D-*arabino*-hexose depend on whether D-glucose or D-mannose was used as the source of carbon in the medium.[205] For growth on D-glucose, the formation of mannan, rather than that of glucan, was inhibited, whereas synthesis of the cell-wall glucan was strongly suppressed in the presence of D-mannose. Preliminary evaluation of the *X*-ray fibre patterns of a (1 → 3)-linked β-D-glucan from *Armillaria mellea* has been reported.[206] The β-D-glucan isolated from corn-smut galls contains *ca.* 90% of the β-D-glucosyl residues in (1 → 6)-linkage and the remainder in (1 → 3)-linkage.[207]

Mannans.—Two classes of mutants of *Kluyveromyces lactis* have been isolated and used to study the incorporation of 2-acetamido-2-deoxy-D-glucose into a mannan, which, in the parent yeast, contains side-chains of structure (21).[208] One of the



(21)

mutants lacked the ability to synthesize mannotetraosyl and 2-acetamido-2-deoxy-D-glucosyl-mannotetraosyl side-chains, owing to the absence of an α-(1 → 3)-mannosyltransferase. It was concluded that the mannotetraosyl unit must be formed before the α-(1 → 2)-2-acetamido-2-deoxy-D-glucosyltransferase can act. The second mutant lacked the 2-acetamido-2-deoxy-D-glucosyl-mannotetraosyl determinant, and could not transfer the acetamido-sugar to the mannotetraosyl side-chains.

A 'nearest-neighbour' analysis of the products of partial acetolysis of *S. cerevisiae* mannan has been carried out in an attempt to learn whether the side-chains are in ordered or in random sequences.[209] The results suggested that the

[203] J. H. Sietsma, J. J. Child, L. R. Nesbitt, and R. H. Haskins, *J. Gen. Microbiol.*, 1975, **86**, 29.
[204] C. G. P. Pillai and J. D. Weete, *Phytochemistry*, 1975, **14**, 2347.
[205] Z. Krátký, P. Biely, and S. Bauer, *European J. Biochem.*, 1975, **54**, 459.
[206] J. Jelsma and D. R. Kreger, *Carbohydrate Res.*, 1975, **43**, 200.
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[209] L. Rosenfeld and C. E. Ballou, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 571.

arrangement is non-random, but did not provide unequivocal support for a unique repeating sequence. If a non-random order exists, it implies that some factor(s), perhaps derived from the specificity of various mannosyltransferases, regulates both the number and the order of the mannan side-chains. Mild degradation of *S. cerevisiae* X2180 wild-type mannan with alkali resulted in the release of fragments (D-mannose, manno-biose, -triose, and -tetraose) identical with those obtained from the mannan by acetolysis.[210] A mutant lacking α-(1 → 3)-mannosyltransferase did not possess the ability to form the tetrasaccharide units that are normally present as part of a large mannan polymer and also attached as short oligosaccharides to serine and threonine residues. After heating the mannan recovered from a β-elimination reaction with alkaline borohydride, a polysaccharide containing 2-acetamido-2-deoxy-D-glucitol was recovered, indicating that the mannan is probably linked to the protein by way of a 2-acetamido-2-deoxy-D-glucosyl residue. An attempt was made to identify the linkage region of the mannan-protein by using a *S. cerevisiae* mutant that synthesized an apparently linear (1 → 6)-linked α-D-mannan.[211] Hydrolysis of the unbranched portion of the chain of this mannan by an *endo*-α-(1 → 6)-mannanase left a material possessing a branched inner core that represents the linkage region of the mannan-protein. An *endo*-2-acetamido-2-deoxy-β-D-glucosyl hydrolase then released the inner core from the protein, and the resulting fragment was characterized as a branched oligosaccharide containing *ca.* 12 D-mannosyl residues and with 2-acetamido-2-deoxy-D-glucose occupying the reducing end. This fragment was shown to have close similarities in structure with the type I glycopeptides of γM-immunoglobulin. When baker's yeast was grown in the presence of 2-deoxy-D-*arabino*-hexose, *ca.* 90% of the deoxy-sugar was incorporated into the side-chains, and 10% into the main (1 → 6)-linked chain, of the cell-wall mannan.[212] Degradation of this mannan by an α-mannosidase was arrested at the 2-deoxy-D-*arabino*-hexosyl residues.

Polysaccharides composed of D-mannosyl and phosphate residues (5 : 1) have been isolated and purified from cells of *Candida intermedia* and *Torulopsis famata*.[213] D-Mannose 6-phosphate was released on mild hydrolysis with acid, possibly by cleavage of a phosphodiester bond linking it to C-1 of an adjacent D-mannosyl residue.

Chitin.—The effect of wheatgerm agglutinin in inhibiting germination of the spores of *Trichoderma viride* appears to result from binding of the lectin to chitin at the tip of the emerging hyphal tube, rather than to the spore coat.[214] Evidence was presented to show that such binding inhibits the synthesis of chitin, the growth of hyphal tubes, and the germination of spores. Chitin synthase (UDP-2-acetamido-2-deoxy-D-glucosyltransferase, E.C. 2.4.1.16) has been reported to be present in the microsomal cell walls and in mitochondrial and soluble cytoplasmic fractions of the mycelia of *Cunninghamella elegans*; it was shown to have similar kinetic properties to chitin synthases found in other membranes of the

[210] T. Nakajima and C. E. Ballou, *J. Biol. Chem.*, 1974, **249**, 7679.
[211] T. Nakajima and C. E. Ballou, *J. Biol. Chem.*, 1974, **249**, 7685.
[212] P. Biely, Z. Krátký, and S. Bauer, *Biochim. Biophys. Acta*, 1974, **352**, 268.
[213] G. M. Streshinskaya and I. B. Naumova, *Biokhimiya*, 1974, **39**, 1030.
[214] D. Mirelman, E. Galun, N. Sharon, and R. Lotan, *Nature*, 1975, **256**, 414.

Mucorales, but, unlike the other chitin synthases, it did not require a primer and intermediate oligosaccharides were not formed.[215] The production of the enzyme was correlated with the growth of the fungus, and maximum enzymic activity was found during the late exponential phase of growth. Microsomal fractions obtained from *Coprinus cinereus* have been reported to contain a highly specific chitin synthase, which was solubilized by treatment of the fractions with digitonin.[216] The preparations required a bivalent cation and UDP-2-acetamido-2-deoxy-D-glucose for the synthesis of chitin, but did not require a primer. Preparations of chitin synthase from *C. cinereus* were activated by UDP-2-acetamido-2-deoxy-D-glucose, which appears to be an allosteric activator, and by such sugars as 2-acetamido-2-deoxy-D-glucose, di-*N*-acetylchitobiose, and D-glucose.[217] Purified preparations of chitin synthase, which were capable of forming microfibrils *in vitro*, have been isolated from *Mucor rouxii*.[218] The enzyme was obtained either by substrate-induced liberation of the bound enzyme or by isolation of the free enzyme present in cell-free extracts. Electron-microscopic examination of the morphology of the enzyme from both sources raised the question of whether or not membranes are directly involved in the elaboration of chitin microfibrils. An enzyme that catalyses the hydrolysis of the *N*-acetyl groups of chitin derivatives has been isolated from *M. rouxii*; the occurrence of this enzyme could account for the formation of chitosan in fungi.[219] Most of the chitin synthase zymogen of *S. cerevisiae* has been found to be associated with the plasma membranes.[220] The chitin synthase in *S. cerevisiae* showed reproducible changes during the course of batch cultivation.[221] A new model for regulation of the enzyme, which accounts for its specific location and involvement in terminating the synthesis of chitin during the budding cycle, was presented.

Cellulin granules (polysaccharide inclusions found only in oomycetous fungi of the order Leptomitales) free from cell-wall and cytoplasmic contaminants have been shown to contain chitin (60%) and (1 → 3 and 6)-linked β-D-glucan (39%).[222] Changes in the chitin–glucan complex at the site of future budding have been followed by electron microscopy during differentiation of the cell wall of *S. cerevisiae*.[223] Nucleated protoplasts capable of cell-wall regeneration have been prepared from *Schizophyllum commune*.[224] Inhibition and kinetic studies suggested that the synthesis of a (1 → 3 and 6)-linked β-D-glucan is necessary for the initiation of hyphal morphogenesis. Protoplasts from *Trichoderma viride* were unable to synthesize chitin, although regenerated cell walls still contained a (1 → 3 and 6)-linked β-D-glucan.[225] Loss of chitin synthase during release of the

[215] P. M. Moore and J. F. Peberdy, *Microbios*, 1975, **12**, 9.
[216] G. W. Gooday and A. De Rousset-Hall, *J. Gen. Microbiol.*, 1975, **89**, 137.
[217] A. De Rousett-Hall and G. W. Gooday, *J. Gen. Microbiol.*, 1975, **89**, 146.
[218] J. Ruiz-Herrera, V. O. Sing, W. J. Van der Woude, and S. Bartnicki-Garcia, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2706.
[219] Y. Araki and E. Ito, *European J. Biochem.*, 1975, **55**, 71.
[220] A. Durán, B. Bowers, and E. Cabib, *Proc. Nat. Acad. Soc. U.S.A.*, 1975, **72**, 3952.
[221] A. Hasilik, *Arch. Mikrobiol.*, 1974, **101**, 295.
[222] H. Y. Lee and J. M. Aronson, *Arch. Mikrobiol.*, 1975, **102**, 203.
[223] O. Seichertová, K. Beran, Z. Holan, and V. Pokorny, *Folia Microbiol.*, 1975, **20**, 371.
[224] O. M. H. De Vries and J. G. H. Wessels, *Arch. Mikrobiol.*, 1975, **102**, 209.
[225] T. Benitez, T. G. Villa, and I. Garcia Acha, *Arch. Mikrobiol.*, 1975, **105**, 277.

protoplasts could explain this finding, since this enzyme may be situated between the membrane and the cell wall.

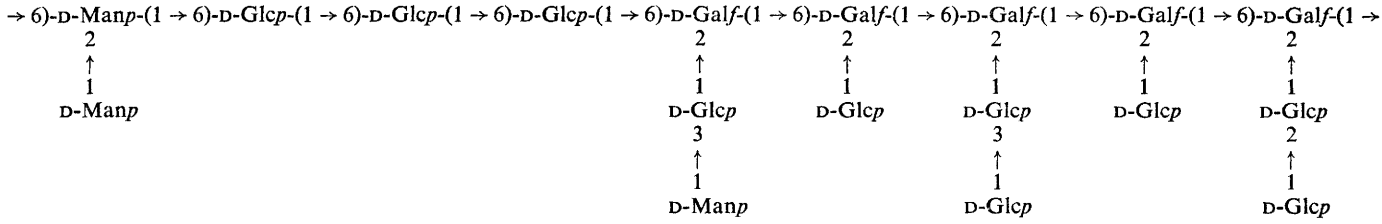
Miscellaneous Polysaccharides

The nature of the cross-reactivities of five galactomannans synthesized by *Aspergillus fumigatus*, *A. niger*, *Trichophyton rubrum*, *Cladosporium werneckii*, and *Hormodendrum pedrosoi* towards anti-(*H. pedrosoi*) sera has been discussed.[226] Partial hydrolysis with acid and cross-precipitation reactions suggested that the galactomannan from *H. pedrosoi* possesses two types of side-chains: one consisting of acid-labile D-galactofuranosyl residues, which are solely responsible for the immunological activity of the galactomannan, and the other of relatively stable α-D-mannopyranosyl residues. Autolytic enzymes in the hyphae of *A. nidulans* are responsible for the liberation of D-glucose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and soluble oligosaccharides.[227] The digestion proceeded at a linear rate until ~3% of the cell-wall polymers were hydrolysed, whereafter it slowed down appreciably. The change in the rate of autolysis is not due to loss of enzymic activity, but is caused by the disappearance of a cell-wall fraction susceptible to digestion; radiolabelling indicated that this fraction consists of newly formed cell wall. In an attempt to establish how the chemical structure is related to the taxonomy, three extracellular polysaccharides, two of which are soluble in water, have been isolated from *Fusarium solani* and purified.[228] The polysaccharides (22)—(24) were shown to be heteroglycans containing D-glucosyl, D-galactosyl, and D-mannosyl residues in different proportions; a combination of periodate oxidation, Smith degradation, and methylation analysis was used to elucidate the principal structural features of these polysaccharides.

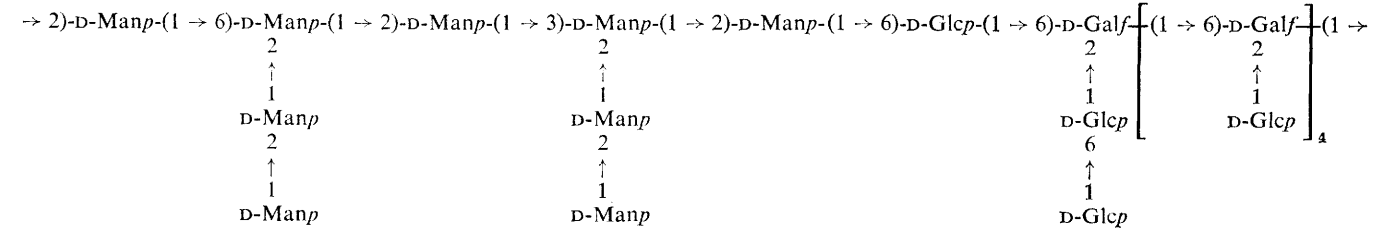
A purified extracellular heteroglycan obtained from cultures of *Cladosporium tricoides* is composed of D-galactosyl, D-glucosyl, D-mannosyl, and L-rhamnosyl residues.[229] Periodate oxidation, Smith degradation, and methylation analysis indicated that the polysaccharide (25) is highly branched.

Cytochemical staining, by a method involving oxidation with periodate and reaction with thiosemicarbazide and osmium tetroxide, has revealed the presence of polysaccharide lamellae on the surface of the cell membrane of *Claviceps purpurea*.[230] The membranous clusters in vacuolar bodies of the endoplasmic reticulum membranes, tonoplasts, and cytoplasmic membranes are covered with these lamellae. Somatic and metabolic polysaccharides composed of D-mannose and L-rhamnose have been isolated from *Certocystic stenoceras* and *Sporotrichum schenckii*; chemical and immunological analyses indicated close similarities in their structures.[231] When pseudoplasmodia of *Dictyostelium discoideum* were incubated with 2-acetamido-2-deoxy-D-[^{3}H]glucose, the label was incorporated into a polysaccharide in the slime sheath.[232] The incorporated label disappeared

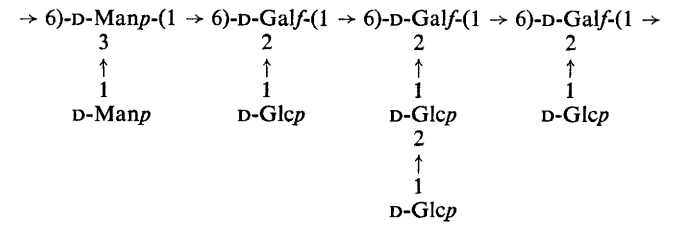
[226] S. Suzuki and N. Takeda, *Carbohydrate Res.*, 1975, **40**, 193.
[227] Y. Polacheck and R. F. Rosenberger, *J. Bacteriol.*, 1975, **121**, 332.
[228] T. Miyazaki and Y. Naoi, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1752.
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[230] J. Vořišek and V. Pokorný, *Arch. Mikrobiol.*, 1975, **102**, 293.
[231] C. Toriello and F. Mariat, *Ann. Microbiol.*, 1974, **125A**, 287.
[232] D. J. Watts and T. E. Treffry, *F.E.B.S. Letters*, 1975, **52**, 262.


→ 6)-D-Manp-(1 → 6)-D-Glcp-(1 → 6)-D-Glcp-(1 → 6)-D-Glcp-(1 → 6)-D-Galf-(1 → 6)-D-Galf-(1 → 6)-D-Galf-(1 → 6)-D-Galf-(1 → 6)-D-Galf-(1 →
2
1
D-Manp
2
1
D-Glcp
3
1
D-Manp
2
1
D-Glcp
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D-Glcp

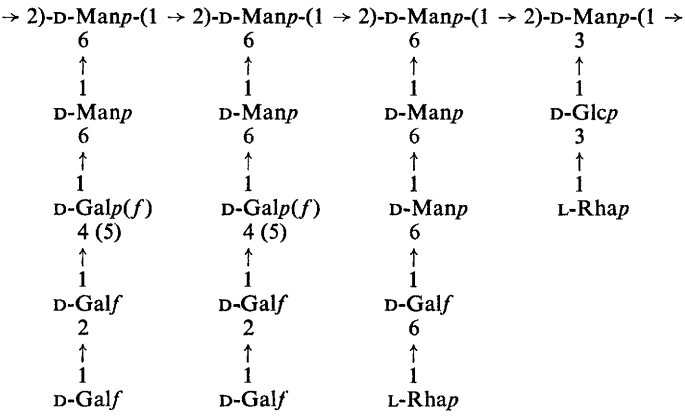
(22)


→ 2)-D-Manp-(1 → 6)-D-Manp-(1 → 2)-D-Manp-(1 → 3)-D-Manp-(1 → 2)-D-Manp-(1 → 6)-D-Glcp-(1 → 6)-D-Galf-(1 → 6)-D-Galf-(1 →
2
1
D-Manp
2
1
D-Manp
2
1
D-Manp
2
1
D-Manp
2
1
D-Glcp
6
1
D-Glcp
2
1
D-Glcp
4

(23)



(24)



(25)

when the incubation was continued in the presence of the unlabelled acetamido-sugar, since an extracellular enzyme in the slime both incorporated and hydrolysed the terminal 2-acetamido-2-deoxy-D-glucosyl residues of the polysaccharide under non-physiological conditions.

Changes have been reported in the contents of the component sugars of intra- and extra-cellular polysaccharides synthesized during the growth phase of *D. discoideum*.[233] The principal cell-wall component of *Fusarium sulphureum* has been identified as chitin, while D-glucose, D-mannose, D-galactose, and D-glucuronic acid account for 30% of the constituents of the cell wall.[234] A polysaccharide (mol. wt. 1.7×10^5) from *Kobayashia nipponica* containing D-glucuronic acid residues was shown to have a linear structure consisting mainly of (1 → 4)-linkages.[235] A water-soluble heterogalactan containing D-galactosyl, L-fucosyl, and D-mannosyl residues has been isolated from fruit bodies of the edible mushroom *Lentinus edodes*; structural studies indicated that it consists of a main chain of (1 → 6)-linked α-D-galactopyranosyl residues, some of which are substituted at C-2 with either single L-fucopyranosyl or D-mannopyranosyl residues, although consideration must be given to a possible alternative structure of a

[233] H. Yamada, T. Yadomae, and T. Miyazaki, *Biochim. Biophys. Acta*, 1974, **362**, 167.
[234] L. R. Barran, E. F. Schneider, P. J. Wood, C. Madhosingh, and R. W. Miller, *Biochim. Biophys. Acta*, 1975, **392**, 148.
[235] T. Miyazaki, T. Yadomae, T. Terui, H. Yamada, and T. Kikuchi, *Biochim. Biophys. Acta*, 1975, **385**, 345.

branched D-galactan in which most of the side-chains are terminated with L-fucosyl or D-mannosyl residues.[236]

The surface peptido-phosphonogalactomannan of *Cladosporium werneckii* has been separated into four principal components by ion-exchange chromatography.[237] Sequential degradations with alkali and with acid showed that the heterogeneity of the components is due to differences in the proportions of three basic types of chain: *viz.* galactomannan units linked by phosphodiester bonds to form long chains of molecular weight 5×10^4 (type a), short D-mannosyl chains (type b), and long galactomannan chains (type c). The antigenicities of the peptido-phosphonogalactomannan of *C. werneckii* and of carbohydrate and peptide moieties isolated therefrom have been studied in rabbits.[238] Antisera produced against whole *C. werneckii* cells reacted only with the carbohydrate portions of the antigen. Most of the antibody was directed towards the long phosphonogalactomannan chains. *O*-Acetyl groups are important determinants on these chains, since selective removal of these groups abolished any reactivity with the antibody. The determinants responsible for the delayed hypersensitivity towards the antigen were shown to reside in the peptide portion.

The levels of 2-amino-2-deoxy-D-glucose, which probably reflect the levels of chitin, and of 2-amino-2-deoxy-D-galactose have been determined in conidia, germinating conidia, and vegetative mycelia of *Neurospora crassa*.[239] The accumulation of polymers containing 2-amino-2-deoxy-D-galactose does not appear to be necessary for germ-tube formation. The content of 2-amino-2-deoxy-D-glucose throughout different regions of the mycelial mat was shown to be constant for cultures of *N. crassa* grown on the surface of dialysis membranes.[240] However, the content of 2-amino-2-deoxy-D-galactose was always found to be lower in the growing front region than in the older regions of mycelial mats, suggesting that polymers containing this amino-sugar cannot exert any appreciable influence on the shape of the hyphal wall. The hyphal wall of the toxigenic fungus *Penicillium rubrum* has been reported to contain neutral sugars (67%), consisting of D-glucose, D-galactose, and D-mannose (45 : 5 : 0.5), and amino-sugars (15%), including both 2-amino-2-deoxy-D-glucose and -D-galactose.[241] The principal extracellular peptido-phosphonogalactomannan produced by *P. charlesii* was detected in culture filtrates only after the growth medium was depleted of ammonium ions.[242] Data on the extent of incorporation of D-[^{14}C]glucose, L-[^{14}C]threonine, and [^{32}P]phosphate into the peptido-phosphonogalactomannan suggested that it is a product of cellular turnover. The malonogalactan (D-galactose : malonic acid 3 : 1) of *P. citrinum* has been identified as a polymer of (1 → 5)-linked β-D-galactofuranosyl residues, some of which are esterified at O-3 with malonic acid.[243]

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[237] W. L. Lee and K. O. Lloyd, *Arch. Biochem. Biophys.*, 1975, **171**, 613.
[238] W. L. Lee and K. O. Lloyd, *Arch. Biochem. Biophys.*, 1975, **171**, 624.
[239] J. C. Schmit, C. M. Edson, and S. Brody, *J. Bacteriol.*, 1975, **122**, 1062.
[240] J. C. Schmit and S. Brody, *J. Bacteriol.*, 1975, **122**, 1071.
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Four polysaccharides [chitin, a (1 → 3)-β-glucan, an α-glucan, and a xylomannan] have been isolated from *Polyporus tumulosus*.[244] Acetolysis of the α-glucan liberated nigerose (3-*O*-α-D-glucopyranosyl-D-glucose) and a homologous series of α-(1 → 3)-linked oligosaccharides, indicating that α-(1 → 3)-linkages predominate in the polysaccharide. The xylomannan (D-xylose : D-mannose 1 : 1.2) released a homologous series of α-(1 → 3)-linked mannodextrins on partial hydrolysis with acid, which, in conjunction with the results of methylation analysis, indicated the presence of a (1 → 3)-linked α-D-mannopyranosyl backbone to which D-xylosyl residues are attached at C-4. The structure of a fucogalactan produced by *Rhodotorula glutinis* has been investigated by means of sequential oxidation with periodate; for example, Smith degradation furnished a polysaccharide composed of (1 → 3)-linked D-galactopyranosyl residues.[245] Each side-chain of the original polysaccharide was shown to be composed of (1 → 2)- and/or (1 → 6)-linked L-fucosyl residues. A polysaccharide fraction isolated from *Pleurotus ostreatus* has been separated into three components by gel permeation chromatography.[246] One of the components, which exhibited antitumour activity, consists of a skeleton of (1 → 3)-linked β-D-glucosyl residues, probably having branches of D-galactosyl and D-mannosyl residues, and also contains acidic sugars. One of the other polysaccharides also contains a (1 → 3)-linked β-D-glucose chain but a larger porportion of acidic sugars than the former polysaccharide, whereas the third is a heteropolysaccharide composed of α-D-galactosyl and α-D-mannosyl residues. Antisera have been prepared in rabbits against the human pathogenic yeast *Sporothrix schenckii* grown at two different temperatures (25 and 37 °C).[247] Precipitation and inhibition data showed that the former serum has a specificity directed against α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-D-Man-(1 → determinants, whereas the latter has a broad specificity in which either α-L-rhamnosyl or α-L-Rha*p*-(1 → 3)-D-Man → is the immunodominant structure. These results are consistent with data on the structures of the L-rhamno-D-mannans isolated from the organism grown at these temperatures. ^{31}P N.m.r. spectra of a number of purified yeast *O*-phosphonohexoglycans have been recorded.[248] The spectroscopic data were correlated with established chemical aspects of individual and collective polymer structures, permitting conclusions to be drawn regarding the chemical environment of the phosphate groups of these polymers, and the assignment of anomeric configurations to the hexosyl phosphate residues.

Improved methods have been devised for the preparation of highly viscous, pigment-free, extracellular polysaccharides from an unidentified black yeast.[249]

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[246] Y. Yoshioka, M. Emori, T. Ikekawa, and F. Fukuoka, *Carbohydrate Res.*, 1975, **43**, 305.
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5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

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Introduction

The change in the authorship of this chapter has led to a few alterations in its construction. Because of their importance, cell-surface glycoproteins have been reviewed separately; some glycosylated proteins that would have previously been included in the sections extending from microbial to animal polysaccharides may now be lodged in this new section.

The biosynthesis of glycoproteins is reviewed twice: once in connection with glycosaminoglycans and again in a separate section dealing with the assembly and attachment of carbohydrates to proteins to give macromolecules that are not glycosaminoglycans.

Reviews that have appeared during the past year will be found in the appropriate sections.

Microbial Glycoproteins

The principal viral glycoprotein of Friend leukaemia virus has been isolated in pure form; it was shown to have a molecular weight of 5.8×10^4 by centrifugation and of 7.1×10^4 by gel electrophoresis in sodium dodecyl sulphate.[1] One major and two minor glycoproteins (mol. wts. 2.2×10^4, 2×10^4, and 3.2×10^4) with total carbohydrate contents varying from 3.6 to 6.5% have been detected in purified hepatitis B antigen.[2] The amino-terminal sequences of the subunits of the haemagglutinin influenza virus have been established.[3] Previous work had suggested that the glycopeptides are derived from a single glycosylated precursor by proteolytic cleavage, whereas it now appears that differences observed in the molecular weights of the subunits are probably due to differences in the carbohydrate moieties and to variations in the position of cleavage of the precursor molecule. A glycoprotein extracted from inactivated rabies vaccine has been shown to be equally as effective an antigen as the purified virus for vaccination.[4] The glycoprotein (mol. wt. 7×10^4) of the woolly monkey type-C RNA virus has been partially purified and characterized immunologically.[5]

The sequence in which sugars are added to the Sindibis virus glycoprotein has been studied.[6] A glycosylated precursor containing only 2-amino-2-deoxy-D-

[1] V. Moennig, H. Frank, G. Hunsmann, I. Schneider, and W. Schafer, *Virology*, 1974, **61**, 100.
[2] R. Chairez, S. Steiner, J. L. Melnick, and G. R. Dreesman, *Intervirology*, 1973, **1**, 224.
[3] J. J. Skehel and M. D. Waterfield, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 93.
[4] P. Atanasiu, H. Tsiang, P. Perrin, S. Favre, and J. Sisman, *Ann. Microbiol.*, 1974, **125B**, 539.
[5] S. Hino, J. R. Stephenson, and S. A. Aaronson, *J. Immunol.*, 1975, **115**, 922.
[6] B. M. Sefton and B. W. Burge, *J. Virol.*, 1973, **12**, 1366.

glucose, D-mannose, and some D-galactose was converted into the virion glycoprotein both by the addition of D-galactose and L-fucose and by the loss of D-mannose. The apparent extent of glycosylation of each virus-specific protein is not influenced by the host cell. In the presence of 0.1% 2-deoxy-D-*arabino*-hexose, four strains of Herpes simplex type-1 virus synthesized a new, major glycopeptide, whereas four strains of the type-2 virus synthesized an unaltered glycopeptide.[7] The deoxy-sugar inhibited the replication of both types of virus in an identical fashion, producing almost complete loss of activity at the above concentration. Other glycopeptides present in both types of virus did not exhibit this differential effect, suggesting that a different mechanism controls the synthesis of virus-induced glycoproteins.

A review of virion and tumour-cell antigens of type-C RNA tumour viruses, including sections on the analysis and the functions of glycoproteins, has been published.[8]

The phenol-soluble envelope fraction of *Escherichia coli* has been shown to consist of at least three distinct glycoproteins; one of them has an appreciable rate of turnover and is thought to be involved in the transfer of carbohydrates in the envelope.[9] Analysis of the outer- and inner-membrane glycoproteins showed that they are not identical.[10] The principal envelope protein of *Halobacterium salinarium* is also a glycoprotein of molecular weight 1.94×10^5.[11]

A water-soluble glycopeptide has been obtained from the supernatant fluid of cultures of *Mycobacterium tuberculosis*; it appears to be an arabinogalactomannan–peptidoglycan complex.[12]

Partial purification of the efflux-stimulating toxins obtained by extraction of the extracellular material from *Torulopsis glabrata* has shown that they are glycoproteins.[13] Other work has shown that phallolysin, a toxin from the bulbous mushroom *Amanita phalloides*, comprises 25.3% sugars composed of D-galactose (15.6%), D-glucose (6.5%), and D-mannose (3.2%).[14] The cytolytic activity appears to be associated with two components of molecular weight $>3.0 \times 10^4$ and is not diminished by proteases, α- and β-D-glucosidases, β-D-galactosidase, or α-D-mannosidase. The mechanism of phallolysin's action has been attributed to the binding of glycoproteins to 2-acetamido-2-deoxy-D-glucosyl residues on the cell-membrane surface.[15]

The arginine-binding protein of *Neurospora crassa* conidia has been shown to be a glycoprotein containing 113 amino-acids, assuming a minimum molecular weight of 1.36×10^4 for the peptide chain.[16] The principal sugar is D-glucose, but L-rhamnose, L-fucose, D-ribose, D-xylose, 2-deoxyhexose, D-mannose, and D-galactose are also present; no amino-sugar was detected. The carbohydrate–

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[8] H. Bauer, *Adv. Cancer Res.*, 1974, **20**, 275.
[9] G. Kidroni and G. Weinbaum, *Biochim. Biophys. Acta*, 1975, **399**, 428.
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[13] H. Bussey and N. Skipper, *J. Bacteriol.*, 1975, **124**, 476.
[14] H. Faulstich and M. Weckauf-Bloching, *Z. physiol. Chem.*, 1974, **355**, 1489.
[15] H. Faulstich and M. Weckauf-Bloching, *Z. physiol. Chem.*, 1975, **356**, 1187.
[16] W. D. Stuart and A G. DeBusk, *Arch. Biochem. Biophys.*, 1975, **166**, 213.

protein linkage probably involves D-xylose and serine or threonine residues. Lesions in amino-acid transport probably occur at the protein level, and the carbohydrate may be involved in the initial binding of amino-acids.

A second extracellular β-glucosidase (mol. wt. 3.4×10^5) present in *Aspergillus fumigatus* has been purified to homogeneity; it is probably composed of two subunits, and contains 2 residues of 2-amino-2-deoxy-D-glucose and 16 residues of D-mannose.[17] The oligosaccharide moiety, which is attached to the protein by a glucosaminyl linkage, does not appear to be the main antigenic site. The L-rhamnose-containing glycoprotein isolated from cells of the pathogenic fungus *Sporothorix schenckii* has been shown to comprise 87.1% of carbohydrate, consisting of L-rhamnose (21%), D-mannose (57%), and D-galactose (11%), and 12.5% of peptide; traces of D-glucose and 2-amino-2-deoxy-D-glucose were also found.[18] Two components of the extracellular aminopeptidase from *Aspergillus oryzae* have been identified as glycoproteins.[19] The principal sugars found were D-mannose and D-galactose, together with minor proportions of D-xylose, L-arabinose, D-glucose, L-rhamnose, and 2-amino-2-deoxy-D-glucose. An examination of the membrane glycoproteins of *Streptomyces albus* has indicated that they are distributed asymmetrically and located mainly on the external surface.[20]

Particulate preparations of a fungus imperfectus (*Cryptococcus laurentii*) transferred D-mannosyl and D-galactosyl residues from GDP-D-[^{14}C]mannose and UDP-D-[^{3}H]galactose, respectively, to the same endogenous acceptor.[21] β-Elimination reactions suggested that there is a common peptide core possessing three types of oligosaccharide bound to it by way of *O*-glycosidic linkages to threonyl and seryl residues.

The cell-wall and extracellular enzymes of *Saccharomyces cerevisiae* have again received attention. The analysis of mannan structures derived from mutant and wild-type yeasts showed that different enzymes are responsible for the biosynthesis of outer and inner portions of the chain.[22] Mild alkaline hydrolysis of a mannan derived from strain X2180 under conditions that caused β-elimination of substituted threonine and serine residues released D-mannose and manno-biose, -triose, and -tetraose.[23] The oligosaccharides were shown to possess the same structure as those of the polysaccharide component of yeast mannan. Similar treatment of a mutant lacking the mannotetraose unit produced only D-mannose and manno-biose and -triose. Thus, the single mutation altered not only the tetrasaccharide units of the polysaccharide component of the mannan but also those attached to serine and threonine residues. Further alkaline degradation in the presence of sodium borotritide of the mannan residue recovered from β-elimination furnished a protein-free polysaccharide, which yielded labelled 2-acetamido-2-deoxy-D-glucitol on hydrolysis. The original linkage probably involves asparagine.

[17] M. J. Rudick and A. D. Elbein, *J. Bacteriol.*, 1975, **124**, 534.
[18] H. Shimonaka, T. Noguchi, K. Kawai, I. Hasegawa, Y. Nozawa, and Y. Ito, *Infection and Immunity*, 1975, **11**, 1187.
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[21] M. K. Raizada, J. S. Schutzbach, and H. Ankel, *J. Biol. Chem.*, 1975, **250**, 3310.
[22] T. Nakajima and C. E. Ballou, *J. Biol. Chem.*, 1974, **249**, 7685.
[23] T. Nakajima and C. E. Ballou, *J. Biol. Chem.*, 1974, **249**, 7679.

The external, mannan-containing β-fructofuranosidase activities have been purified from three strains of *S. cerevisiae* having cell-wall mannans of related, but different, carbohydrate structures.[24] The immunological properties of the external invertase and cell-wall mannan from each strain were compared using several anti-mannan sera specific for distinct carbohydrate determinants. The invertase activity from each of the three strains was precipitated only by antisera that precipitated the cell-wall mannan. Removal of the D-mannosyl side-chains from the α-(1 → 6)-linked mannan backbone of two strains of the yeast produced an invertase that now precipitated with an antiserum directed against unbranched α-(1 → 6)-linked mannan. Thus, the carbohydrate structures of β-fructofuranosidase mannans of the yeast are similar to those of cell-wall mannan-proteins, which are known to contain an α-(1 → 6)-linked backbone to which side-chains are attached by α-(1 → 2)- and α-(1 → 3)-linkages. A strain lacking D-mannose 1-phosphate in the cell wall was capable of having all of the carbohydrate moiety removed from its invertase. Both the specific activity and the stability towards heat of this mannan-deficient enzyme were the same as those of the glycoprotein. A survey of the mutant yeasts used in these investigations indicated that the presence of external invertase is unaffected by alterations in the fine structure of the side-chains of the mannan core. Examination of the covalently bound phosphate of external invertases demonstrated that three preparations had D-mannose : phosphate ratios equivalent to 24—27 phosphate residues per molecule, whereas another had only seven phosphate residues per molecule.[25] Despite this variation, no correlation between the activity and the phosphate content was apparent. The invertase of low phosphate content was resolved into four components by isoelectric focusing, whereas an enzyme of high phosphate content was appreciably more heterogeneous; chromatography on hydroxyapatite afforded fractions possessing different ratios of mannan and protein, thus accounting for the microheterogeneity.

The encystment of zoospores of *Phytophthora palmivora* increased the binding of concanavalin A to the plasmalemma, an effect that could be inhibited by methyl α-D-mannopyranoside.[26]

Glycoproteins isolated from whole-cell epimastigotic forms of *Trypanosoma cruzi* have been shown to contain 2-deoxy-2-amino-D-glucose, D-galactose, D-glucose, and D-mannose.[27] The presence of D-xylose is doubtful, but traces of sialic acid were detected. Agglutination studies suggested that the glycoprotein complex is located on the cell surface of this protozoan.

Blepharmone, one of two gamones necessary for conjugation of the ciliate *Blepharisma intermedium*, has been shown to be a glycoprotein (mol. wt. 2.0×10^4) containing three residues each of D-mannose and 2-amino-2-deoxy-D-glucose, but no sialic acid.[28]

A carbohydrate-binding protein that participates in intercellular adhesion of the slime mould *Polysphondylium pallidum* has been isolated and found to have a molecular weight of 2.5×10^4.[29] Growing cells of *Dictyostelium discoideum*

[24] W. L. Smith and C. E. Ballou, *Biochemistry*, 1974, **13**, 355.
[25] W. J. Colonna, F. R. Cano, and J. O. Lampen, *Biochim. Biophys. Acta*, 1975, **386**, 293.
[26] V. O. Sing and S. Bartnicki-Garcia, *Experientia*, 1975, **31**, 643.
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[28] V. Braun and A. Miyake, *F.E.B.S. Letters*, 1975, **53**, 131.
[29] S. D. Rosen, D. L. Simpson, J. E. Rose, and S. H. Barondes, *Nature*, 1974, **252**, 128.

were each shown to possess 4×10^7 binding sites, all of equal affinity, for [^{125}I]concanavalin A, whereas aggregating cells appear to have, at least, two types of binding site, although the number of binding sites is only slightly increased.[30]

The compositions of the antitumour glycoproteins from *Lobaria orientalis* have been examined; the carbohydrate moiety consists of a (1 → 6)-glucan and a (1 → 3)-mannan linked to serine and threonine.[31] A glycoprotein present in the cell surface is responsible for the attachment of the protozoan *Trichomonas vaginalis* to glass surfaces; the glycoprotein can be extracted with H_4edta.[32]

Plant Glycoproteins

Many lectins that fall under this heading are considered in the following section.

The Golgi apparatus has been implicated in the synthesis and secretion of hydroxyproline-rich, cell-wall glycoproteins present in the phloem parenchymal cells of carrot roots.[33] Ricin D, a toxic protein extracted from the castor bean (*Ricinus communis* L.), contains 2-amino-2-deoxy-D-glucose (6) and D-mannose (17 residues) distributed among three carbohydrate moieties of the glycoprotein.[34] The sites of all three attachments to the peptide chain involve asparagine, and tri-, tetra-, and penta-peptide sequences at these sites have been established.[35] Three asparagine-oligosaccharides have been isolated from soy-bean 7S-protein by fractionation on Dowex 50-X2 resin; they were shown to consist of residues of asparagine (1), 2-amino-2-deoxy-D-glucose (2), and D-mannose (7, 8 and 9 residues, respectively). 2-Acetamido-1-L-β-aspartamido-1,2-dideoxy-β-D-glucopyranose was also isolated.[36]

The similarities of, and the differences between, peptide maps obtained from peroxidases from turnip and horse-radish have been described, and problems associated with the mapping of glycopeptides were discussed.[37]

Lectins

Lectins are widely used in studies of the compositions and structures of cell surfaces and will also be referred to in a following section on cell-surface glycoproteins. It has been the policy to include the jack-bean meal lectin, concanavalin A, in this Report, although it is not a glycoprotein; references to its use as a probe for membrane surfaces will be found in this section and elsewhere.

The purification of a number of lectins, including concanavalin A, Favin, phytohaemagglutinin, wheat-germ agglutinin, and *Limulus* haemagglutinin, has been achieved by affinity chromatography on Sepharose 4B-fetuin.[38] New lectins

[30] G. Weeks, *J. Biol. Chem.*, 1975, **250**, 6706.
[31] K. Takahashi, T. Takeda, S. Shibata, M. Inomata, and F. Fukuoka, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 404.
[32] P. Cappuccinelli, I. Cagliani, and G. Cavallo, *Experientia*, 1975, **31**, 1157.
[33] M. Gardiner and M. J. Chrispeels, *Plant Physiol.*, 1975, **55**, 536.
[34] S. Nanno, M. Ishiguro, G. Funatsu, and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1645.
[35] S. Nanno, M. Ishiguro, G. Funatsu, and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1651.
[36] F. Yamauchi, M. Kawase, M. Kanbe, and K. Shibazaki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 873.
[37] K. G. Welinder and G. Mazza, *European J. Biochem.*, 1975, **57**, 415.
[38] B.-A. Sela, J. L. Wang, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 7535.

possessing unknown binding specificities were also purified from extracts of small California white beans, Idaho red beans, and white pea beans. Examination of the binding of lectins to mouse-spleen cells indicated that, although the proteins were purified using the same adsorbent, they possessed markedly different binding specificities for cell-surface receptors.

A recent volume in 'Methods in Enzymology' has described techniques for investigating membranes, including the use of lectins; it also contains a section on the isolation and purification of wheat-germ agglutinin.[39]

A report from the Rockefeller group on the amino-acid sequence and the three-dimensional structure of concanavalin A by *X*-ray diffraction at 2 Å resolution is most welcome.[40] These studies have revealed details of the subunit structure and how the subunits interact to form dimers and tetramers, in addition to the interactions of the protein with metal ions. They have also demonstrated differences between the saccharide-binding properties of concanavalin A in solution and in the crystal. The unique arrangement of the β-structure and the role of metal ions in establishing the conformation of the protein make concanavalin A a useful system for a general analysis of polypeptide folding. The binding site of the inhibitor 2-iodophenyl β-D-glucopyranoside was shown to be located in a deep cavity containing distinct hydrophobic and hydrophilic binding subsites.[40–43] Changes in the luminescence and phosphorescence of concanavalin A when it binds methyl α-D-glucopyranoside, and when calcium and manganese ions are removed, indicated that tyrosine-12 and -101 are present at the binding site.[44] The saccharide-binding activity has been shown to be independent of the size of the metal ion in one of the transition-metal-ion binding sites, but to be critically dependent on the size of the ion in the other.[45] The use of stopped-flow n.m.r. spectroscopy to elucidate conformational changes showed an ordered binding of manganese ions, or calcium ions, or sugar before any irreversible changes of conformation take place.[46] In agreement with the observations of Edelman's group, the use of ^{19}F n.m.r. spectroscopy showed that the binding of 2-deoxy-2-(*N*-trifluoroacetamido)-α- and -β-D-glucose to dimers and tetramers of concanavalin A in solution did not involve the hydrophobic pocket previously identified.[47]

A study of the interaction of a number of immunoglobulin-derived glycopeptides with concanavalin A has shown that the binding depends on terminal 2-acetamido-2-deoxy-β-D-glucosyl and α-D-mannosyl residues and on internal D-mannosyl residues substituted at C-2.[48] The saccharide-binding site seems to be capable of binding several sugar residues. The inhibition indexes of several

[39] Y. Nagata, A. R. Goldberg, and M. M. Burger, *Methods Enzymol.*, 1974, **32B**, 611.
[40] J. L. Wang, B. A. Cunningham, M. J. Waxdal, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1490.
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[43] G. N. Reeke, jun., J. W. Becker, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1525.
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[47] G. M. Alter and J. A. Magnuson, *Biochemistry*, 1974, **13**, 4038.
[48] R. Kornfeld and C. Ferris, *J. Biol. Chem.*, 1975, **250**, 2614.

glycosides exhibited a simple relationship with their association constants, illustrating the value of the turbidimetric inhibition test, even for glycosides having a high affinity, and also indicated that all the glycosides compete with polysaccharides for a single binding region.[49]

The transition of the cellular slime mould *Dictyostelium discoideum* from the amoebal phase to the aggregation phase initiates the production of discoidin, a cell-surface lectin.[50] This lectin has been resolved into two components that differ in the way that they are regulated during development.

Inhibition studies on the precipitin reaction between *Pisum sativum* lectin and the phosphomannan from *Pichia pinus* with haptens have shown that the lectin binds to D-mannose, D-glucose, D-fructose, and L-sorbose.[51] Unmodified hydroxy-groups at C-4 and C-6 of the D-glucopyranose ring are essential for binding to the protein. With the exception of gentiobiose, β-linked glycosides were not bound to the lectin, whereas α-linked glycobioses were potent inhibitors. Hybridization of the lectins from pea has been demonstrated, but the biological significance, if any, of this is unknown.[52]

Despite its apparent homogeneity on discontinuous gel electrophoresis, isoelectric focusing, sedimentation equilibration, and immunodiffusion, the lectin from *Dolichos biflorus* seeds could be fractionated into bound and unbound forms by chromatography on concanavalin A-Sepharose.[53] An unbound form and two principal bound forms contain D-mannosyl and 2-acetamido-2-deoxy-D-glucosyl residues. The subunit structure of the dominant form has been characterized, and the features that distinguish the fractions of the lectin probably reside in the D-mannosyl residues.[54] Cyanogen-bromide fragmentation has been used to isolate glycopeptides from this lectin.[55]

Half-gram quantities of lectins have been purified from saline extracts of red-kidney beans (*Phaseolus vulgaris*) by affinity chromatography on porcine thyroglobulin-Sepharose.[56] Extracts of twenty-one cultivars of *P. vulgaris* have been screened for haemagglutinating specificity and for mitogenic activity.[57] Sequence studies of phytohaemagglutinating mitogenic lectins, which comprise a class of five glycoproteins composed of two subunits in tetrameric configuration, have revealed striking similarities between them.[58] In each subunit, the twelfth residue is asparagine glycosylated with an identical carbohydrate moiety. Tryptic peptide mapping of the succinylated subunits showed extensive homology.

The ability of wheat-germ agglutinin to precipitate with a series of synthetic carbohydrate–protein conjugates and with the carcinoembryonic antigen and its Smith-degradation products has shown that the binding site is complementary to

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[50] W. A. Frazier, S. D. Rosen, R. W. Reitherman, and S. H. Barondes, *J. Biol. Chem.*, 1975, **250**, 7714.
[51] J. P. Van Wauwe, F. G. Loontiens, and C. K. De Bruyne, *Biochim. Biophys. Acta*, 1975, **379**, 456.
[52] G. Entlicher and J. Kocourek, *Biochim. Biophys. Acta*, 1975, **393**, 165.
[53] W. G. Carter and M. E. Etzler, *J. Biol. Chem.*, 1975, **250**, 2756.
[54] W. G. Carter and M. E. Etzler, *Biochemistry*, 1975, **14**, 2685.
[55] W. G. Carter and M. E. Etzler, *Biochemistry*, 1975, **14**, 5118.
[56] R. L. Felsted, R. D. Leavitt, and N. R. Bachur, *Biochim. Biophys. Acta*, 1975, **405**, 72.
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a sequence of three (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residues.[59] Studies of the agglutinating ability of wheat-germ agglutinin with cells of *Escherichia coli*, *Micrococcus luteus*, and *Staphylococcus aureus* have been reported.[60] The interactions with a soluble, linear peptidoglycan secreted by *M. luteus* and with the teichoic acid of *S. aureus* were specifically inhibited by low concentrations of chitotriose and a bacterial cell-wall tetrasaccharide [β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Mur*N*Ac-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-D-Mur*N*Ac]. Comparison of the efficacy of different saccharides as inhibitors suggested that secondary, non-specific interactions contribute to the binding of the lectin to the polymers. The binding of wheat-germ agglutinin to fungal cell walls, presumably to chitin, indicated that the lectin protects wheat against chitin-containing phytopathogens.[61]

The four subunits of soy-bean agglutinin have been shown to consist of two types, both of which are glycoproteins.[62] Amino-acid analysis revealed the subunits to be similar but not identical. Periodate oxidation destroyed as many as five of the nine D-mannosyl residues present in each of the subunits, without loss of the haemagglutinating activity.[63] Subsequent reduction with sodium borohydride afforded a fully active lectin that was indistinguishable from the native protein by gel filtration, gel electrophoresis, and affinity chromatography. The immunochemical specificity of purified soy-bean agglutinin has been studied using the blood-group substances and periodate-oxidized and partially hydrolysed products thereof, submaxillary mucins, and streptococcal C polysaccharides.[64] The activity was attributed to a terminal 2-acetamido-2-deoxy-D-galactopyranosyl residue or to an α- or β-D-galactopyranosyl residue. The agglutinating activity and aggregation of soy-bean agglutinin increased on storage of freeze-dried samples.[65]

The compositions of the lectins abrin and ricin from seeds of *Abrus precatorius* L. and the castor bean *Ricinus communis* L., respectively, have been studied, and certain homologous sequences of amino-acids were shown to exist.[66] The B-chains of abrin and ricin each contain similar proportions of D-mannose and 2-amino-2-deoxy-D-glucose; the A-chain of ricin contains some carbohydrate, whereas that of abrin does not appear to be a glycoprotein. The conformational change induced when sugars bind to the lectins of *R. communis* has been investigated by c.d.; this change was shown to take place in the amino-acid side-chains, but not in the polypeptide backbone.[67] Immobilization of the lectin, by binding to concanavalin A, did not appear to impair its sugar-binding properties; for example, the number of binding sites and apparent association constant for lactose remained unaltered.[68]

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The separation of lectins from lima beans (*Phaseolus lunatus*) on a column of concanavalin A-Sepharose has been reported; binding studies with methyl 2-acetamido-2-deoxy-α-D-galactopyranoside showed that the association constants of the lectins are virtually identical.[69]

A new, non-specific phytohaemagglutinin from lentil (*Lens esculentia*) seeds has been shown to be a glycoprotein of molecular weight 5.3×10^4.[70] A lectin found in the seeds of *Vicia ervilla* has a molecular weight of 6×10^4, and consists of four subunits composed of two types of peptide chain.[71] It has a similar specificity to that of concanavalin A, and also contains no carbohydrate. A lectin from *Vicia graminea* has been described.[72] A lectin from *Vistaria floribunda*, which possesses haemagglutinating activity but no mitogenic activity against normal lymphocytes, has been shown to possess four identical subunits (mol. wt. 3.5×10^4) and to contain about 5% carbohydrate, comprising D-mannose, 2-amino-2-deoxy-D-glucose, L-fucose, and some D-xylose.[73] Its binding specificity is directed towards D-galactose. A phytohaemagglutinin (approximate mol. wt. 1.27×10^5) containing 2-amino-2-deoxy-D-glucose and unidentified neutral sugars has been separated from the mixture of lectins present in the fleshy arils of seeds of the spindle tree (*Evonymus europaea* L.).[74]

A simple method for the purification of carbohydrate-binding proteins by adsorption on to formalinized erythrocytes has been used to isolate the haemagglutinin from the haemolymph of *Limulus polyphemus*.[75] This glycoproteinaceous agglutinin (mol. wt. 4.6×10^5) contains 2-amino-2-deoxy-D-glucose (4%) and is composed of subunits of molecular weight 2.2×10^4. *N*-Acetylneuraminic acid and D-glucuronic acid inhibited its agglutination. Comparative studies on different species and subspecies of haemagglutinins derived from fish eggs [76] and newt eggs [77] have been reported. The agglutinin (tridacnin) from the elongate clam (*Tridacna maxima*) has been isolated and purified; inhibition experiments indicated that the agglutinin is specific for 2-acetamido-2-deoxy-D-galactopyranosyl residues, probably in β-linkage.[78–80]

An anti-B haemagglutinin from a *Streptococcus* species has been reported.[81]

2-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-D-mannose, methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside, and methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannofuranoside have been used to demonstrate subtle differences in the binding specificities of lectins from *Lens culinaris* and *Pisum sativum* and of concanavalin A.[82]

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Blood-group Substances

Glycoprotein antigens with ABH and Lewis blood-group specificities, as well as their immunogenic properties, biosynthesis, and occurrence in malignant cells and tissues, have been reviewed.[83] The chemistry of the MN, P, I, J, and Rh antigens was also discussed. A survey of studies on the immunochemistry of water-soluble A, B, H, Le^a, and Le^b antigens and their precursors has also appeared.[84]

In an examination of blood-group modifications, the α-D-galactosyltransferase present in fresh B or AB sera was found to convert O red cells into B-active cells in the presence of UDP-D-galactose.[85] The same level of transferase activity was demonstrated in B sera of both secretors and non-secretors, but not in A or O sera. A method for extracting the ABO antigens from erythrocyte membranes using sodium deoxycholate and H_4edta has been described.[86] A sulphated heteropolysaccharide exhibiting blood-group A activity has been isolated from oyster viscera.[87]

Blood-group substances of Le^a specificity from ovarian cyst fluids have been separated into eight fractions by equilibrium-density centrifugation; comparison of these fractions with fractions obtained by solvent extraction showed them to be more homogeneous.[88] A correlation was noted between the buoyant density and the chain length of the oligosaccharide moieties, but not with the extent of glycosylation. The L-fucose contents of active blood-group substances isolated from a range of salivas have been correlated with the Lewis activity, and were shown to decrease for non-secretors.[89]

The principal erythrocyte sialoglycoprotein associated with MN activity has been purified and shown not to carry ABO(H), I, and Ss antigens; the Ss activity is thought to be associated with a minor glycoprotein.[90] The MM and NN antigens are very similar, differing by only one residue of sialic acid for each immunodominant group. Removal of this residue, with consequent exposure of the penultimate β-D-galactosyl residue, converted the MM antigen into the NN antigen.[91] The NN activity was removed by a specific β-D-galactosidase, as well as by removal of the remaining sialic acid residue; desialylation produced T-antigenic activity, which was transformed into Tn activity by β-D-galactosidase. Structural differences have been reported in the amino-acid sequences of the peptide chains of M and N antigens, in addition to differences reported in the carbohydrate moieties.[92] The significance of this class of blood-group antigen extends to human breast cancer, where MN antigens have been found in benign and malignant glands, whereas their presursors occur only in cancerous tissue.[93]

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The incorporation of tryptic peptides derived from MN glycoproteins into phospholipid liposomes and black membranes produced a significant increase in the permeability of the liposomes to potassium ions and of the membranes to water and potassium ions.[94] These observations were interpreted in the light of the knowledge that MN glycoprotein penetrates the cell membrane *via* a non-polar segment of its polypeptide chain. Care must be exercised in the preparation of the MN–antigen complex, since its molecular weight can vary from 3×10^4 to 5.3×10^4, depending on the method of extraction.[95] The MN and Ss antigens have been separated by extraction with sodium deoxycholate.[96] It was suggested that differences in their structures are such that they cannot be considered to exhibit the homology found in the ABO antigens.

Comparative studies of the blood-group A substances of invertebrates have been reported,[97] and the results of an examination of tissues from pigs, horses, baboons, Rhesus monkeys, and humans are consistent with, but do not unequivocally confirm, the microheterogeneity of glycoproteins exhibiting blood-group activity.[98]

Collagens

The meaning of the term 'post-ribosomal' is becoming less clear with regard to the modification of proteins. Using chicken-embryo fibroblasts, it appeared that glycosylation of the peptide-bound hydroxylysine residues of collagen is initiated while the polypeptide chain is still being assembled on the ribosomes.[99] Other studies have indicated that this event occurs earlier; thus, use of a transferase prepared from chicken embryo showed that glycosylation of D-galactosylhydroxylysyl sequences does not occur once the triple-helix conformation has been assembled.[100] UDP-Glucose:D-galactosylhydroxylysine collagen glucosyltransferase has been purified fifty-fold from calf arterial tissues; its molecular weight is 7.2×10^4, and it requires manganese ions for activity.[101] The protein can be resolved into two active components, and showed greater affinity for calf-skin collagen than for the α-1 or β-12 components. A procedure for the assay of both D-galactosyl- and D-glucosyl-transferase activities has used the transfer of ^{14}C-labelled hexoses from the corresponding uridine nucleotides to hydroxylysyl or D-galactosylhydroxylysyl residues.[102] The galactosyltransferase from chicken-embryo cartilage could not utilize free hydroxylysine as an acceptor, and manganese ions were found to be the most efficient metal-ion cofactor.

The biosynthesis of basement membrane has been studied *in vitro* using rat parietal yolk sac.[103] There appears to be both temporal and cellular compart-

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[103] C. C. Clark, E. A. Tomichek, T. R. Koszalka, R. R. Minor, and N. A. Kefalides, *J. Biol. Chem.*, 1975, **250**, 5259.

mentalization involved in the biosynthesis of collagen and glycoprotein, with the more rapid synthesis of glycoprotein followed by the secretion of membrane collagen. Interaction, perhaps through disulphide bonds, might occur extracellularly to form a functional basement membrane.

Collagen in which the carbohydrate had been oxidized by periodate ion no longer caused the release of serotonin nor did it polymerize.[104] The effect of proteoglycans on the formation of collagen fibrils has been discussed.[105] The proposal that the organization of collagen fibrils is related to the amount and type of proteoglycan is supported by the observation that precipitation of the fibrils is markedly retarded in the presence of proteoglycan.

Collagens derived from pleural and parenchymal regions of bovine lung have been compared with those from basement-membrane tissues; collagens from bovine lung contain both D-galactosyl and D-glucosyl-D-galactosyl side-chains, whereas only disaccharide side-chains occur in collagens from basement membranes.[106] The glycoprotein contents of pleural and parenchymal regions were found to be 4% and 15.5%, respectively; both tissues contain heterogeneous mixtures of glycoproteins, which are similar in amino-acid and carbohydrate compositions and resemble those present in other tissues.[107] Some collagen fractions isolated from the renal glomerulus of steer kidney contained more D-glucose than D-galactose, implying that D-glucose is also present in a form other than D-galactosyl-D-glucosylhydroxylysine.[108] The extent of glycosylation of hydroxylysine in human-skin collagen was shown to decrease during maturation and then to increase with age.[109]

The sequence of 37 residues at the *C*-terminus of the α-1-CB5 glycopeptide prepared from chicken-skin collagen has been determined, and the *N*-terminal hydroxylysyl residue was shown to be glycosylated.[110] Denatured forms of this glycopeptide and of the α1-chain have been shown to bring about platelet aggregation.[111] The hydroxylysine-linked carbohydrate on the α2-chain of guinea-pig skin collagen is also present on the α2-CB4B fragment; analyses are compatible with the presence of single mono- and di-saccharide residues in this region.[112] However, the precise function of the carbohydrate and the reason why it is restricted to the *N*-terminus of the α-chain are not yet known.

Glycogen

A method for the determination of glycogen in the presence of an excess of D-glucose has been reported; the procedure involves degradation of D-glucose with alkali prior to digestion of the polysaccharide with amyloglucosidase.[113] Determination of the glycogen content of a single neutrophil leucocyte using the periodic acid–Schiff reagent has been described, and a mean value of

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13.3×10^{-12} g of glycogen was obtained for normal human neutrophils.[114] Quantitative and qualitative diagnoses of glycogen metabolism have been reported, including a detailed account of a procedure for isolating leucocytes.[115]

Examination of the metabolic actions of vasopressin, glucagon, and adrenalin in intact rats has revealed a rapid response to vasopressin by hepatic glycogenolysis, whereas adrenalin had a multiple and prolonged action.[116] Changes in phosphorylase and glycogen synthase activities during perfusion of rat liver have been studied in conjunction with the responses to added epinephrine and glucagon.[117] The phosphorylase activity in fed or fasted rats fell rapidly whether or not D-glucose was present, but the addition of epinephrine or glucagon restored the activity. The I-form of glycogen synthase in fed rats increased rapidly in the presence of 10 mM D-glucose, but this effect was prevented by either epinephrine or glucagon; glycogen synthase in fasted rats did not respond to either of these additives. The glucocorticoids were shown to play a permissive role by increasing the affinity of liver phosphorylase for epinephrine and glucagon. A study of the influence of hormones on the myocardial metabolism of glycogen in fed and fasted rats indicated that adrenal hormones exert a stabilizing effect on the levels of glycogen in fed animals, but are not necessary for synthesis to occur.[118] Hypophysectomy eliminated the increase in the level of glycogen that occurs in fasting animals, whereas an insulin deficiency led to increased storage of glycogen in both fed and fasted animals. The rise in the level of rabbit-liver phosphorylase on stimulation of the splanchnic nerve, unlike that induced by epinephrine or glucagon, is not mediated by adenosine cyclic 3′,5′phosphate(cyclic AMP) and consequent activation of dephosphophosphorylase kinase, but rather by the inactivation of phosphorylase phosphatase.[119] From investigations of the regulation of glycogen metabolism by sympathetic and parasympathetic systems in rat submaxillary gland, it was concluded that norepinephrine and acetylcholine do not act antagonistically.[120] Rather, it seems that the mechanism regulating the conversion of phosphorylase *b* into *a* in rat submaxillary gland is similar to that existing in skeletal muscle.

The antagonism between insulin and selective adrenergic stimulation of the interconversion of glycogen synthases and phosphorylases has been investigated in isolated rat diaphragms.[121] The results indicated that the sensitivities of the synthase and phosphorylase systems to insulin differ, and suggested that noradrenaline and terbutaline influence the metabolism of glycogen by different mechanisms. The relation between the levels of plasma corticosterone and cardiac glycogen in rats suggested that physical exertion and the presence of steroids can produce changes, perhaps local, that affect the levels of cardiac glycogen for some hours after exercise.[122] The glycogenolytic effect of glucagon has been

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studied in foetal hepatocytes cultured in the presence of 10 mM cortisol.[123] The initial minute amount of glycogen present at transplant increased to a high level over 3—4 days. This glycogen could be mobilized by adenosine cyclic 3′,5′-phosphate, and the administration of glucagon to the cells was quickly followed by an accumulation of adenosine cyclic 3′,5′-phosphate. Metabolic modulation of the sensitivity of the control mechanism for phosphorylase has been examined in rat heart and diaphragm.[124] The control mechanism was shown to be active when glucagon and catecholamines were used to stimulate cardiac tissue, but only catecholamines stimulated the mechanism in the diaphragm.

The effects of D-glucose, insulin, and glucagon in controlling the metabolism of glycogen have been studied in isolated rat-liver parenchymal cells.[125] It was concluded either that the capacity of the cells to synthesize glycogen is already maximal in the absence of insulin or that some other factor essential for insulin to act is missing. Although D-glucose stimulated the synthesis of glycogen from D-fructose, it was not utilized; this finding supports the suggestion that D-glucose regulates the synthesis of glycogen in the cells.

Treatment of rats with 100 μg of L-thyroxine resulted in an appreciable increase of liver glucose-6-phosphatase and a depletion of glycogen.[126] Hypothyroid animals showed a corresponding decrease in the phosphatase and a significant accumulation of glycogen. Testosterone has been shown to alter the levels of glycogen in perineal and skeletal muscles by two distinct mechanisms.[127] The effects of reserpine and the anti-depressive drug chlorimipramine on the levels of glycogen in rat brain have been examined.[128]

The mechanism of *de novo* synthesis of glycogen in the absence of added primer continues to be explored actively. Biosynthesis under these conditions in rat liver has been shown to take place in at least two stages.[129] The first stage could be initiated by an enzyme that transfers D-glucose from UDP-D-glucose to an acceptor glycoprotein; the transfer takes place *in vitro* only in the presence of high concentrations of certain salts. The complex product of this reaction acts as a precursor for the synthesis of glycogen. The acceptor glycoprotein may contain the bound, synthesized α-1,4-glucan as its carbohydrate moiety. Another group has reported that rat-liver microsomes are capable of mediating the transfer of D-glucose from UDP-D-glucose to endogenous glycoprotein or to exogenous glycogen or collagen.[130] Triton X-100 inhibited the transfer of D-glucose to glycogen or glycoprotein, but activated its transfer to collagen. Prior treatment of the microsomal membranes with α-amylase was claimed to remove any endogenous glycogen. It is of interest to note that a non-sedimentable fraction from potato tubers catalysed the transfer of D-glucose from D-glucose 1-phosphate to an endogenous protein acceptor in the absence of added primer.[131] The product appears to be a glycoprotein.

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[131] J. Tandecarz, N. Lavintman, and C. E. Cardini, *Biochim. Biophys. Acta*, 1975, **399**, 345.

Glycogen synthase can use D-glucose as an acceptor. Using very high concentrations of D-glucose, it has been demonstrated that maltose is synthesized from UDP-D-glucose by a highly purified, skeletal-muscle glycogen synthase.[132] In questioning whether or not rabbit-muscle glycogen synthase acts by a 'ping-pong mechanism', the same authors have indicated that the kinetics are compatible with a sequential mechanism for the synthase utilizing either glycogen or maltose as an acceptor.[133] The relation between phosphate incorporation and the interconversion of glycogen synthases has been examined.[134] The complete conversion of rabbit skeletal-muscle glycogen synthase from the I-form into the D-form requires the incorporation of 2 mol of phosphate for each enzyme subunit (mol. wt. 9×10^4). Incubation of the I-form with low concentrations of cyclic-AMP-dependent protein kinase (10 units ml^{-1}) and magnesium-ATP resulted in incorporation of 1 mol of phosphate per subunit, with a concomitant decrease in the synthase activation ratio (*i.e.* (±)-glucose 6-phosphate) from 0.85 to 0.25. Further incubation or addition of protein kinase to a concentration of 150 units ml^{-1} did not produce any change; however, an increase in the concentration of protein kinase to 10 000 units ml^{-1} effected the incorporation of a second mole of phosphate per subunit, and the synthase activation ratio decreased to $\leqslant 0.05$. Further slow and non-specific phosphorylation, not associated with activity changes, was also observed. The role of magnesium ions in controlling the activity of bovine-heart glycogen synthase and glycogen synthase phosphatase has been considered.[135] They are suggested to stimulate the phosphatase reaction by generating conformers of the D-form having a much higher reactivity as substrates for the synthase phosphatase, as well as much greater catalytic activity. Double-reciprocal plots of the reaction velocity *versus* the concentration of D-glucose 6-phosphate were biphasic, suggesting the presence of two kinetic forms, each with a binding site of different affinity for D-glucose 6-phosphate. The hormonal regulation of liver synthase interconversions has been discussed in terms of dominant control by the phosphatase, the kinase being considered to play a subordinate role.[136] Both I- and D-forms of synthase have been partially purified from pig brain.[137] Studies with perfused livers of streptozotocin-diabetic rats have demonstrated a marked inherent impairment in the synthesis of glycogen by starved animals; this effect can be rapidly reversed *in vivo*, but not in perfused livers.[138] Liver-glycogen synthesis does not appear to be sensitive either to the short-term, direct action of insulin or to long-term deprivation of insulin *in vivo*.

The molecular basis for the hormonal control of phosphorylase kinase in rabbit-skeletal muscle has been discussed. Two tryptic phosphopeptides, which contain the sites on the α- and β-subunits of the kinase phosphorylated by a cyclic-AMP-dependent protein kinase, have been isolated, and the sequence

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[135] C. Nakai and J. A. Thomas, *J. Biol. Chem.*, 1975, **250**, 4081.
[136] H. L. Segal, in 'Isozymes', ed. C. L. Markert, Academic Press, New York, San Francisco, and London, 1975, Vol. 3, p. 691.
[137] J. V. Passonneau, J. P. Schwartz, and D. A. Rottenberg, *J. Biol. Chem.*, 1975, **250**, 2287.
[138] P. D. Whitton and D. A. Hems, *Biochem. J.*, 1975, **150**, 153.

around the phosphorylated serine residue has been determined.[139] It is apparent that the cyclic-AMP-dependent kinase is extremely specific in its action. The *in vivo* response of this kinase to adrenalin has also been investigated.[140] The activated and non-activated forms were found to be indistinguishable on dodecyl sulphate disc-electrophoresis. Digestion with trypsin showed that, as *in vitro*, identical amino-acid segments of the α- and β-subunits of the kinase are phosphorylated and that the degree of phosphorylation of each site is at least 50%. A novel form of enzyme control was established whereby the kinase becomes susceptible to dephosphorylation only after further phosphorylation. Thus, phosphorylase kinase *a* is converted rapidly into a derivative by phosphorylation of the β-subunit. Dephosphorylation of the kinase, and the reverse of this process, depend on phosphorylation of the α-subunit.

The changes in the quaternary structure of rabbit-muscle phosphorylase *b* at 4 °C have been followed by measuring the reactivity of the thiol group.[141] A slow, concerted transition between the two dimeric forms was shown to take place, the rate of which is affected by adenosine monophosphate. A phosphopeptide containing 14 residues, including the phosphorylated serine residue, derived from the amino-terminus of rabbit skeletal-muscle phosphorylase has been shown to induce the enzymic properties of phosphorylase *a* in phosphorylases *b* and b^1 (a modified form in which the phosphorylated site has been removed by limited digestion with trypsin).[142] Thus, these enzymes become partially active in the absence of adenosine monophosphate.

Although not within the remit of this section, it is of interest to note that threonine is phosphorylated on activation of the phosphorylase from *Saccharomyces cerevisiae*.[143] The sequences of amino-acids around this site and around pyridoxal 5′-phosphate were determined; the latter, but not the former, sequence is closely homologous to that found in vertebrate phosphorylases.

High concentrations of D-glucose and potassium ions in the incubation medium greatly increased the rate of inactivation of the phosphorylase and activated the glycogen synthase in isolated rat hepatocytes and perfused rat liver.[144]

Conditions for the assay of liver phosphorylases *a* and *b* have been reviewed, and procedures were suggested for the estimation of these enzymes.[145] For example, both forms can be estimated at pH 6.5 in the presence of 1% glycogen, 0.05M-D-glucose 1-phosphate, and 0.15M-sodium fluoride. This assay can be made specific for phosphorylase *a* by the addition of either caffeine or maleate. The total activity is best measured in the presence of 1mM-adenosine monophosphate and 0.5M-sulphate.

Purified rabbit-liver phosphorylase phosphatase appears to be a single polypeptide chain having an approximate molecular weight of 3.4×10^4; it has an optimum pH of 6.9—7.4 and a K_m for rabbit-muscle phosphorylase *a* of 2 μM.[146] The purification procedure is also applicable to rabbit-muscle phosphorylase

[139] P. Cohen, D. C. Watson, and G. H. Dixon, *European J. Biochem.*, 1975, **51**, 79.
[140] S. J. Yeaman and P. Cohen, *European J. Biochem.*, 1975, **51**, 93.
[141] M.-H. Buc-Caron and H. C. Buc, *European J. Biochem.*, 1975, **52**, 575.
[142] T. J. Carty, J.-I. Tu, and D. J. Graves, *J. Biol. Chem.*, 1975, **250**, 4980.
[143] K. Lerch and E. H. Fischer, *Biochemistry*, 1975, **14**, 2009.
[144] L. Hue, F. Bontemps, and H.-G. Hers, *Biochem. J.*, 1975, **152**, 105.
[145] W. Stalmans and H.-G. Hers, *European J. Biochem.*, 1975, **54**, 341.
[146] H. Brandt, Z. L. Capulong, and E. Y. C. Lee, *J. Biol. Chem.*, 1975, **250**, 8038.

phosphatase. Rabbit liver has been shown to contain a heat-stable, non-dialysable inhibitor of phosphorylase phosphatase.[147] The effect of the inhibitor is destroyed by trypsin, and the kinetics of inhibition are non-competitive with respect to phosphorylase *a*. Other work has indicated that the control of rabbit-muscle phosphorylase phosphatase is effected by a cyclic-AMP-dependent protein kinase and a manganese-stimulated phosphatase, which together control the phosphorylation and dephosphorylation of a protein that functions as a phosphatase inhibitor.[148]

Following the discovery that increased glycogenolysis occurs during platelet aggregation, despite a fall in the levels of adenosine cyclic 3′,5′-phosphate, it was found that low levels of calcium ions activate the phosphorylase *b* kinase isolated from human platelets.[149]

Marked alterations in the patterns of glycogen phosphorylase isoenzymes have been observed in a series of transplanted rat hepatomas.[150] The binding site of rabbit-muscle phosphorylase *b* is thought to be hydrophobic.[151] A test for the early differential diagnosis of acute myocardial infarction has been devised, based on the levels of glycogen phosphorylase in blood sera.[152]

An investigation of the metabolism of glycogen in foetal rat liver has established a relationship between the size of the glycogen particles and amylo-1,6-glucosidase activity.[153] The molecular weight of glycogen is low when debranching activity is high, and *vice versa*. The molecular weight of amylo-1,6-glucosidase from rabbit muscle has been determined as $1.6—1.7 \times 10^5$ under denaturing and non-denaturing conditions.[154] The mammalian enzyme, which appears to be a single polypeptide chain containing two enzymic activities,[154] has been purified to homogeneity.[155] The method of preparation gave yields 200 mg of protein from 1000 g of muscle, which represents an overall yield of 60%. Evidence was presented to show that the debranching enzyme is monomeric at concentrations existing in the muscle. Glycogen particles isolated from skeletal muscle contain seven major protein-staining components (polyacrylamide gel electrophoresis); five of the components were identified as the branching enzyme, the α- and β-subunits of phosphorylase kinase, glycogen phosphorylase, and glycogen synthase. A new titrimetric assay was used to demonstrate that the activity of the debranching enzyme is unaffected by mechanisms that lead to activation of glycogen phosphorylase and phosphorylase kinase.

The synthesis of glycogen from pyruvate, glutamate, and hydrogen carbonate has been demonstrated to take place in slices of guinea-pig brain, but gluconeogenesis appears to be important only under abnormal conditions.[156] The levels of glycogen in adult rat brain appear to be highest in the medulla oblongata and lowest in the frontal lobes.[157] A recent review has dealt with the morphological

[147] H. Brandt, E. Y. C. Lee, and S. D. Killilea, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 950.
[148] F. L. Huang and W. H. Glinsmann, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3004.
[149] A. R. L. Gear and W. Schneider, *Biochim. Biophys. Acta*, 1975, **392**, 111.
[150] K. Sato, T. Sato, H. P. Morris, and S. Weinhouse, in ref. 136, p. 951.
[151] G. Soman and G. Philip, *Biochem. J.*, 1975, **147**, 369.
[152] E.-G. Krause, H. Will, M. Böhm, and A. Wollenberger, *Clinica Chim. Acta*, 1975, **58**, 145.
[153] R. Vaillant, *F.E.B.S. Letters*, 1975, **52**, 22.
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[155] C. Taylor, A. J. Cox, J. C. Kernohan, and P. Cohen, *European J. Biochem.*, 1975, **51**, 105.
[156] M. E. Phillips and R. V. Coxon, *Biochem. J.*, 1975, **146**, 185.
[157] R. V. Živković, B. B. Mršulja, and B. M. Djuričić, *Experientia*, 1975, **31**, 404.

form of glycogen and with alterations in the levels of glycogen in pathological and experimentally induced conditions of the central nervous system.[158] Reports on the metabolism of glycogen in the turtle (*Lacerta sicula*),[159] common frog,[160] and scorpion[161] have appeared.

An examination of the catabolism of glycogen by an acid α-1,4-glucosidase present in cultured skin fibroblasts of normal cells and in those derived from patients with Cori Type II glycogen-storage disease has indicated the presence of an extra-lysosomal glycogen that is more amenable to degradation.[162]

Other reports on glycogen and glycogen-metabolizing enzymes are referred to in Chapters 4 and 6, respectively.

Glycosaminoglycans

Analytical Methods.—The detection and estimation of glycosaminoglycans in body organs and fluids have been reviewed.[163] A simple, non-destructive colorimetric method for the determination of both sulphate groups and carboxy-groups in heparins and the chondroitin sulphates has been developed.[164] A spectrophotometric method using pseudoisocyanin can be used to distinguish between sulphated glycosaminoglycans on the basis of the spatial arrangement of the negative charge with respect to the rest of the molecule.[165] A 3-hydroxydiphenyl reagent, requiring 1 ml of serum, is reported to measure the hexuronic acid content (0—15 μg) of mucopolysaccharides.[166] Sub-microgram quantities of acid glycosaminoglycans can be determined as the Alcian Blue complexes, following electrophoretic fractionation on membranes of cellulose acetate.[167] The use of g.l.c. for the determination of iduronic and glucuronic acids has been reported.[168] A method using orcinol has been developed for the determination of dermatan sulphate (0—100 μg) in the presence of chondroitin 4- and 6-sulphates.[169] A simple, rapid, one-stage assay of heparin in plasma, based on the heparin-dependent neutralization of activated factor X, has been described; the lower limit of detection is about 0.05 units of heparin per ml.[170] Hyaluronate (0—100 μg) has been determined by a method that does not require its prior separation from other glycosaminoglycans; the method is based on the specificity of a *Streptomyces* hyaluronidase.[171]

The molecular weights of acid glycosaminoglycans have been measured by t.l.c. on Sephadex G-100 and cellulose.[172] A rapid electrophoretic assay for

[158] J. Koizumi, *Progr. Histochem. and Cytochem.*, 1974, **6**, 1.
[159] L. Landmann and W. Villiger, *Experientia*, 1975, **31**, 967.
[160] P. Koskela and S. Pasanen, *Comp. Biochem. Physiol.*, 1975, **50A**, 723.
[161] K. Satyanarayana, V. R. Selvarajan, V. Chanrasekharam, R. Ramamurthi, and K. S. Swami, *Current Sci.*, 1975, **44**, 127.
[162] C. W. Seiter and G. K. Summer, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 945.
[163] H. Greiling, in ref. 115, p. 944.
[164] B. Casu and U. Gennaro, *Carbohydrate Res.*, 1975, **39**, 168.
[165] V. Vilter, C. Da Lage, and F. Gaudin-Harding, *Compt. rend.*, 1974, **279**, *D*, 815.
[166] P. Niebes and M. H. Schifflers, *Clinica Chim. Acta*, 1975, **62**, 195.
[167] D. J. Newton, J. E. Scott, and P. Whiteman, *Analyt. Biochem.*, 1974, **62**, 268.
[168] S. Inoue and M. Miyawaki, *Analyt. Biochem.*, 1975, **65**, 164.
[169] T. Kinoshita, K. Kanada, and A. Tsuji, *J. Biochem. (Japan)*, 1975, **77**, 399.
[170] A. L. Chen, E. J. Hershgold, and D. E. Wilson, *J. Lab. Clin. Med.*, 1975, **85**, 843.
[171] Y. Hatae and A. Makita, *Analyt. Biochem.*, 1975, **64**, 30.
[172] G. Tortolani and E. Romagnoli, *Analyt. Biochem.*, 1975, **66**, 29.

compounds containing ^{35}S-sulphate has been adapted to measure glycosaminoglycans in skin fibroblasts.[173]

Occurrence, Isolation, and Structure.—Acid glycosaminoglycans in human platelets and leucocytes have been identified by using chondroitinase and *Streptomyces* hyaluronidase.[174] The principal components are the chondroitin sulphates, with smaller amounts of hyaluronic acid, whereas heparan sulphate occurs only in leucocytes, possibly as a membrane component. Normal human kidney contains mainly hyaluronic acid and dermatan sulphate, with smaller proportions of chondroitin 4- and 6-sulphates and an over-sulphated chondroitin sulphate.[175] A family of high-molecular-weight chondroitin sulphates possessing different degrees of sulphation has been identified in the nuclei of cultured mouse melanoma cells; a minor component appears to be heparan sulphate.[176]

A magnesium chloride extract of bovine Achilles tendon has been fractionated into three components by means of affinity chromatography on concanavalin A-Sepharose and Sephadex G-200.[177] One of the components was characterized as a proteodermatan sulphate of low molecular weight and high protein content (45—50%). A new procedure for fractionating glycosaminoglycans is based on counter-current distribution using an aqueous, polymer two-phase system.[178] Pevikon (a copolymer of vinyl chloride and vinyl acetate) has been used in a preparative electrophoretic method for the fractionation of hyaluronic acid, heparan sulphate, dermatan sulphate, and chondroitin sulphate.[179] A method has been reported for the quantitative recovery of glycosaminoglycans from cellulose-acetate membranes after removal of the stain used to detect them.[180] Complexes of glycosaminoglycans and hexadecylpyridinium chloride were fractionated into sulphate-free and mono- and poly-sulphated materials on partitioning between water and butanol.[181] A procedure designed to minimize any degradation has been used to isolate the proteoglycans of bovine corneal stroma; extraction with 4M-guanidinium chloride was followed by chromatography on DEAE-cellulose and precipitation with ethanol.[182] Conditions for the cleavage of keratan sulphate with alkaline borohydride have been studied in an effort to reduce the degradation of 2-acetamido-2-deoxy-D-galactosyl residues linked to the peptide chain.[183]

A method for preparing tritium-labelled oligosaccharides of hyaluronic acid has used the Wilzbach recoil technique; the tritiated oligosaccharides are of potential use in studies of the mechanism of hyaluronidases.[184] A four-fold helical form of hyaluronic acid has been detected using the data from fibre-

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[178] D. G. Pritchard, R. M. Halpern, J. A. Halpern, B. C. Halpern, and R. A. Smith, *Biochim. Biophys. Acta*, 1975, **404**, 289.
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[181] G. C. Jennings and R. E. Hurst, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1208.
[182] I. Axelsson and D. Heinegård, *Biochem. J.*, 1975, **145**, 491.
[183] F. J. Kieras, *Carbohydrate Res.*, 1975, **41**, 339.
[184] S. Highsmith and D. M. Chipman, *Analyt. Biochem.*, 1974, **61**, 557.

diffraction experiments.[185] Other *X*-ray studies on an extended conformation of sodium and calcium hyaluronates, having three residues per turn, showed that the chains pack in a number of two-dimensional arrays.[186] The hexad distribution of the chains might be maintained by a co-operative interaction between the three-fold hyaluronate chains and a network of water molecules, which are hydrogen-bonded to one another and to the hyaluronate chains. Purified samples of hyaluronic acid from human umbilical cord were found to contain minor proportions of fucose, arabinose, and glucose; silicon was also detected.[187] The minor constituents may be involved in cross-linkages. A study of the intrinsic viscosities of hyaluronic acids of low (1—7.2 × 10^4) and high (3.1—15 × 10^5) molecular weights has shown that the rheological behaviour differs for molecules of molecular weight $<2.1 \times 10^4$ and $>5.7 \times 10^4$.[188]

Analysis of the amino-acid sequence in the linkage region of chondroitin sulphate from bovine tracheal cartilage has indicated that the smallest sequon necessary for glycosylation is -Ser-Gly-.[189] Comparison of the Fourier transform ^{13}C n.m.r. spectra of the proteoglycan subunit and complex of bovine nasal cartilage and of the intact cartilage with that of chondroitin 4-sulphate has indicated that neither the carbohydrate–protein linkage nor the association of proteoglycan with collagen significantly alters the structure and motion of the polysaccharide chains.[190]

The distribution of the antigenic determinants in the products obtained following hydrolysis of bovine nasal proteoglycan with chondroitinase and trypsin has been determined.[191] An examination of various layers of calf knee-joint cartilage has shown that, although the relative proportions of chondroitin 4- and 6-sulphates and the ratios of hexosamine, uronic acid, and sulphate do not alter, the average molecular weight of the chondroitin sulphates increases as the distance from the articular surface increases.[192] The glycosaminoglycans in different layers of bovine articular cartilage from calves, heifers, and cows have been examined.[192a] Chondroitin 4-sulphate is the principal glycosaminoglycan in all layers of the cartilages of calves and heifers, whereas chondroitin 6-sulphate predominates in cows. Increased concentrations of glycosaminoglycans were found as the distance from the articular surface increases; the increase in calves and heifers is due to chondroitin 4-sulphate, whereas it is due to keratan sulphate in cows.

A chondroitinase from *Flavobacterium heparinum* acted on dermatan sulphate to yield an oligosaccharide that was susceptible to the action of chondroitinase AC, thus providing further evidence that dermatan sulphate is a hybrid structure containing both D-glucuronic and L-iduronic acids, and that disaccharides containing D-glucuronic acid are confined to a specific region of the molecule.[193]

[185] J. M. Guss, D. W. L. Hukins, P. J. C. Smith, W. Twinter, S. Arnott, R. Moorhouse, and D. A. Rees, *J. Mol. Biol.*, 1975, **95**, 359.
[186] J. K. Sheehan, E. D. T. Atkins, and I. A. Nieduszynski, *J. Mol. Biol.*, 1975, **91**, 153.
[187] R. Varma, R. S. Varma, W. S. Allen, and A. H. Wardi, *Biochim. Biophys. Acta*, 1975, **399**, 139.
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[189] M. Isemura and T. Ikenaka, *Biochim. Biophys. Acta*, 1975, **404**, 11.
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[192] I. L. Jones and R. Lemperg, *Biochim. Biophys. Acta*, 1975, **392**, 310.
[192a] R. Lemperg, S. E. Larsson, and S. O. Hjertquist, *Calcified Tissue Res.*, 1974, **15**, 237.
[193] Y. M. Michelacci and C. P. Dietrich, *Biochem. J.*, 1975, **151**, 121.

A copolymeric structure has been proposed for a proteoglycan complex of dermatan sulphate and chondroitin 4- and 6-sulphates occurring in bovine aorta.[194] Digestion of the chondroitin sulphate from bovine tracheal cartilage with trypsin and chymotrypsin yielded peptide fragments containing from one to eight polysaccharide chains.[195] All the fragments contained keratan sulphate (3—11%), indicating that either about half of the keratan sulphate chains in the original proteoglycan are located close to the chondroitin sulphate chains or some of the peptides contain a large number of keratan sulphate chains.

The heparin extracted from bovine-liver capsule with 4M-guanidinium chloride has a molecular weight of 8.8×10^4.[196] Since only 15% of the polysaccharide chains are linked to protein and the method of extraction does not involve extensive degradation, the absence of a linkage region may result from *in vivo* modification, possibly by an *endo*-glucuronidase. It was suggested that heparin is synthesized as a glycoprotein, which is converted into the biologically active molecule by removal of the protein. A trimethylsilylated derivative of heparin has been reported.[197] The total degradation of heparin by the combined actions of heparinase and heparinitase from *Flavobacterium heparinum* has been reported.[198] Analysis of the released di- and tetra-saccharides suggested that di- and tri-sulphated residues are linked alternately in the ratio of 3 : 1, respectively. Two sulphated, unsaturated disaccharides have been isolated following treatment of whale heparin with heparinase; they were identified as 2-deoxy-4-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid 2-sulphate)-2-sulphamido-D-glucose and 2-deoxy-4-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid 2-sulphate)-2-sulphamido-D-glucose 6-sulphate.[199] The high yield of the first disaccharide suggested that it is derived from one of the principal repeating units of this heparin.

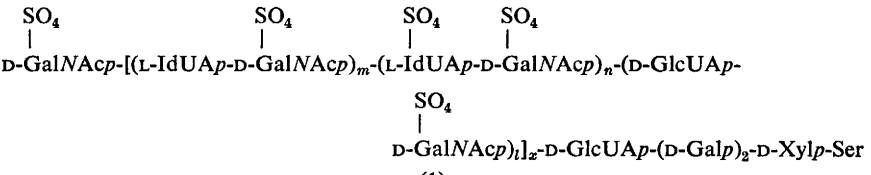
A change has been noted in the content of heparan sulphate present at the surface of transformed mouse cells, whereas that of hyaluronic acid and of chondroitin sulphate remained unaltered.[200] Heparan sulphate fractions from beef lung that contain a wide range of sulphate contents have been degraded with heparinase and with nitrous acid.[201] The polysaccharide appears to be composed of uninterrupted blocks of disaccharides containing 2-acetamido-2-deoxy-D-glucose, large blocks (mol. wt. 5000—6000) that include the *N*-acetyl block but do not contain heparinase-sensitive linkages, and segments where *N*-acetylated, *N*-sulphated, and some disulphated residues alternate in the chain. Five disaccharides, all containing a 4,5-unsaturated uronic acid residue, have been isolated after degradation of heparan sulphate by heparinase and heparitinase.[202] They were identified as a trisulphated disaccharide that appears to be identical to a disaccharide repeating unit of heparin, a disulphated disaccharide – that seems unique for heparan sulphate – containing 2-deoxy-2-sulphamido-D-

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glucose and sulphated uronic acid residues, a non-sulphated disaccharide containing a 2-acetamido-2-deoxy-D-glucose residue, and monosulphated disaccharides containing either a sulphated 2-acetamido-2-deoxy-D-glucose residue or a 2-deoxy-2-sulphamido-D-glucose residue. ^{1}H N.m.r. spectroscopy of heparan sulphate from beef-lung tissue indicated a high degree of heterogeneity.[203]

N-Deacetylation occurred when keratan sulphate was treated with dilute sodium hydroxide, but the glycosylamine linkage of corneal proteoglycan was shown to be stable to 1.5M-sodium hydroxide in the presence of sodium borohydride at 80—83 °C.[204]

The repeating unit of dermatan sulphate from larvae of the sea urchin (*Pseudocentrotus depressus*) has been identified as 2-acetamido-2-deoxy-3-*O*-(β-L-iodopyranosyluronic acid)-D-galactose 4,6-disulphate.[205] A variety of chemical and enzymic procedures has shown that each chain of pig-skin dermatan sulphate contains five or six disaccharide units having the structure L-IdUA*p*(SO$_4$)-D-Gal*NAcp*.[206] Among monosulphated disaccharides isolated were L-IdUA*p*-D-Gal*NAcp*(SO$_4$), L-IdUA*p*(SO$_4$)-D-Gal*NAcp*, and D-GlcUA*p*-D-Gal*NAcp*(SO$_4$). A general structure (1) for pig-skin dermatan sulphate was proposed.



(1)

2-Acetamido-2-deoxy-3-*O*-D-galactopyranosyl-D-galactitol has been identified following treatment of the linkage region of keratan sulphate from bovine nasal septum with alkaline borohydride.[207] The isolation of a relatively large amount of galactitol and 2-acetamido-2-deoxy-D-galactitol suggested that monomeric side-chains are also present. An *endo*-galactosidase from a *Pseudomonas* sp. has been purified and used to examine differences between the keratan sulphates of human nucleus pulposus and shark cartilage.[208]

The protein moieties of porcine and bovine cartilage proteoglycans have been examined to see whether or not the polypeptide core is homogeneous.[209] Larger proteoglycans appear to consist of an invariant region containing the binding site for hyaluronic acid and a variable region to which other glycosaminoglycans are attached. Most complex proteoglycans are able to aggregate and to interact with hyaluronic acid, whereas a few proteoglycans of simple structure do not.

Examination of the proteoglycans of guinea-pig and bovine hyaline cartilages by electron microscopy has supported the view that they consist of a central protein core to which are attached clusters of oligosaccharide side-chains.[210]

[203] C. P. Dietrich, H. B. Nader, and A. S. Perlin, *Carbohydrate Res.*, 1975, **41**, 334.
[204] S. Hirano, Y. Akimoto, and M. Ishigami, *Carbohydrate Res.*, 1975, **41**, 323.
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[207] F. J. Kieras, *J. Biol. Chem.*, 1974, **249**, 7506.
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[209] E. Baxter and H. Muir, *Biochem. J.*, 1975, **149**, 657.
[210] J. Thyberg, S. Lohmander, and D. Heinegård, *Biochem. J.*, 1975, **151**, 157.

Electron microscopy of proteoglycans stained with cytochrome *c* has been used to investigate the molecular architecture of aggregates isolated from bovine articular cartilage.[211] The proteoglycan aggregate consists of proteoglycan subunits arising laterally at fairly regular intervals (20—30 nm) from opposite sides of an elongated, filamentous structure; the stoicheiometry of the binding of subunits to the filament is close to that reported for the binding of hyaluronic acid.

Variations in the aggregation capacity, composition, and molecular weight of the proteoglycans of guinea-pig costal cartilage have been examined.[212] The amounts of proteoglycan selectively extracted from bovine articular cartilage with 3M-magnesium chloride or 3M-guanidinium hydrochloride have been compared.[213] Large amounts of a proteoglycan–collagen complex occur in joints, where the direction of stress on the cartilage is constantly changing. The proteoglycans of human intervertebral discs have been shown to be more polydisperse than those in bovine nasal cartilage; in contrast to that in the cartilage, most of the hexuronate of the discs could be extracted at low shear with either water or dilute salt solutions.[214] Reports describing the comparative biochemistry of invertebrate glycosaminoglycans have appeared.[215–217]

An initial study of the charge distributions and torsional potentials and a steric energy map for hyaluronic acid, chondroitin 4- and 6-sulphates, dermatan sulphate, and keratan sulphate have been reported.[217a]

Biosynthesis.—In studies of the biosynthesis of heparin, methods have been developed that enable the *N*- and *O*-sulphotransferase activities in an enzyme preparation from mouse mastocytoma to be determined separately.[218] The sulphation of heparin has been shown to be a rapid process involving a limited number of polysaccharide chains at any one time.[219] The biosynthesis of heparin may involve a pathway whereby the formation of an *N*-sulphated intermediate preceeds the introduction of *O*-sulphate groups.

A particulate, cell-free system from rat fibrosarcoma has been used to investigate the biosynthesis of hyaluronic acid.[220] The transfer of D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose from UDP-nucleotides proceeded at a rate of 60 nmol h^{-1} mg^{-1} of protein. The activity of this process was optimal at pH 7—7.5, with very little activity at pH 8, and magnesium ions were required for maximum activity. Myxoma cells growing in tissue culture have been used to study the biosynthesis of glycosaminoglycans.[221] The biosynthesis of hyaluronic acid in sheep and rabbit articular cartilages has been investigated using [1-^{14}C]acetate; the rate of turnover of hyaluronic acid appears to be less than that of chondroitin sulphate.[222]

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It has been shown that inversion of the configuration at C-5 of D-glucuronic acid, to give L-iduronic acid, occurs at the polymer level in the biosynthesis of dermatan sulphate and is promoted by the presence of adenosine phosphophosphoryl sulphate.[223] Inversion of the configuration at isolated sites can take place in the absence of sulphated hexosamines, but the formation of repeating blocks containing L-iduronic acid residues is associated with the sulphation of adjacent hexosamine residues at O-4.

The rate of incorporation of [^{3}H]acetate into the chondroitin sulphates synthesized in whole tibias and femurs of chicken embryos was stimulated by D-xylose, β-D-xylosides, and β-D-galactosides, which appear to act as artificial initiators.[224] The chain-initiating enzyme, *viz.* UDP-D-xylose:core protein β-D-xylosyltransferase, has been purified from chicken cartilage and shown to be a glycoprotein (approximate mol. wt. 9.5×10^4) consisting of two pairs of dissimilar subunits.[225] The transferase from rat chondrosarcoma has been purified and compared with that from chicken cartilage.[226] The molecular weights and subunit patterns appear to be similar, but the interactions with D-galactosyltransferase are different. Comparisons of the activities and the subcellular distributions of the transferases involved in the biosynthesis of chondroitin sulphate in normal cells and in rat chondrosarcoma cells showed that the level of UDP-D-xylose:core protein β-D-xylosyltransferase is elevated in the chondrosarcoma.[227] Solubilization of embryonic chicken-cartilage transferases has been achieved using a non-ionic detergent (Nonidet P-40) in a solution of 0.5M-potassium chloride.[228] The transferases in the brains of 13-day-old chicken embryos appear to be distributed mainly in the synaptosomes as membrane-bound forms.[229] An increased rate of synthesis of chondroitin sulphate with increasing age has been reported from an investigation of the incorporation of [^{35}S]sulphate into bovine articular cartilage.[230] Although the absolute amount of chondroitin sulphate falls with age, there is an increase in the levels of keratan sulphate. The addition of serum to stationary chicken-embryo fibroblasts stimulated the synthesis of glycosaminoglycans by 300%, indicating that serum controls the biosynthetic processes *in vitro*.[231] Direct evidence has been obtained that the Golgi apparatus is the primary site of biosynthesis of chondroitin sulphate in cultured mast cells.[232] Trimethylcolchicinic acid (4 μg ml^{-1}) stimulated the production of the chondroitin sulphates in tissue-cultured L-cells, whereas it inhibited the synthesis of dermatan sulphate and had no effect on the synthesis of hyaluronic acid.[233] Cultures of chicken-embryo chondrocytes were able to utilize 4-methylumbelliferyl

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β-D-xylopyranoside as an acceptor, producing a fluorogenic chondroitin sulphate.[234] The synthesis of glycosaminoglycans in normal and transformed cells was also stimulated by the addition of a number of β-D-xylopyranosides.[235]

Somatomedins, which stimulate the incorporation of sulphate into glycosaminoglycans, are thought to be produced peripherally in response to growth hormones.[236] The hormonal response could be simulated in hypophysectomized rats by the *in vitro* addition of 3,3′,5-tri-iodo-L-thyronine at physiological concentrations. Somatotrophin appears to have little direct effect on costal cartilage.[237] The rates of turnover of sulphated glycosaminoglycans were found to decrease in three related lines of mouse cells transformed with Simian virus and having different degrees of density-dependent inhibition.[238] Amyloid fibrils have been shown to stimulate the synthesis of glycosaminoglycans in normal fibroblasts.[239] L-Ascorbic acid 2-sulphate is considered not to be involved in the sulphation of glycosaminoglycans.[240] In cultures of fibroblast-like cells derived from bovine aorta, the synthesized glycosaminoglycans were shown to be distributed between extracellular and pericellular pools, and to possess different metabolic characteristics.[241] The subcellular localization of xylosyltransferases in epiphysial plate cartilage has been investigated.[242]

Degradation.—The mixture of hyaluronidase, β-glucuronidase, and β-acetamidodeoxyglucosidase present in synovial fluids from rheumatoid knee joints may be responsible for degradation of the glycosaminoglycans.[243] These *exo*-polysaccharidase activities were shown to be appreciably elevated in rheumatoid synovial fluids in comparison with those in osteoarthritic synovial fluids and normal sera. Explants of the articular cartilage of growing pigs have been grown in culture; the extent of degradation of glycosaminoglycans depended on whether or not the explants were cut so as to include invading bone marrow.[244] As much as three-quarters of the proteoglycans were lost in media containing retinol, whether bone marrow was present or not. Purified preparations of heparinase and heparitinase from *Flavobacterium heparinum* have been used to show that heparan sulphate on the surface of cultured endothelial cells is susceptible to enzymic hydrolysis.[245] The turnover of sulphated glycosaminoglycans in rat aortic wall has been examined.[246] Specificities have been demonstrated for the

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pinocytosis of various proteoglycans in cultured arterial fibroblasts.[247] The half-lives of sulphated glycosaminoglycans in rat-kidney cortex, medulla, and papilla are comparable to those in other organs and tissues, but hyaluronate has a somewhat faster turnover.[248]

The onset of mineralization in the cartilages of guinea pigs and Beagle puppies was found to be associated with considerable losses of proteoglycan.[249] Increases in the mineral content of the tissues led to increases in the ratio of protein to polysaccharide, of chondroitin sulphate to keratan sulphate, and of chondroitin 4-sulphate to chondroitin 6-sulphate.

Functions.—It has been suggested that the high amounts of hyaluronic acid present in very young rat brain may be responsible for the high content of water, and that the hydrated complex acts as a matrix through which neurons migrate during early development.[250] The sorptive and retentive properties of three corneal proteoglycans containing different proportions of keratan sulphate and chondroitin 4-sulphate have been investigated.[251] If sufficient chondroitin 4-sulphate is present, the proteoglycan can form a tight network that possesses limited uptake, but high retention, of water. On the other hand, when chondroitin 4-sulphate is a minor component, keratan sulphate creates an open network possessing a large swelling capacity, but with little retentive power.

Thrombin probably undergoes a change of conformation when it binds heparin, thereby facilitating the formation of a complex between thrombin and antithrombin.[252] The interaction of heparin and plasminogen has been studied using Sepharose-lysine and -cadaverine.[253] The anticoagulant activity of heparin has been found to be affected by its molecular weight, and by the content and distribution of sulphate groups.[254] Whereas sequences of single, repeating 2-acetamido-2-deoxy-D-glucosyl residues separated by adjacent residues of 2-sulphamido-2-deoxy-D-glucose do not affect the anticoagulant activity of heparin, the presence of multiple 2-acetamido-2-deoxy-D-glucosyl residues in sequence decreases the biological activity. The L-iduronic acid content does not appear to influence the biological activity of heparin, but this assessment did not take into account the possible role and distribution of sulphated L-iduronic acid residues. Heparin interacted specifically with the factors initiating protein synthesis in cell-free extracts of rabbit reticulocytes.[255] The rate of secretion of aldosterone was inhibited by heparin and by a heparinoid (*N*-formylchitosan polysulphate).[256]

Interactions between human-plasma glycosaminoglycans and low-density lipoproteins are considered to be important in maintaining the rheological

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properties of plasma lipoproteins.[257] The absence of dermatan sulphate and heparan sulphate in the complexes formed between glycosaminoglycans and lipoproteins of low and very low density in the tissues of human atherosclerotic aortas contrasts with the occurrence of such complexes in atherosclerotic lesions.[258] Heparin has been coupled to 1,4-diaminobutane-Sepharose 4B, and the immobilized form was used to study the interactions of heparin with human-serum lipoproteins in the presence of calcium, magnesium, and manganese ions.[259] Concanavalin A–heparin complexes have been employed in similar studies.[260]

The copper-catalysed, thermal aggregation of human γ-globulin was augmented by hyaluronic acid.[261]

The ability of certain polymers to diffuse through solutions of hyaluronate suggested that asymmetric or coiled molecules preferentially penetrate the network.[262]

Glycosaminoglycans have been suggested to modify lysosomal functions by the formation of complexes with lysosomal enzymes.[263] Collagen from rat-tail tendon on attachment to Sepharose 4B was able to bind the proteoglycan subunits of bovine nasal cartilage; the interaction appears to depend on the subunit structure of the protein core.[264] Mouse-bone collagenase was bound tightly by a heparin–agarose complex at low ionic strength, although it was eluted at high ionic strength.[265] This system could be used as a model to study the enhancement of collagenase activity by heparin. The ability of salts of hyaluronic acid to transduce pressures into electrical potentials has been discussed.[266] Further work with Kurloff-cell proteoglycans has indicated that they stimulate the synthesis of proteins in macrophages.[267] Fibrillation was enhanced when connective-tissue proteoglycan interacted with α-elastin.[268] A proteoglycan from bovine nasal cartilage has been shown to bind to type I collagen, the highest affinity being for the α_2- and β_{12}-components.[269]

Pathology.—The enzymic deficiencies, corrective factors, and clinical correlations of the mucopolysaccharidoses have been reviewed.[270] The recognition of mucopolysaccharidoses using four screening tests, including a refinement of the albumin turbidity test, and the differentiation of mucopolysaccharidoses by electrophoretic separation of urinary glycosaminoglycans have been reported.[271]

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The electrophoretic forms of human-liver α-L-fucosidase and their relation to fucosidosis (mucopolysaccharidosis F) have been examined.[272] One patient had 4% of normal liver fucosidase activity with all the electrophoretic forms deficient, but with K_m and pH optimum indistinguishable from normal values. Characterization of the mucopolysaccharidoses has been reported in a study of the urinary glycosaminoglycans from 46 patients.[273] The analysis was based on the ratios of 2-amino-2-deoxy-D-glucose to 2-amino-2-deoxy-D-galactose, in conjunction with molecular-weight profiles obtained by chromatography on Sephadex-G200. Urinary heparan sulphates from normal individuals have been compared with those from patients with either Hunter's or Sanfillipo syndrome.[274] Whereas normal heparan sulphate is polydisperse, that from the patients is monodisperse and has a lower molecular weight. Heparan sulphate from the urine of patients with Hurler's syndrome appears to be composed of two populations; the molecular size of one population corresponds to that of heparan sulphate in normal urine, whereas the molecular size of the other is characteristic of Hunter's syndrome. Skin grafts transplanted on a 4-year-old boy with Hunter's syndrome produced increases in the uronic acids and the relative proportions of oligosaccharides of low molecular weight excreted, suggesting that sufficient corrective factor had been released to induce these changes.[275] Increased desulphation of low-molecular-weight glycosaminoglycans also supported this view. A deficiency of 2-acetamido-2-deoxy-D-galactose-4-sulphatase has been shown to be responsible for Maroteaux–Lamy disease (mucopolysaccharidosis VI) subtype A.[276] Patients with Kniest's disease have been shown to possess anomalous proteoglycans in the growth cartilage.[277] The hyaluronic acid content in the urine of patients with Werner's syndrome was found to be higher than normal.[278] Morquio's syndrome has been shown to result from a deficiency of chondroitin sulphate:2-acetamido-2-deoxyhexose sulphate sulphatase.[279]

Artificially induced obesity in mice produced a reduction in the total content of glycosaminoglycans over a 4-month period; there was a marked reduction in the level of hyaluronic acid, whereas that of chondroitin sulphate increased.[280] The principal glycosaminoglycan isolated from the urine of a patient with epidermolysis bullosa dystrophica et albo-papuloidea (Pasini) has been identified as partly degraded dermatan sulphate. Moreover, the amounts of partly degraded chondroitin 4- and 6-sulphates, the principal glycosaminoglycans in normal urine, are lower than average in this disease.[281] Fractions containing glycoproteins and/or glycosaminoglycans have been separated from capsular and

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caseous tissues of patients with human pulmonary tuberculosis.[282] The amounts of these materials are greater than those in normal tissues, and there is a marked increase in the sialoglycopeptide fraction.

Mammalian Cell and Tissue Glycoproteins

References to macromolecules present in cell surfaces have been grouped together in a separate section (see p. 303).

Slight variations have been detected in the heterogeneity of sialoglycopeptides from sclerotic and non-sclerotic tissues of the intima media of human aorta.[283] Epithelial cells from the colons of humans and rats contain glycoproteins composed, *inter alia*, of *O*-acetylated sialic acid residues.[284] Human gastric mucosa has been shown to contain a higher content of sialic acid, and a lower content of total hexose, than jejunal mucosa; no chemical differences were apparent between gastric biopsy specimens from patients with or without peptic ulcers.[285] Human secretory component has been purified from colostrum; it was shown to contain carbohydrate (18.6%) composed of L-fucose (2.1%), D-mannose (3.6%), D-galactose (4.1%), 2-acetamido-2-deoxy-D-glucose (4.8%), and *N*-acetylneuraminic acid (4.0%).[286] Eight of the first ten residues of the *N*-terminal portion were sequenced, but no homology was found with immunoglobulin light- or heavy-chains. The chemical composition of meconium has been investigated.[287] It possesses a similar distribution of blood-group activities to that found in adults, but the blood-group activity is specific to the infant. Meconium can be separated into water-soluble and -insoluble fractions; chromatography of the water-soluble fraction on Sepharose 2B separated the H activity from the A and B activities.

The carbohydrate moiety of horse-spleen apoferritin is composed of hexose (25), hexosamine (3), and L-fucose (10 residues per molecule); this ratio did not vary for iron-poor or -rich ferritins.[288] Nine sialoglycopeptides prepared from intima media of bovine aorta exhibited heterogeneity in both the carbohydrate and peptide moieties; all contain D-galactose, D-glucose, D-mannose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, L-fucose, and sialic acid, although one of them did not contain 2-amino-2-deoxy-D-galactose.[289] Dopamine β-hydroxylase present in bovine adrenal medulla has been shown to be a glycoprotein containing sialic acid.[290] The properties of two insoluble, non-collagenous glycoproteins from puppy-rib cartilage have been examined.[291] Both of them contain high proportions of aspartic and glutamic acids, but only a low proportion of hydroxyproline, and hexose, uronic acid, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and some sialic acid. Electron microscopy suggested that these glycoproteins may be associated with collagen fibres. The

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glycosaminoglycans and glycoproteins of rabbit femur have been examined.[292] The lysosomal enzymes (acid phosphatases, aryl sulphatases, β-glucuronidases, and β-acetamidodeoxyhexosidase) from rat kidney have been separated by isoelectric focusing and shown to be glycoproteins containing sialic acid.[293] The principal neuraminidase activity present in rat liver has been located in the mitochondria and in the lysosomes.[294] A new neuraminidase that hydrolysed sialylated trisaccharides, but not glycoproteins or gangliosides, was detected in the Golgi apparatus. Fractionation of the glycoproteins present in rat-liver mitochondria has shown that they differ from those present in hepatic microsomes.[295]

The functions, distribution, metabolism, physiology, and pathology of brain glycolipids and glycoproteins have been reviewed.[296] It has been found that, during early post-natal development (2—15 days), the size of the oligosaccharide chains of brain glycoproteins rapidly increases.[297] The possibility of purifying brain glycoproteins by means of affinity chromatography on immobilized concanavalin A has been investigated.[298] A group of highly soluble glycoproteins has been extracted from normal human brain and liver and from brain tumours, and their electrophoretic and chromatographic properties were compared.[299]

A glycoprotein from rat brain has been purified by affinity chromatography on concanavalin A-Sepharose, and was shown to contain D-mannose (6) and 2-acetamido-2-deoxy-D-glucose (2 residues).[300] The distribution of glycoproteins and glycosaminoglycans in whole rat-brain tissue has been examined.[301] Approximately 90% of the glycoproteins occurs in the particulate fraction of the homogenates, with high amounts in synaptic and microsomal membranes; treatment of synaptic membranes with Triton X-100 solubilized 70% of the glycoproteins. In other studies, the subcellular and anatomical distributions of D-mannose- and sialic-acid-rich glycoproteins in rat brain have been reported, and their levels in white and grey matter, myelin, synaptosomes, and glial tissues have been compared.[302] Tritiated L-fucose, but neither D-galactose nor D-glucose, was incorporated into neural-like components (mol. wt. 1×10^4) in rat brain, which appear to be intragranular components distinct from neurophysin.[303] Tubulin, the subunit protein of microtubules, was labelled after incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose; it is probably a glycoprotein.[304]

Glycopeptide fragments, that are not detected in normal cells, have been isolated from cultured skin fibroblasts of patients with one of four lysosomal

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[298] E. G. Brunngraber, J. P. Susz, J. Javaid, A. Aro, and K. Warecka, *J. Neurochem.*, 1975, **24**, 805.
[299] K. G. Haglid, L. Rönnbäck, and D. Stavrou, *J. Neurochem.*, 1975, **24**, 1053.
[300] J. I. Javaid, H. Hof, and E. G. Brunngraber, *Biochim. Biophys. Acta*, 1975, **404**, 74.
[301] R. K. Margolis, R. U. Margolis. C. Preti, and D. Lai, *Biochemistry*, 1975, **14**, 4797.
[302] E. G. Brunngraber and J. I. Javaid, *Biochim. Biophys. Acta*, 1975, **404**, 67.
[303] C. W. Jones and R. W. Swann, *J. Endocrinol.*, 1974, **63**, 53P.
[304] H. Feit and M. L. Shelanski, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 920.

disorders.[305] The fragment from patients with type I G_{M1}-gangliosidosis contains D-galactose, D-mannose, and 2-acetamido-2-deoxy-D-glucose in the ratio of 1.8 : 2.8 : 4.0; those from patients with type II G_{M2}-gangliosidosis or mannosidosis contain D-mannose and 2-acetamido-2-deoxy-D-glucose in ratios of 2.7 : 4.0 and 3.4 : 1.0, respectively, and that in fucosidosis contains L-fucose and 2-acetamido-2-deoxy-D-glucose in equimolar ratio. The relative homogeneity of the storage material confirmed that the same oligosaccharide unit is common to most of the glycoproteins synthesized by the cell. The action of an *endo*-galactosidase on a glycopeptide isolated from the liver of a patient with G_{M1}-gangliosidosis liberated a trisaccharide containing the sequence D-galactose → 2-acetamido-2-deoxy-D-glucose → D-galactose.[306] This and related studies established the structure (2) for part of this glycopeptide, which probably represents

$$\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-}[\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Gal}p]_2\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}N\text{Ac}p\text{-Thr}$$

(2)

the desulphated linkage region of skeletal keratan sulphate. Other glycopeptides and oligosaccharides from the liver of a patient with type I G_{M1}-gangliosidosis have been characterized.[307] A marked elevation in the levels of brain glycoproteins has been reported in two cases of infantile gangliosidosis.[308] Sulphated glycoproteins having blood-group H activity have been isolated from the sputum of a child with cystic fibrosis.[309] Treatment with alkaline borohydride revealed that the carbohydrate moieties are heterogeneous with respect to charge and size. Neutral glycoprotein chains and incomplete chains, containing only one residue of 2-acetamido-2-deoxy-D-galactose, possessed H activity. The minimum size of chains containing neuraminic acid was shown to be less than that of sulphated chains, the size of which increases with the degree of sulphation. The sulphate groups are attached at O-6 of D-galactosyl residues.

Immunological techniques have demonstrated that carcinoembryonic antigen which had been partially degraded in both protein and carbohydrate moieties did not contain the tumour-associated antigen.[310] The antigen (mol. wt. 1.8×10^5) has been studied by electron microscopy. Treatment of carcinoembryonic antigen with neuraminidase, followed by reduction and alkylation, afforded a relatively compact random coil, whereas a less compact structure resulted when the treatment with neuraminidase was omitted.[311] Oxidation of carcinoembryonic antigen with periodate revealed that 2-acetamido-2-deoxy-D-glucose is linked to asparagine, and that L-fucose and *N*-acetylneuraminic acid are present at the non-reducing termini; the latter residue is attached to C-3 of D-galactose, which, together with D-mannose, occupy internal positions in the

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chain.[312] The molecular biology and clinical significance of carcinoembryonic antigen have been reviewed.[313]

A number of Herpes simplex virus-induced antigens of BHK cells have been identified as glycoproteins by virtue of their binding to insolubilized concanavalin A and subsequent elution with methyl α-D-mannopyranoside.[314] The immunology and biochemistry of spontaneously metastasizing and non-metastasizing rat mammary carcinomas and of the plasma membranes thereof have been examined.[315] The surface of highly immunogenic, non-metastasizing cells is covered with an acid glycoprotein, that cannot be removed entirely by neuraminidase, whereas that of non-immunogenic, spontaneously metastasizing cells is not. Low- and high-molecular-weight glycopeptides and heparan sulphate have been isolated from the plasma membrane of an ascites hepatoma AH66.[316] Homologies between the structures of the oligomannosyl cores of cellular and non-membrane glycopeptides have been established in SV40-transformed rat fibroblasts.[317] Three glycoproteins in the fluid of Ehrlich ascites tumours in mice have been purified by discontinuous gel electrophoresis; they were shown to be rich in glutamic and aspartic acids and to contain D-galactose, D-mannose, L-fucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid, which vary from 17.7 to 37.3% of the total weight.[318] Chemical and immunological differences have been established between the mucoprotein antigens of normal and tumour colons.[319] Less threonine, proline, valine, and isoleucine were found in the tumour mucoprotein.

Cell-surface Glycoproteins

The nature, location, and functions of complex carbohydrates occurring at the surface of mammalian cells have been reviewed.[320] In particular, the functions of complex carbohydrates as receptors and in the recognition of asialylated glycoproteins were discussed. Two volumes of 'Methods in Enzymology' have dealt with techniques for preparing and analysing membranes and the components thereof.[321, 322] The purification of wheat-germ agglutinin[39] and assays for agglutination with lectins have been described.[323] The functions of glycoproteins in cell adhesion and in intracellular processes have been discussed.[324] Correlations

[312] J. E. Coligan and C. W. Todd, *Biochemistry*, 1975, **14**, 805.
[313] A. Fuks, C. Banjo, J. Shuster, O. Freedman, and P. Gold, *Rev. Cancer*, 1975, **2**, 123.
[314] M. Ponce de Leon, H. Hessle, and G. H. Cohen, *J. Virol.*, 1973, **12**, 766.
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[319] D. Gold and F. Miller, *Nature*, 1975, **255**, 85.
[320] R. C. Hughes, *Essays in Biochem.*, 1975, **11**, 1.
[321] 'Methods in Enzymology, Biomembranes A', ed. S. Fleischer and L. Packer, Academic Press, 1974, Vol. 31.
[322] 'Methods in Enzymology, Biomembranes B', ed. S. Fleischer and L. Packer, Academic Press, 1974, Vol. 32.
[323] M. M. Burger, ref. 322, p. 615.
[324] R. B. Kemp, C. W. Lloyd, and G. M. W. Cook, in 'Progress in Surface and Membrane Science', ed. J. F. Danielli, M. D. Rosenberg, and D. A. Cadenhead, Academic Press, New York and London, 1973, Vol. 7, p. 271.

between the binding of lectins, agglutinability, and the transformed state, and also alterations of the cell surface during growth and changes in the cell cycle have been reviewed.[324a]

Purified membranes from human T-mycoplasmas contain 1—7% carbohydrate by weight, consisting of D-mannose, D-galactose, and D-glucose.[325] Rabbit antisera have been prepared towards membrane glycoproteins obtained from cultures of human B-cell lines; the antisera reacted only with Ig-bearing, peripheral blood lymphocytes and completely blocked the reaction with mixed lymphocytes *in vitro*.[326] Investigations of changes in the growth-dependent patterns of L-fucose-containing glycopeptides have been complemented by similar investigation of D-mannose-containing glycopeptides at the surfaces of growing and non-growing human diploid cells (KL-2).[327] A change in the size of D-mannose-containing acid glycopeptides was observed, but neuraminidase failed to arrest this change completely. The accumulation of adenosine cyclic 3′,5′-phosphate in human peripheral blood lymphocytes was stimulated by concanavalin A.[328] The receptor sites for this lectin on the surfaces of specific populations of embryonic cells have been discussed.[329] Direct activation of the D-glucose transport system of fat cells by concanavalin A has been studied by means of a rapid filtration procedure.[330] This and other investigations with neuraminidase suggested that the sialic acid residues of components of fat-cell membranes have important roles in events involved in lectin-mediated agglutination and in activation of the hexose transport system. The role of the liver plasma-membrane galactosyltransferase in cell adhesion has been revised.[331] It is proposed that cells remain attached to one another through complexes formed between transferases and oligosaccharides produced by their action.

In an evaluation of the evidence for the localization of glycosyltransferases within eukaryotic cells, it was concluded that there is no valid basis for the suggestion that the transferases involved in membrane biogenesis are exclusively located on the surface of mammalian cells, so that assignment of roles to the transferases is premature.[332]

A receptor glycopeptide (minimum mol. wt. 1.2×10^4) for concanavalin A has been isolated from calf thymocytes; it contains neutral sugars (10.4%) consisting of D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, D-glucose, and L-fucose, and sialic acid in the ratio 3 : 2 : 2 : 1 : 1 : 1, respectively.[333]

A galactosyltransferase present in the cell surface of rabbit erythrocytes has been purified and shown to be a glycoprotein containing D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, sialic acid, and a trace of L-fucose.[334] Some,

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[326] P. Cresswell and S. S. Geier, *Nature*, 1975, **257**, 147.
[327] C. Ceccarini, T. Muramatsu, J. Stang, and P. H. Atkinson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3139.
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[333] J. Pospišilová, C. Haškovec, G. Entlicher, and J. Kocourek, *Biochim. Biophys. Acta*, 1974, **373**, 444.
[334] D. K. Podolsky and M. M. Weiser, *Biochem. J.*, 1975, **146**, 213.

but not all, of the D-mannosyl residues were removed by α-mannosidase. Non-agglutinable human erythrocytes were agglutinated by concanavalin A following treatment with the galactosyltransferase. Concanavalin A inhibited the uptake of iron, but did not impair that of transferrin, by rabbit reticulocytes.[335] This effect was ascribed to binding of the lectin by cell-surface glycoproteins.

Inhibition of the chemotactic migration of germ cells in chickens by concanavalin A has demonstrated the importance of glycoproteins in this process.[336] Following the discovery of a new plasma-membrane protein in chicken, human, and mouse fibroblasts, a similar glycoproteinaceous antigen has been found in chicken serum.[337] The antigen, which disappears from tumour- or virus-transformed cells, was shown to contain three principal peptides of molecular weights 2.1×10^5, 1.45×10^5, and 4.5×10^4. A rapid procedure for analysis of the cell-surface carbohydrates of chicken lymphocytes has been reported.[338] Cells from thymus, bursa, spleen, and blood were characterized by the relative proportions of D-glucose, 2-amino-2-deoxy-D-galactose, L-fucose, and sialic acid. Since equimolar proportions of D-mannose, 2-amino-2-deoxy-D-glucose, and D-galactose were found for all lymphocyte preparations, the carbohydrate compositions could be used to distinguish between different cell preparations. A cell-surface glycoprotein that constitutes up to 3% of the total cell protein of chicken-embryo fibroblasts was shown to decrease after viral transformation.[339] The agglutinating activity of this glycoprotein is sensitive to proteases and required bivalent cations.

A comparison of the cell-surface glycoproteins from wild-type, Chinese-hamster ovarian cells with those resistant to the lectin from *Phaseolus vulgaris* suggested that they differ in the carbohydrate moiety of the membrane glycoproteins.[340]

Use of concanavalin A and a ferritin-conjugated derivative thereof has revealed a high density of lectin-binding sites on the cell surface of the submandibular glands of rats in the vicinity of cisternae of the rough endoplasmic reticulum; these sites are where newly synthesized membrane glycoproteins are inserted.[341] Glycopeptides and glycosaminoglycans from the plasma membranes of normal and regenerating rat livers have been isolated and characterized.[342] Liver plasma membrane has been shown to be the principal location for binding of desialylated glycoproteins, but the binding ability was diminished by prior treatment of the membranes with phospholipase A or C.[343] However, the sensitivity to phospholipase-induced inhibition of binding was abolished on solubilization of the membrane-associated binding protein. Thus, phospholipids do not appear to constitute an integral part of the membrane–glycoprotein binding complex, so that phospholipase-induced inhibition of binding probably arises from non-specific disruption of lipid constituents of the membrane surface. Concanavalin A

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[339] K. M. Yamada, S. S. Yamada, and I. Pastan, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3158.
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[341] T. Amakawa and T. Barka, *J. Histochem. and Cytochem.*, 1975, **23**, 607.
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[343] J. Lunney and G. Ashwell, *Biochim. Biophys. Acta*, 1974, **367**, 304.

is thought to induce blast transformation of rat lymphocytes by an indirect mechanism.[344] ^{125}I-Labelled membrane glycoproteins that bind concanavalin A have been isolated from the surface of mouse lymphocytes. Six principal components, with apparent molecular weights 1.2×10^5, 7.3×10^4, 4.0×10^4, 3.4×10^4, 2.7×10^4, and 2.3×10^4, were obtained, whereas immunoglobulin IgM constitutes a minor component.[345] In addition to a protein of molecular weight 2.0×10^5, a glycoprotein of molecular weight 3.0×10^4 has been detected on the surface of T-cells.[346] A cell-surface glycoprotein, which is a receptor for concanavalin A, has been isolated from mouse L-cells.[347] This glycoprotein can be purified from plasma membranes of L-cells, and is labelled on the exterior surface of intact cells following treatment of the cells with D-galactose oxidase and sodium borotritide. It migrates on sodium dodecyl sulphate gels as a single component of molecular weight 1×10^5 and contains carbohydrate moieties at five or more sites on the polypeptide chain. The presence of species-specific receptors on the surface of cells of *Dictyostellium discoideum* for the associated lectin (discoidin) has suggested a basis for the species-specific cohesion.[348] Concanavalin A has been used to detect changes on the cell surface during the differentiation of this slime mould.[349]

Binding of concanavalin A to the papilla of *Blastocladiella emersonii* is thought to prevent access of some enzymes in the culture fluid to the zoosporangia.[350]

The use of ^{125}I-labelled wheat-germ agglutinin and other lectins for labelling the surfaces of plasma membranes has been discussed.[351]

Cells contain less hexosamine and sialic acid during myoblast differentiation, but D-glucose and D-galactose accumulate: the increase in these hexoses reflects the presence of components containing D-glucosyl-D-galactosylhydroxylysine on the cell surface as the myoblast reaches confluency.[352]

Tritiated cells of cultured lens epithelium have been used to study the biosynthesis of glycoproteins therein.[353] Most of the labelled proteins were exposed on the cell surface, and were released on digestion with trypsin. A smaller proportion of labelled proteins was removed from intact cells with H_4edta in the absence of trypsin. Relationships were suggested between the biosynthesis of glycoproteins by lens epithelial cells in culture and the dual biological function (capsule synthesis and differentiation into fibre cells) of these cells *in vivo*. An identical glycoprotein (mol. wt. 7.5—9.0 $\times 10^4$) was found in dodecyl sulphate extracts of lens capsules and in H_4edta extracts of epithelial cells. Current work is exploring the possibility that this loosely attached material may be a capsular glycoprotein in the process of being secreted from epithelial cells. The cell-aggregating factor prepared from neural retinal cells of chicken embryos is

[344] C. F. Beyer and W. E. Bowers, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3590.
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[347] R. C. Hunt, C. M. Bullis, and J. C. Brown, *Biochemistry*, 1975, **14**, 109.
[348] R. W. Reitherman, S. D. Rosen, W. A. Frazier, and S. H. Barondes, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3541.
[349] C. Weeks and G. Weeks, *Exp. Cell Res.*, 1975, **92**, 372.
[350] E. M. Lemos and W. R. Lodi, *Exp. Cell Res.*, 1975, **94**, 437.
[351] K.-J. Chang, V. Bennett, and P. Cuatrecasas, *J. Biol. Chem.*, 1975, **250**, 488.
[352] R. Winand and D. Luzzati, *Biochimie*, 1975, **57**, 765.
[353] R. C. Hughes, M. Laurent, M.-O. Lonchampt, and Y. Courtois, *European J. Biochem.*, 1975, **52**, 143.

thought to be a tissue-specific ligand; this glycoprotein (mol. wt. 5.0×10^4) contains carbohydrate (10—15%) composed of 2-acetamido-2-deoxy-D-glucose (10), D-mannose (10), D-galactose (10), and sialic acid (1 or 2 residues per molecule).[354] [^{125}I]Concanavalin A has been used to map the receptor sites on the surface membrane of retina cells of 19-day-old chicken embryos.[355]

The interactions of concanavalin A and *Ricinus communis* agglutinin with purified influenza and vesicular stomatitis viruses and with virus-infected cells have been examined.[356] There appears to be a close correlation between the number of lectin-receptor sites on influenza virus and the number of viral spikes. BHK cells infected with either virus showed an increased agglutinability with concanavalin A within 30—120 min, although the number of binding sites remained constant during infection. The agglutinability of the cells with *R. communis* lectin was also enhanced, but, in this case, the number of binding sites increased six-fold during infection with influenza virus. The insertion of fowl-plague, virus-specific haemagglutinins and neuraminidase glycoproteins into the plasma membranes of infected chicken cells took place without apparent displacement of membrane proteins of the host cell.[357] Synthesis of these polypeptides is closely associated with the rough endoplasmic reticulum, where glycosylation occurs; they are then transferred to the plasma membrane *via* the smooth endoplasmic reticulum. The activity of sialyltransferase on the surfaces of normal and transformed chicken-embryo fibroblasts has been shown to be modulated by the environment (*e.g.* pH, ionic strength, temperature, and cell density).[358] Care must be taken, therefore, in assessing any changes in the activity of this enzyme. Surface galactosyl- and sialyl-transferases in the plasma membrane of SV40-transformed mouse cells were inhibited by 0.5mM-butyryl adenosine cyclic 3′,5′-phosphate, whereas those in the plasma membrane of normal cells were not.[359] Confluently grown BHK cells and cells transformed with polyoma virus have been treated with neuraminidase and then assayed for their agglutinability with lectins and for the quantitative binding of *R. communis* and wheat-germ agglutinins and concanavalin A.[360] It appears that the release of sialic acid exposes D-galactopyranosyl residues, probably β-linked. More of these new receptors were uncovered in neuraminidase-treated transformed cells than in normal cells treated similarly. The appearance of lectin-receptor sites on the surface of transformed ovary cells of hamsters and Chinese hamsters has been examined during the cell cycle.[361]

The induced clustering of membrane components has been explored. Concanavalin-A induced clustering of normal and transformed cells to the same extent, whereas wheat-germ agglutinin induced no clustering.[362] Differences in

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[358] A. C. Spataro, H. R. Morgan, and H. B. Bosmann, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 486.
[359] T. Sudo and K. Onodera, *Exp. Cell Res.*, 1975, **91**, 191.
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[361] J. Garrido, *Exp. Cell Res.*, 1975, **94**, 159.
[362] E. Roos and J. H. M. Temmink, *Exp. Cell Res.*, 1975, **94**, 140.

the lectin-induced agglutination between normal and transformed cells could be ascribed to differences in the clustering of lectin-receptor sites.

Biochemical evidence has provided a link between the G_{Ix} thymocyte-surface antigen and the 69/71 envelope glycoprotein of murine leukaemia virus.[363]

The interaction of lectins with D-galactose-containing glycoproteins present in hamster fibroblasts and polyoma-transformed derivatives thereof has been studied.[364] Association of either *R. communis* lectin or concanavalin A with normal fibroblasts involves a glycoprotein of molecular weight 2×10^5, whereas a glycoprotein of molecular weight 1.3×10^5 is involved in the transformed cell. Labelling of the cell surface by means of galactose oxidase and sodium borotritide was enhanced by low concentrations of lectins.

Following the observation that proteins located on the external surface of human erythrocyte membranes contain carbohydrate, lactoperoxidase-catalysed iodination has been used to label the external surface of human tumour KB cells.[365] Only a relatively small number of protein subunits of the plasma membrane were accessible to enzymic iodination, and most, if not all, of these molecules appear to be glycosylated. A cell-surface glycoprotein containing a D-galactosyl residue at the non-reducing terminus has been isolated from culture fluids of 129/J mouse ascites teratoma cells, and it may be responsible for cell-to-cell adhesion.[366]

Changes in the surface glycopeptides of tumour cells obtained from the peripheral blood of patients with active leukaemia, leukaemic transformations, or lymphosarcoma have been reported.[367] With the exception of cells obtained from patients with chronic myelocytic leukaemia, increases in the levels of neuraminidase-labile sialic acid were found. This difference has been noted in a great many transformed cells.

Tritiated L-fucose has been used to study the mode of assembly of plasma membranes in HeLa cells.[368] Pulse-chase experiments and inhibition of protein synthesis revealed that there is an internal pool of completed fucosylated glycoprotein, which takes not less than 12 min to deplete. Newly synthesized glycoprotein moves from this pool to the plasma membrane with a transit time of 12 min, and little was found in the cell.

The structure of epiglycanin, a glycoprotein component (mol. wt. 5×10^5) of TA3-Ha cancer cells, has been studied by immunochemical and chemical methods.[369] The results suggested the presence of six different carbohydrate chains of one to five residues in length, having as terminal groups *N*-acetylneuraminic acid, D-galactose, and 2-acetamido-2-deoxy-D-galactose. All the chains are attached to a single polypeptide chain by *O*-glycosyl linkages involving a 2-acetamido-2-deoxy-D-galactosyl residue and either a serine or a threonine residue. It was suggested that the native molecule of epiglycanin is composed of more than 500 carbohydrate chains attached to a single polypeptide chain

[363] Y. Obata, H. Ikeda, E. Stockert, and E. A. Boyse, *J. Exp. Med.*, 1975, **141**, 188.
[364] C. G. Gahmberg and S.-I. Hakomori, *J. Biol. Chem.*, 1975, **250**, 2447.
[365] T. D. Butters and R. C. Hughes, *Biochem. J.*, 1975, **150**, 59.
[366] S. B. Oppenheimer, *Exp. Cell Res.*, 1975, **92**, 122.
[367] W. P. Van Beek, L. A. Smets, and P. Emmelot, *Nature*, 1975, **253**, 457.
[368] P. H. Atkinson, *J. Biol. Chem.*, 1975, **250**, 2123.
[369] J. F. Codington, K. B. Linsley, R. W. Jeanloz, T. Irimura, and T. Osawa, *Carbohydrate Res.*, 1975, **40**, 171.

containing about 1300 amino-acid residues. A large, mucin-type surface glycoprotein located on TA3-Ha mammary carcinoma ascites cells of strain A mice acted as a receptor for the lectin from *Vicia graminea*.[370] Release and purification of this glycoprotein showed it to be heterogeneous.

Glycoproteins solubilized from plasma-cell membranes of murine tumours have been shown to contain sialic acid residues that function as antigenic determinants.[371] Differences have been demonstrated in the cell-surface glycoproteins of sarcoma 180 tumour cells grown *in vitro* in cell culture or *in vivo* as an ascites form in mice.[372]

Treatment of the intact walls of Novikoff ascites hepatoma cells with papain released a surface glycopeptide possessing receptor sites for concanavalin A and wheat-germ agglutinin.[373] Further digestion of the isolated receptors with pronase produced sialoglycopeptides with potent and specific binding activity for concanavalin A. Although the carbohydrate contents of glycoprotein and glycolipid components of these cells are lower than those of normal rat cells, important differences in their distribution were noted.[374] A reduction in the carbohydrate content, notably in 2-amino-2-deoxy-D-galactose, L-fucose, and sialic acid, has also been noted in tumour tissues from human colonic adenocarcinoma.[375]

A study of the incorporation of 2-amino-2-deoxy-D-glucose, UDP-2-acetamido-2-deoxy-D-glucose, and CMP-sialic acid into Ehrlich ascites cells suggested that the surface acceptor for CMP-sialic acid added exogenously is not identical to that for the surface sialoglycopeptide synthesized endogenously.[376]

Several laboratories have demonstrated that there is an enrichment of high-molecular-weight glycopeptides in virus-transformed and rapidly growing cells.[377] Such increases are dependent on the rate of growth and are unrelated to the transformation. Thus, differences in size distribution may not be adequate criteria on which to base any evaluation of growth-dependent alterations in cell-surface glycoproteins.

Hormonal Glycoproteins

Reviews of the experimental methods used in hormone research have been published. One volume is concerned with steroid hormones, and includes references to steroid-binding glycoproteins in sera and to cytoplasmic receptors.[378] A second volume deals with the methodology associated with peptide hormones, including procedures for tritium-labelling of sialylated glycoproteins.[379] A

[370] J. F. Codington, A. G. Cooper, M. C. Brown, and R. W. Jeanloz, *Biochemistry*, 1974, **14**, 855.
[371] M. Prat, S. Landolfo, and P. M. Comoglio, *F.E.B.S. Letters*, 1975, **51**, 351.
[372] J. W. Huggins, R. W. Chesnut, N. N. Durham, and K. L. Carraway, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 497.
[373] G. Neri, D. F. Smith, E. B. Gilliam, and E. F. Walborg, *Arch. Biochem. Biophys.*, 1974, **165**, 323.
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[378] 'Methods in Enzymology, Hormone Action. Part A. Steroid Hormones', ed. B. W. O'Malley and J. G. Hardman, Academic Press, 1975, Vol. 36.
[379] 'Methods in Enzymology, Hormone Action. Part B. Peptide Hormones', ed. B. W. O'Malley and J. G Hardman, Academic Press, 1975, Vol. 37.

general review of hormonal proteins and peptides discusses the chemistry of glycoproteins and the roles of the carbohydrate moieties of pituitary thyrotrophin and human chorionic gonadotrophin, *etc.*[380]

The α- and β-chains of human luteinizing hormone have been isolated using acidified urea, followed by ion-exchange chromatography.[381] Two major and two minor components of luteinizing hormone have been isolated and shown to be glycoproteins containing L-fucose, D-mannose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and *N*-acetylneuraminic acid.[382] The carbohydrate portions of the α-subunits of porcine luteinizing, follicle-stimulating, and thyrotrophin-stimulating hormones are significantly different, suggesting that the biosynthesis of the subunits occurs in different cells of the pituitary gland.[383]

Desialylated human chorionic gonadotrophin was found to be twice as effective as the native hormone in competing for the binding site of receptors in rat testis.[384] However, it has been pointed out that caution must be exercised when radio-ligand receptors are used to assay preparations of human chorionic gonadotrophin of different sialic acid contents.

The chemistry, biology, immunology, biochemistry, and clinical chemistry of the gonadotrophins have been comprehensively reviewed; several sections deal specifically with the structures and functions of the carbohydrate moieties of gonadotrophins.[385]

Polyacrylamide gel electrophoresis has been used to separate five active thyrotrophins from human pituitary glands.[386] Treatment of each thyrotrophin with neuraminidase afforded more basic components without change in the overall pattern, supporting the view that differences between them cannot be attributed to variations in the sialic acid contents.

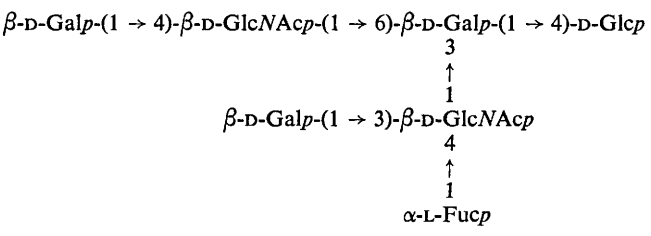
Changes in the ratio of tri-iodothyronine to thyroxine in female guinea-pigs treated with thyroid-stimulating hormone were not apparent in the extents of glycosylation.[387] Sialic acid was incorporated only into nascent thyroglobulin, but not into molecules already secreted into the follicular lumen.[388] The incorporation of 2-amino-2-deoxy-D-mannose into the sialic acid of thyroglobulin has been examined in bovine thyroid gland; 2-amino-2-deoxy-D-mannose 6-phosphate, 2-acetamido-2-deoxy-D-mannose, and 2-acetamido-2-deoxy-D-mannose 6-phosphate were identified as metabolic precursors of sialic acid.[389] Studies on cold and hot nodules from human thyroid gland have demonstrated that cold nodules are able to synthesize a thyroglobulin that is deficient in carbohydrate.[390] Incomplete glycosylation may be important in maturation and in migration of thyro-

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globulins to the iodinating site of the cell. Human kininogen (mol. wt. 5×10^4) was found to contain 8.3 residues of sialic acid.[391]

Milk Glycoproteins

A new heptasaccharide, lacto-*N*-fucoheptaose, isolated from human milk, has the structure (3).[392] Anion-exchange and paper chromatography have been used



(3)

to prepare thirty oligosaccharides in the molecular-weight range 998—3113 from a non-dialysable fraction of human milk.[393] The oligosaccharides do not originate from glycoproteins or glycolipids, and their biosynthesis may be initiated by the activation of glycosyltransferases related to the blood-group substances.

A soluble glycoprotein (mol. wt. 1.39×10^5) containing 30% carbohydrate has been prepared from the milk-fat globule membrane of cows; the sugar moieties consist of hexose (13.9%), hexosamine (8.1%), sialic acid (8.0%), and fucose (0.8%).[394] Discontinuous gel electrophoresis indicated heterogeneity. Sialic acid could be completely released from the membrane without disruption of the fat globule.[395] Sialoglycopeptides released from the surface were fractionated into five components that showed many similarities with those derived from erythrocyte membranes.

κ-Casein has been purified and shown to be a glycoprotein (mol. wt. 2×10^4) containing sialic acid (0.3%), mainly as the *N*-glycolyl derivative, and a small proportion of 2-acetamido-2-deoxy-D-galactose and D-galactose.[396] Ion-exchange chromatography on DEAE-cellulose established the heterogeneity of κ-casein.

The carbohydrate moieties of the galactosyltransferase component of lactose synthetase have been shown to be heterogeneous, and are probably linked to the protein by both *N*- and *O*-glycosidic linkages.[397]

Serum Glycoproteins

A review of the role of carbohydrates, particularly sialic acid, in regulating the survival time of plasma glycoproteins in sera has appeared.[398] A short review has discussed the molecular structures and functions of plasma proteins.[398a]

[391] J. C. Londesborough and U. Hamberg, *Biochem. J.*, 1975, **145**, 401.
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[397] E. D. Lehman, B. G. Hudson, and K. E. Ebner, *F.E.B.S. Letters*, 1975, **54**, 65.
[398] G. Ashwell and A. G. Morrell, *Adv. Enzymol.*, 1974, **41**, 99.
[398a] H. G. Schwick, *Naturwiss.*, 1974, **61**, 484.

The function of the D-galactosyl units present on bovine factor V has been studied using galactose oxidase and β-galactosidase.[399] The results suggested that those D-galactose residues most susceptible to oxidation by galactose oxidase are necessary for activity; these residues can be protected by complexation with diarochidonyl-lecithin. Other results indicated the presence of more than one type of linkage between sialic acid and D-galactosyl residues. Bovine factor VII (proconvertin) has been purified (approximately 5×10^5-fold) with an overall yield of 30%; it consists of a single polypeptide chain having a molecular weight of 4.55×10^4 (by sedimentation equilibration) or 5.4×10^4 (by gel electrophoresis).[400] Hexose (14.1), 2-acetamido-2-deoxy-D-glucose (6.2), and *N*-acetylneuraminic acid (7.9 residues) were found for a molecule of weight 4.55×10^4. Factor IX from human plasma has been purified approximately 10^5-fold; the electrophoretically homogeneous form consists of a single polypeptide chain (mol. wt. 7.0×10^4) containing 54.4 mol of hexose per mol of protein.[401] The molecular events associated with the activation of bovine factor IX (Christmas factor) by factor XI_a (activated plasma-thromboplastin antecedent) have been shown to involve the scission of two peptide bonds to give a glycoprotein (mol. wt. 9×10^3) and factor IX_a.[402] The bovine glycoprotein factor X (Stuart factor) was converted into factor X_a by a number of proteases, which cleaved a unique arginyl–leucyl bond in the precursor heavy-chain, with the release of a glycopeptide (mol. wt. 1.1×10^4) and factor $X_{a\alpha}$ (a glycoprotein of mol. wt. 4.0×10^4).[403] Further proteolysis led to factor $X_{a\beta}$, with the release of all the carbohydrate as a glycoprotein (mol. wt. 4×10^3). Determination of the amino-acid sequences of the heavy-chain of bovine factor X_1 and of activated factor X_{1a} has revealed homologies with other hepatic and pancreatic serine proteases.[404]

Digestion of bovine prothrombin with thrombin and trypsin released three glycopeptides from the *N*-terminus.[405] Two of the fragments containing carbohydrate did not bind calcium ions. Although the molecular weights of the glycopeptides differed, they had almost identical proportions of carbohydrate and essentially the same distribution of sialic acid, D-mannose, 2-amino-2-deoxy-D-glucose, and D-galactose. The purity of bovine prothrombin obtained by affinity chromatography on anti-(prothrombin immunoglobulin) immobilized on Sepharose is claimed to be comparable to existing preparations.[406] In studies of the renaturation activity of bovine thrombin, the sialic acid residue of the B-chain carbohydrate was used to bind the protease to a solid support before the A-chain was removed.[407] Evidence for the formation of a carboxylic ester bond in the complex formed between thrombin and heparin cofactor has been presented.[408]

[399] S. Saraswathi and R. W. Colman, *J. Biol. Chem.*, 1975, **250**, 8111.
[400] W. Kisiel and E. W. Davie, *Biochemistry*, 1975, **14**, 4928.
[401] B. Østerud and R. Flengsrud, *Biochem. J.*, 1975, **145**, 469.
[402] K. Fujikawa, M. E. Legaz, H. Kato, and E. W. Davie, *Biochemistry*, 1974, **13**, 4508.
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[406] R. Wallin and H. Prydz, *F.E.B.S. Letters*, 1975, **51**, 191.
[407] T. C. Hageman, G. F. Endres, and H. A. Scheraga, *Arch. Biochem. Biophys.*, 1975, **171**, 327.
[408] W. G. Owen, *Biochim. Biophys. Acta*, 1975, **405**, 380.

Desialylation of both major forms of rabbit-plasma plasminogen resulted in the abolition of charge differences, whereas the binding to anti-fibrinolytic amino-acids (*e.g.* ε-aminocaproic acid) appeared to be unaffected.[409] The metabolic survival times of the desialylated materials did not differ greatly, but were appreciably lower than normal. These observations suggest that charge differences between the two forms of plasminogen are incidental to differences in their functions.

Bovine fetuin has been isolated by precipitation with ethanol in the presence of heavy-metal ions.[410] Digestion of this fetuin with pronase yielded two distinct series of glycopeptides, although microheterogeneity is manifest in each series. The more abundant type-A series contained 80% of the hexose originally present in fetuin, and consisted of D-mannose (3 or 4), D-galactose (4—6), and 2-acetamido-2-deoxy-D-glucose (5—7 residues), probably linked to asparagine in at least three positions on the peptide chain. The type-B series contained D-galactose (2—5), 2-acetamido-2-deoxy-D-galactose (2 or 3), and *N*-acetylneuraminic acid (0—3 residues); the carbohydrate–protein linkage involves serine and 2-acetamido-2-deoxy-D-galactose. The carbohydrate moieties of bovine fetuin have been compared with those present in red-blood cells.[411] Fetuin complexed strongly with phytohaemagglutinins and isomitogens H-PHAP, but not with L-PHAP which does not bind to red cells. However, fetuin was able to inhibit the transformation of lymphocytes induced by H- and L-PHAP; this effect may result from masking of the cell membrane in a manner analogous to that of other immunosuppressive α-globulins in sera.

Comparisons have been made between the carbohydrate compositions of human, porcine, and bovine transferrins and duck ovotransferrin.[412] Each yielded two glycopeptides which appeared to have identical compositions, although differing in the sequences of amino-acids. The carbohydrate moieties are located on asparagine residues, which are linked to either a basic residue or a hydrophobic residue. One of the carbohydrate moieties in human transferrin is located in the neighbourhood of the *C*-terminus of the peptide chain. Asialotransferrins from bovine, canine, and porcine sources were not removed from rabbit plasma by way of the Ashwell–Morell pathway that operates in human plasma.[413] Part of the explanation for this discrepancy may be found in the observations that bovine, porcine, canine, and rabbit transferrins contain roughly half the amount of carbohydrate found in human transferrin, and that – unlike human asialotransferrin – D-galactose is not released on treatment with galactosidase. This is consistent with the view that the rapid removal of transferrins depends on the presence of accessible D-galactosyl residues.

The glycoprotein α2H-globulin, which is normally present in trace quantities in the sera of healthy subjects, has been shown to increase in some pathological disorders, especially in malignant diseases.[414] Its effect on lymphocyte response has been investigated. The plasma glycoprotein α_2HS has been shown to be a

[409] G. E. Siefring and F. J. Castellino, *J. Biol. Chem.*, 1974, **249**, 7742.
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[412] I. Graham and J. Williams, *Biochem. J.*, 1975, **145**, 263.
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[414] D. Buffe and C. Rimbaut, *Immunology*, 1975, **29**, 175.

component of the matrices of both foetal and adult human bone; its incorporation into and concentration in bone presumably involves an interaction with one of the components of the mineralizing matrix.[415]

The response of rat liver to inflammation is manifested by an increased production of α_1-acid glycoprotein.[416] The carbohydrate moieties of human-plasma lipoproteins have been analysed for hexose, hexosamine, and sialic acid.[417] A major plasma glycoprotein (cold-insoluble globulin) contains sialic acid (1.2%), hexose (1.8%), and hexosamine (2.1%).[418]

A progesterone-binding globulin present in the pooled sera of pregnant guinea-pigs has been purified to constant binding activity; it was shown to be appreciably heterogeneous by gel filtration and gel electrophoresis.[419] Two arbitrary fractions of high carbohydrate content (62—72%) contained neutral hexose, 2-acetamido-2-deoxyhexoses, sialic acid, and some L-fucose. The amino-acid content and peptide mapping indicated that differences in the carbohydrate moieties are responsible for the heterogeneity of this globulin.

The cell-type-specific glycoproteinaceous antigen from human fibroblasts also appears in plasma; it interacts with fibrin and appears in the serum cryoglobulin fraction associated with cryofibrinogen.[420]

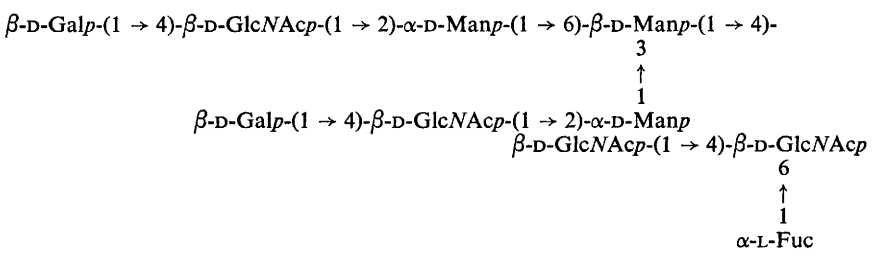
Immunoglobulins

The distribution, isolation, determination, physicochemical properties, structure, biosynthesis, metabolism, *etc.* of immunoglobulin A have been reviewed.[421] The use of affinity labelling to explore the topography of the antibody-combining sites of immunoglobulins [422] and the specificities of antibodies [423] have also been reviewed.

The structure (4) of the asparagine-linked carbohydrate moiety of bovine IgG immunoglobulin has been determined.[424, 425] IgG immunoglobulin from human placenta has been shown to contain 11 mol of hexose per mol of protein.[426]

Determination of the amino-acid sequence of a portion of the heavy-chain containing the carbohydrate moiety of a polymeric IgA_2 myeloma protein has revealed homologies with a number of other subclasses of human IgA immunoglobulin.[427] The carbohydrate structure of a human-serum immunoglobulin IgA

[415] I. R. Dickson, A. R. Poole, and A. Veis, *Nature*, 1975, **256**, 430.
[416] J. C. Jamieson, K. E. Morrison, D. Molasky, and B. Turchen, *Canad. J. Biochem.*, 1975, **53**, 401.
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[422] D. Givol, *Essays in Biochem.*, 1974, **10**, 73.
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[424] S. Ito, T. Muramatsu, and A. Kobata, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 938.
[425] T. Tai, S. Ito, K. Yamashita, T. Muramatsu, and A. Kobata, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 968.
[426] E. Byszewska and K. Zakrzewski, *Acta Biochim. Polon.*, 1974, **21**, 365.
[427] C. Wolfenstein-Todel, B. Frangione, and E. C. Franklin, *Biochim. Biophys. Acta*, 1975, **379**, 627.



(4)

(α_1-subtype) from a myeloma protein has been elucidated; five carbohydrate moieties are located on the heavy-chain, and are probably linked *O*-glycosidically by way of 2-acetamido-2-deoxy-D-galactose and serine.[428] Digestion of IgA_1 with pronase released four major glycopeptides, but only one of them was homogeneous.[429] The structures of the heterogeneous glycopeptides are probably closely related, differing, for example, by a sialic acid residue linked to a terminal D-galactosyl residue. L-Fucose is present in one of the glycopeptides, and appears to be α-(1 → 6)-linked to a 2-acetamido-2-deoxy-D-glucosyl residue attached to the peptide chain. The finding that the complex oligosaccharides of immunoglobulins IgG, IgA, IgE, and, probably, also the oligosaccharides of IgM with a high D-mannose content have a common core structure accords with the view that oligosaccharide–lipid intermediates are involved in the synthesis of the core. Human immunoglobulin IgA from myeloma sera has also been examined using the lectin from *Phaseolus vulgaris*; binding to the IgA_2 subclass, but not to most of the IgA_1 subclass, was observed.[430] The presence of 2-acetamido-2-deoxy-D-galactosyl residues in IgA_1 proteins, but not in IgA_2 proteins, suggested the presence of a second type of oligosaccharide. L-Fucose was present only in IgA_2 proteins.

The immunoglobulin IgM heavy-chain sialoglycopeptides associated with asparagine-170, -332, and -395 have been isolated, and core glycopeptides containing 2-acetamido-2-deoxy-6-*O*-L-fucopyranosyl-D-glucose were obtained from them by digestion with an *endo*-β-acetamidodeoxyglucosidase.[431] In addition to the core peptides, a high yield of a tetrasaccharide, $(D\text{-Man})_3$-D-GlcNAc, was obtained. The structures of the core peptides appear to be similar to those of most other asparagine-linked peptides, except for the presence of L-fucose. Modification of the carbohydrate moieties of the reduced chains of human IgM immunoglobulins with glycosidases greatly inhibited the re-assembly process.[432]

A study of cell-surface immunoglobulins in rats for a three month period after birth revealed that a change from IgM to IgD immunoglobulin takes place during the differentation of virgin B-lymphocytes in the spleen.[433]

[428] J. Baenziger and S. Kornfeld, *J. Biol. Chem.*, 1974, **249**, 7270.
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[433] E. S. Vitetta, U. Melcher, M. McWilliams, M. E. Lamm, J. M. Phillips-Quagliata, and J. W. Uhr, *J. Exp. Med.*, 1975, **141**, 206.

The structure and function of J-chain, a glycoprotein component (mol. wt. 1.5×10^4) of polymeric immunoglobulins, have been reviewed.[434]

The interaction of homogeneous, murine myeloma immunoglobulins with such polysaccharide antigens as (2 → 6)- and (2 → 1)-β-D-fructans, (1 → 6)-β-D-galactans, dextrans, and pneumococcal C polysaccharides has been discussed.[435] The binding energies of proteins derived from BALB/c mouse myelomas with members of the isomaltodextrin series have been examined.[436] The heterogeneity of the carbohydrate-containing light-chains synthesized by murine myeloma MOPC-46 has been examined.[437] The secreted chains are of three types, each containing 2-acetamido-2-deoxy-D-glucose, D-mannose, D-galactose, L-fucose, and *N*-glycolylneuraminic acid. Two unusual features of the κ light-chain of human myeloma protein are the presence of carbohydrate bound covalently to asparagine-28 and the depletion of one amino-acid residue at position-95.[438]

Periodate oxidation of purified horse anti-diphtheria immunoglobulin T destroyed six of the hexosyl residues and all of the L-fucosyl and sialic acid residues; the ability of the oxidized material to precipitate with the toxin was significantly reduced.[439]

Erythrocyte Glycoproteins

The sequence of glycophorin—the principal sialoglycoprotein present in human-erythrocyte membranes—containing 131 amino-acid residues and, on average, 16 oligosaccharide chains, has been determined. Fifteen of the carbohydrate moieties are linked either to serine or to threonine, whereas the other residue is attached to asparagine.[440] The amino-acids appear to be organized into three distinct regions. The report that glycophorin could be resolved by electrophoresis on dodecyl sulphate gel into three glycoproteins (mol. wts. 9.6×10^4, 4.5×10^4, and 2.6×10^4) can be explained only by aggregation.[441] A group of major sialoglycopeptides has been separated into two fractions by gel filtration in the presence of a non-ionic detergent (Ammonyx-LO).[442] The principal component (designated glycophorin A) comprised 75% of the total sialoglycopeptides and contained 131 amino-acids. Another group of glycopeptides of high molecular weight was eluted in two fractions and was able to form aggregates. One of the principal glycoproteins (mol. wt. 6×10^4) of human-erythrocyte membranes has been located on the outer surface of the membrane by means of the lacto-peroxidase technique; a minor glycoprotein (mol. wt. 2.5×10^4) appears to be accessible to the reagent on both sides of the membrane.[443] The α-helix content of erythrocyte-membrane proteins from human and bovine sources has been investigated by c.d.[444] The c.d. spectra of the proteins were similar, whether or

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[435] C. P. J. Glaudemans, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 313.
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[442] H. Furthmayr, M. Tomita, and V. T. Marchesi, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 113.
[443] E. Reichstein and R. Blostein, *J. Biol. Chem.*, 1975, **250**, 6256.
[444] R. V. Decker and K. L. Carraway, *Biochim. Biophys. Acta*, 1975, **386**, 52.

not dodecyl sulphate was present, and the apparent helix content rose from 18% to 65% in non-polar solvents. The chemical and immunological properties of two minor glycoproteins (GP-II and -III) have been reported; GPII contains hexose (4.9%), hexosamine (5.4%), and sialic acid (7.8%), whereas GPIII contains hexose (7.6%), hexosamine (7.2%), and sialic acid (8.1%).[445] The amino-acid compositions of both glycoproteins are similar, but differ from the MN glycoprotein. Both glycoproteins are associated with blood-group Ss, I, and A activities.

Concanavalin A bound to human erythrocytes can be visualized directly by freeze-etching.[446] This technique revealed that about 1×10^5 molecules of lectin are bound to the cell in a random manner.

The carbohydrate moieties of erythrocytes from patients with Tn-syndrome have been shown to contain less D-galactose and sialic acid.[447, 448] The work suggested that alkali-labile carbohydrate chains of these erythrocytes consist solely of 2-acetamido-2-deoxy-D-galactosyl residues linked either to serine or to threonine.

I-Active receptors have been suggested to be located on different ABH- or MN-active substances rather than on unique I-substances.[449] An I-active antigen isolated from human red cells treated with papain was shown to be a glycoprotein rather than a glycolipid.[450] The I-immunodeterminants, which are linked *N*-glycosidically to the protein, contain D-galactosyl and 2-acetamido-2-deoxy-D-glucosyl residues.

'Bombay' erythrocytes were not transformed into erythrocytes phenotypically resembling O cells on treatment with GDP-L-fucose and an α-(1 → 2)-fucosyltransferase obtained from the gastric mucosa of O individuals.[451] However, this transformation was achieved after prior treatment of 'Bombay' erythrocytes with neuraminidase.

Studies on the binding of the blood-group B-specific lectin from *Bandeiraea simplicifolia* to α-D-galactopyranosyl residues have demonstrated that each subunit of the tetrameric protein contains a single carbohydrate-binding site, which appears to be homogeneous and non-interacting.[452] The number of lectin-receptor sites on human B erythrocytes varied from 0.72×10^5 to 1.34×10^5. Release of D-galactose from the B determinant, by treatment with coffee-bean α-galactosidase, resulted in the disappearance of B activity and the enhancement of H activity.[453]

The nature of erythrocyte receptor sites for several agglutinins has been examined; the results obtained with snail anti-A agglutinins suggested that human-erythrocyte glycoproteins contain a peptide-linked disaccharide composed of 2-acetamido-2-deoxy-D-galactosyl and sialic acid residues.[454]

[445] S. Fujita and H. Cleve, *Biochim. Biophys. Acta*, 1975, **382**, 172.
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The density of HL-A antigens (a group of serologically defined cell-surface glycoproteins) present on the peripheral blood lymphocytes of a single donor was significantly increased either after stimulation with phytohaemagglutinin or after transformation with Epstein–Barr virus.[455]

The Rh(D) antigen of human red-blood cell membranes has been isolated as an immunologically homogeneous protein possessing a molecular weight in the region of $1—2 \times 10^4$.[456] The removal of *N*-acetylneuraminic acid from human erythrocytes reduced the half-life of the erythrocytes in the blood.[457]

The principal glycoproteins of equine and porcine erythrocyte membranes have been examined.[458] The principal equine glycoprotein (mol. wt. 3.3×10^4), which contains 53.8% carbohydrate, is associated with the activity of the infectious mononucleosis heterophile antigen. Two principal groups of porcine glycoproteins were isolated; one (mol. wt. 4.62×10^4) contains 65.8% carbohydrate, and the other (mol. wt. 2.9×10^4) contains 49.6% carbohydrate. They differ in activity towards the lectin from *Phaseolus vulgaris*.

The amoeba *Acanthamoeba castellani* has been shown to bind to equine erythrocytes by interaction with carbohydrates on the surface of the red cells.[459]

4-Nitrophenyl tri-*N*-methyltyrosinate [^{125}I]iodide has been used to label the outer surface of mature chicken erythrocytes. Two component glycoproteins, with subunits of molecular weight 5×10^4 and 1×10^5, were identified.[460]

Neuraminidase-treated rat and rabbit erythrocytes were shown to be rapidly sequestered by the liver.[461] Chemical studies of glycoproteins obtained from erythrocyte membranes from a number of sources have been reported.[462]

Salivary and Mucous Glycoproteins

Sputum has been used as a source of bronchial glycoproteins.[463] Ultracentrifugation of sputum produced a supernatant or sol phase containing lactoferrin, albumin, α_1-acid glycoprotein, immunoglobulin A, and a high-molecular-weight glycoprotein, which was also isolated from the gel phase. This glycoprotein contains 58—69% carbohydrate composed of L-fucose, D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and *N*-acetylneuraminic acid. The carbohydrate–protein linkage probably involves 2-acetamido-2-deoxy-D-galactose and threonine and/or serine. Chromatography of bronchial glycoproteins on DEAE-Sephadex A-25 afforded fractions enriched in sulphate and sialic acid, but individual fuco-, sialo-, and sulpho-glycoproteins were not obtained. Alkaline borohydride released a complex mixture of oligosaccharides from bronchial glycoproteins, but sulphated oligosaccharides free of sialic acid were not isolated. Separation of these oligosaccharides gave two

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fractions of different size; the sulphate content was greatest in the larger moieties, whereas the sialic acid content was greatest in the smaller moieties. A glycoprotein (mol. wt. 6.2×10^4) that precipitates with calcium ions has been isolated from amicrobial saliva collected from 6—16-year-old children; the carbohydrate moiety (10%) of this glycoprotein is composed of D-galactose, L-fucose, and D-mannose in the ratio 6 : 3 : 1.[464] The electrophoretic mobility of the glycoprotein on polyacrylamide gel indicated that it is similar to the principal glycoprotein present in the matrix of dental plaque.

Mucins isolated from human submandibular glands have been shown to be glycoproteins containing sialic acid, L-fucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and traces of D-glucose and D-mannose.[465] The molecular weights and carbohydrate and amino-acid compositions have been determined for five glycoproteins isolated from human parotid glands.[466] The carbohydrate portion of one of these glycoproteins contains peripheral D-galactosyl residues, whereas D-mannosyl and 2-acetamido-2-deoxy-D-glucosyl residues are present in the core.[467] Mucinous glycoproteins may afford protection against infectious agents by acting instead of the receptors present on buccal epithelial cells.[468]

Mucus from human gastric mucosa had been purified to immunological and electrophoretic homogeneity.[469] The purified material exhibited blood-group activity.

The binding of calcium ions by the mucins of rat small intestines probably involves sialic acid and carboxy-groups, which co-operate to produce a contraction or folding of the molecule on binding of the cation.[470]

The enzymic removal of sialic acid from bovine cervical mucus has been used in an attempt to establish a molecular basis for the rheological properties.[471] The storage modulus, viscosity, and c.d. spectrum were essentially unchanged, indicating that the removal of terminal sialic acid residues does not affect the physical structure of the glycoprotein to any appreciable extent.

The binding of cholera toxin by salivary mucin was inhibited by free sialic acid or by treatment of the mucin with neuraminidase, whereas no inhibition was achieved in parallel experiments with gastric and intestinal mucins.[472]

Urinary Glycoproteins

A new type of carbohydrate–protein linkage has been detected in a glycopeptide from normal human urine.[473] Sugar and amino-acid analyses, methylation, mass spectrometry, and optical rotational data suggested the structure (5) for the linkage region.

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β-D-Glc*p*-(1 → 3)-α-L-Fuc*p*-Thr
(5)

The amounts of different hydroxylysine-containing compounds present in the urine of patients up to forty years of age have been examined.[474] The urinary excretion of hydroxylysine glycosides, free hydroxylysine, and peptide-bound hydroxylysine increased from the age of six months to puberty.

Procedures have been described for the assay of protein-bound carbohydrate in sera and urine from normal children and from patients with sialuria.[475] It was concluded that sialuria is not associated with an anomalous metabolism of glycoproteins, but with an anomalous biosynthesis of sialic acid.

A marked deficiency of α-mannosidase has been demonstrated in the fibroblasts obtained from a skin-biopsy specimen of a child with mannosidosis.[476] Oligosaccharides present in the urine could also be isolated from extracts of these cells. The glycopeptide nature of the storage material isolated was attributed to a low level of *N*-aspartyl-β-glycosaminidase in the cells.

The β-acetamidodeoxyhexosidase present in normal human urine has been purified by affinity chromatography: the enzyme is a glycoprotein containing residues of sialic acid, D-galactose, D-glucose, D-mannose, and unidentified hexosamines.[477]

Miscellaneous Glycoproteins and Chitin

A homogeneous preparation of reduced and carboxymethylated β-ovomucin has been shown to contain a subunit of molecular weight 1.12×10^5, sulphate (4.24%), and carbohydrate (60%) consisting largely of D-galactose (22%), 2-amino-2-deoxy-D-galactose (8.9%), and sialic acid (10.6%).[478] A soluble, fibrous component extracted from crude ovomucin with 5M-guanidinium chloride contained 70% β-ovomucin.

Chromatography of glycopeptides derived from ovomucoid by acetolysis and partial hydrolysis with acid has revealed slight differences in the sequences of sugars in the carbohydrate moieties of ovomucoid.[479] Precipitation with salt and chromatography on sulphoethyl-Sephadex has been used to prepare hen ovomucoid (mol. wt. 2.83×10^4) in an electrophoretically homogeneous form containing 2-acetamido-2-deoxy-D-glucose (15), D-mannose (10), D-galactose (1), unidentified hexose (1), and acetate (16 residues), in addition to a small proportion of sialic acid.[480] Hydrodynamic data suggested that hen ovomucoid adopts a non-globular conformation under normal conditions. Further studies have been reported on the structure of a sulphated glycoprotein (mol. wt. 2.6×10^4) isolated from chick allantoic fluid following infection with influenza virus.[481] Methylation analysis, mass spectrometry, digestion with an

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[481] H. U. Choi and K. Meyer, *Carbohydrate Res.*, 1975, **40**, 77.

α-galactosidase, oxidation with periodate, and Smith degradation were used to investigate the structure of the highly heterogeneous carbohydrate chains, which are linked to the peptide backbone by way of *O*-glycosidic linkages from 2-acetamido-2-deoxy-D-glucosyl residues. The main chains are composed of D-galactosyl residues linked at C-3 and 2-acetamido-2-deoxy-D-glucosyl residues linked at C-4, whereas L-fucosyl and sialic acid residues are present at non-reducing termini.

Light-scattering studies have demonstrated a decrease in the effective hydrodynamic radius of the 'anti-freeze' glycoproteins from Antarctic fish on cooling to −0.2 °C.[482] The carbohydrate moiety of the 'anti-freeze' glycoprotein from *Trematomus borchgrevinki* was shown to be β-D-Gal*p*-(1 → 3)-D-Gal*N*Ac by g.l.c.; agglutination studies indicated that the carbohydrate–protein linkage has the α-configuration.[483] The presence of the Thomsen–Friedenreich antigen (T-antigen) on the 'antifreeze' glycoprotein and its relation to tumour-cell surfaces were briefly discussed.

A glycoprotein that comprises 77% dry-weight of grasshopper (*Locusta migratoria*) eggs has been found to contain D-mannose (14) and 2-amino-2-deoxy-D-glucose (2 residues per molecule), with the carbohydrate moiety linked to an asparagine residue.[484]

Purification of the anticoagulant principle in the venom of *Trimeresurus gramineus* has revealed it to be a glycoprotein (mol. wt. 1.95×10^4).[485]

Hydroxyproline has been tentatively identified as the linkage residue in a number of apparently homogeneous proteoglycans obtained from rice bran.[486]

Exhaustive digestion of the jelly-coat of toad (*Bufo vulgaris*) eggs with pronase yielded two glycopeptides containing different proportions of sialic acid.[487]

Three morphologically and functionally distinct layers of the jelly-coat of *Zenopus laevis* eggs have been shown to contain carbohydrate moieties linked *O*-glycosidically.[488]

Structural studies have revealed that the fucogalactan of egg masses from snail (*Ampullarius*) species is highly branched, with branches at C-3 and C-6 of the D-galactosyl residues.[489]

Glycoproteins from the intercellular matrix of sponges have been investigated.[490]

Conjugates between polysaccharides (*e.g.* dextrans, starch, and dextrin) and proteins (*e.g.* lysozyme, chymotrypsin, β-glucosidase, casein, and serum albumin) have been prepared with the aid of cyanogen bromide.[491] Loss of enzymic activity was offset by increases in the thermal stability and the resistance to proteolytic digestion. Glutaminase-asparaginase from Acinetobacter has been

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glycosylated with glycopeptides from human fibrin and γ-globulin; the artificial glycoprotein exhibited enhanced thermal stability, and its half-life in mice, rats, and rabbits increased appreciably.[492]

The absence of chitin in the endosternite (an internal skeletal support from prosomal muscles) of chelicerates has been noted.[493] There is some evidence that chitin isolated from crayfish (*Astacus fluviatilis*) is bound covalently to protein.[494]

Various acid anhydrides have been used to *N*-acylate chitosan.[495]

New derivatives of sialic acids and methods for identifying *O*-acetyl-*N*-glycoloyl- and *N*-acetyl-*O*-glycoloyl-neuraminic acids have been reported.[496]

Analysis of Glycoproteins

Discontinuous gel electrophoresis in the presence of dodecyl sulphate has been used to determine the molecular weights of membrane glycoproteins.[497] However, there are uncertainties regarding the binding of the detergent and the relation of the Stoke's radius to the molecular weight compared with other protein standards used.[498] Bovine rhodopsin has been examined using this technique. Two-dimensional electrophoresis on polyacrylamide gel has afforded improved resolution in the detection of proteins and glycoproteins extracted from erythrocyte membranes.[499] The greater resolving power of isoelectric focusing in gels has been used to examine glycoproteins.[500] A linear relationship was obtained when the electrophoretic mobilities of dansylated glycopeptides derived from hen-egg ovotransferrin were plotted against the logarithm of the molecular weights of these derivatives.[501]

Hydrazinolysis, deamination, and methylation analysis have demonstrated that α_1-acid glycoprotein, fetuin, lactotransferrin, and transferrin possess structural features in common.[502]

Anion-exchange chromatography has been used to separate sugar–borate complexes of D-mannose, L-fucose, D-galactose, D-glucose, D-xylose, L-arabinose, 2-amino-2-deoxy-D-mannose, 2-amino-2-deoxy-D-galactose, 2-deoxy-D-*arabino*-hexose, 2-deoxy-D-*lyxo*-hexose, and lactose.[503] This technique was used to study the composition of the carbohydrate moieties of ovomucoid, lichenin, and *Collocalia* mucoid.

A method for determining the ratio of glycosylated serine to threonine residues in mucins is based on the relative rates of β-elimination of olefinic amino-acids therefrom.[504] The elimination reaction is faster with glycosylated serine residues.

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T.l.c. on silica gel has been used for the rapid detection of urinary oligosaccharides.[505]

The lectin from *Ricinus communis* coupled to Sepharose 4B has been used as an affinity-chromatography matrix in the purification of glycoproteins containing terminal D-galactosyl residues.[506]

L-Fucose has been estimated by fucose-kinase-catalysed transfer of [^{32}P]phosphate from ATP to L-[^{3}H]fucose, followed by electrophoretic separation of the labelled product.[507]

Glycoproteins containing sialic acid may be detected by staining with a cationic carbocyanine dye before and after digestion with neuraminidase: a change in the colour of the stain from blue to red–purple indicates the presence of sialic acid.[508] The ^{13}C n.m.r. spectra of *N*-acetylneuraminic acid and its methyl ester in DMSO and in water have indicated that the acid exists predominantly in the β-form in solution.[509] The finding supports the assumption that *N*-acetylneuraminic acid, produced by the enzymic cleavage of its α-ketosides, leaves the catalytic site of *Vibrio cholerae* neuraminidase as the β-anomer.

A review dealing with cell-surface phenomena, the structures and biosynthesis of tissue proteoglycans, the regulation of glycogen metabolism, and inborn errors of carbohydrate metabolism has appeared.[510]

Biosynthesis of Glycoproteins

The biosynthesis and functions of the membrane glycoproteins of eukaryotic cells have been reviewed.[511] The general features of the biosynthesis of these glycoproteins closely resemble those involved in the biosynthesis of complex bacterial glycans, especially the O-antigen of lipopolysaccharides. Syntheses of polyisoprenyl β-D-mannopyranosyl phosphate[512] and P^1-di-*N*-acetylchitobiosyl P^2-dolichyl pyrophosphate have been reported.[513] A preparation from human-lymphocyte membranes was able to transfer D-mannose from GPD-D-mannose to the chitobiosyl derivative, whereas dolichyl D-mannosyl phosphate did not act as a D-mannosyl donor. It appears that D-mannosyl residues at various positions of the glycoprotein have different anomeric configurations, so that different D-mannosyl donors are required in their synthesis.

A particulate fraction from calf thyroid catalysed the transfer of D-mannose from GDP-D-mannose to exogenous glycopeptides and to methyl or aryl glycosides to form sequences of α-D-mannopyranosyl-D-mannose.[514] Transfer to the simple glycosides required a single, non-reducing D-mannosyl residue

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linked to a lipophilic aglycone (*e.g.* methyl, phenyl, 4-methylumbelliferyl). Glycopeptides containing sequences of D-mannose acted as acceptors in the transfer reaction, but only after dinitrophenylation of the peptide portion. GDP-D-Mannose proved to be a better glycosyl donor than either ADP- or UDP-D-mannose. Investigations with the transferases in thyroid slices showed that D-mannosylation of exogenous substrates is accomplished by two sequential enzymic reactions: the first reaction involves the transfer of D-mannose from the nucleotide precursor to an endogenous acceptor to form dolichyl mannosyl phosphate, which donates D-mannose to an exogenous substrate in the second reaction.[515] This two-step glycosylation sequence, which results in the formation of α-D-mannopyranosyl-D-mannose linkages, involves a double inversion of configuration at the anomeric centre of D-mannose. Dolichyl D-mannosyl phosphate present in calf pancreatic microsomes has been shown to have the β-configuration of the hexose residue.[516] Polyisoprenyl D-mannosyl phosphate and polyisoprenyl oligosaccharyl pyrophosphates have been implicated in the biosynthesis of the glycoproteins of bovine aorta [517] and pig-liver microsomes.[518] An oligosaccharyl-lipid involved in the biosynthesis of hen-oviduct glycoproteins has been found to contain 7—9 monosaccharide residues, with a D-mannosyl residue at the non-reducing terminus and a 2-acetamido-2-deoxy-D-glucosyl residue at the reducing terminus.[519] In the absence of GDP-D-mannose, incubation of membrane preparations from hen oviduct with UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose yielded a [^{14}C]chitobiosyl-lipid, but little oligosaccharide. The addition of GDP-D-mannose produced an oligosaccharide containing labelled amino-sugar residues. Incubation of the partially purified oligosaccharyl-lipid with oviduct membranes resulted in the formation of glycoprotein. A polypeptide (mol. wt. 2.5×10^4) obtained from hen oviduct was also shown to accept the oligosaccharyl-lipid.[520] Mannosyltransferases present in the mitochondria of rat liver [521] and brain [522] have been examined, and dolichyl D-mannosyl phosphate has been implicated in the biosynthesis of brain glycoproteins.[523]

Inhibition by 2-deoxy-D-*arabino*-hexose of the biosynthesis of the cell-wall mannan in *Saccharomyces cerevisiae* appears to involve a complex mechanism that disrupts the synthesis of protein rather than glycosylation.[524] Studies using a particulate enzyme from *Mycobacterium smegmatis* have demonstrated that polyisoprenyl D-mannosyl phosphates are obligatory donors,[525] while preparations obtained from *Hansenula holstii* have shown that a D-mannosyl-lipid is involved in the synthesis of mannosylated serine (or threonine) residues.[526]

A preparation from pig-liver microsomes catalysed the formation of P^2-dolichyl P^1-2-acetamido-2-deoxy-D-glucosyl and P^1-2-acetamido-2-deoxy-D-mannosyl

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pyrophosphates from UDP-2-acetamido-2-deoxy-D-glucose.[527] Epimerization appears to take place while 2-acetamido-2-deoxy-D-glucose is bound to the lipid. Crude extracts from rabbit liver catalysed the *in vitro* transfer of 2-acetamido-2-deoxy-D-glucose from the corresponding UDP-derivative to bovine pancreatic ribonuclease A (the non-glycosylated ribonuclease).[528] The enzymic activity, which is contained in the rough endoplasmic reticulum, has an absolute requirement for such bivalent cations as Co^{2+}, Mn^{2+}, and Ni^{2+}, but not for Mg^{2+}. Glycosylation occurred only at asparagine-34, supporting the view that the sequence Asn-*X*-Thr(Ser) is necessary – but not sufficient – for attachment of 2-acetamido-2-deoxy-D-glucose to the protein. Clones of Chinese hamster ovary cells that are resistant to the cytotoxity of the phytohaemagglutinin from *Phaseolus vulgaris* are deficient in UDP-2-acetamido-2-deoxy-D-glucose:glycoprotein acetamidodeoxyglucosyltransferase activity.[529] However, a resistant clone showing less ability to bind [^{125}I]phytohaemagglutinin, but without a deficiency of this transferase activity, was also isolated. A similar observation has been made by another group, who also noted reduced contents of sialic acid, D-galactose, and 2-acetamido-2-deoxy-D-glucose in the glycoproteins.[530] Incubation of membrane fractions obtained from *Saccharomyces cerevisiae* with UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose catalysed the transfer of the sugar to an endogenous lipid fraction consisting of a mixture of P^2-dolichyl P^1-2-acetamido-2-deoxy-D-glucosyl and P^1-di-*N*-acetylchitobiosyl pyrophosphates.[531]

Lipid intermediates have also been implicated in the incorporation of D-galactose into microsomal preparations from beef liver,[532] although they may not be similarly involved in preparations from the mucosal lining of canine trachea.[533] The D-galactosyltransferase activity of the tracheal preparation was subject to control by such polycationic compounds as putrescine, putreanine, cadaverine, protamines, histones, and polylysine at suboptimal concentrations of Mg^{2+} ions.[534] Galactosyltransferases from rat-liver microsomes exhibited appreciable activity when the nucleotide pyrophosphatase was subjected to control by various uridine nucleotides.[535]

The incorporation of *N*-acetylneuraminic acid from the CMP-nucleotide derivative into neuraminidase-treated human lipoproteins of very low density was catalysed by preparations from porcine liver microsomes.[536] The activity of the CMP-*N*-acetylneuraminic acid:glycoprotein sialyltransferase present in rat-liver microsomes has been shown to be stimulated by UDP, but to be inhibited by CMP.[537] The functions of glycosyltransferases in the processes of recognition and binding of asialoglycoproteins in liver have been investigated.[538]

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Glycoproteins rich in sialic acid were formed in the epithelium of cultured explants of rabbit trachea.[539]

A lipid-linked oligosaccharide serving as the donor of carbohydrate moieties to proteins in hen-oviduct membranes has been identified as (α-D-Man*p*)$_n$-β-D-Man*p*-(1 → 4)-β-D-Glc*NAcp*-(1 → 4)-D-Glc*NAcp*.[540]

Glycosyl:glycoprotein transferases involved in the transfer of 2-acetamido-2-deoxy-D-glucose, D-galactose, and L-fucose have been shown to be located mainly in the Golgi apparatus of mouse and rat testis.[541]

The addition of dibutyryl adenosine cyclic 3′,5′-phosphate and theophylline to normal or virus-transformed 3T3 mouse fibroblasts in concentrations sufficient to affect the growth characteristics produced no qualitative change in the pattern of incorporation of 2-amino-2-deoxy-D-glucose into glycoproteins at the periphery.[542] The metabolism of amino-sugars in murine cells has been investigated using 2-deoxy-2-(2-fluoroacetamido)-α-D-glucopyranose; effects on the biosynthesis of glycolipids and of glycoproteins were sought.[543] In the presence of the fluoro-sugar, the accumulation of UDP-2-acetamido-2-deoxy-α-D-glucose and CMP-*N*-acetylneuraminic acid was inhibited.

Glycosylation and sulphation of the proteins in rat-brain myelin[544] and sciatic-nerve myelin[545] have been investigated using [^{35}S]sulphate and tritium-labelled L-fucose and leucine. Six of the seven glycosyltransferases present in homogenates of embryonic chicken brain were located mainly in the particulate (synaptosome-rich) fraction, whereas the other (UDP-D-galactose:glycoprotein galactosyltransferase) was found in particulate and soluble fractions of young embryonic chicken brain, but only in the particulate fraction of older embryos.[546]

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6
Enzymes

BY J. F. KENNEDY

Introduction

A recent volume in the series 'Methods in Enzymology' deals with theories of, and standard methodologies for, the purification and isolation of enzymes by affinity chromatography.[1] Although methods for specific enzymes are reported, the emphasis is on providing general information on the design of affinity systems. Articles on a number of carbohydrate-oriented enzymes are included. The use of immobilized biochemicals in the purification and isolation of enzymes by affinity chromatography has been described briefly.[2]

Recent progress in the production, isolation, and use of enzymes as industrial catalysts has been reported.[3]

Other volumes in 'Methods in Enzymology' have reviewed enzymic procedures that have appeared in the literature since 1965, with particular reference to dissimilatory reactions between disaccharides and pyruvate.[4, 5]

The well-known series 'Methods of Enzymatic Analysis' has been expanded and updated in a new edition that includes recent developments in this area.[6] In addition to providing pertinent experimental details, the work deals with the availability, stability, purity, and physicochemical properties of coenzymes, metabolites, and other biochemical reagents required for assays. Problems relating to quantities and units in enzymology have been discussed.[7]

A simple procedure for determining the specificity of β-D-glucan hydrolases involves the use of pneumococcal type III polysaccharide as a substrate.[8] Whereas cellulase, laminarinase, and lichenase are inactive towards this polysaccharide, the two last enzymes hydrolyse its reduced and esterified form. Comparisons of the activities of the enzymes towards these and other substrates (*e.g.* laminarin, lichenin, carboxymethylcellulose) permit them to be distinguished, provided the oligosaccharides released are identified.

[1] 'Methods in Enzymology', Vol. XXXIV, ed. W. B. Jakoby and M. Wilchek, Academic Press, New York and London, 1974.
[2] 'Immobilized Biochemicals and Affinity Chromatography', ed. R. B. Dunlop, Plenum Press, New York, 1974.
[3] J. Konecny, *Chimia* (*Switz.*), 1975, **29**, 95.
[4] 'Methods in Enzymology', Vol. 41B, ed. W. A. Wood, Academic Press, New York and London, 1975.
[5] 'Methods in Enzymology', Vol. 42C, ed. W. A. Wood, Academic Press, New York and London, 1975.
[6] 'Methods of Enzymatic Analysis', 2nd English edn., ed. H. U. Bergmeyer, Academic Press, New York and London, 1974.
[7] R. Dybkaer, *Enzyme*, 1975, **20**, 46.
[8] M. A. Anderson and B. A. Stone, *F.E.B.S. Letters*, 1975, **52**, 202.

Enzymes involved in the metabolism of glycogen, and details of the enzymic assays used in the diagnosis of inborn errors of metabolism have been reviewed.[9]

Enzymes commonly exist in multiple molecular forms, even in a single organism; a series of four volumes has dealt with the molecular structures, physiological functions, developmental biology, and genetic aspects of isoenzymes.[10]

A series placing particular emphasis on physical, chemical, and kinetic methods used in investigations of enzymes has appeared.[11] The applications of spin-labelling techniques to conformational studies of enzymes,[12] 'half-of-the-sites' reactivity, and the role of subunit interactions in enzymic reactions[13] have been discussed.

The degradation of polysaccharides by *endo*- and *exo*-enzymes has been discussed from a theoretical viewpoint, both for a single enzyme and for mixtures of enzymes.[14]

The possibility that the rate-limiting step in the enzymic hydrolysis of aryl glycosides involves a change of conformation has been considered.[15] Particular attention was given to glycoside hydrolases, since no correlation is found between k_{cat} and the acidity of the aglycone for these enzymes.

The mechanisms of enzymic action are discussed briefly in a paperback in the Oxford Biology Readers series,[16] while a new book on enzyme kinetics has covered a number of topics, including one-substrate mechanisms, the steady-state rate law, methods of graphical and statistical assessment, inhibitors and activators, homotropic branching and linear mechanisms, co-operative interactions, *etc.*[17]

Evidence has been presented in support of the view that glycosyl–proton interchange underlies the actions catalysed by glycoside hydrolases and glycosyltransferases; these enzymes form a group of glycosylases that form one glycosyl bond at the expense of another.[18] The synthesis of α-D-gluco-oligosaccharides from β-D-glucopyranose and β-maltose by glucoamylase and β-amylase, respectively, confirmed this model, and showed that glycosyl transfer is more general than the term – which implies biochemical action on glycosidic bonds – suggests.

A recent edition of 'Enzymes in Food Processing' includes contributions both on chemical and technological aspects of the uses of enzymes in food processing.[19] Information on industrial preparations of microbial enzymes and the applications of enzymes in the Food Industry are accorded particular attention. Carbo-

[9] 'Principles and Methods', Vol. II, *Clinical Biochem.*, ed. H. C. Curtius and M. Roth.
[10] 'Isozymes', Vols. I—IV, ed. C. L. Markert, Academic Press, New York and London, 1975.
[11] 'Progress in Bio-organic Chemistry', Vol. 3, ed. E. T. Kaiser and F. J. Kezdy, Wiley, Chichester, 1974.
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[13] M. Lazdunski, in ref. 11, p. 81.
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[17] J. Tze-Fei Wong, 'Kinetics of Enzyme Mechanisms', Academic Press, New York and London, 1975.
[18] 'Carbohydrates in Solution', ed. R. F. Gould, *Adv. in Chem. Series*, No. 117, Amer. Chem. Soc., Washington D.C., 1973, p. 309.
[19] 'Enzymes in Food Processing', 2nd edn., ed. G. Reed, Academic Press, New York and London, 1975.

hydrases (particularly cellulases and pectolytic enzymes) and other enzymes used in food processing have been further discussed.[20, 21]

The uses of enzymes in the structural analysis of polysaccharides have been reviewed.[22]

Principles relating to the technology of immobilized enzymes have been discussed,[23] and details of the techniques used in the preparation of immobilized enzymes and in the associated enzyme engineering have appeared.[2, 24–27] The preparation, properties, and uses of immobilized enzymes have also been reviewed for non-specialists.[28] A collection of papers on the production of immobilized enzymes for use in industrial reactors has outlined some of the many problems encountered in the initial research, development, and engineering programmes.[29] Carriers, immobilization techniques, cofactor requirements, the design and operation of reactors, the properties of free and immobilized forms of enzymes, and immobilized coenzymes are discussed in detail. The use of immobilized enzymes for the degradation of milk and starch, and methods for the *in vitro* synthesis of edible carbohydrates have been discussed.[30] The progress made in the use of immobilized enzymes as industrial catalysts has been discussed.[31]

Certain natural and synthetic polymers have been selected for the immobilization of enzymes, since they best maintain the physical and chemical properties of the catalytically active protein.[32] Alkyl and aryl derivatives of agarose have been proposed as general supports for the immobilization of glycoside and polysaccharide hydrolases; the enzymes are bound by hydrophobic interactions, which can be varied by changing the hydrocarbon ligand.[33] Since the enzymes are not covalently linked to the matrix, they are readily recovered. Unlike ion-exchange chromatography, adsorption by hydrophobic interaction decreased with decreasing ionic strength and decreasing temperature.

The influence of the solvent on the motion of molecules 'immobilized' on polystyrene matrices and glass surfaces has been studied.[34] The effects of limited

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[25] 'Bonded Stationary Phases in Chromatography', ed. E. Grushka, Ann Arbor Science, Wiley, Chichester, 1974.
[26] 'Insolubilized Enzymes', ed. M. Salmona, C. Saronio, and S. Garattini, Raven Press, New York, 1974.
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diffusion[30] and internal diffusion[35] on Lineweaver–Burk plots for immobilized enzymes have been investigated. Diffusion has been shown to influence the apparent rate of denaturation of gel-entrapped enzymes.[36]

Many of the theoretical problems relating to immobilized enzymes can be resolved by collocation methods.[37] A graphical procedure that is rapid and sufficiently accurate for most practical purposes has been used to determine the 'effectiveness factor' of an immobilized enzyme.

Engasser and Horvath have discussed the action of inhibitors on the kinetic behaviour of enzymes in heterogeneous media. The effect of diffusional limitations was analysed for the inhibition of bound enzymes by substances that do not participate in the reaction,[38] by the product,[39] and by the substrate.[40] This analysis is discussed more fully in Chapter 8.

Acetamidodeoxygalactosidases, Acetamidodeoxyglucosidases, and Acetamidodeoxyhexosidases

The affinity chromatography of α-D-acetamidodeoxygalactosidase and β-D-acetamidodeoxyglucosidase on an agarose derivative that had been allowed to react with diazophenyl oxamate has been investigated.[41] The binding of the enzymes to the matrix, which hitherto has been considered to be specific for neuraminidase, is now thought to be non-specific, and it is doubtful whether the association is of a true affinity nature.

Various aspects of the electron microscopy of enzymes, particularly β-D-acetamidodeoxyglucosidases, have been reviewed.[42]

The purification of both forms (A and B) of β-D-acetamidodeoxyhexosidase from human sources has allowed their catalytic, immunological, physical, and genetic properties to be examined in detail; several models were proposed to explain the relation between the various forms of β-D-acetamidodeoxyhexosidases and the involvement of these forms in the G_{M_2}-gangliosidoses.[43] The catabolism of gangliosides in adults who are deficient in β-D-acetamidodeoxyhexosidase A has been investigated, and the findings were discussed with reference to current views on the A and B forms of this enzyme.[44]

A technique for resolving and determining the activities of the components of β-D-acetamidodeoxyhexosidase, based on micro-column chromatography on DEAE-cellulose, has been described.[45] The column effluent is led directly into the flow-stream of an autoanalyser and mixed with a buffered solution of a fluorogenic substrate (4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside) to produce a continuous trace of the elution profile of the enzymic

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[45] R. B. Ellis, J. U. Ikonne, and P. K. Masson, *Analyt. Biochem.*, 1975, **63**, 5.

activity. Good separation of four components of β-D-acetamidodeoxyhexosidase from human tissues was achieved in 30 min on 5 μl samples of 1% (w/v) homogenates. Immunological distinction between β-D-acetamidodeoxyhexosidases A and C present in human tissues, *etc.*, has been reported.[46]

β-D-Acetamidodeoxyhexosidase C has been separated from human-brain material by immunoadsorption of the A and B forms on a column of the immobilized antibody, followed by starch-block electrophoresis.[47] Some differences in the properties of β-D-acetamidodeoxyhexosidase C after each of these stages were revealed by comparison of the heat-inactivation characteristics and by filtration through molecular sieves. The C form (mol. wt. 2×10^5; pH optimum 6—7) possesses properties appreciably different from the A and B isoenzymes at all stages of purification, and was strongly adsorbed by DEAE-cellulose and Sephadex G-200, even at high ionic concentrations. The results support the view that the C form is under separate genetic control.

The levels of α-D-acetamidodeoxyglucosidase and β-D-acetamidodeoxyhexosidase activities of human-skin fibroblasts in patients with Maroteaux–Lamy disease were shown to be normal.[48]

Antisera have been raised to the A and B isoenzymes of β-D-acetamidodeoxyhexosidase from human liver.[49] A protein that cross-reacted with the enzyme was detected by an antibody-consumption method, and a cross-reacting protein of low molecular weight (2×10^4) was isolated from normal human liver and characterized; this protein is also present in the livers of patients with either Tay–Sachs or Sandhoff's diseases. β-D-Acetamidodeoxyhexosidases A and B gave immunological reactions almost identical with those of the low-molecular-weight protein. The possibility that this cross-reacting protein may be a subunit of β-D-acetamidodeoxyhexosidase was discussed.

The levels of activity and properties of β-D-acetamidodeoxy-galactosidase and -hexosidases from diploid WI-38 fibroblasts from embryonic human lung have been investigated.[50]

Chromatography of a crude extract of human placenta on concanavalin A immobilized on macroporous agarose has achieved purifications of β-D-acetamidodeoxyhexosidase up to 300-fold in a single step.[51] Since the capacity of immobilized concanavalin A for the enzyme is high, the method should be suitable for purification of the enzyme on a large scale. It was concluded that the enzyme is a glycoprotein.

Methods of preparing highly purified β-D-acetamidodeoxyhexosidases A and B from human placenta have been described.[52] The purified isoenzymes possess identical molecular weights, exhibit similar kinetics with synthetic fluorogenic substrates, and catalyse the hydrolysis of Tay–Sachs ganglioside and the asialo-derivative thereof. Although both isoenzymes consist of four subunits (each of

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mol. wt. 3.3 × 10^4), they differ in the way the subunits interact to form the fully associated enzymes, *i.e.* by hydrophobic association and by the formation of disulphide bonds. Isoenzyme A was converted into isoenzyme B by carefully controlled heating; since this conversion does not involve the removal of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues from the carbohydrate moieties, it was concluded that a change of conformation takes place. A model relating the β-D-acetamidodeoxyhexosidases in normal tissues with those in clinical conditions characterized by the absence of one or more of these enzymes (particularly Tay–Sachs disease and its variant forms) has been presented.

β-D-Acetamidodeoxyhexosidases have been obtained from human placenta by extensive use of gel filtration and ion-exchange chromatography.[53] The purified A and B isoenzymes migrated as single bands, which retained all the enzymic activity, on polyacrylamide disc electrophoresis. The placental enzymes differed electrophoretically and antigenically from the β-D-acetamidodeoxyhexosidases of human fibroblasts and liver. Neuraminidase did not convert the heat-labile A form into the heat-stable B form.

Human placental β-D-acetamidodeoxyhexosidases A and B exhibited maximum activity against 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galactopyranoside at pH 4.4, and the kinetics of the enzymes have been examined in detail.[54] On disc electrophoresis in the presence of sodium dodecyl sulphate, the *S*-carboxymethylated B isoenzyme (mol. wt. 1.0—1.4 × 10^5) gave one principal subunit (mol. wt. 1.7—1.8 × 10^4), whereas the A isoenzyme (mol. wt. 1.0—1.4 × 10^5) yielded a subunit of similar size and two additional protein bands (mol. wt. 3.5 × 10^4 and 5.5 × 10^4). The amino-acid compositions and *N*-termini of the subunits were compared.

β-D-Acetamidodeoxyhexosidase C from human placenta has been completely separated from the A and B forms.[55] The C isoenzyme differs from the A and B isoenzymes in the following ways: it is present in the supernatant fraction, rather than in the lysosomal fraction, of the extract; it has a neutral pH optimum and a higher molecular weight; and it lacks immunological relationships and activity on glycosides derived from 2-acetamido-2-deoxy-D-galactopyranose. β-D-Acetamidodeoxyhexosidase C was shown to be active in patients deficient in β-D-acetamidodeoxyhexosidases A and B, and it can be identified by its characteristic electrophoretic mobility. It was suggested that the genetic origin of placental isoenzyme C differs from that of the other isoenzymes.

The uptake of β-D-acetamidodeoxyglucosidase by cells and the intracellular fate and metabolic effect of this enzyme have been investigated with fibroblasts from normals and from patients with Sanfillipo B syndrome.[56] The uptake for both genotypes was specific, highly efficient, and constant over a period of at least six days; the enzyme may be taken up by adsorptive pinocytosis, and evidence was presented for the localization of the pinocytosized enzyme in the lysosomes. The results were evaluated with regard to enzyme-replacement

[53] S. K. Srivastava, C. A. Yogesh, A. Yoshida, and E. Beutler, *J. Biol. Chem.*, 1974, **249**, 2043.
[54] S. K. Srivastava, A. Yoshida, Y. C. Awasthi, and E. Beutler, *J. Biol. Chem.*, 1974, **249**, 2049.
[55] E. Penton, L. Poenaru, and J. C. Dreyfus, *Biochim. Biophys. Acta*, 1975, **391**, 162.
[56] K. von Figura and H. Kresse, *J. Clin. Invest.*, 1974, **53**, 85.

therapy, and it was concluded that doses previously used in attempts to correct Sanfillipo disease were insufficient to produce measurable effects.

Knowledge of the genetic relationships between β-D-acetamidodeoxyhexosidases A and B may aid in an understanding of the deficiency of β-D-acetamidodeoxyhexosidase associated with G_{M_2}-gangliosidosis.[57] A combination of immunological and electrophoretic techniques was used to identify human β-D-acetamidodeoxyhexosidases A and B with certainty in cell hybrids. Man–mouse somatic cell hybrids revealed that a gene involved in expression of β-D-acetamidodeoxyhexosidase is linked to genes coding for D-mannose phosphate isomerase and pyruvate kinase.

A dodecasaccharide that was obtained by hydrolysis of dermatan sulphate with testicular hyaluronidase, chondroitin lyase AC, and β-D-glucuronidase yielded a sulphated 2-amino-2-deoxy-D-galactose derivative when treated with human serum at pH 4.5 or 7.0.[58] No further degradation of the substrate occurred, suggesting that normal human serum possesses an *exo*-β-D-acetamidodeoxyhexosidase that is active towards dermatan sulphate.

β-D-Acetamidodeoxyhexosidase A in normal human urine has been purified by means of ion-exchange chromatography and affinity chromatography on concanavalin A immobilized on macroporous agarose.[59] The enzyme possessed K_m values of 0.51 and 0.28 mmol l^{-1} and pH optima of 4.4—4.5 and 4.4—4.5, respectively, for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides. The enzyme is a glycoprotein containing residues of 2-amino-2-deoxyhexose, D-galactose, D-glucose, D-mannose, and 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid.

The impaired catabolism of glycosaminoglycans in fibroblasts obtained from patients with Sandhoff's disease can be normalized by the addition of isoenzyme A of β-D-acetamidodeoxyhexosidase from normal human urine to the culture media.[60] However, if homogenates of the fibroblasts from either normals or patients with Tay–Sachs disease are used as sources of the enzyme, metabolic correction can still be achieved. Testing of individual A and B isoenzymes of β-D-acetamidodeoxyhexosidase obtained from normal fibroblasts showed that the A form possesses five times the corrective activity of the B form when their activities towards a synthetic substrate are compared. Although the B enzyme participates in the degradation of sulphated glycosaminoglycans in cultured cells, only homogenates of fibroblasts containing the A form released 2-acetamido-2-deoxy-D-glucose *in vitro* from oligosaccharides derived from hyaluronic acid.

Preparative discontinuous electrophoresis on polyacrylamide gels has been used to separate the A and B forms of β-D-acetamidodeoxyglucosidase present in bovine spleen.[61] Each of the separated forms was free from other glycoside hydrolase activities and gave a single band on disc electrophoresis. Each of the isoenzymes cleaved phenyl, 4-nitrophenyl, and 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides, but neither released glycopeptides

[57] P. A. Lalley, M. C. Rattazzi, and T. B. Shows, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1569.
[58] J. Singh, G. V. Coppa, and N. Di Ferrante, *Enzyme*, 1975, **19**, 15.
[59] D. K. Banerjee and D. Basu, *Biochem. J.*, 1975, **145**, 113.
[60] M. Cantz and H. Kresse, *European J. Biochem.*, 1974, **47**, 581.
[61] E. Werries and L. Fressmann, *Z. physiol. Chem.*, 1974, **355**, 1479.

from hen-egg albumen, although it contains terminal 2-acetamido-2-deoxy-β-D-glucosyl residues. The physical properties and biological activities of two forms of α-D-acetamidodeoxyglucosidase from bovine spleen have been investigated.[62]

2-Acetamido-2-deoxy-D-glucono-1,4- and -1,5-lactones and 2-acetamido-2-deoxy-D-gluconic acid have been examined as inhibitors of the β-D-acetamido-deoxyglucosidase from bovine epididymis.[63] Similar tests with 2-acetamido-2-deoxy-5,6-*O*-isopropylidene-D-glucono-1,4-lactone and 2-acetamido-2-deoxy-4,6-*O*-isopropylidene-D-glucono-1,5-lactone, which are protected against inter-conversion of the lactones, showed the latter compound to be appreciably more active.

β-D-Acetamidodeoxyhexosidase from commercial bovine serum albumin was inhibited by D-glucoheptono-, D- and L-glucono-, D-galactono-, D-fucono-, L-arabinono-, D-ribono-, and L-mannono-lactones, and L-ascorbic acid.[64] The kinetics of inhibition of the hydrolysis of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides with the purified enzyme and with rat-liver lysosomes were carefully examined. The lactones of the corresponding acetamido-sugars were found to be competitive inhibitors. D-Gluconolactone and L-ascorbic acid gave 'mixed'-type inhibition with the first substrate, whereas they behaved as non-competitive inhibitors with the other substrate.

The B isoenzyme of the *exo*-β-D-acetamidodeoxyglucosidase present in porcine epididymis has been purified by a new systematic procedure that yields a preparation possessing a specific activity as good as that obtained by an empirical procedure.[65] Three steps are involved: (i) initial fractionation of the proteins according to their molecular size, (ii) ion-exchange chromatography to isolate proteins of charge closely similar to that of the enzyme, and (iii) resolution based on the isoelectric points of the remaining proteins.

The subcellular distribution of β-D-acetamidodeoxyhexosidase in rabbit liver has been determined.[66] The distribution of β-D-acetamidodeoxyglucosidase has been studied in cultured, murine peritoneal macrophages stimulated with thioglycolate.[67] The effects of streptozotocin-induced diabetes on the β-D-acetamido-deoxyglucosidase activity in various organs and sera of rats have been investigated.[68]

The K_m values of β-D-acetamidodeoxy-galactosidase and -glucosidase activities in distal colonic tumours induced in rats by 1,2-dimethylhydrazine were shown to differ significantly from those of the same enzymes present in the colonic mucosa of control and tumour-bearing rats and in proximal colonic tumours.[69]

β-D-Acetamidodeoxyglucosidase has been partially purified from epididymal spermatozoa and testes of mature rats; sedimentation analysis revealed the presence of three isoenzymes (9.2, 8.1, and 6.4*S*, respectively).[70] Hormonal

[62] G. Mersmann, K. von Figura, and E. Buddecke, *Biochim. Biophys. Acta*, 1974, **364**, 88.
[63] M. Pokorny, E. Zissis, H. G. Fletcher, jun., and N. Pravdić, *Carbohydrate Res.*, 1974, **37**, 321.
[64] J. N. Kanfer and C. H. Spielvogel, *Biochim. Biophys. Acta*, 1974, **327**, 405.
[65] H. K. Robinson and D. H. Leaback, *Biochem. J.*, 1974, **143**, 143.
[66] R. T. Dean, *Biochem. J.*, 1974, **138**, 407.
[67] S. Gordon, J. Todd, and Z. A. Cohn, *J. Exp. Med.*, 1974, **139**, 1228.
[68] P. D. Rosenblit, R. P. Metzger, and A. N. Wick, *Proc. Soc. Exp. Biol. Med.*, 1974, **145**, 244.
[69] N. Mian, D. G. Herries, D. M. Cowen, and E. A. Batte, *Biochim. Biophys. Acta*, 1975, **391**, 179.
[70] G. C. Majumder and R. W. Turkington, *Biochemistry*, 1974, **13**, 2857.

regulation of the isoenzymes of β-D-acetamidodeoxyglucosidase during spermatogenesis in rats has been studied.[71] β-D-Acetamidodeoxyglucosidase present in fowl semen has been examined.[72]

The α- and β-D-acetamidodeoxyglucosidase activities of limpets (*Patella vulgata*) have been ascribed to the presence of two enzymes: one (mol. wt. 2.17×10^5) hydrolysed both the α- and β-glycosides, and is referred to as an αβ-D-acetamidodeoxyglucosidase, whereas the other (mol. wt. 1.36×10^5) hydrolysed only the β-glycoside.[73] Both enzymes were isolated and shown to be glycoproteins containing 12% of hexose (principally D-galactose). The enzymes have similar amino-acid and 2-amino-2-deoxyhexose contents, but they differ insofar as the β-D-acetamidodeoxyglucosidase contains 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (9%).

Extracts of *Actinobacillus* and *Pseudomonas* species exhibiting symbiotic growth in media containing keratan sulphate as the only source of carbon were shown to contain, *inter alia*, an *exo*-β-D-acetamidodeoxyglucosidase, which, together with other enzymes, catalysed extensive degradation of corneal keratan sulphate.[74] The distribution of the various enzymic activities and the substrate specificity of the *exo*-β-D-acetamidodeoxyglucosidase were determined.

The inhibition constants (K_i) of 4-nitro- and 4-amino-phenyl 2-acetamido-2-deoxy-1-thio-β-D-gluco- and -galacto-pyranosides for the β-D-acetamidodeoxyglucosidase obtained from *Aspergillus niger* have been determined using the 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glycopyranosides as substrates.[75]

A procedure for the isolation of mutants of an *exo*-β-D-acetamidodeoxyglucosidase from *Bacillus subtilis* has used a plate assay incorporating a fluorescent substrate.[76] A procedure for screening clones for alterations in the products of specific genes has been applied to mutagenized cultures of *Dictyostelium discoideum*.[77] Six strains containing less β-D-acetamidodeoxyglucosidase activity than in the wild type were recovered. The enzyme in another four strains was temperature sensitive and possessed an altered substrate affinity in one of the strains.[78] A minor isoenzyme component of the β-D-acetamidodeoxyglucosidase present in growing cells of *D. discoideum* has been purified; it differs from the principal isoenzyme in being stage-specific and accumulating shortly after development had been initiated. Its pH optimum, thermostability, and sensitivity to heavy-metal ions also distinguished it from the principal isoenzyme.

Antibodies have been raised against a purified β-D-acetamidodeoxyhexosidase obtained from *D. discoideum*.[79] Ouchterlony tests produced single precipitin bands with both β-D-acetamidodeoxy-glucosidase and -galactosidase activities, which were co-precipitated in identical proportions in quantitative immunoprecipitation reactions. It was concluded that one species of enzyme catalyses the hydrolysis of both 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides.

[71] G. C. Majumder, S. Lessin, and R. W. Turkington, *Endocrinology*, 1975, **96**, 890.
[72] Y. Kannan, *J. Reproduction Fertility*, 1974, **40**, 227.
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[79] D. Every and J. M. Ashworth, *Biochem. J.*, 1974, **143**, 785.

'*endo*-β-D-Acetamidodeoxyglucosidases' have been identified in *Diplococcus pneumoniae*[80] and *Streptomyces griseus*.[81, 82]

α-L-Arabinofuranosidases

The α-L-arabinofuranosidase activity towards 4-methylumbelliferyl α-L-arabinofuranoside in fibroblasts from patients with Gaucher's disease is appreciably less than normal.[83] Further investigation indicated that a single enzyme exhibits the α-L-arabinofuranosidase, β-D-glucosidase, and β-D-xylosidase activities found in the fibroblasts. The β-D-galactosidase of human liver has been shown to cleave α-L-arabinofuranosyl linkages.[84]

β-D-Fructofuranosidases

The properties of tomato β-D-fructofuranosidase have been investigated.[85] Stabilization of β-D-fructofuranosidase at the cell-wall site of cycad pollens occurs by the formation of complexes with pectic substances.[86]

Changes in the β-D-fructofuranosidase activity in tobacco leaves during curing have been related to the degradation of carbohydrates present.[87]

The synthesis of an endocellular β-D-fructofuranosidase by a constitutive mutant of *Bacillus subtilis* can be induced by sucrose.[88] After purification, the enzyme (mol. wt. 5.5×10^4) migrated as a single band on polyacrylamide gel electrophoresis, either in the absence or the presence of sodium dodecyl sulphate; it was activated by potassium ions but was inhibited by 4-hydroxymercuribenzoate.

The mechanisms of *in vivo* and *in vitro* inhibition of the β-D-fructofuranosidase of *Saccharomyces cerevisiae* by mercury compounds were found to be similar with respect to the effective concentrations of the inhibitors.[89] Contrary to previous reports, the enzyme neither dimerized in the presence of mercury compounds nor did it exhibit competitive inhibition. Another inconsistency with previous work appears to be the marked dependencies of the inhibition on pH and the concentration of anions.

The molecular forms of yeast (*Saccharomyces*) β-D-fructofuranosidase have been studied.[90] Gel filtration demonstrated the presence of not only a carbohydrate-free light form of the enzyme and a heavy form containing 50% of carbohydrate, but also a continuous spectrum of molecular forms that probably arise from sequential addition of D-mannose to the light form during the secretion process, culminating in formation of the enzyme outside the cytoplasmic mem-

[80] S. Ito, T. Muramatsu, and A. Kobata, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 938.
[81] A. L. Tarentino and F. Maley, *J. Biol. Chem.*, 1974, **249**, 811.
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[83] B. Hultberg, S. Sjöblad, and P. A. Öckerman, *Clinica Chim. Acta*, 1973, **49**, 93.
[84] A. G. W. Norden, L. L. Tennant, and J. S. O'Brien, *J. Biol. Chem.*, 1974, **249**, 7969.
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[87] T. Kakie and M. Makinoda, *J. Agric. Chem. Soc. Japan*, 1973, **47**, 659.
[88] F. Kunst, M. Pascal, J. A. Lepesant, J. Walle, and R. Dedonder, *European J. Biochem.*, 1974, **42**, 611.
[89] D. K. Kidby, *J. Gen. Microbiol.*, 1974, **84**, 343.
[90] F. Moreno, A. G. Ochoa, S. Gascón, and J. R. Villanueva, *European J. Biochem.*, 1975, **50**, 571.

brane. Separation of these forms by ion-exchange chromatography and isoelectric focusing was facilitated by removal, by precipitation with ammonium sulphate, of the heavy form. The effects of 2-deoxy-D-*arabino*-hexose and cycloheximide on the biosynthesis of the molecular forms of this β-D-fructofuranosidase were examined.

β-D-Fructofuranosidase (mol. wt. 1.6×10^5) has been obtained from *Streptococcus mutans* by means of gel filtration and ion-exchange chromatography; only one band was observed when the purified enzyme was subjected to polyacrylamide gel electrophoresis and immunodiffusion.[91] The enzyme exhibited maximum activity at pH 5.25, and its properties were compared with those of an associated dextransucrase. The β-D-fructofuranosidase and dextransucrase of *S. mutans* have been examined by immunodiffusion, using antisera directed against each enzyme.[92] The cross-reactions observed depended on the strain of the microorganism used.

Active immobilized forms of β-D-fructofuranosidase have been obtained by embedding the enzyme within a polyacrylamide gel, by adsorbing the enzyme on carboxymethylcellulose,[85] and by trapping the enzyme in spherical pellets of agar.[93]

α-L-, α-1,2-L-, and β-D-Fucosidases

The affinity chromatography of α-1,2-L-fucosidase on an agarose derivative that had reacted with diazophenyl oxamate has been investigated[41] (see also p. 330). Starch-gel electrophoresis has been used to study the α-L-fucosidases present in cultured skin fibroblasts, leucocytes, long-term lymphoid cell lines, plasma, and sera.[94] The enzyme from lymphoid cell lines was shown to consist of up to six isoenzymes and a diffuse region that was of low staining intensity. The enzymes from leucocytes and fibroblasts were less clearly resolved, but they appeared to contain components of mobilities similar to those of the lymphoid cells, whereas each of the enzymes from sera or plasma showed only a diffuse region of activity with a mobility slightly greater than that of the lymphoid isoenzymes.

A study of human-liver α-L-fucosidase by starch-gel electrophoresis and isoelectric focusing revealed the presence of six forms (p*I*'s 6.9, 6.5, 6.1, 5.9, 5.7, and 5.5) of the enzyme.[95] Treatment of the enzyme with neuraminidase reduced the relative proportions of the two forms of highest acidity. Liver tissues from a patient with fucosidosis contained 4% of the normal level of α-L-fucosidase activity, and were deficient in all forms of the enzyme. The K_m values for the enzymes from normal and pathological tissues are 0.085 and 0.058 mmol l^{-1}, respectively, using 4-methylumbelliferyl α-L-fucopyranoside as substrate; the pH–activity profiles of both enzymes are very similar, with pH optima at pH 5.4 and 5.3, respectively.

[91] K. Fukui, Y. Fukui, and T. Moriyama, *J. Bacteriol. Rev.*, 1974, **118**, 796.
[92] K. Fukui, Y. Fukui, and T. Moriyama, *Infection and Immunity*, 1974, **10**, 985.
[93] K. Toda and M. Shoda, *Biotechnol. and Bioeng.*, 1975, **17**, 481.
[94] B. M. Turner, N. G. Beratis, V. S. Turner, and K. Hirschhorn, *Clinica Chim. Acta*, 1974, **57**, 29.
[95] J. A. Alhadeff, A. L. Miller, D. A. Wenger, and J. S. O'Brien, *Clinica Chim. Acta*, 1974, **57**, 307.

A method for the assay of α-L-fucosidase has been based on the use of 4-methylumbelliferyl α-L-fucopyranoside; this substrate was hydrolysed more readily than the corresponding 4-nitrophenyl glycoside by human-liver α-L-fucosidase, where human-serum α-L-fucosidase showed approximately the same activity towards both substrates.[96] The pH–activity profiles of the enzyme are similar for both substrates, and both isoenzymes of human α-L-fucosidase are active against these substrates. The greater sensitivity of the fluorescence method enhances its use in the diagnosis of fucosidosis (mucopolysaccharidosis F).

The β-D-galactosidase of human liver has been shown to cleave β-D-fucosides.[84]

A sensitive fluorometric assay of α-L-fucosidase, which also uses 4-methylumbelliferyl α-L-fucopyranoside, has been reported for sera and plasma.[97] Several normal individuals were found to have an α-L-fucosidase of very low activity; this enzyme has a different pH–activity profile from, but a K_m value of the same order of magnitude as, the enzyme of high activity.

The elution profiles obtained on gel filtration of a β-D-fucosidase and a unique β-D-galactosidase present in porcine kidney were very similar.[98] Investigation of these enzymes showed that they possess similar pH optima and stabilities, thermostabilities, and susceptibilities to inhibitors, and it was concluded that mammalian β-D-galactosidase is responsible for β-D-fucosidase activity.

The effects of streptozotocin-induced diabetes on the α-L-fucosidase activities of various organs and sera of rats have been investigated.[68]

The K_m values of α-L-fucosidase of distal colonic tumours induced in rats by 1,2-dimethylhydrazine differed significantly from those for the enzyme in the colonic mucosa of control and tumour-bearing rats and in proximal colonic tumours.[69]

D-Galactosidases, β-D-Galactosyl- and β-D-Galactosyl-D-glucosylceramidases, and β-D-Galactosylsphingosidases

A derivative of agarose carrying octyl groups has been used in the purification of β-D-galactosidase by affinity chromatography; the interaction between the enzyme and affinant is hydrophobic in nature, and depends on the length of the alkyl chain.[33] The desired specificity can be achieved by optimization of the length of the alkyl chain. The affinity chromatography of β-D-galactosidase on an agarose derivative that had been treated with diazophenyl oxamate, a matrix hiterto considered to be specific for neuraminidase, has been investigated [41] (see also p. 330).

Both human and canine globoid cell leukodystrophy are caused by deficiencies in β-D-galactosyl-ceramidase and -sphingosidase, although different mutations underlie the enzymic defects.[99]

β-D-Galactosidases in the brains of patients with G_{M_1}-gangliosidoses types 1 and 2 have pH optima of 4.0 and 5.5, respectively, compared with a value of pH 4.0 for the normal enzyme; the liver enzymes possess pH optima of 5—6 in both types of gangliosidosis, compared with a value of pH 4.5 for the normal

[96] D. Robinson and R. Thorpe, *Clinica Chim. Acta*, 1974, **55**, 65.
[97] S. Wood, *Clinica Chim. Acta*, 1975, **58**, 251.
[98] G. J. Wiederschain, *Doklady Akad. Nauk S.S.S.R.*, 1973, **211**, 974.
[99] Y. Suzuki, T. Miyatake, T. F. Fletcher, and K. Suzuki, *J. Biol. Chem.*, 1974, **249**, 2109.

enzyme.[100] The enzymes in pathological livers were more stable than the normal enzyme at 42 °C, and different thermostabilities were observed for the brain enzymes. Gel filtration of normal liver β-D-galactosidase activity yielded two peaks, both exhibiting activity at pH 3.6 and 6.5. Similar fractionation of the enzyme from type-1 liver gave two peaks corresponding to the normal isoenzymes; however, one was active at pH 3.6 but not at pH 6.5, and *vice versa*. The enzyme from type-2 liver gave only one peak that was active at pH 3.6 but not at pH 6.5.

The activities of β-D-galactosyl- and β-D-galactosyl-D-glucosyl-ceramidases in homogenates of human fibroblasts and leucocytes have been determined.[101] It was possible to improve the sensitivities of the assay by using new conditions for incubation. The activity of β-D-galactosyl-D-glucosylceramidase was much higher than that of β-D-galactosylceramidase, and this finding should provide a sensitive method for the diagnosis of globoid cell leukodystrophy (Krabbe's disease). In fact, prenatal diagnosis of this disease has been achieved already.

The levels of β-D-galactosidase have been measured in jejunal biopsy specimens from normals and from sibling cases involving a deficiency of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase–isomaltase').[102]

The α-D-galactosidase in human liver active against 4-methylumbelliferyl α-D-galactopyranoside has been separated into four components (p*I* values 4.9, 4.5, 4.2, and 3.9) by isoelectric focusing.[103] Although differences were noted in the thermostabilities and the inhibition (by *myo*-inositol) of these components, the ratios of the activities against 4-methylumbelliferyl α-D-galactopyranoside and a trihexosylceramide were similar. This similarity was also observed for an α-D-galactosidase present in human kidney. The α-D-galactosidase activities in the kidneys and livers of cases of Fabry's disease are approximately 2% and 20% of normal, respectively. Isoelectric focusing of the residual α-D-galactosidase in both organs furnished a single component possessing activity towards 4-methylumbelliferyl α-D-galactopyranoside and trihexosylceramide. Kidney and urinary α-D-galactosidases have been obtained from patients with Fabry's disease; comparison of the enzymes with that from normal urine indicated that no cross-reacting material is present in the pathological specimens.[104] The glycosphingolipid-specific β-D-galactosidases present in normal human liver have been investigated using radiolabelled D-galactosylceramide, lactosylceramide, G_{M_1}-ganglioside, and asialo-G_{M_1}-ganglioside as substrates.[105] Chloride ions were required to stimulate and stabilize the reactions. Isoelectric focusing fractionated the β-D-galactosidase activities into three components (p*I* values 4.1—4.2, 4.5—4.6, and 4.8—4.9) that were active against 4-methylumbelliferyl β-D-galactopyranoside, lactosylceramide, and asialo-G_{M_1}-ganglioside; two of them also showed activity against D-galactosylceramide and G_{M_1}-ganglioside.

[100] T. Yutaka, S. Okada, K. Mimaki, T. Sugita, and H. Yabuuchi, *Clinica Chim. Acta*, 1975, **59**, 283.
[101] D. A. Wenger, M. Sattler, C. Clark, and H. McKelvey, *Clinica Chim. Acta*, 1974, **56**, 199.
[102] H. Preiser, D. Menard, R. K. Crane, and J. J. Cerda, *Biochim. Biophys. Acta*, 1974, **363**, 279.
[103] I. Kano and T. Yamakawa, *Chem. and Phys. Lipids*, 1974, **13**, 283.
[104] P. J. G. M. Rietra, J. L. Molenaar, M. N. Hamers, J. M. Tager, and P. Borst, *European J. Biochem.*, 1974, **46**, 89.
[105] Y. Suzuki and K. Suzuki, *J. Biol. Chem.*, 1974, **249**, 2098.

β-D-Galactosidase has been purified (1.7×10^4-fold) from human liver; the purified enzyme appeared as a single band on polyacrylamide gel electrophoresis.[84] Double immunodiffusion of rabbit anti-(β-D-galactosidase) antiserum against either the supernatant fluid of liver homogenates or the purified enzyme gave a single precipitin band. The apparent molecular weight of the enzyme is $6.5—7.5 \times 10^4$ (by gel filtration), whereas that of the denatured, reduced, and carboxymethylated enzyme is 7.2×10^4 (by gel electrophoresis in sodium dodecyl sulphate). The purified enzyme liberated D-galactose from synthetic chromogenic and fluorogenic β-D-galactosides, and from lactose, 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose, and desialylized fetuin. The same catalytic site(s) appears to be responsible for the hydrolysis of G_{M_1}-ganglioside and 4-methylumbelliferyl β-D-galactopyranoside. The enzyme also cleaved β-D-fucosides and α-L-arabinosides.

The livers of patients with G_{M_1}-gangliosidosis do not contain a β-D-galactosidase capable of hydrolysing 4-methylumbelliferyl β-D-galactopyranoside, lactosylceramide, G_{M_1}-ganglioside, and asialo-G_{M_1}-ganglioside, whereas the activities against β-D-galactosylceramidase are abnormally high.[106] The residual activity against 4-methylumbelliferyl β-D-galactopyranoside exhibited an abnormal isoelectric point (p*I* 4.6—4.7). It was concluded that, in addition to the depletion of certain glycosphingolipid-specific β-D-galactosidases, other complex abnormalities of the β-D-galactosidases are involved in the disease. Differences between the isoelectric-focusing patterns of the β-D-galactosyl- and lactosylceramidases provide another means of differentiating between G_{M_1}-gangliosidosis types 1 and 2.

The distribution of β-D-galactosidases active against lactosylceramide and asialo-G_{M_1}-ganglioside in the livers of patients with globoid cell leukodystrophy was found to be relatively normal by isoelectric focusing.[107] However, the distribution of the activity against 4-methylumbelliferyl β-D-galactopyranoside is abnormal, since, instead of three peaks, only one principal peak (p*I* 4.6—4.8) was detected. The residual β-D-galactosidase activity against D-galactosylceramide possessed p*I* values of 4.6—4.8, which corresponded with those of the non-glycosphingolipid-specific β-D-galactosidase. These data indicate that the genetic mutation in globoid cell leukodystrophy causes more than a deficiency of a single glycosphingolipid-specific β-D-galactosidase.

The α- and β-D-galactosidases extracted from human placenta have been purified, by as much as 300-fold, in a single step by chromatography on concanavalin A immobilized on macroporous agarose.[51] Their interaction with the affinant indicated that the enzymes are glycoproteins, but the fractionation procedure is sufficiently selective to separate them from a number of other glycoproteinaceous enzymes present in placenta. Since immobilized concanavalin A has a high capacity for the enzymes, the method is suitable for large-scale purification.

The concentrations, latencies, pH optima, and heparin-induced inhibitions of β-D-galactosidases in neutrophils and eosinophils of equine blood have been determined.[108]

[106] Y. Suzuki and K. Suzuki, *J. Biol. Chem.*, 1974, **249**, 2113.
[107] Y. Suzuki and K. Suzuki, *J. Biol. Chem.*, 1974, **249**, 2105.
[108] R. A. Heyneman, D. Monbaliu-Bawens, and R. E. Vercauteren, *Comp. Biochem. Physiol.*, 1975, **50B**, 463.

Four forms of porcine kidney β-D-galactosidase have been separated by means of gel filtration; all forms were active against 4-nitrophenyl β-D-galactopyranoside, but only three of them were active against lactose.[98] The lactose-inactive form showed the highest activity against 4-nitrophenyl β-D-galactopyranoside, and also exhibited β-D-fucosidase activity. The β-D-fucosidase and β-D-galactosidase activities appear to be manifest by a single enzyme.

The principal β-D-galactosidase (p*I* 6.3) of rabbit brain has been purified and shown to be active against several gangliosides.[109] The activities towards G_{M_1} ganglioside and D-galactosyl-D-glucosylceramide were not separated by polyacrylamide gel electrophoresis in two buffer systems: pH optima of 4.3 and 4.5 and K_m values of 78 and 17 μmol l^{-1} were observed for the two substrates, respectively, in acetate buffer. Hydrolysis of natural and synthetic substrates by the enzyme was inhibited by D-galactono-1,4-lactone, D-galactose, and lactose. These and other data implied that the activities against the substrates tested are manifest by a single enzyme.

The subcellular distribution of β-D-galactosidase in rabbit liver has been determined.[66]

Inbred strains of mice (typified by C3H/HeJ) have twice as much β-D-galactosidase activity in their tissues as that of such strains as DBA/2J.[110] The difference in activity is determined by a locus-designated β-D-galactosidase on chromosome nine. Studies of the pH optima, thermal stability, molecular weight, and intracellular location of the β-D-galactosidase activity in crude homogenates suggested that a single enzyme is present. Partially purified β-D-galactosidases from each group could not be distinguished by their molecular weights, thermal stabilities, and K_m values for 4-nitrophenyl β-D-galactopyranoside.

The distribution of β-D-galactosidase has been studied in cultured murine peritoneal macrophages stimulated with thioglycolate.[67]

The effects of streptozotocin-induced diabetes on the activities of β-D-galactosidase in various organs and sera of rats have been investigated.[68] The β-D-galactosidase in rat brain has been partially purified and characterized; it was active towards various glycosphingolipids.[111]

The K_m value of the β-D-galactosidase present in distal colonic tumours induced in rats by 1,2-dimethylhydrazine was found to be different from those of β-D-galactosidases present in the colonic mucosa of control and tumour-bearing rats and in proximal colonic tumours.[69]

β-D-Galactosidase has been partially purified from the testes and epididymal spermatozoa of mature rats; two isoenzymes (9.9*S* and 6.4*S*) were identified.[70] Hormonal regulation of the isoenzymes of β-D-galactosidase during spermatogenesis in rats has been investigated.[71]

The urinary excretion of β-D-galactosidase during nephrotoxic serum glomerulonephritis and mercuric chloride-induced tubulonephritis in rats has been compared with that of lactate dehydrogenase, alkaline and acid phosphatases, and aspartate aminotransferase.[112]

[109] J. W. Callahan and J. Gerrie, *Biochim. Biophys. Acta*, 1975, **391**, 141.
[110] J. Felton, M. Meisler, and K. Paigen, *J. Biol. Chem.*, 1974, **249**, 3267.
[111] T. Miyatake and K. Suzuki, *J. Biol. Chem.*, 1975, **250**, 585.
[112] M. Sternberg, I. Szlamka, M. Moisy, P. Rebeyrotte, and G. Lagrue, *Z. klin. Chem. klin. Biochem.*, 1974, **12**, 543.

The α- and β-D-galactosidase activities in fowl semen have been investigated.[72]

The properties and functions of α-D-galactosidases in higher plants have been reviewed.[113] The separation of α- and β-D-galactosidases from *Cajanus indicus* has been reported, and various properties of the enzymes were investigated.[114]

Coffee-bean α-D-galactosidase has been purified by affinity chromatography on macroporous agarose substituted with α-D-galactopyranosylamine residues.[115] The enzyme was separated by polyacrylamide gel electrophoresis into three components, each of which is enzymically active.

Protein bodies and spherosomes isolated from mature, but ungerminated, seeds of *Sorghum bicolor* have been shown to contain β-D-galactosidase activities.[116]

Extracts of *Actinobacillus* and *Pseudomonas* species exhibiting symbiotic growth in media containing keratan sulphate as the only source of carbon contained an *exo*-β-D-galactosidase.[74] This enzyme, in combination with an *endo*-glycosidase, a sulphatase, and an *exo*-β-D-acetamidodeoxyglucosidase, catalysed extensive cleavage of corneal keratan sulphate. The *Actinobacillus exo*-β-D-galactosidase is exceptionally active.

The ability of mould fungi to synthesize β-D-galactosidase has been studied.[117] The most active producers, *Alternaria tenuis* and *Aspergillus awamori*, were examined in detail; the synthesis of the enzyme by *A. awamori* depended on the nitrogen content of the medium. The ability of four strains of *Escherichia coli* to synthesize an intracellular β-D-galactosidase when grown on media containing lactose has been investigated. The highest activity was produced in a strain that also synthesized colibacterin.

Several derivatives of porous glass have been examined as affinity-chromatography matrices for purification of the β-D-galactosidase from *Aspergillus niger*.[118] An azelaic acid derivative of alkylamino-glass possessed good load capacity and efficiency after coupling to 4-aminophenyl 1-thio-β-D-galactopyranoside, a competitive inhibitor of the enzyme; malonic acid derivatives were also effective. Hydrophobic interactions between the spacer arm and the enzyme appear to contribute to the separations achieved.

A procedure reported for the lysis of bacterial cells enables the β-D-galactosidase to be measured.[119] The method which involves the use of sodium dodecyl sulphate, toluene, manganous sulphate, and 2-mercaptoethanol, is more reliable and reproducible, and gives higher yields than currently used techniques. Possible inhibition of the enzyme by the reagents is prevented by appropriate dilution of the lysate, and a correction curve is used when sufficient dilution cannot be achieved.

The defect in the synthesis of proteins that occurs when *Escherichia coli* is grown at low temperatures has been studied.[120] For β-D-galactosidase activity, transcription and translocation were found to take place slowly, but normally,

[113] J. B. Pridham and P. M. Dey, in 'Plant Carbohydrate Biochemistry', ed. J. B. Pridham, Academic Press, New York and London, 1974, p. 83.
[114] P. M. Dey and M. Dixon, *Biochim. Biophys. Acta*, 1974, **370**, 269.
[115] N. Harpaz, H. M. Flowers, and N. Sharon, *Biochim. Biophys. Acta*, 1974, **341**, 231.
[116] C. A. Adams and L. Novellie, *Plant. Physiol.*, 1975, **55**, 7.
[117] A. S. Tikhomirova, A. K. Kulikova, and R. V. Feniksova, *Mikrobiologia*, 1974, **43**, 257.
[118] G. Baum, *J. Chromatog.*, 1975, **104**, 105.
[119] S. L. Putnam and A. L. Koch, *Analyt. Biochem.*, 1975, **63**, 350.
[120] W. A. Anderson, *J. Bacteriol.*, 1975, **121**, 907.

at 5 °C. Purification of the repressor β-D-galactosidases of *E. coli*, and determination of the molecular weights of the subunits, *etc.*, indicated that the *lac*-repressor may be fused on to β-D-galactosidase.[121]

The reaction of *E. coli* β-D-galactosidase with 2-nitrophenyl β-D-galactopyranoside has been investigated over the temperature range −30 to +25 °C in 50% aqueous DMSO.[122] At temperatures below −10 °C, the turnover became very slow, and a surge of 2-nitrophenol was observed, indicating that the breakdown of a D-galactosyl-enzyme intermediate is rate-limiting.

β-D-Galactopyranosyl pyridinium salts have proved to be well-behaved substrates for *E. coli* β-D-galactosidase, catalysis occurring by interaction of the salt with the active site of the enzyme.[15] Values of $\log k_{cat}$ for a series of pyridinium salts showed a linear relationship with the pK_a of the parent pyridines. The β-D-galactopyranosyl derivatives of pyridine and 4-bromoisoquinoline each exhibited an α-deuterium kinetic isotope effect, indicating that the formation of a D-galactopyranosyl cation is rate-limiting. The behaviour of the pyridinium salts contrasts with that of aryl β-D-galactopyranosides.

The inhibition of *E. coli* β-D-galactosidase by D-galactal has been shown to be reversible and time-dependent.[123] Rate constants observed for the binding (2.7×10^2 s^{-1} mol^{-1}) and the release (4.6×10^{-3} s^{-1}) of D-galactal in the presence of imidazole and hydrochloric acid are consistent with an apparent K_i of 1.4×10^{-5} mol l^{-1} for the glycal as a competitive inhibitor. Attempts to trap or to analyse for a D-galactal-enzyme intermediate failed, although the rate of formation of this intermediate was significantly reduced in the presence of deuterium oxide. The use of deuterium oxide instead of water also raised the apparent K_i for D-galactal by a factor of 2.1. By contrast, a conventional competitive inhibitor (isopropyl 1-thio-β-D-galactopyranoside) exhibited no detectable lag in binding or release, and its K_i was unaffected in deuterium oxide. The rate of release of D-galactal from an enzyme–inhibitor complex (formed by incubation of the β-D-galactosidase with D-galactal before hydrolysis of 2-nitrophenyl β-D-galactopyranoside) could be accounted for by its rate of conversion into 2-deoxy-D-*lyxo*-hexose. Incubation of 2-deoxy-D-*lyxo*-hexose with *E. coli* β-D-galactosidase yielded 3,6-anhydro-2-deoxy-D-*lyxo*-hexose ('D-isogalactal') (2%), which is considered to result from cyclization of 2,3-dideoxy-D-*threo*-hex-2-enose ('D-pseudogalactal') formed from the 2-deoxy-D-*lyxo*-hexopyranosyl cation.[124] This pathway accords with the view that the cyclic carboxonium ion results from the action of β-D-galactosidase on appropriate aldohexo-pyranoses and -pyranosides.

Incubation of 2-nitrophenyl β-D-galactopyranoside with adenosine and crystalline *E. coli* β-D-galactosidase afforded adenosine 2′(3′)- and 5′-β-D-galactopyranosides.[125] A method for determining the binding constant of the activating antibody towards one of the determinants of *E. coli* β-D-galactosidase has been reported.[126] The binding of *E. coli* β-D-galactosidase by cells obtained from germ-free piglets deprived of colostrum has been investigated.[127]

[121] B. Müller-Hill and J. Kania, *Nature*, 1974, **249**, 561.
[122] A. L. Fink and K. J. Angelides, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 701.
[123] D. F. Wentworth and R. Wolfenden, *Biochemistry*, 1974, **13**, 4715.
[124] J. Lehmann and E. Schröter, *Carbohydrate Res.*, 1974, **36**, 303.
[125] Y. Suzuki, K. Uchida, and S. Fujimori, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 605.
[126] F. Celada, A. J. L. Macario, and E. C. de Macario, *Immunochemistry*, 1973, **10**, 797.
[127] J. M. Decker, Y.-B. Kim, and A. Miller, *J. Immunol.*, 1974, **113**, 1599.

Ten strains of *Propionibacterium shermanii* have been tested for β-D-galactosidase activity against 2-nitrophenyl β-D-galactopyranoside; the relation between the production of the enzyme and the stage of growth was examined.[128] The enzyme was inhibited by blocking of its sulphydryl groups, but this inhibition was partly reversed with 1,4-dithiothreitol.

Studies have been carried out on the β-D-galactosidase activity in *Vibrio cholerae* in an attempt to understand why the micro-organism is unable to use lactose for growth.[129]

Active immobilized forms of β-D-galactosidase have been produced by binding the enzyme to chitin *via* glutaraldehyde,[130] by encapsulation in collodion microcapsules,[131] and by adsorption onto alumina- and titania-treated stainless steel.[132]

D-Glucosidases, β-D-Glucocerebrosidases, and β-1,6-D-Glucosidases

Derivatives of macroporous agarose carrying alkyl side-chains have been used for the purification of β-D-glucosidase by affinity chromatography [33] (see p. 329).

The electron microscopy of enzymes has been reviewed, with particular reference to β-D-glucosidases.[133]

A book on cellular, biological, and enzymological aspects of the therapy of lysosomal storage diseases has considered β-D-glucocerebrosidase as a model for enzymic action in a membrane.[134]

The kinetic properties of β-D-glucosidase from human-brain tissues have been investigated.[135]

The β-D-glucosidase activities in cultured fibroblasts from controls and from patients with Gaucher's disease have been measured using 4-methylumbelliferyl β-D-glucopyranoside as the substrate; low activities were exhibited by the pathological specimens.[83] The K_m value for the fibroblastic enzyme was higher than that of liver β-D-glucosidase with this substrate. The activity exhibited at pH 4.0, but not at pH 5.0—5.5, depended on the ionic strength of the buffer; this finding accounts for discrepancies observed between earlier work. Further studies indicated that the β-D-glucosidase, β-D-xylosidase, and α-L-arabinofuranosidase activities of fibroblasts are manifest by a single enzyme.

The activities and properties of α- and β-D-glucosidases of embryonic human-lung (diploid W1-38) fibroblasts have been investigated.[50] The levels of α-D-glucosidase in jejunal biopsy specimens from normals and from sibling cases of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase–isomaltase') deficiency have been measured.[102]

Human intestinal α-D-glucosidase has been shown to consist of 30—40% of carbohydrate composed principally of L-fucose, D-galactose, 2-amino-2-deoxy-D-

[128] J. C. Hartley and E. R. Vedamuthu, *Appl. Microbiol.*, 1975, **29**, 74.
[129] G. Bhattacharya, P. G. Dastidar, A. M. Sinha, P. K. Majumder, and G. C. Chatterjee, *Canad. J. Microbiol.*, 1974, **20**, 897.
[130] W. L. Stanley, G. G. Watters, B. Chan, and J. M. Mercer, *Biotechnol. and Bioeng.*, 1975, **17**, 315.
[131] M. A. Paine and R. G. Carbonell, *Biotechnol. and Bioeng.*, 1975, **17**, 619.
[132] M. Charles, R. W. Coughlin, E. K. Paruchuri, B. R. Allen, and F. X. Hasselberger, *Biotechnol. and Bioeng.*, 1975, **17**, 203.
[133] See ref. 42, p. 77.
[134] M. W. Ho, in 'Cell Biological and Enzymological Aspects of the Therapy of Lysosomal Storage Diseases', Elsevier, Amsterdam, 1974.
[135] J. J. W. Lisman and G. J. M. Hooghwinkel, *Neurobiology*, 1974, **4**, 337.

glucose, and 2-amino-2-deoxy-D-galactose, and some D-mannose and D-xylose.[136] The enzyme resisted digestion by papain, and a purified form exhibited potent blood-group antigenicity. Rat intestinal α-D-glucosidase contains 15—20% of carbohydrate, but does not exhibit blood-group activity. It was concluded that human intestinal glycoside hydrolases are glycoproteins containing blood-group activity in oligosaccharide side-chains covalently linked to the protein. These enzymes may be responsible for part of the insoluble blood-group activity found in intestinal tissues.

α-D-Glucosidase in extracts of human placenta has been purified by chromatography on concanavalin A immobilized on macroporous agarose; the method is advocated for the large-scale preparation of this enzyme.[51]

The activities of β-D-glucosidase (towards 4-methylumbelliferyl β-D-glucopyranoside) and β-D-glucocerebrosidase in the leucocytes of adults with chronic Gaucher's disease have been compared.[137]

The hydrolytic and transglycosylation activities of calf-spleen β-D-glucosidase have been investigated.[138]

An 'acid' α-D-glucosidase (mol. wt. 1.6×10^5) has been purified from porcine spleen.[139] The preparation, which was homogeneous on disc gel electrophoresis, cleaved reducing α-D-glucosyl disaccharides and dextrans containing (1 → 3)- and (1 → 6)-linkages, whereas (1 → 2)-linked dextrans were only partially hydrolysed. The effects of pH, temperature, and inhibitors on the kinetics of the enzyme, and the enzyme's activity against both poly- and oligo-saccharides, confirmed that there are multiple substrate-binding sites.

Treatment of closed, right-side-out vesicles of porcine, rabbit, and rat jejunum brush borders with Triton X-100 and papain released α-D-glucosidase bound to the membranes without grossly affecting the lipid bilayers limiting the vesicles.[140] It was concluded that intestinal α-D-glucosidase is attached to the outside of the membrane.

The subcellular distribution of β-D-glucosidase in rabbit liver has been determined.[66]

O-β-D-Glucopyranosyl-*N*-hexylsphingosine completely inhibited β-D-glucosidase in cells of cultured rat astrocytoma (RGC-6) and mouse neuroblastoma (NB41A and NB2a) cells, so providing a model for the nerve system of children with Gaucher's disease.[141]

The α- and β-D-glucosidases present in fowl semen have been investigated.[72]

The properties of an α-D-glucosidase activity located at the tip of the labellar chemosensory setae of blowflies (*Phormia regina*) have been examined.[142] The enzymic activity was independent of pH over a wide range (3.0—8.0), and was competitively inhibited by tris(hydroxyamino)methane and low concentrations of Ca^{2+} ions. Comparison with α-D-glucosidase isoenzymes in an extract from the proboscis showed that the properties of the intact enzyme differ from those of

[136] J. J. Kelly and D. H. Alpers, *J. Biol. Chem.*, 1973, **248**, 8216.
[137] C. Klibansky, J. Hoffman, R. Zaizov, and Y. Matoth, *Biomedicine*, 1974, **20**, 24.
[138] J. N. Kanfer, S. S. Raghaven, and R. A. Mumford, *Biochim. Biophys. Acta*, 1975, **391**, 129.
[139] M. E. Preobrazhenskaya, A. L. Minakova, and E. L. Rosenfeld, *Carbohydrate Res.*, 1974, **38**, 267.
[140] D. Louvard, S. Maroux, C. Vannier, and P. Desnuelle, *Biochim. Biophys. Acta*, 1975, **375**, 236.
[141] G. Dawson, A. C. Stoolmiller, and N. S. Radin, *J. Biol. Chem.*, 1974, **249**, 4638.
[142] O. Koizumi, H. Kijima, and H. Morita, *J. Insect Physiol.*, 1974, **20**, 925.

the soluble form. Similarities were noted between the properties of the intact enzyme and those of the labellar sugar-receptor.

A number of active sites exist in two forms of β-D-glucosidase from sweet-almond emulsin.[143] Inhibition studies demonstrated that the ring oxygen of lactone inhibitors cannot be replaced by other sterically equivalent groups, since it is involved in binding to the enzyme.

The β-D-glucosidase of potato tubers has been studied,[144] and changes in the levels of α-D-glucosidase in tobacco leaves during curing have been related to the degradation of constituent carbohydrates.[87]

Both α- and β-D-glucosidase activities have been detected in protein bodies and spherosomes isolated from ungerminated seeds of *Sorghum bicolor*.[116]

The properties of bacterial β-D-glucosidases have been compared with those of D-thioglucosidases from various sources.[145]

A radioactive hexadecapeptide has been cleaved with pepsin from the active site of *Aspergillus wentii* β-D-glucosidase after labelling with [^{3}H]conduritol-B epoxide.[146] Sequence data in the region of the active site were obtained.

A homogeneous α-D-glucosidase has been isolated from *Bacillus cereus*.[147] The purified enzyme (1.4 *S*; mol. wt. 1.2×10^4) hydrolysed maltose, maltotriose, and phenyl α-maltoside, but showed little or no activity towards other oligosaccharides (except nigerose) or phenyl and methyl α-D-glucopyranosides, amylose, amylopectin, glycogen, and soluble starch. The enzyme (pH optimum 6.8—7.3) also possesses α-D-glucosyltransferase activity; thus, it yielded maltotriose from maltose and transferred an α-D-glucosyl residue from maltose to riboflavin. Inhibition studies with diazonium-1*H*-tetrazole, Rose Bengal, and 4-chloromercuribenzoate indicated that L-histidine and L-cysteine residues are located at the active site of the enzyme.

Various properties of a partially purified β-D-glucosidase from *Erwinia herbicolor* have been reported.[148]

An intracellular β-D-glucosidase (mol. wt. 5.9×10^4; pH optimum 5.8; temperature optimum 40 °C) has been prepared from a *Flavobacterium* species by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[149] The enzyme readily hydrolyses (1 → 6)-β-D-glucosidic linkages, with K_m values of 2.8×10^{-4}, 3.0×10^{-4}, 4.2×10^{-4}, and 4.6×10^{-4} mol l^{-1} for gentio-biose, -triose, -tetraose, and -pentaose, respectively. Its substrate specificity, transglycosylation properties, and inhibition by D-glucono-1,5-lactone indicated that the enzyme is a β-1,6-D-glucosidase. The enzyme was also inhibited by Hg^{2+}, Cu^{2+}, Ag^+, and Fe^{3+} ions, 4-chloromercuribenzoate, *N*-ethylmaleimide, D-glucose, and tris(hydroxyamino)methane, but not by H_4edta.

The properties of α-D-glucosidase activities detected in a number of strains of *Klebsiella* (*Aerobacter*) *aerogenes* have been investigated.[150]

[143] G. Legler and F. Witassek, *Z. physiol. Chem.*, 1974, **355**, 617.
[144] N. P. Korableva, L. V. Popova, É. V. Morozova, and L. V. Metlitskii, *Biochemistry* (*U.S.S.R.*), 1974, **39**, 755.
[145] N. Tani, M. Ohtsuru, and T. Hata, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 1623.
[146] E. Bause and G. Legler, *Z. physiol. Chem.*, 1974, **355**, 438.
[147] Y. Yamasaki and Y. Suzuki, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 443.
[148] A. Garibaldi and L. N. Gibbons, *Canad. J. Microbiol.*, 1975, **21**, 513.
[149] K. Sano, A. Amemura, and T. Harada, *Biochim. Biophys. Acta*, 1975, **377**, 410.
[150] G. C. Hope and A. C. R. Dean, *Biochem. J.*, 1974, **144**, 403.

A stable enzyme–D-glucose intermediate has been obtained in the hydrolysis of methyl α-D-[^{14}C]glucopyranoside by an α-D-glucosidase from *Saccharomyces oviformis*.[151] Phenol was used to terminate the reaction and to trap the intermediate in a rapid-flow technique. The intermediate appears to be covalently linked, since continuous washing of the denatured protein failed to remove the radiolabel, which was also retained by a tryptic peptide isolated by gel filtration. Treatment of the intermediate with acid released D-[^{14}C]glucose. The radiolabel was not bound when the enzyme was replaced with bovine albumin or when β-D-glucosylamine (a potent inhibitor of α-D-glucosidase) was added with the enzyme.

The hydrolytic and transglycosylation reactions of two α-D-glucosidases from *Saccharomyces oviformis* have been compared.[152] A 'maltase' that hydrolysed both maltose and isomaltose also catalysed the formation of these disaccharides from D-glucose, whereas an 'isomaltase' that hydrolysed isomaltose, but not maltose, catalysed the formation of isomaltose only from D-glucose. Both enzymes hydrolysed 4-nitrophenyl α-D-glucopyranoside, releasing α-D-glucose, which was used for disaccharide synthesis. The results are consistent with a double-displacement mechanism for the actions of the glycoside hydrolases, and with the proposal that a D-glucosyl–enzyme complex is involved in the reaction. Both α-D-glucosidases were inhibited by α- and β-D-glucose.

A β-D-glucosidase (mol. wt. 4.7×10^4) has been isolated from a commercial preparation of cellulase (obtained from culture filtrates of *Trichoderma viride*) by fractional precipitation with ammonium sulphate, gel filtration, ion-exchange chromatography, and isoelectric focusing.[153] The purified, homogeneous enzyme (p*I* 5.74) was inactive against microcrystalline cellulose and carboxymethylcellulose. K_m Values of 0.28, 1.5, and 0.35 mmol l^{-1} were obtained for 4-nitrophenyl β-D-glucopyranoside, cellobiose, and cellotetraose, respectively.

β-D-Glucuronidases

The molecular structure, transformation, and subcellular translocation of multiple forms of β-D-glucuronidase have been reviewed.[154]

The levels of β-D-glucuronidase in cultured human-skin fibroblasts were found to be influenced by the conditions of growth.[155] Significant increases in the levels of the enzyme occurred on increasing the cell density. Despite such variations, the fibroblasts of homozygous-deficient patients contained very low levels of β-D-glucuronidase activity. Although the levels of β-D-glucuronidase activity normally found in sera, plasma, and urine show appreciable variations, fluorometric measurement of the enzymic activity in sera or plasma is recommended as a general screening procedure for patients with hyperglycosaminoglycanuria (mucopolysaccharidosis). Lysates of normal leucocytes showed little variation in the levels of β-D-glucuronidase, but lysates of leucocytes from heterozygous

[151] H.-Y. L. Lai, L. G. Butler, and B. Axelrod, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 635.
[152] H.-Y. L. Lai and B. Axelrod, *Biochim. Biophys. Acta*, 1975, **391**, 121.
[153] L. E. F. Berghem and L. G. Pettersson, *European J. Biochem.*, 1974, **46**, 295.
[154] C. Lin, in 'Isoenzymes I – Molecular Structure', ed. C. L. Markert, Academic Press, New York and London, 1975, p. 637.
[155] J. H. Glaser and W. S. Sly, *J. Lab. Clin. Med.*, 1972, **82**, 969.

carriers showed a level between those of normal and homozygous-deficient patients.

The level of β-D-glucuronidase activity in human-skin fibroblasts from patients with Maroteaux–Lamy syndrome has been shown to be normal.[48] The activities and properties of β-D-glucuronidase in embryonic human-lung (diploid W1-38) fibroblasts have been reported.[50]

The non-phagocytic release of β-D-glucuronidase from human neutrophils by various reagents, including inhibitors of the enzyme, has been investigated.[156]

A simple procedure for the isolation of β-D-glucuronidase from extracts of human placenta has used chromatography on concanavalin A immobilized on macroporous agarose.[51]

A non-competitive inhibitor (mol. wt. 3.8×10^5) of β-D-glucuronidase has been isolated from human saliva by ion-exchange chromatography and gel filtration.[157] It contains fucose (4.9%), hexose (16.6%), 2-amino-2-deoxyhexose (15.7%), 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (12.8%), and protein (46.7%). The inhibitor either is derived from serum or simulates the antigenicity of components therein.

The concentrations, latencies, pH optima, and heparin-induced inhibition of β-D-glucuronidases in neutrophils and eosinophils of equine blood have been determined.[108]

A mammalian system has been used to determine the fate *in vivo* of bovine β-D-glucuronidase administered intravenously to mice that are deficient in the enzyme; sensitive and reliable discrimination between bovine-liver and residual, murine-tissue β-D-glucuronidase activities was achieved by inactivation of the former with heat.[158] The bovine activity was cleared rapidly from circulation following injection, and was recovered almost exclusively in the liver, where 72% is localized in the lysosomes. The mammalian system provides an *in vivo* model that enables the protection and delivery of an exogenous enzyme to be evaluated prior to replacement trials in patients with inherited diseases caused by an enzyme deficiency.

Two non-competitive inhibitors (each of mol. wt. 3.4×10^5) of β-D-glucuronidase from porcine [sublingual and submaxillary glands have been purified by ion-exchange chromatography, *etc.*, and shown to contain hexose (468, 402), L-fucose (199, 128), 2-amino-2-deoxy-D-galactose (181, 407), 2-amino-2-deoxy-D-glucose (208, 11), and 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (338, 329 mol, respectively).[159]

β-D-Glucuronidase in rabbit liver is localized in microsomal and lysosomal fractions.[66] The multiple forms of the enzyme in both fractions were shown to be immunologically identical. The presence of an inactive enzyme was also demonstrated in some preparations.

β-D-Glucuronidase from rat liver has been purified to homogeneity by autolysis, fractional precipitation with ammonium sulphate, ion-exchange chromatography,

[156] L. J. Ignarro, *Arthritis and Rheumatism*, 1974, **17**, 25.
[157] W. Sakamoto, O. Nishikaze, and T. Sugimura, *Arch. Oral Biol.*, 1974, **19**, 703.
[158] S. R. Thorpe, M. B. Fiddler, and R. J. Desnick, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 1464.
[159] W. Sakamoto, O. Nishikaze, and E. Sakakibara, *Biochim. Biophys. Acta*, 1974, **343**, 409.

gel filtration, sucrose density-gradient sedimentation, and isoelectric focusing.[160] Electron microscopy revealed that ferritin is the main contaminant in later stages of the purification, and that the enzyme exists as aggregates. The purified enzyme was homogeneous on polyacrylamide gel electrophoresis, both under non-dissociating conditions and in the presence of sodium dodecyl sulphate, and on Ouchterlony gel diffusion and immunoelectrophoresis against specific antisera. Molecular weights of 3.0×10^5 and 4.4×10^5 were estimated by sedimentation and gel filtration, respectively, and subunits (mol. wt. 7.5×10^4) were observed on gel electrophoresis in the presence of sodium dodecyl sulphate and on gel filtration in the presence of urea. The enzyme was active against 4-nitrophenyl β-D-glucopyranuronoside (K_m value 0.6 mmol l^{-1}), and was inhibited by lactones and Hg^{2+} ions. Multiple forms (p*I* values 4.5—5.8) of the pure β-D-glucuronidase were observed by isoelectric focusing, the subunits also exhibiting heterogeneity. It was concluded that the multiple forms are not artefacts, but that neuraminidase converts some of the forms of lower p*I* into other forms.

The distribution of β-D-glucuronidase has been investigated in cultured peritoneal macrophages of mice after stimulation with thioglycolate.[67]

Microsomal rat-liver β-D-glucuronidase has been characterized, and its behaviour on irradiation with *X*-rays has been examined.[161] The release of β-D-glucuronidase from rat liver following administration of diazinon (an organophosphorus insecticide) has been studied.[162]

Rat-kidney lysosomal β-D-glucuronidase underwent autolysis, and its pH value increased, under mild acid conditions.[163] This finding is considered to bear on the origin of the molecular heterogeneity of the lysosomal enzyme.

The expression of β-D-glucuronidase during embryogenesis in mice has been examined.[164] An electrophoretic variant of β-D-glucuronidase has been detected in certain lines of inbred mice; the variant simultaneously affected the mobilities of lysosomal and microsomal forms of the enzyme.[165] The difference is inherited as a single gene that occurs on chromosome 5, confirming that β-D-glucuronidase at both intracellular sites is coded for by the same structural gene.

The β-D-glucuronidase activity of fowl semen has been investigated.[72]

The effects of high-meat and meat-free diets on intestinal, bacterial β-D-glucuronidases in humans have been examined.[166] The enzymic activity was significantly higher in individuals on high-meat diets, so that the intestinal flora were able to hydrolyse D-glucuronoside conjugates more readily. This might raise the amounts of such substances as carcinogens within the colonic lumen.

Strains of streptococcal groups B and D have been tested for the presence of β-D-glucuronidase: none of the D strains, but most of the B strains, contain β-D-glucuronidase activity.[167]

[160] R. T. Dean, *Biochem. J.*, 1974, **138**, 395.
[161] M. Durante, A. Covi, and L. Barsanti, *Ital. J. Biochem.*, 1974, **23**, 292.
[162] Y. Suzuki, H. Kikuchi, and M. Uchiyama, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 886.
[163] A. Goldstone and H. Koenig, *Biochem. J.*, 1974, **141**, 527.
[164] V. M. Chapman and L. Wudl, in 'Isoenzymes III – Development Biology', ed. C. L. Markert, Academic Press, New York and London, 1975, p. 57.
[165] P. A. Lalley and T. B. Shows, *Science*, 1974, **185**, 442.
[166] B. S. Reddy, J. H. Weisburger, and E. L. Wynder, *Science*, 1974, **183**, 416.
[167] T. O. Röd, R. H. Haug, and T. Midtvedt, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 533.

β-D-Glycylamidodeoxyglucosidases

An enzyme that hydrolyses 4-nitrophenyl 2-deoxy-2-glycylamido-β-D-glucopyranoside has been detected in marine invertebrates.[168] The ophiurus *Amphipholis kohii* contains appreciable activity (pH optimum 7.0), but there is no relation between the β-D-glycylamidodeoxyglucosidase activity and the β-D-acetamidodeoxyglucosidase activity. The distribution and properties of β-D-glycylamidodeoxyglucosidase in mushrooms have been investigated.[169]

α-L-Iduronidases

Skin fibroblasts obtained from patients with Maroteaux–Lamy syndrome exhibited normal levels of α-L-iduronidase activity.[48]

D-Mannosidases

The activities and properties of an α-D-mannosidase of diploid W1-38 fibroblasts from embryonic human-lung tissues have been reported.[50] Mannosidosis is a genetic disorder that can be recognized by a deficiency of a specific α-D-mannosidase in cultured fibroblasts.[170]

The affinity-chromatographic properties of human-liver α-D-mannosidase have been investigated using β-D-mannopyranosylamine covalently attached to derivatives of macroporous agarose.[171] The enzyme was shown to exist in multiple forms, which displayed different affinities for the affinants. These forms, which occur in normal human liver, were designated as 'acidic', 'neutral', and 'intermediate' activities (pH optima 4.25, 6.5, and 5.5, respectively).[172] The heat-stable 'acid' enzyme was separated into two interconvertible forms by ion-exchange chromatography. The significance of the multiple forms of α-D-mannosidase was discussed in relation to mannosidosis.

A single passage of a crude extract of human placenta through a column of concanavalin A immobilized on macroporous agarose effected the purification of an α-D-mannosidase by 300-fold.[51] The nature of the interaction with concanavalin A indicated that the α-D-mannosidase is a glycoprotein.

The distribution and concentration of α-D-mannosidase in cellular fractions of neutrophils and eosinophils of equine blood have been reported.[108] The latency, pH requirements, and inhibition response to heparin of the enzyme were examined.

α-D-Mannosidase in normal calf tissues exists in at least three forms (A, B, and C) that are separated by ion-exchange chromatography or starch-gel electrophoresis.[173] The A and B forms exhibit maximum activity between pH 3.75 and 4.75, whereas the C form possesses a pH optimum of 6.6; the forms differ in molecular weight and sensitivity to H_4edta and Co^{2+}, Mn^{2+}, and Zn^{2+} ions. Active A and B forms are absent from tissues of calves with mannosidosis, the residual activity arising from the C form. The D form of α-D-mannosidase activity present in normal calf plasma is absent from mannosidosis calf plasma,

[168] N. V. Molodtsov and M. G. Vafina, *Internat. J. Biochem.*, 1974, **5**, 235.
[169] N. V. Molodtsov and M. G. Vafina, *Internat. J. Biochem.*, 1974, **5**, 239.
[170] H. A. Taylor, G. H. Thomas, A. Aylsworth, R. E. Stevenson, and L. W. Reynolds, *Clinica Chim. Acta*, 1975, **59**, 93.
[171] D. Robinson, N. C. Phillips, and B. G. Winchester, *F.E.B.S. Letters*, 1975, **53**, 110.
[172] N. C. Phillips, D. Robinson, and B. G. Winchester, *Clinica Chim. Acta*, 1974, **55**, 11.
[173] N. C. Phillips, D. Robinson, B. G. Winchester, and R. D. Jolly, *Biochem. J.*, 1974, **137**, 363.

the residual activity being at a maximum at pH 5.50—5.75. Although the D form is probably related to the A and B forms, they can be distinguished by ion-exchange chromatography and gel filtration. It was concluded that mannosidosis in Angus cattle is caused by a deficiency of lysosomal 'acid' α-D-mannosidase activity.

The effects of streptozotocin-induced diabetes on the levels of α-D-mannosidase activities of various organs and sera of rats have been studied.[68]

The α- and β-D-mannosidase activities of fowl semen have been investigated.[72] The purification and properties of a purified β-D-mannosidase from malted barley have been reported.[174]

α-D-Mannosidase in an aqueous extract from Jack-bean meal has been obtained free from β-D-acetamidodeoxyglucosidase by fractional precipitation with ammonium sulphate, gel filtration, and ion-exchange chromatography.[175] Jack-bean α-D-mannosidase has been identified as a zinc metalloenzyme.[176]

Purification of the α-D-mannosidase (mol. wt. $\sim 2.2 \times 10^5$) from the seeds of *Medicago sativa* has been achieved by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[177] The enzyme was inactivated at pH < 7; this process could be prevented by Zn^{2+} ions, whereas H_4edta accelerated it. The influence of pH on the K_m value and V_{max} was determined using 4-nitrophenyl α-D-mannopyranoside. 4-Chloromercuribenzoate and other sulphydryl reagents did not inhibit the enzyme, and it appears that a dissociable group of $pK_a \sim 6$, which functions as a proton donor, is necessary for catalytic activity. Studies of the glycone specificity revealed strict requirements at C-3—C-5 of the D-mannopyranosyl moiety, whereas a *trans*-diaxial arrangement of the *C*-2-hydroxy-group and the aglycone is necessary for hydrolysis but not for binding. Strong inhibition by D-mannono-1,5-lactone indicated that the glycone portion of the substrate is bound in a half-chair conformation.

Neuraminidases

A simple method for preparing commercial neuraminidase (sialidase) free from proteases has been reported.[178]

The effects of neuraminidase and of anti-neuraminidase sera on the properties of cell surfaces have been investigated.[179] The activities and properties of diploid W1-38 fibroblasts from embryonic human-lung tissues have been compared with those of other 'acid' glycoside hydrolases from the same source.[50]

The removal of residues of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid from particulate glycoproteins of calf brain by endogenous, membrane-bound neuraminidase has been studied.[180]

Neuraminidase has been found in porcine gastric mucus; the enzyme probably originates from bacteria present in mucous secretions, and is considered to affect the balance of gastric glycoproteins.[181]

[174] C. W. Houston, S. B. Latimer, and E. D. Mitchell, *Biochim. Biophys. Acta*, 1974, **370**, 276.
[175] C. Y. T. Chu and M. D. Turner, *Biochem. J.*, 1974, **139**, 469.
[176] S. M. Snaith, *Biochem. J.*, 1975, **147**, 83.
[177] J. De Prijcker, A. Vervoort, and C. De Bruyne, *European J. Biochem.*, 1974, **47**, 561.
[178] M. W. C. Hatton and E. Regoeczi, *Biochim. Biophys. Acta*, 1973, **327**, 114.
[179] P. Sachtleben, R. Gsell, and J. N. Mehrishi, *Vox Sanguinis*, 1973, **25**, 519.
[180] A. Preti, A. Lombardo, G. Tettamanti, and V. Zambotti, *J. Neurochem.*, 1973, **21**, 1559.
[181] A. Allen and B. J. Starkey, *Biochim. Biophys. Acta*, 1974, **338**, 364.

The pathology of microbial neuraminidases has been reviewed; numerous sources of the enzyme were considered, and diseases associated with neuraminidases were tabulated.[182]

An affinity matrix consisting of macroporous agarose carrying glycyl-L-tyrosyl groups derivatized with an oxamic acid has been used in the purification of neuraminidase from *Clostridium perfringens*.[183] Haemoglobin, haemolysin, and phospholipase C were also bound at pH 5.5 to this matrix and to agarose carrying glycyl-L-tyrosine groups. The latter effect is attributed to ion-exchange phenomena, since non-specific adsorption of haemoglobin, haemolysin, and phospholipase C did not occur at pH 7.5 although the neuraminidase was still adsorbed. It was concluded that these affinity-chromatographic materials are unsuitable for the purification of neuraminidase, since they fail to reject non-specific proteins, *etc.*

Preliminary results using a new, flat-bed, gel isoelectric focusing method to study the heterogeneity of neuraminidases have been reported.[184] The method has high resolution and the capacity to analyse many samples in a single experiment. The isoelectric points recorded were in good agreement with those obtained by other methods.

A *Corynebacterium haemolyticum* neuraminidase has been shown to cleave α-(2 → 3)- and α-(2 → 6)-linkages joining 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues to adjacent carbohydrate chains, but it was appreciably less active against 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulosonyl-(2 → 8)-5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid linkages.[185] K_m Values of 4.0×10^{-4} mol l^{-1} for α-(2 → 3)- and α-(2 → 6)-linkages and α_1-acid glycoprotein, and of 1.0×10^{-3} mol l^{-1} for α-(2 → 8)-linkages, were reported. The relation between the neuraminidase activity and pathogenicity of the organisms was discussed.

The neuraminidase activities of oral strains of Haemophili found in humans have been investigated.[186]

The neuraminidase present in a *Streptococcus* sp. has been purified (1500-fold) by fractional precipitation with ammonium sulphate, followed by affinity chromatography on agarose derivatives carrying either oxamic acid or 2-acetamido-2-deoxy-1-thio-β-D-glucopyranosyl groups.[187] The affinity matrices bound β-D-acetamidodeoxyhexosidase and β-D-galactosidase, and the former matrix also bound neuraminidase. The chromatographic procedures effectively removed any glycoside hydrolases. The neuraminidase (mol. wt. 1.1×10^5) was stimulated slightly by Ca^{2+}, Co^{2+}, Mg^{2+}, and Mn^{2+} ions, but was inhibited by heavy-metal ions, and its specificity is similar to that of the enzymes from *Clostridium perfringens* and *Vibrio cholerae*.

Certain strains of *Streptomyces* contain neuraminidases.[188] The neuraminidases purified (1000-fold) from two such strains had similar molecular weights

[182] H. E. Müller, *Des. Med. Wochenschr.*, 1974, **99**, 1933.
[183] J. I. Rood and R. G. Wilkinson, *Biochim. Biophys. Acta*, 1974, **334**, 168.
[184] N. P. Groome and G. Belyavin, *Analyt. Biochem.*, 1975, **63**, 249.
[185] H. von Nicolai, H. E. Müller, and F. Zilliken, *Z. physiol. Chem.*, 1975, **356**, 105.
[186] J. E. Tuyau and W. Sims, *Arch. Oral Biol.*, 1974, **19**, 817.
[187] T. Kiyohara, T. Terao, K. Shioiri-Nakano, and T. Osawa, *Arch. Biochem. Biophys.*, 1974, **164**, 575.
[188] S. Kunimoto, T. Aoyagi, T. Takeuchi, and H. Umezawa, *J. Bacteriol.*, 1974, **119**, 394.

(3.2×10^4) and substrate specificities, and exhibited similar behaviour towards ions; they differed slightly in pH optima, thermostabilities, and kinetic properties. The neuraminidases differ from *Clostridium perfringens* neuraminidase (mol. wt. 5.7×10^4) in substrate specificity, and were not inhibited by 4-chloromercuribenzoate.

Purification of *Vibrio cholerae* neuraminidase has been achieved by affinity chromatography on a series of derivatives of macroporous agarose carrying tyrosyl or tyraminyl groups.[41] Since adsorption of the enzyme was unaffected by 4-diazophenyloxamic acid coupled to the phenolic groups of the affinity materials, it was suggested that earlier biological data (Vol. 5, p. 356) obtained with neuraminidase should be re-evaluated. Other glycoside hydrolases from the same organism were also adsorbed and eluted from the affinity materials under the same conditions, so that the mechanism originally proposed for the affinity chromatography of neuraminidase is no longer tenable. A new mechanism was proposed.

The affinity chromatography of *V. cholerae* neuraminidase on macroporous agarose bearing derivatized 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues has been examined.[189] Whereas residues attached by amide linkages did not adsorb the enzyme significantly, matrices bearing 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid residues bound the enzyme strongly, as did those in which the units were linked α-ketosidically. The effects of *V. cholerae* neuraminidase on the synthesis of collagen and proteins in mammalian tissues,[190] and on the immunogenicity of viral-associated and private antigens of mammary carcinomas,[191] have been investigated.

β-D-Phosphogalactosidases

A number of strains of *Propionibacterium shermanii* have been tested for β-D-phosphogalactosidase activity; the levels of activity were related to the growth of the organism, and were also compared to the levels of β-D-galactosidase also present.[128]

A β-D-phosphogalactosidase (mol. wt. 6.76×10^4) present in cell-free extracts of *Streptococcus cremoris* has been purified to homogeneity by gel electrophoresis.[192] Use of 2-nitrophenyl β-D-galactopyranoside 6-phosphate as a substrate showed that the enzyme is relatively heat-resistant and maximally active at pH 5.0—8.0 and 45—52 °C. 1,4-Dithiothreitol, H_4edta, and citrate ion stimulated the β-D-phosphogalactosidase activity, but Mg^{2+} and Li^+ ions and 4-hydroxymercuribenzoate were inhibitors. The amino-acid composition of this enzyme is similar to that of other β-D-phosphogalactosidases.

β-D-Phosphoglucosidases

Two β-D-phosphoglucosidases in extracts of *Escherichia coli* have been purified to apparent homogeneity.[193] Both enzymes are specific for substrates containing

[189] L. Holmquist, *Acta Chem. Scand.* (*B*), 1974, **28**, 1065.
[190] M. Aalto, T. Rönnemaa, and E. Kulonen, *Biochim. Biophys. Acta*, 1974, **342**, 247.
[191] R. L. Simmonds and A. Rios, *J. Immunol.*, 1973, **111**, 1820.
[192] K. G. Johnson and I. J. McDonald, *J. Bacteriol.*, 1974, **117**, 667.
[193] G. Wilson and C. F. Fox, *J. Biol. Chem.*, 1974, **249**, 5586.

β-D-glucopyranosyl 6-phosphate, liberating D-glucose 6-phosphate. Although the enzymes have similar molecular weights and appear to be dimers containing identical subunits, their behaviour during purification, and their amino-acid compositions, kinetic parameters, and thermostabilities, differ appreciably.

Sucrose α-D-Glucohydrolases

Sucrose α-D-glucohydrolase ('sucrase') present in human intestines contains 30—40% of carbohydrate consisting of L-fucose, D-galactose, 2-amino-2-deoxy-D-galactose, and 2-amino-2-deoxy-D-glucose, and some D-mannose and D-xylose.[136] The enzyme resisted digestion by papain and exhibited potent blood-group antigenicity in purified form. The corresponding enzyme in rat intestines contains 15—20% of carbohydrate of similar composition, but it was not digested by papain nor did it exhibit blood-group activity. These and other studies indicated that human intestinal glycoside hydrolases are glycoproteins containing oligosaccharide side-chains possessing blood-group activity. These enzymes may account for part of the insoluble blood-group activity found in intestinal tissues.

Highly purified sucrose α-D-glucohydrolase from human small intestines has been shown to act on α-D-glucopyranosides.[194] The hydrolyses of sucrose and palatinose proceeded with net retention of configuration at C-1 of the D-glucopyranosyl residues. Scission of the glycosidic linkage occurs between C-1 of the D-glucose ring and the glycosidic oxygen atom, so that, for example, D-fructose is released as β-D-fructofuranose from sucrose. Transglycosylation also takes place; for example, methyl α-D-glucopyranoside was formed in the presence of methanol. Non-stoicheiometric amounts of D-glucose and D-fructose were produced from sucrose, turanose, and palatinose, the amount of D-glucose released being less than expected.

Electrophoresis on polyacrylamide gels of materials from brush-border membranes of siblings with a deficiency of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase – isomaltase') did not reveal the normal protein band associated with the complex.[102]

Treatment of closed, right-side-out vesicles of porcine, rabbit, and rat jejunum brush borders with papain and Triton X-100 released a sucrose α-D-glucohydrolase that had been bound to the membrane without grossly affecting the lipid bilayers limiting the vesicles.[140] It was concluded that intestinal sucrose α-D-glucohydrolase is attached to the outside of the membrane.

β-D-Xylosidases

Use of 4-methylumbelliferyl β-D-xylopyranoside as a substrate showed that the levels of β-D-xylosidase activity, as well as those of α-L-arabinofuranosidase and β-D-glucosidase, are significantly reduced in the fibroblasts of patients with Gaucher's disease.[83] These activities appear to be manifest by a single enzyme.

exo-β-D-Acetamidodeoxyglucanases and *exo*-β-D-Acetamidodeoxygalactanases

A dodecasaccharide (obtained from dermatan sulphate) containing a terminal, non-reducing and also internal 2-acetamido-2-deoxy-D-galactosyl residues

[194] B. Zagalak and H. C. Curtius, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 503.

released the terminal residue when treated with human serum, which must therefore contain an *exo*-β-D-acetamidodeoxyglycanase.[58]

The B isoenzyme of the *exo*-β-D-acetamidodeoxyglucanase in porcine epididymis has been purified by standard procedures.[65]

Extracts of *Actinobacillus* and *Pseudomonas* species exhibiting symbiotic growth in media containing keratan sulphate as the only source of carbon contain an *exo*-β-D-acetamidodeoxyglucanase that, in combination with other enzymes, extensively hydrolysed corneal keratan sulphate.[74]

α-Amylases

The ability of α-amylases to synthesize malto-oligosaccharides from α-D-glucosyl and α-maltosyl fluorides has been reviewed.[18]

The problem of measuring α-amylase in the presence of β-amylase has been overcome.[195] Thus, coupling of a chromogen to starch inhibited the action of the *exo*-enzyme but not that of the *endo*-enzyme. Cibachron-F3GA starch (Phadebas starch) was used as the chromogenic substrate; since the diameter of the chromogen is larger than that of the amylose helix and the distance between adjacent spirals of the helix, the chromogen must be located on the outside of the helix.

Amylases labelled with ^{125}I have been prepared with the aid of immobilized lactoperoxidase.[196] The catalytic activity of the labelled enzymes (measured against Phadebas starch) appeared to depend on the ratio of ^{125}I to amylase. When the concentration of amylase in canine plasma was increased artificially, parallel curves were obtained for the disappearance of the label and α-amylase activity.

The amylase activities in aqueous extracts obtained from various species of mammals, birds, and insects and from marine species have been determined quantitatively.[197] Extracts from insects that destroy wheat grain and stored-wheat products contained much more amylase activity than did those from other insects. The inhibition of the amylases in these extracts and in those from a number of cereals (*e.g.* immature and germinating wheat kernels) by three albumin fractions (mol. wts. 6.0×10^4, 2.4×10^4, and 1.25×10^4) from mature wheat kernels was investigated. Amylases were classified into three types depending on whether they were susceptible, partly susceptible, or resistant to inhibition.

The specific activities of purified preparations of human parotid and pancreatic amylases towards Amylose Azure, Remazol Brilliant Blue starch, phosphate-derivatized starch, and cross-linked starch have been measured.[198] The activities varied with the physical state of the substrate; the parotid enzyme exhibited a higher specific activity towards all soluble starches, whereas the pancreatic amylase was more active towards insoluble chromogenic starches. Variations in the enzyme–substrate interactions support the view that there is a configurational difference between the amylases; this may be useful in determining the proportions of these amylases in normal and pathological body fluids.

[195] S. Scharpé, A. Lauwers, and W. Cooreman, *Experientia*, 1973, **29**, 1572.
[196] M. D. O'Donnell and K. F. McGeeney, *Z. klin. Chem. klin. Biochem.*, 1974, **12**, 551.
[197] V. Silano, M. Furia, L. Gianfreda, A. Macri, R. Palescandolo, A. Rab, V. Scardi, E. Stella, and F. Valfré, *Biochim. Biophys. Acta*, 1975, **391**, 170.
[198] D. J. Stiefel and P. J. Keller, *Clinical Chem.*, 1975, **21**, 343.

Proteinaceous inhibitors of α-amylase in aqueous extracts of seeds of *Triticum* and related species have been characterized according to their molecular weights, electrophoretic mobilities, and specificities in inhibiting α-amylases from human saliva and *Tenebrio molitor* larvae.[199]

The isoenzymes of an α-amylase present in the sera of patients with cystic fibrosis have been separated by microelectrophoresis and compared with those from normal sera.[200] Whereas 86% of normal sera contain α-amylases of pancreatic and salivary origin and 14% contain the pancreatic enzyme only, the distribution among the sera of the patients was pancreatic α-amylase only, 38%; salivary amylase only, 50%; or both, 12%.

The properties of an amylase of abnormally large size from three cases of macroamylasemia have been examined.[201] The isoenzyme patterns of these amylases differed from normal, but the macroamylases dissociated into normal amylase on gel filtration at pH 3.4. Proteins bound to the macroamylase were identified as immunoglobulin IgG in one case and as immunoglobulin IgA in the others. It appears that immunoglobulins IgG and IgA bind to amylase in the pathological condition, but the amylase is not defective in any way.

The effects of individual bile salts on the hydrolysis of Cibachron Blue starch by α-amylase at pH 6.0 have been studied.[202] Measurements with cholate, deoxy-, tauro-, taurodeoxy-, taurochenodeoxy-, chenodeoxy-, and lithio-cholates demonstrated that these salts can either inhibit or enhance the α-amylase activity. Since the pH of the jejunum is ~6.0, this finding may be of physiological significance.

α-Amylase from porcine pancreas has been shown to possess one binding site for chloride ion, with a dissociation constant of 3×10^{-4} at 25 °C.[203] The anion functions as an activating effector, and increases k_{cat} towards starch and 4-nitrophenyl maltoside; no change in the K_m value was observed for either substrate. Bromide, iodide, nitrite, nitrate, perchlorate, azide, and cyanate anions did not abolish the enzymic activity, but k_{cat} decreased as the radius of the anions increased. The binding of chloride ion induced a subtle conformational change in the molecule, which was indicated by suppression of the exchange of twenty-six protons and an increase in the binding constant of α-amylase–Ca^{2+} from 8.3×10^8 to 2×10^{11} l mol^{-1}. However, this conformational change is slight, since it was not detected by c.d. spectroscopy, protein fluorescence, and specific probes attached to the enzyme. The ability of a small structural change to induce a significant acceleration of the catalytic activity of the enzyme was discussed.

Porcine pancreatic α-amylase has been shown to contain two sulphydryl groups and one tightly bound Ca^{2+} ion.[204] Derivatization of the enzyme with 5,5′-dithio-bis(2-nitrobenzoic acid), iodoacetamidonaphthol, and Hg^{2+} ions did not abolish the enzymic activity, indicating that both of the sulphydryl groups are not

[199] C. Bedetti, A. Bozzini, V. Silano, and L. Vittozzi, *Biochim. Biophys. Acta*, 1974, **362**, 299.
[200] H. Van Husen, H.-C. Dominick, U. Gerlach, and D. Kamanabroo, *Z. klin. Chem. klin. Biochem.*, 1974, **12**, 214.
[201] K. Harada, T. Nakayama, M. Kitamura, and T. Sugimoto, *Clinica Chim. Acta*, 1975, **59**, 291.
[202] M. D. O'Donnell, K. F. McGeeney, and O. Fitzgerald, *Enzyme*, 1975, **19**, 129.
[203] A. Levitzki and M. L. Steer, *European J. Biochem.*, 1974, **41**, 171.
[204] M. L. Steer, N. Tal, and A. Levitzki, *Biochim. Biophys. Acta*, 1974, **334**, 389.

required for activity. The first two reagents reacted only after removal of the Ca^{2+} ions, whereas this was not necessary in the formation of mercury(II) derivatives. The pH–activity profiles of the enzyme derivatives were similar to those for the parent molecule, although the specific activity at each pH was slightly depressed. Since the reaction of the enzyme with an equimolar amount of Hg^{2+} gave an —S—Hg—S— bridge, the two sulphydryl groups must lie close to one another. The suitability of such Hg^{2+} derivatives for *X*-ray crystallographic studies was noted. Both sulphydryl groups of porcine pancreatic α-amylase are masked by the adjacent structure, but are unmasked with 5,5′-dithiobis(2-nitrobenzoic acid) in the presence of H_4edta and/or urea by a pseudo-unimolecular process.[205] The reactivity of the unmasked groups was studied by prior incubation of the enzyme with H_4edta only; the subsequent reaction of both groups with 5,5′-dithio-bis(2-nitrobenzoic acid) was first-order in enzyme concentration, and complete reaction abolished all enzymic activity. The kinetic investigation and inhibition of the derivatization with maltotriose indicated that one of the sulphydryl groups of this α-amylase is not located at the catalytic site, although it is close by.

Replacement of the primary hydroxy-groups of the D-glucopyranosyl residues in amylose by amino-groups caused changes in the nature of the enzyme–substrate fit, so that 6-amino-6-deoxy-D-glucose was not detected on hydrolysis with amylases from porcine pancreas and *Bacillus subtilis* and with a mould amylase that also contained glucoamylase.[206] However, the enzymes appear to hydrolyse the rest of the modified amylose, provided the d.s. is low. Hydrolysis products of 6-amino-6-deoxyamyloses by *B. subtilis* amylase included 6^2-amino-6^2-deoxymaltotriose and 6′-amino-6′-deoxymaltose.

Measurements of the levels of α-amylase in canine sera using saccharogenic, amyloclastic, and Phadebas starch methods have shown that the first procedure gives abnormally high values, owing to the presence of glucoamylase.[207] Since Phadebas starch is not hydrolysed by glucoamylase, it is recommended for use.

The isolation and purification, by ion-exchange chromatography and gel filtration, of amylase from the parotid gland of rats (*Rattus norvegicus albinus*) have been reported.[208] Disc gel electrophoresis showed that the preparation contains one principal and two minor amylases. The activity of the pancreatic amylase and the osmo-sensitivity of its zymogen have been investigated in rats receiving ethanol.[209]

A study of the amylase isoenzymes during viral infection of the salivary glands and pancreas of mice has shown that Coxsackie B virus decreased the level of pancreatic amylase, whereas that of serum amylase increased.[210] The level of amylase in the pancreas remained appreciably lower than normal for several months. Polyacrylamide gel electrophoresis of the serum-amylase isoenzymes indicated that hyperamylasemia was predominantly pancreatic in origin during the acute phase of infection; the level of serum amylase subsequently returned to

[205] G. Pommier, P. Cozonne, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1974, **350**, 71.
[206] C. E. Weill, J. B. Nickel, and J. Guerrera, *Carbohydrate Res.*, 1975, **40**, 396.
[207] M. D. O'Donnell and K. F. McGeeney, *Comp. Biochem. Physiol.*, 1975, **50B**, 269.
[208] W. D. Ball, *Biochim. Biophys. Acta*, 1974, **341**, 305.
[209] J. Espinoza, E. Navia, H. Orrego, and A. Feres, *Arch. Biol. Med. Exp.*, 1973, **9**, 1.
[210] M. E. Ross, R. O. Wolf, and A. L. Notkins, *J. Lab. Clin. Med.*, 1974, **83**, 750.

normal, and analysis of the isoenzymes showed a significant decrease in the pancreatic forms. The effect of Freund's adjuvant on the level of blood amylase in laboratory mice has been investigated by testing the enzymic activity against 'Amylochrome' Roche (an amylose that has been dyed blue).[211]

The effect of constant temperature on digestive enzymes, including amylase, of the midgut of *Morimus funereus* larvae has been investigated.[212]

Two isoenzymes of an α-amylase present in germinating seeds of three types of barley have been compared by crossed immunoelectrophoresis.[213] The method permitted the amounts of the corresponding isoenzymes to be determined, and activator control, *de novo* synthesis, and degradation of the enzyme protein to be distinguished.

Changes in the levels of enzymes, including that of α-amylase, have been investigated during the curing of tobacco leaves.[87]

An active bacterial amylase that is stable in starch solutions at temperatures above 100 °C has been reported.[214] New liquefaction procedures using this enzyme are envisaged, since the enzyme is active over broad ranges of temperature and pH, and is less dependent on Ca^{2+} ions than other amylases in commercial use. Pilot-plant experiments have been carried out, using wheat and maize starches.

An α-amylase obtained from *Aspergillus batatae* exhibited maximum activity in the pH range 4—5, and did not yield oligosaccharides in the initial stages of the hydrolysis of starch.[215]

The amylase activities of the mycelia have been measured at various stages of growth for nine strains of *Aspergillus nidulans*, and synthesis of the enzyme was correlated with the production of a (1 → 3)-linked α-D-glucan.[216]

Possible synergistic effects in the combined actions of an α-amylase (from *Aspergillus oryzae*) and a glucoamylase (from *A. awamori*) have been investigated.[217]

Factors regulating the synthesis of α-amylase in *Bacillus licheniformis* have been examined.[218] The amounts of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses produced by the action of amylases from *Bacillus macerans* and *B. megaterium* on uniformly labelled [^{14}C]starch have been measured.[219] Two fractions of enzymic activity from *B. megaterium* afforded the cycloamyloses in the proportions 1 : 2.4 : 1, whereas the proportions for *B. macerans* amylase were 2.7 : 1 : 1. Further studies on the *B. megaterium* amylase showed that the proportion of cycloamyloses produced is largely independent of pH, and that cyclohexa- and cyclo-octa-amyloses are formed directly from starch and not from cyclohepta-amylose. D-[^{14}C]Glucose was principally released from malto-oligosaccharides

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[219] S. Kitahata and S. Okada, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 2413.

(DP 2—8) labelled at the reducing end by the action of *B. megaterium* amylase, whereas *B. macerans* amylase acted on malto-oligosaccharides of DP > 3 to yield labelled maltose.

Genetic control of the synthesis of α-amylase by *B. subtilis* has been studied.[220] It was shown that the rate of synthesis of α-amylase by *B. subtilis* is regulated by a gene (amy R) located near a structural gene for the enzyme.[221] Related studies with a strain of *B. subtilis* have examined how the synthesis of α-amylase depends on a long-lived mRNA.[222] The effects of D-glucose and cyclic nucleotides on transcription of the synthesis of α-amylase mRNA by *B. subtilis* showed that D-glucose-repressed synthesis is unaffected by adenosine and guanosine 3′,5′-cyclic phosphates.[223] The influence of phytates on the synthesis of *B. subtilis* amylase has been investigated.[224]

Aspects of the action of *B. subtilis* α-amylase on starch have been examined.[225] The properties of four heat-sensitive mutants of *B. subtilis* α-amylase have been compared with those of the parent enzyme: two of the mutants differed therefrom only in thermosensitivity, whereas the other two exhibited different catalytic and immunological properties and molecular weights.[226]

A small, but significant, α-amylase activity has been detected in cells of *B. subtilis* Marburg; the level of this activity remained constant, regardless of the level of any extracellular α-amylase activity.[227] One of the three components separated from the cell-bound enzyme by gel filtration exhibited properties identical with those of the extracellular α-amylase; another component (mol. wt. $<7.0 \times 10^4$) was degraded with trypsin, but it remained larger than the third component. A mutant of the organism lacking extracellular α-amylase did not contain cell-bound α-amylases, suggesting that they are precursors of the extracellular enzyme. It appears that the α-amylases of *B. subtilis* Marburg are controlled by the same gene, whether the enzyme is inside or outside the cell.

The production of amylolytic activity by a large number of Basidiomycetes has been investigated.[228] Aphyllophorales and several strains of Trametinae and Coriolinae, but only a few strains of Agaricales, showed high amylolytic activity. These amylolytic activities did not correlate with αα-trehalase activity.

The effect of the source of carbon on the synthesis of α-amylase by *Clostridium acetobutylicum* has been studied.[229]

An intracellular α-amylase (mol. wt. 9.6×10^4) from a *Pseudomonas* species has been purified to homogeneity by fractional precipitation, ion-exchange chromatography, and gel filtration.[230] This *endo*-enzyme (pH optimum 5.5,

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[221] K. Yamaguchi, Y. Nagata, and B. Maruo, *J. Bacteriol.*, 1974, **119**, 416.
[222] J. Sekiguchi, N. Takada, and H. Okada, *J. Bacteriol.*, 1975, **121**, 688.
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[230] K. Kato, T. Sugimoto, A. Amemura, and T. Harada, *Biochim. Biophys. Acta*, 1975, **391**, 96.

temperature optimum 50 °C) hydrolysed malto-oligosaccharides faster than amylose, releasing principally α-D-glucose and α-maltose.

Amylase from *Rhizopus javanicus* is a glycoprotein containing D-mannose (27) and 2-acetamido-2-deoxy-D-glucose (4 mol).[231] Three glycopeptides isolated (by gel filtration and ion-exchange chromatography) after digestion of the heat-denatured enzyme with Pronase have the following compositions: L-asparagine, glycine, D-mannose, and 2-acetamido-2-deoxy-D-glucose, 1 : 1 : 6 : 2; L-asparagine, L-threonine, D-mannose, and 2-acetamido-2-deoxy-D-glucose, 1 : 1 : 9 : 2; L-threonine, L-serine, L-proline, L-alanine, and D-mannose, 6 : 2 : 2 : 2 : 12. The amino-acid sequences (1) and (2) and the peptide–

Gly-L-Asn-carbohydrate (1)

L-Thr-L-Asn-carbohydrate (2)

carbohydrate linkages of the first two glycopeptides were established; the linkage, which involves L-asparagine and 2-acetamido-2-deoxy-D-glucose, is of the glycosylamine type.[232]

Screening of numerous *Streptomyces* species and strains revealed that a strain of *Streptomyces hygroscopicus* produced the highest amylase activity, which was purified to homogeneity by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[233] The enzyme hydrolysed amylose and amylopectin to maltose, but is not a β-amylase; it exhibits maximum activity at pH 5—6 and 50—55 °C, is thermostable, and is not inhibited by pentachloronitrobenzene.

Active immobilized forms of α-amylase have been prepared by reaction of the enzyme with a cyclic imidocarbonate derivative of carboxymethylcellulose,[225] with anti-(human parotid α-amylase) antibody immunoglobulin IgG attached to glass beads,[234] and with 5,5′-dithio-bis(2-nitrobenzoic acid) or 3-maleimidobenzoic acid, whereafter the products were coupled with the thiol groups of human erythrocytes.[235] α-Amylase was also immobilized by copolymerization of acrylate and acrylamide monomers in its presence.[236]

β-Amylases

α-Amylase can be determined in the presence of β-amylase using Phadebas starch as the substrate, since the action of β-amylase is inhibited.[195]

A brief report on the interaction of β-amylase with substrates and inhibitors has commented on Koshland's induced-fit theory.[237] Rebuttals were made to criticism of the proposed binding mechanism on the grounds that it is not supported by kinetic or other data. The finding that α-D-gluco-oligosaccharides are synthesized from β-maltose has confirmed that glycosyl-proton interchange

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[236] K. Kawashima and K. Umeda, *Biotechnol. and Bioeng.*, 1975, **17**, 599.
[237] J. A. Thoma, *European J. Biochem.*, 1974, **44**, 139.

underlies all the reactions catalysed by glycosyl hydrolases and glycosyltransferases.[18]

The inhibition of plant and bacterial β-amylases has been studied.[238]

Polymorphism of β-amylase in the mature grains of cultivars of barley is closely associated with the proportion of free to total (*i.e.* free and latent) β-amylase rather than with the total β-amylase.[239]

The ratio of reactive to non-reactive enzyme–substrate encounters for sweet-potato β-amylase has been measured for the degradation of amylose (DP 250) labelled with D-[^{14}C]glucose at the non-reducing end: 14.6 bonds are cleaved for each effective enzyme–substrate encounter, but only 0.405 bonds are cleaved when inactive encounters are also included.[240] The rate constants for formation and dissociation of both reactive and non-reactive enzyme–substrate complexes were also determined.

Changes in the levels of β-amylase activity during curing of tobacco leaves have been related to the degradation of constituent carbohydrates.[87]

Wheat β-amylase has been claimed to contain four sulphydryl groups: two are free and two are hidden.[241] In the absence of a substrate, both 4-chloromercuribenzoate and *N*-ethylmaleimide reacted with the sulphydryl groups, causing almost complete inhibition, which, for the former compound, is non-competitive. The inhibitory effect of these reagents is vastly decreased in the presence of a substrate; the enzyme then undergoes certain conformational changes, due to 'induced adaptation', that render the internal sulphydryl groups inaccessible to the reagents. The sulphydryl groups on the surface were shown to play no significant role in reactions catalysed by the enzyme.

Active immobilized forms of β-amylase have been prepared by the reaction of the enzyme with agarose cyclic imidocarbonate and its derivatives,[242] and by hydrophobic interaction with alkyl derivatives of agarose cross-linked with epichlorohydrin.[243]

Arabinanases

Culture fluids of a fungal plant pathogen (*Ceratocystis paradoxa*) contained at least five extracellular hemicellulases when commercial hemicellulose was present in the growth medium.[244] One of the enzymes was purified to homogeneity by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and isoelectric focusing. The purified enzyme degraded hemicellulose B (an arabino-4-*O*-methylglucuronoxylan) and arabinoxylan to arabinose, D-xylose, 4-*O*-β-D-xylopyranosyl-D-xylose, and a mixture of xylo-oligosaccharides (DP 3—5) and arabinosyl derivatives thereof; arabinose was slowly released from the arabinoxylo-oligosaccharides. It was concluded that the enzyme is an *endo*-hydrolase, but the arabinanase and xylanase activities are not manifest by the same active site. The arabinanase possesses a pH optimum of 5.5, a temperature optimum of 40 °C, and a K_m value for hemicellulose of 4.24 mg ml^{-1}.

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[244] R. F. H. Dekker and G. N. Richards, *Carbohydrate Res.*, 1975, **39**, 97.

Cellulases

Cellulases used in food processes have been reviewed.[21]

The purification and characterization of two cellulases from auxin-treated pea epicotyls have been reported.[245] Cellulase in hyphae of *Achyla ambisexualis* is localized in dicytosomes, in vesicles, around the periphery of storage bodies, and between the plasmalemma and the cell wall.[246] The enzyme is also present at the outer surface of the cell wall of the male strain of *A. ambisexualis* following treatment with antheridiol.

Most Basidiomycetes synthesize C_x activity and are therefore able to hydrolyse sodium carboxymethylcellulose.[228] Only a few Basidiomycetes (*e.g. Irpex lacteus, Trametes sanguinea, Fomitopsis cytisina, Lampteromyces japonicus, Pholiota adiposa*, and *Stropharia cubensis*) produce an enzyme (C_2 activity) able to hydrolyse filter paper; the enzyme appears to be inducible.

Studies on the adaptive nature of the cellulolytic enzyme from *Chaetomium aureum* have shown that cellulase is induced only in media supplemented with cellulose.[247] Multiple cellulases produced by a soft-rot bacterium (*Erwinia carotovora*) have been investigated, and some fractionation of these enzymes was achieved.[248] A cellulolytic bacterium (*Micromonospora ruminantium* sp. novo) has been found in sheep rumen.[249]

The extracellular cellulase from a *Myxobacter* species has been purified until it was homogeneous on electrophoresis and by velocity sedimentation.[250] Although L-valine was detected at the *N*-terminus, the enzyme also possesses chitosanase activity; these enzymic activities decreased at the same rate during denaturation.

The cellulase activities C_1 and C_x have been detected in filtrates of germinating conidiospores and in growing mycelia of pathogenic and non-pathogenic strains of *Pyricularia oryzae*.[251] Comparisons of these activities for various strains of *P. oryzae* grown on rice-plant powder showed that the C_1 activity is related to the pathogenicity.

The release of cell-bound cellulase from mycelia of *Rhizoctonia solani* can be achieved, to various degrees, with salts and detergents, *etc.*, although the enzyme was inactivated when sodium dodecyl sulphate was used.[252]

Five *endo*-cellulases (mol. wt. 2.83—3.75 $\times$ 10^4) have been isolated from culture filtrates of the rot fungus *Sporotrichum pulverulentum* (formerly *Chrysosporium lignorum*) grown on cellulose powder as the only source of carbon.[253] Flat-bed isoelectric focusing was used to identify the individual enzymes, and a new zymogram technique, which is useful for studying carbohydrate hydrolases, was developed. The molecular activities and K_m values of the enzymes were also determined.[254]

[245] H. Byrne, N. V. Christou, D. P. S. Verma, and G. A. Maclachlan, *J. Biol. Chem.*, 1975, **250**, 1012.
[246] R. A. Nolan and A. K. Bal, *J. Bacteriol.*, 1974, **117**, 840.
[247] B. K. Ghora and K. L. Chaudhuri, *Experientia*, 1975, **31**, 147.
[248] T. C. Tseng, *Bot. Bull. Acad. Sci.*, 1974, **15**, 49.
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[250] A. Hedges and R. S. Wolfe, *J. Bacteriol.*, 1974, **120**, 844.
[251] T. Sudo, H. Nagayama, and K. Tamari, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1651.
[252] N. K. Lisker, J. Katan, I. Chet, and Y. Henis, *Canad. J. Microbiol.*, 1975, **21**, 521.
[253] K.-E. Eriksson and B. Pettersson, *European J. Biochem.*, 1975, **51**, 193.
[254] K. E. Almin, K.-E. Eriksson, and B. Pettersson, *European J. Biochem.*, 1975, **51**, 207.

A derivatized form of pneumococcal type III polysaccharide has been used to distinguish between three β-D-glucan hydrolases (including cellulase) from a *Streptomyces* species.[8] Oligosaccharides also released from carboxymethylcellulose, lichenin, and oat glucan by the enzymes were identified, allowing the substrate specificities of the enzymes to be deduced.

Cellulase C_1 component, free from associated activities of the cellulase complex, has been isolated from *Trichoderma homingii*.[255] The enzyme released terminal cellobiose units from cellulose, the extent of its action being determined principally by the natures of the substrate and the product. Cellulase C_x component was not required for the C_1 component to act. A synergistic effect was produced in the presence of β-D-glucosidase, thereby extending the extent of hydrolysis of native cellulose and less complex forms thereof to $\geqslant 70\%$. The relatively unstable C_1 enzyme (pH optimum 5.0, K_m value for cellulose 0.05 mg ml^{-1}) was inhibited by the product of its action, from which it was released by β-D-glucosidase, and by Cu^{2+}, Zn^{2+}, Fe^{3+}, and phenylmercuric ions; D-glucose did not inhibit the enzyme at concentrations at which cellobiose was effective. The relationship of the C_1 enzyme to the entire cellulase complex of *T. homingii* was discussed.

The synthesis of cellulase from barley straw by *T. viride* has been investigated,[256] and the cellulase has been purified by affinity chromatography on aryl or alkyl derivatives of agarose.[33]

Chitinases (*endo*-β-D-Acetamidodeoxyglucanases)

An enzyme active against glycol-chitin (pH optimum 1.65), but without lysozyme activity, occurs in the sera of sheep, pigs, goats, cows, and hens, but not in the sera of humans, monkeys, horses, dogs, cats, rabbits, guinea-pigs, or hamsters.[257] The enzyme (p*I* 4.85, mol. wt. 6.0×10^4) from goat serum degraded colloidal chitin (pH optimum 5.5) and was not inhibited by goat anti-(human lysozyme) antibodies. Chitinase also occurs in other tissues, the highest activities being found in the stomachs of goats and cows.

The chitinase activity of moulting *Manduca sexta* larvae is localized in older parts of the cuticle; it could be extracted therefrom by homogenization.[258] This chitinase exhibited high activity at the pH of the moulting fluid and preferentially utilized the endogenous chitin in the cuticles as a substrate, indicating that it is physiologically active in the moulting of larvae.

An enzyme (chitinase or *endo*-β-D-acetamidodeoxyglucanase) from *Diplococcus neumoniae* released such oligosaccharides as (3) from immunoglobulin G glyco-

β-D-Gal*p*-β-D-Glc*N*Ac*p*-α-D-Man*p*-β-D-Man*p*-D-Glc*N*Ac
↑
α-D-Man*p*

(3)

peptides that had already been treated with β-D-acetamidodeoxyglucosidase.[80] Digestion of the glycopeptides with β-D-acetamidodeoxyglucosidase and α-D-mannosidase rendered them resistant to this *endo*-β-D-acetamidodeoxyglucanase,

[255] G. Halliwell and M. Griffin, *Biochem. J.*, 1973, **135**, 587.
[256] N. Peitersen, *Biotechnol. and Bioeng.*, 1975, **17**, 361.
[257] G. Lundblad, B. Hederstedt, J. Lind, and M. Steby, *European J. Biochem.*, 1974, **46**, 367.
[258] M. L. Bade, *Biochim. Biophys. Acta*, 1974, **372**, 474.

which appears to require the presence of an unsubstituted α-D-mannopyranosyl residue in the substrate. Chitinase has been isolated from culture fluids of *Staphylococcus aureus* by affinity chromatography on chitin.[259] The enzyme was identified as a lipoprotein containing 80% lipid, and, after repeated extraction with ether–ethanol, a preparation (mol. wt. 1.45×10^4) of pronounced hydrophobic character was obtained.

An enzyme (chitinase or *endo*-β-D-acetamidodeoxyglucanase) that hydrolysed di-*N*-acetylchitobiosyl linkages in oligosaccharides and glycoproteins has been obtained from culture filtrates of *Streptomyces griseus*.[81] The enzyme (mol. wt. $2.72 \pm 0.02 \times 10^4$) consists of a single polypeptide chain, is thermostable, and does not interact with a number of other proteolytic enzymes. Treatment of the glycopeptides (4)—(6) yielded the fragments shown. [^{3}H]Dansylated L-asparagine derivatives of (3)—(5) were also rapidly hydrolysed, and were used as

$$(\text{D-Man})_6\text{-}(\text{D-Glc}N\text{Ac})_2\text{-L-Asn} \longrightarrow (\text{D-Man})_6\text{-D-Glc}N\text{Ac} + \text{D-Glc}N\text{Ac-L-Asn}$$

(4)

$$(\text{D-Glc}N\text{Ac})_2\text{-}(\text{D-Man})_6\text{-}(\text{D-Glc}N\text{Ac})_2\text{-L-Asn} \longrightarrow (\text{D-Glc}N\text{Ac})_2\text{-}(\text{D-Man})_6\text{-D-Glc}N\text{Ac} + \text{D-Glc}N\text{Ac-L-Asn}$$

(5)

$$(\text{D-Glc}N\text{Ac})_2\text{-}(\text{D-Man})_6\text{-}(\text{D-Glc}N\text{Ac})_2 \longrightarrow (\text{D-Glc}N\text{Ac})_2\text{-}(\text{D-Man})_6\text{-D-Glc}N\text{Ac} + \text{D-Glc}N\text{Ac}$$

(6)

$$\text{D-Man-}(\text{D-Glc}N\text{Ac})_2\text{-L-Asn}$$

(7)

substrates in an assay for the enzyme. The glycopeptide (7) was not hydrolysed by this enzyme, but was hydrolysed by another chitinase isolated from *S. griseus* by gel filtration. The ability of a homogeneous chitinase (*endo*-β-D-acetamidodeoxyglucanase H) isolated from culture filtrates of *S. griseus* to hydrolyse oligosaccharides derived from various glycoproteins has been examined.[82] Complete and rapid release of oligosaccharides occurred from sulphitolysed ovalbumin, bovine pancreatic deoxyribonucleases I and II, and a β-D-fructofuranosidase from *Saccharomyces cerevisiae*. Since all of these substrates contain the sequence (8), the enzyme appears to cleave between adjacent 2-acetamido-2-deoxy-D-glucosyl residues distal and proximal to the L-asparagyl residue.

$$(\text{D-Glc}N\text{Ac})_y\text{-}(\text{D-Man})_x\text{-}(\text{D-Glc}N\text{Ac})_2\text{-L-Asn}$$

(8)

Chitosanases

An extracellular enzyme obtained from a *Myxobacter* species has been shown to possess both chitosanase and cellulase activities.[250] The following values were determined when the enzyme acted on chitosan: pH optima 5.0 and 6.8, temperature optimum 70 °C, K_m 0.30 mg ml^{-1}, and V_{max} 7.5×10^{-10} mg ml^{-1}.

[259] V. A. Leonenko, N. A. Kravchenko, I. A. Cherkasov, T. I. Afanas'eva, and Z. V. Ermol'eva *Biochemistry* (*U.S.S.R.*), 1974, **39**, 912.

Chondroitin Sulphate Lyases ABC

Values for K_m and V_{max} have been determined for the degradation of chondroitin 4- and 6-sulphates, chondroitin 4-sulphate proteoglycan, dermatan sulphate, and hyaluronic acid by a chondroitin sulphate lyase ABC obtained from *Proteus vulgaris*.[260] The hydrolysis of chondroitin 4-sulphate by the enzyme was inhibited by hyaluronic acid, but not by keratan sulphate. The findings were discussed in connection with the use of chondroitin sulphate lyase ABC as a reagent for the degradation of tissue glycosaminoglycans.

Chondroitin Sulphate Lyases AC

Arthrobacter aurescens secretes an enzyme that hydrolyses chondroitin sulphate.[261] The enzyme crystallized after purification and exhibited the following properties: pH optimum 6.0, temperature optimum 50 °C, and pH stability 4.9—7.4; it was stable below 45 °C and hydrolysed chondroitin 4- and 6-sulphates, chondroitin, and hyaluronic acid (ratio of initial rates 1.0 : 1.1 : 1.95 : 3.2). The hydrolyses of chondroitin 4- and 6-sulphates furnished 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4- and 6-sulphates, respectively. Since the enzyme is inactive against dermatan sulphate, it is a chondroitin sulphate lyase AC.

Chondroitin 4- and 6-Sulphate Lyases

Enzymes that depolymerize chondroitin 4- and 6-sulphates have been found in mucosal scrapings from the stomachs of rats.[262] Hydrolysis of chondroitin 4-sulphate yielded 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulphate. The stabilities of fractions enriched in the lyase(s) were investigated.

Dextranases

An enzyme (mol. wt. 1.6×10^5) purified to homogeneity (disc gel electrophoresis) from porcine spleen was found to hydrolyse disaccharides containing α-D-glucopyranosyl residues; it degraded dextrans containing (1 → 3)- and (1 → 6)-linkages, but those containing (1 → 2)-linkages were only partly degraded.[139] The kinetics for hydrolysis of several substrates under various conditions of pH, temperature, and inhibition confirmed that the enzyme possesses many substrate-binding sites.

An agar medium containing Blue Dextran 2000 has been used in detecting micro-organisms producing dextranase in human dental plaque.[263] Although preliminary characterization of several dextranase-producing micro-organisms revealed a mixture of cell types of different morphological and biochemical characteristics, a number of the bacteria were identified as *Actinomyces* and *Bacteroides* species. The dextranases are produced extracellularly by these

[260] C. F. Thurston, T. E. Hardingham, and H. Muir, *Biochem. J.*, 1975, **145**, 397.
[261] K. Hiyama and S. Okada, *J. Biol. Chem.*, 1975, **250**, 1824.
[262] Y. H. Liau and M. I. Horowitz, *Proc. Soc. Exp. Biol. Med.*, 1973, **144**, 13.
[263] R. H. Staat, T. H. Gawronski, and C. F. Schachtele, *Infection and Immunity*, 1973, **8**, 1009.

organisms, and a cell-free preparation from one of them caused extensive cleavage of high-molecular-weight dextrans.

A strain of *Aspergillus ustus* synthesizes a dextranase in amounts which are influenced by the quantity and nature of dextrans present during growth; inhibition of the formation of this dextranase was also studied.[264] The dextranase (mol. wt. 3.5×10^4; pH optimum 5.7; temperature optimum 50 °C) was purified to homogeneity by fractional precipitation, ion-exchange chromatography, and gel filtration; it was stable at pH 5.5—8.5 and at temperatures <40 °C, but 50% of the activity was lost on heating at 50 °C for 15 min at pH 7.0.[265] However, the enzyme was more stable to heat in the presence of Cr^{3+} and Al^{3+} ions, but was inhibited by Hg^{2+}, Ag^{+}, and MnO_4^{-} ions.

Dextranase has been obtained from a strain of *Brevibacterium fuscum* by fractional precipitation, gel filtration, and ion-exchange chromatography.[266] The enzyme (pH optimum 7.0—7.5) was stable over a wide pH range (5.0—11.0) and was activated by L-cysteine and H_4edta, whereas it was inhibited by iodine, mercuric chloride, *N*-bromosuccinimide, and cupric sulphate. Since the hydrolysis of dextran by the enzyme afforded principally isomaltotriose, the enzyme appears to be a new *exo*-dextranase.

The action pattern and kinetics of a dextranase obtained from *Penicillium funiculosum* have been studied.[267] Whereas chelated cobalt compounds did not activate the enzyme, Co^{2+} ions and cobalt species bound by the enzyme enhanced its activity, with a concomitant decrease in the activation energy and an increase in V_{max}; K_m was not affected.[268] From modifications to L-lysine, L-arginine, L-histidine, and L-cysteine residues and carboxy-groups in the enzyme, it was concluded that an L-cysteine residue located outside the active site is responsible for the activation. It appears that conformational changes induced when cobalt ions combine with this amino-acid residue make it easier for the enzyme to accept dextran and for the enzyme–substrate complex to dissociate.

Oxidized dextran containing a carbonyl group at C-3 of some of the D-glucopyranosyl residues inhibited the action of a dextranase from *P. funiculosum* on the glucosidic bond of the substituted residue and on the adjacent bond nearer to the reducing terminus.[269] However, hydrolysis of the derivatized residue as the aglycone was allowed. No explanation could be offered for the decreased rate of enzymic hydrolysis brought about by a low degree of oxidation.

The distributions of products resulting from the action of *P. lilacinum* dextranase on end-labelled malto-oligosaccharides have been determined.[270] D-Glucose was released only from the reducing end of isomaltotriose, whereas higher members of the series were hydrolysed at more than one of the other linkages.

Differences in the dextranases of *P. funiculosum* and *P. lilacinum* have been revealed by treatment of the products of their actions on dextran with an *exo*-1,6-

[264] K. Hanada, A. Hirose, and E. Ninomiya, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 17.
[265] K. Hanada, A. Hirose, and E. Ninomiya, *J. Agric. Chem. Soc. Japan*, 1974, **48**, 23.
[266] M. Sugiura, A. Ito, and T. Yamaguchi, *Biochim. Biophys. Acta*, 1974, **350**, 61.
[267] M. Sugiura and A. Ito, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2941.
[268] M. Sugiura, A. Ito, T. Ogiso, and K. Kato, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2953.
[269] R. G. Brown and B. Lindberg, *Carbohydrate Res.*, 1974, **38**, 369.
[270] G. J. Walker and M. D. Dewar, *Carbohydrate Res.*, 1975, **39**, 303.

α-D-glucosidase from *Streptococcus mitis*.[271] Both dextranases hydrolysed dextran B-512, yielding branched oligosaccharides that retained side-chains of one or two residues present in the parent polysaccharide.

An active immobilized form of *Penicillium funiculosum* dextranase has been prepared by reaction with agarose cyclic imidocarbonate.[267] Conditions have been established for the reaction of dextranase and concanavalin A with glutaraldehyde to furnish a soluble, cross-linked derivative retaining both enzymic and agglutination properties, although the action pattern of the immobilized form differed from that of the free enzyme.[272]

endo-Galactanases

An extracellular *endo*-galactanase produced by *Aspergillus flavus* hydrolysed a polysaccharide occurring in peach-tree (*Prunus persica*) gum, yielding fragments containing terminal, non-reducing D-galactosyl residues.[273]

endo-β-D-Galactanases

A keratan-sulphate-degrading enzyme from *Escherichia freundii* has been shown to hydrolyse milk oligosaccharides and desialylized porcine colonic mucin; for example, the tetrasaccharide (9) from milk and a reduced form thereof

β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*
(9)

released D-glucose and D-glucitol, respectively, whereas 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose and 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl 6-sulphate)-D-galactose were released from colonic mucin.[274] Thus, the enzyme is an *endo*-β-D-galactanase of broad specificity.

exo-β-D-Galactanases

An extracellular *exo*-D-galactanase has been shown to be produced by *Aspergillus flavus* [273] (see also above).

Extracts of an *Actinobacillus* sp. and a *Pseudomonas* sp. contain an *exo*-β-D-galactanase (*exo*-β-D-galactosidase) which, in combination with other enzymes, caused extensive degradation of keratan sulphate.[274] The purified enzyme degraded corneal keratan sulphate to a series of oligosaccharides; characterization of two principal oligosaccharides indicated that enzymic attack is limited to internal linkages involving non-sulphated D-galactosyl residues. Unlike other *exo*-β-D-galactanases, the enzyme did not catalyse the hydrolyses of phenyl β-D-galactopyranoside and the tetrasaccharide (9).[275] Other keratan sulphates were degraded more slowly than, and in a different way from, corneal keratan sulphate.

[271] G. J. Walker and A. Pulkownik, *Carbohydrate Res.*, 1974, **36**, 53.
[272] S. A. Barker, A. G. Giblin, C. J. Gray, and W. H. Bowen, *Carbohydrate Res.*, 1974, **36**, 23.
[273] A. Kardošová, J. Rosik, and J. Kubala, *Phytochemistry*, 1975, **14**, 341.
[274] M. Fukuda and G. Matsumura, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 465.
[275] K. Nakazawa and S. Suzuki, *J. Biol. Chem.*, 1975, **250**, 912.

endo-α-1,3-D-Glucanases

The *endo*-α-1,3-D-glucanase activities of the mycelia of nine mutants of *Aspergillus nidulans* have been measured at various stages of growth.[216] The synthesis of the enzyme was correlated with that of a 1,3-α-D-glucan.

endo-β-1,3-D-Glucanases

An *endo*-β-1,3-D-glucanase has been purified (~100-fold) from malted barley; the preparation, which appeared to be physicochemically homogeneous, possesses a molecular weight of 1.28×10^4, and is highly specific for (1 → 3)-β-D-glucosidic linkages.[276]

A β-1,3-D-glucanase has been obtained from a *Bacillus* sp. by ion-exchange chromatography and gel filtration, and was crystallized from ammonium sulphate solution.[277] The crystalline form (mol. wt. 3.6×10^4) was homogeneous on examination by polyacrylamide gel electrophoresis, ultracentrifugation (3.2*S*) and isoelectric focusing (p*I* 4.1). The pH optimum of the enzyme (pH 8.5) is higher than those of other β-1,3-D-glucanases. It was classified as an *endo*-β-1,3-glucanase, since it hydrolysed laminaritetraose to D-glucose and laminaritriose, and laminarin to D-glucose, laminaribiose, laminaritriose, and higher oligosaccharides.

An extracellular *endo*-β-1,3-D-glucanase was produced when *B. circulans* was grown on yeast cell walls as the only source of carbon, although lower levels of the enzyme were obtained for growth on laminarin, pustulan, or D-mannitol.[278] The enzyme was separated from any *endo*-β-1,6-D-glucanase activity by gel filtration, and was further purified by ion-exchange chromatography. Although the enzyme is specific for (1 → 3)-β-D-glucosidic linkages, it did not hydrolyse laminaribiose and hydrolysed laminaritriose only slowly; (1 → 4)-linkages having the β-D-glucosyl residue substituted at C-3 were also cleaved. The kinetics of hydrolysis of laminarin (pH optimum 5.0) were complex, but hydrolysis followed Michaelis–Menten kinetics at low substrate concentrations. The enzyme is devoid of sulphydryl groups, did not require metal ions, and was inhibited by Hg^{2+} ions and D-glucono-1,5-lactone. In contrast to the crude culture fluids, the enzyme had a limited action only on the cell walls of yeast or *Schizosaccharomyces pombe*.

A crystalline β-D-glucanase capable of disrupting the cells of living yeasts has been prepared from a species of *Fungi imperfecti*.[279] This enzyme rapidly degraded yeast D-glucan and laminarin, producing only little reducing sugar; thus, it appears to be a 'random'-type of *endo*-β-1,3-D-glucanase analogous to the C_1 component of cellulase.

Cell walls of all species of the fission yeast *Schizosaccharomyces* underwent autolysis at pH 5.0, releasing β-1,3-D-glucanases that could be separated (by ion-exchange chromatography) into *endo*- and *exo*-activities.[280] The *endo*-β-1,3-D-glucanase (mol. wt. 9.7×10^4) was further purified to homogeneity; it hydro-

[276] D. J. Manners and G. Wilson, *Carbohydrate Res.*, 1974, **37**, 9.
[277] K. Horikoshi and Y. Atsukawa, *Biochim. Biophys. Acta*, 1975, **384**, 477.
[278] G. H. Fleet and H. J. Phaff, *J. Bacteriol.*, 1974, **119**, 207.
[279] S. Nagasaki, J. Fukuyama, S. Yamamoto, and R. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 349.
[280] G. H. Fleet and H. J. Phaff, *J. Biol. Chem.*, 1974, **249**, 1717.

lysed (1 → 3)-β-D-glucans randomly to oligosaccharides, which were then hydrolysed to D-glucose and laminaribiose. Laminaritriose was hydrolysed much more rapidly by these *endo*-D-glucanases than by those from other organisms. The enzyme (pH optimum 5—6, K_m 0.33 mg ml^{-1} for laminarin) was inhibited by D-glucono-1,5-lactone (K_i 95 mmol l^{-1}).

endo-β-1,6-D-Glucanases

Extracellular *endo*-β-1,6-D-glucanase was produced in high concentration when *Bacillus circulans* was grown on yeast cell walls [278] (see also p. 368). This enzyme was separated from the *endo*-β-1,3-D-glucanase by gel filtration, and was then purified by ion-exchange chromatography. The enzyme exhibited maximal activity at pH 5.5 but, in contrast to the crude culture fluid, had only a limited action on the cell walls of yeast or *Schizosaccharomyces pombe*.

An *endo*-β-1,6-D-glucanase (mol. wt. ~3 × 10^4) has been isolated from culture filtrates of an organism resembling *Rhizopus chinensis* by ion-exchange chromatography and gel filtration.[281] The enzyme (pH optimum 5.5—6.0, temperature optimum 60 °C) was active towards luteose, required no metal ions, and hydrolysed gentio-oligosaccharides of DP > 4 to gentio-biose and -triose; laminarin was degraded at one third of the rate of luteose.

exo-α-1,6-D-Glucanases

The action of an *exo*-α-1,6-D-glucanase (*exo*-α-1,6-D-glucosidase) from *Streptococcus mitis* on α-D-gluco-oligosaccharides containing (1 → 6) and other α-linkages has been investigated.[271] Whereas the enzyme hydrolysed α-(1 → 6)-linkages from the non-reducing end of the substrate, including those linkages adjacent to α-(1 → 2, 3, or 4)-linkages, (1 → 6)-linkages at branch points were not hydrolysed. Moreover, the enzyme could neither hydrolyse nor by-pass linkages other than α-(1 → 6)-D-glucosidic ones.

exo-β-1,3-D-Glucanases

An *exo*-β-D-glucanase (*exo*-1,3-D-glucosidase) present in the digestive juices of snails (*Helix pomatia*) has been purified by gel filtration and ion-exchange chromatography.[282] The homogeneous preparation (mol. wt. 8.2 × 10^4) rapidly hydrolysed oligo- and poly-saccharides containing (1 → 3)-linked β-D-glucopyranosyl residues to yield D-glucose. Mixed (1 → 3,4)-linked β-D-glucans were also extensively degraded, whereas (1 → 6)- and (1 → 4)-linked β-D-glucans were hydrolysed slowly. The enzyme (pH optimum 4.3, temperature optimum 50—55 °C) has a broader substrate specificity than other *exo*-β-D-glucanases.

The mechanism of action of an *exo*-β-1,3-D-glucosidase from a Basidiomycetes species has been examined with unbranched (1 → 3)-linked β-D-glucans that had been subjected to periodate oxidation and then reduced.[283] The amount of glycerol released indicated the number of individual chains attacked, and the amount of D-glucose indicated the extent of initial attack on a chain. The ratio

[281] S. Yamamoto, R. Kobayashi, and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1493.
[282] J. J. Marshall and R. J. A. Grand, *Arch. Biochem. Biophys.*, 1975, **167**, 165.
[283] T. E. Nelson, *Biochim. Biophys. Acta*, 1975, **377**, 139.

of D-glucose : glycerol released on enzymic hydrolysis indicated a multiple-attack mechanism in which four D-glucosyl residues are successively removed from the non-reducing terminus of the substrate on each encounter.

exo-β-1,4-D-Glucanases

An *exo*-β-1,4-D-glucanase (*exo*-β-1,4-D-glucosidase) isolated from almond seeds acted on β-D-glucans and β-D-gluco-oligosaccharides by a mechanism similar to that of glucoamylase.[284] A multiple (two)-subsite model was suggested for the active enzyme–carbohydrate complex on the basis of substrate specificities and rate data.

An *exo*-β-1,4-D-glucanase present in the culture fluids of *Sporotrichum pulverulentum* (formerly *Chrysosporium lignorum*) has been separated from contaminating *endo*-cellulases.[253] A five-step procedure, involving ion-exchange chromatography, gel filtration, activation on Dowex-2 resin, and affinity chromatography, on immobilized concanavalin A, was necessary to achieve homogeneity.[285] The enzyme (p*I* 4.3, mol. wt. 4.86×10^4) contains no carbohydrate and was inactive against carboxymethylcellulose.

D-Glucanases (Miscellaneous)

The formation of mono- and oligo-saccharides on enzymic hydrolysis of starch has been discussed.[286]

A glycoprotein from the cell wall of *Saccharomyces cerevisiae* reacted with agarose cyclic imidocarbonate to form an immobilized derivative which preferentially adsorbed *exo*-β-D-glucanase from crude mixtures also containing D-glucosidase.[287] The specificity of the adsorbed enzyme towards laminaribiose, higher laminarioligosaccharides, and other polysaccharides containing (1 → 3)-linked β-D-glucosyl residues was investigated.

Yeast-cell lytic enzymes have been differentiated serologically, using antibodies elicited towards an *endo*-β-1,3-D-glucanase from a species of *Fungi imperfecti*; this technique revealed the absence of any immunological common structure.[279]

D-Glucanases have been shown to be synthesized independently of other lytic enzymes in the thermotolerant *Actinomyces griseinus*, although their formation is sensitive to the composition of the growth medium.[288] A purified D-glucanase isolated from culture fluids of an *Arthrobacter* species was found to be active against D-glucans, pachymans, curdlans, and laminarins.[289] The principal product from *Saccharomyces cerevisiae* D-glucan was laminaripentaose, whereas (1 → 6)-linked β-D-glucosidic bonds were unaffected.

An enzyme (mol. wt. 2.45×10^4) that degrades yeast D-glucan and yeast cells has been crystallized from culture filtrates of an organism strain belonging to the *Fungi imperfecti*; it hydrolysed laminarin, pachyman, and yeast D-glucan, producing a mixture of laminaridextrins.[290]

[284] J. H. Pazur, D. J. Dropkin, and C. E. Hetzler, in ref. 18, p. 374.
[285] K.-E. Eriksson and B. Pettersson, *European J. Biochem.*, 1975, **51**, 213.
[286] T. Komaki, *J. Japan. Soc. Starch Sci.*, 1974, **21**, 34.
[287] M. Edward and R. J. Sturgeon, *L'Actualité Chimique*, 1973, No. 4, p. 67.
[288] S. A. Konovalov, S. Pvorotilo, and L. A. Ziukova, *Mikrobiologia*, 1974, **43**, 261.
[289] K. Doi, A. Doi, and T. Fukui, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 1619.
[290] S. Yamamoto, J. Fukuyama, and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 329.

An intracellular α-D-glucanase (mol. wt. 9.6×10^4) from a *Pseudomonas* species has been purified to homogeneity by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[230] The enzyme (maximum activity at pH 5.5 and 50 °C) hydrolysed malto-triose and -tetraose more rapidly than amylose, but it did not hydrolyse maltose. Cyclo-amyloses and structures containing (1 → 6)-linked α-D-glucopyranosyl residues were also hydrolysed, the latter only slowly. The enzyme cleaved the substrates by an *endo*-action, which ultimately yielded equimolar amounts of D-glucose and maltose from most substrates. The products were released from the enzyme in the α-configuration.

A β-1,3-D-glucanase (mol. wt. 2.2—2.4 × 10^4) that degrades the cells of living yeasts has been crystallized following its isolation from culture filtrates of a micro-organism resembling *Rhizopus chinensis*.[291] Although the pattern of bond cleavage of the enzyme was not established, it appears to be either a 'random' or a 'debranching' β-1,3-D-glucanase.

β-1,3-D-Glucanase was released during autolysis of the cell walls of all species of a fission yeast of the *Schizosaccharomyces* genus at pH 5.0. Ion-exchange chromatography afforded an *exo*-D-glucanase, in addition to an *endo*-β-1,3-D-glucanase [280] (see p. 368). The *exo*-enzyme was further purified and its properties were compared with those of other *exo*-D-glucanases.

A thermophilic *Streptomyces* species producing a β-1,3-D-glucanase has been grown on a chemically defined medium.[292] The enzyme is semi-constitutive and subject to catabolic repression by metabolizable carbon substrates. Surprisingly, synthesis of the enzyme was induced by gentiobiose, which bears little resemblance to normal substrates for the enzyme.

Glucoamylases

The finding that α-D-gluco-oligosaccharides can be synthesized from β-D-glucose has supported the view that the reactions catalysed by glycoside hydrolases and glycosyltransferases are almost identical.[18]

An assay of glucoamylase activity in microbial materials is based on the determination of D-glucose released from soluble starch by means of the D-glucose oxidase–peroxidase reagent.[293] The method is simple, reproducible (standard deviation ±2%), and unaffected by the presence of α-amylase.

The levels of glucoamylase activity in jejunal biopsy specimens from patients with a deficiency of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase–isomaltase') have been determined.[102]

Assessment of the levels of α-amylase in canine sera has shown that Phadebas starch is not hydrolysed by a glucoamylase that is also present.[207]

The action of a mixture of mould enzymes containing glucoamylase activity on 6-amino-6-deoxyamyloses has been studied.[206]

The glucoamylases of *Aspergillus awamori* have been separated into four fractions by adsorption onto corn starch and by ion-exchange chromatography.[217]

[291] S. Yamamoto, R. Kobayashi, and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1563.
[292] G. Lilley and A. T. Bull, *J. Gen. Microbiol.*, 1974, **83**, 123.
[293] M. Kujawski and A. Zajac, *Stärke*, 1974, **26**, 93.

One of the fractions possessed a strong debranching activity that rapidly degraded raw starch; this process was accelerated in the presence of α-amylase or isoamylase from a *Pseudomonas* species, but the extent of acceleration depended on the source of the substrate. Another fraction was less active in digesting and debranching raw starch, but its action was accelerated by the addition of α-amylase.

A study of the amylolytic enzymes isolated from *A. batatae* has shown that the activity of glucoamylase increases as the chain-length of the substrate increases.[215]

A. niger glucoamylase acted on α-D-glucans and α-D-gluco-oligosaccharides by a multi-chain mechanism that results in the liberation of D-glucose from the non-reducing end in a manner akin to that of almond-seed *exo*-β-1,4-glucosidase [284] (see p. 370).

The properties of glucoamylase activities occurring in a number of strains of *Klebsiella* (*Aerobacter*) *aerogenes*[150] and *Rhizopus* species[294] have been investigated.

Active immobilized forms of glucoamylase have been prepared by the reactions with a copolymer of a diaminobenzene and glutaraldehyde [295] and macroreticular beads of derivatized polystyrene,[296] and by coupling to alumina, stainless steel treated with titania, and nickel–nickel oxide sinters.[132]

Glycanases

Extracts of *Actinobacillus* and *Pseudomonas* species contain an *endo*-glycanase, which, in combination with *exo*-β-D-galactosidase, *exo*-β-D-acetamidodeoxyglucosidase, and sulphatase, catalysed extensive cleavage of corneal keratan sulphate.[74] The *Pseudomonas* extract was particularly rich in the *endo*-glycanase activity.

Infection of *Klebsiella aerogenes* with K-2 phage induced the *de novo* synthesis of a glycanohydrolase that is active against the capsular polysaccharide of the host organism.[297] The enzyme was present in the lysates as phage-bound and soluble forms; the homogeneous, soluble form (mol. wt. 4.04×10^5) consists of eight subunits composed of equimolar proportions of two non-identical subunits (mol. wt. 7.0×10^4 and 3.0×10^4). Digestion of the native enzyme with trypsin completely degraded the smaller subunits without loss of enzymic activity, and the substrate specificities and kinetics of the residual tetramer (mol. wt. 2.8×10^5) were unchanged.

Three fungal pathogens (*Ceratocystis paradoxa*, *Cephalosporium sacchari*, and *Marasmius sacchari*) grown on bagasse hemicellulose B or a crude xylan have been screened for the synthesis of hemicellulases.[298]

A depolymerase active towards *Klebsiella* type 11 capsular polysaccharide was induced by the action of bacteriophage II on a *Klebsiella* species.[299] The enzyme catalysed the hydrolyses of β-D-glucopyranuronosyl-(1 → 3)-D-glucuronic

[294] S. Ueda and S. Kano, *Stärke*, 1975, **27**, 123.
[295] V. Krasnobajew and R. Böniger, *Chimia* (*Switz.*), 1975, **77**, 123.
[296] G. Baum, *Biotechnol. and Bioeng.*, 1975, **17**, 253.
[297] T. N. Oeltmann and E. C. Heath, *Carbohydrate Res.*, 1974, **37**, 59.
[298] R. F. H. Dekker and G. N. Richards, *Carbohydrate Res.*, 1974, **38**, 257.
[299] W. Bessler, E. Freund-Mölbert, H. Knufermann, C. Rudolph, H. Thurow, and S. Stirm, *Virology*, 1973, **56**, 134.

acid linkages and some (~1 in 20) D-galactopyranuronosyl-(1 → 3)-D-glucose linkages in the capsular polysaccharide. This glycanase consists of dimeric club- or drop-shaped particles composed of two dissimilar polypeptide chains (mol. wt. 6.25×14^4 and 9.40×10^4).

Hyaluronidases

The purification of tritium-labelled oligosaccharides derived from hyaluronic acid has been improved, using a specific hyaluronidase.[300]

A simple overlay technique has been reported for the detection of hyaluronidase activity following electrophoresis on cellulose acetate membranes.[301] This sensitive method appears to be specific for *endo*-β-D-acetamidodeoxyglucanase activity.

The level of hyaluronidase activity in female sera has been shown to rise during pregnancy.[302] This increase correlates with a qualitative change in the enzyme, suggesting that it is of placental origin; the enzymic activity does not appear to be under hormonal control.

Factors affecting the release of hyaluronidase from guinea-pig spermatozoa during the normal reactions of acrosomes *in vitro* have been investigated.[303] The effects of hyaluronidase on the glycogen contents of parenchymal cells isolated from rat livers have been reported.[304]

Hyaluronidase has been shown to be present in crude extracts of the testes of honey bees (*Apis mellifera*) and oriental hornets (*Vespa orientalis*).[305] The enzyme hydrolysed hyaluronic acid and chondroitin 6-sulphate (pH optimum 4.5—5.0 for both substrates), and its activity increased throughout the temperature range 37—45 °C (*cf.* bovine and ovine testicular hyaluronidases, which are gradually deactivated).

The formation and release of hyaluronidase by cultures of *Streptococcus mitis* in a stirred fermentor at constant pH have been studied.[306] A good correlation was demonstrated between the degree of autolysis and the release of hyaluronidase, indicating that the enzyme is bound to the cells. Freeze-pressing and autolysis were found to be the most effective methods for disintegration of *S. mitis* and subsequent extraction of the hyaluronidase.[307] The synthesis of free and cell-bound hyaluronidases in cultures of *S. mitis* grown under anaerobic conditions was completely repressed by D-glucose during the exponential growth-phase.[308] Rapid synthesis of the enzyme ensued when such cells were transferred to a medium containing peptone but no fermentable carbohydrate.[309] The repressive effect of D-glucose has been shown to be dependent on concentration.[310] The isoenzyme profiles of staphylococcal hyaluronidase have been reviewed.[311]

[300] S. Highsmith and D. M. Chipman, *Analyt. Biochem.*, 1974, **61**, 557.
[301] J. K. Herd, J. Tschida, and L. Motycka, *Analyt. Biochem.*, 1974, **61**, 133.
[302] M. L. Salkie and B. E. Lambert, *Enzyme*, 1975, **19**, 5.
[303] P. Talbot and L. E. Franklin, *J. Reproductive Fertility*, 1974, **39**, 429.
[304] S. R. Wagle and W. R. Ingerbretsen, jun., *Proc. Soc. Exp. Biol. Med.*, 1974, **147**, 581.
[305] D. Allalouf, A. Ber, and J. Ishay, *Comp. Biochem. Physiol.*, 1975, **50B**, 331.
[306] L. Linder, T. Holme, and G. Frostell, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 521.
[307] L. Linder, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 593.
[308] L. Linder, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 608.
[309] L. Linder, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 615.
[310] L. Linder and M. L. Sund, *Acta Pathol. Microbiol. Scand.*, 1974, **82B**, 625.
[311] C. Abramson, *Ann. New York Acad. Sci.*, 1974, **236**, 495.

Isoamylases

Possible synergistic effects resulting from the actions of a *Pseudomonas* isoamylase and *Aspergillus awamori* glucoamylases have been investigated.[217]

Keratan Sulphate Hydrolases

Extracts of *Actinobacillus* and *Pseudomonas* species each catalysed extensive hydrolysis of corneal keratan sulphate, due to the combined actions of a 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase, an *exo*-D-galactanase, an *exo*-β-D-acetamidodeoxyglucanase, and an unidentified *endo*-glycosidase.[74]

Laminarinases

The levels of laminarinase in the mycelia of nine mutants of *Aspergillus nidulans* have been measured at various stages of growth; synthesis of the enzyme was correlated with that of a 1,3-α-D-glucan.[216] The laminarinase isolated from *Rhizopus arrhizus* hydrolysed laminarin, carboxymethyl-laminarin, lichenin, oat glucan, and a reduced pneumococcal type III polysaccharide, whereas it was inactive against carboxymethylcellulose and the original type III polysaccharide; these substrate specificities distinguished it from lichenase and cellulase.[8] The products of enzymic action were investigated and the reduced pneumococcal polysaccharide was particularly useful for the identification of laminarinase, *etc.*

Lichenases

Bacillus subtilis lichenase hydrolysed lichenin, oat glucan, and reduced pneumococcal type III polysaccharide.[8] Unlike laminarinase, the enzyme did not degrade laminarin and carboxymethyl-laminarin.

Lysozymes

A reference work on lysosymes has dealt with their structures, the structural bases of enzymic and non-enzymic characteristics, and biological and clinical investigations.[312]

Human monocytes and mouse peritoneal macrophages have been shown to synthesize and secrete large amounts of lysozyme.[67] Synthesis of the enzyme was rapidly inhibited by cycloheximide and less rapidly by colchicine. The physicochemical properties of this lysozyme were reported.

Lysozyme in human sera, cerebrospinal and amniotic fluids, urine, saliva, gastric juice, bile, tears, milk, and sperm have been examined by means of diffusion in agarose gel.[313] The enzyme is found in most body fluids, the highest activity occurring in tears. The level of lysozyme activity in human sera has been shown to increase after exercise, but to drop to the original level during recovery.[314] Possible mechanisms for these changes were discussed.

Density-gradient centrifugation of dissociative extracts of bovine nasal cartilage has yielded a proteoglycan that forms an ionic complex with cartilagenous

[312] 'The Enzymes', Vol. 10, 3rd edn., ed. P. D. Boyer, Academic Press, New York and London, 1974.
[313] J. Hankiewicz and E. Swierczek, *Clinica Chim. Acta*, 1974, **57**, 205.
[314] G. Haralambie and F. J. Cerny, *Clinica Chim. Acta*, 1974, **55**, 217.

lysozyme.[315] Human cartilagenous and urinary lysozymes and hen egg-white lysozyme behaved similarly. The lysozyme–proteoglycan complex was solubilized by the addition of salt or alkali, but a higher concentration of salt was required than for a lysozyme–chondroitin sulphate complex. It was suggested that cartilagenous lysozyme may form part of an ionic complex involving proteoglycans *in vivo*, although it is unlikely to play a major role in maintaining the structural integrity of cartilage.

Lysozyme has been isolated from bovine and canine cartilages and from canine sera by a variety of chromatographic procedures, including affinity chromatography on carboxymethylchitin.[316] The composition of the bovine lysozyme is similar to that of other mammalian lysozymes. Anti-(canine lysozyme) antibodies cross-reacted with bovine lysozyme, but with neither hen egg-white lysozyme nor embryonic chicken-cartilage lysozyme. Electron microscopy has shown that lysozyme in canine epiphyseal plates is localized extracellularly, mainly in the vicinity of the chondrocytes. Evidence that lysozyme has a physiological role in normal endochondral calcification has been presented.[317]

The 'g' types of lysozyme found in goose egg-white have also been detected in the egg-whites of species representing nine orders of birds, whereas the 'c' type found in chicken egg-white has been detected only in the egg-whites of two orders.[318]

The ^{19}F n.m.r. spectrum of *N*-trifluoroacetylated chitotriose has been measured in the presence of turkey egg-white lysozyme.[319] The ^{19}F n.m.r. spectrum of the complex did not show any marked dependence upon pH, and was very similar at all pHs to that obtained when the trisaccharide complexes with hen egg-white lysozyme at low pH, when L-aspartic acid-101 is protonated. The replacement of this residue by glycine in turkey egg-white lysozyme is considered to account for differences in the pH dependence of the spectra, and a pK_a of 4.2 was obtained for L-aspartic acid-101 in hen egg-white lysozyme. By contrast to that of chitotriose, the binding of chitobiose and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside is the same for turkey and hen egg-white lysozymes.[15] It appears that L-aspartic acid-101 does not contribute as much as previously suggested to the binding energy of the disaccharide. Both enzymes possessed identical activities against cells of *Micrococcus lysodeikticus*.

Although all species of bird previously studied produce only one form of lysozyme in the egg-white, two distinct lysozymes have been isolated from the whites of black swans' (*Cygnus atratus*) eggs.[320] The molecular weight, amino-acid sequence, specific activity, and immunological cross-reactivity of one of them are similar to those of the egg-white lysozymes of hen, duck, and many other birds, whereas those of the other form (mol. wt. 2.05×10^4) are not. However, the *N*-terminal amino-acid sequence and immunological cross-reactivities are

[315] R. A. Greenwald and C. E. Schwartz, *Biochim. Biophys. Acta*, 1974, **359**, 66.
[316] H. L. Guenther, N. Sorgente, H. E. Guenther, R. Eisenstein, and K. E. Kuettner, *Biochim. Biophys. Acta*, 1974, **372**, 321.
[317] K. E. Kuettner, N. Sorgente, R. L. Croxen, D. S. Howell, and J. C. Pita, *Biochim. Biophys. Acta*, 1974, **372**, 335.
[318] E. M. Prager, A. C. Wilson, and N. Arnheim, *J. Biol. Chem.*, 1974, **249**, 7295.
[319] N. Arnheim, F. Millett, and M. A. Raftery, *Arch. Biochem. Biophys.*, 1974, **165**, 281.
[320] N. Arnheim, A. Hindenburg, G. S. Begg, and F. J. Morgan, *J. Biol. Chem.*, 1973, **248**, 8036.

identical to those of the lysozyme present in Embden goose egg-white. Bone-marrow tissues of Embden geese and polymorphonuclear leucocytes of chickens contain two forms of lysozyme, although only one form is present in the egg-whites.[321]

The levels of lysozyme in nuclei isolated from chicken oviducts have been determined.[322]

It has been shown that 35% of the enzymic activity is retained when all eleven L-arginine residues of hen egg-white lysozyme are derivatized with cyclohexanedione.[323] The function of L-arginine residues in the molecule was discussed in the light of this evidence.

Treatment of hen egg-white lysozyme first with 2,3-epoxypropyl β-di-*N*-acetylchitobioside and then with sodium borohydride and 1,4-dithiothreitol resulted in replacement of L-aspartic acid-52 by a homoserine residue; this product was enzymically inactive, although the reduced (sodium borohydride) enzyme retained its activity.[324] Since the size of the side-chain of homoserine is similar to that of L-aspartic acid, the loss of activity must result from chemical modification of L-aspartic acid-52.

Studies of the structures and antigenicities of the regions 1—27 and 122—129 of hen egg-white lysozyme by derivatization (*e.g.* reduction and alkylation) have indicated that long-range interactions between the *N*- and *C*-termini help to stabilize the molecule.[325] Lysozyme and α-lactalbumin (a structurally related molecule) have been compared in an attempt to understand the greater stability of lysozyme.[326] Digestion of hen egg-white lysozyme with pepsin under controlled conditions has yielded large fragments corresponding to regions 57—107, 57—83, and 91—107.[327] Inhibition of the lysozyme–anti-lysozyme antibody reaction showed that the region 84—90 influences the antigenicity of fragment 57—107. One of the regions of antigenicity, which reacted with 10% of the total anti-lysozyme antibodies, appears to involve the amino-acid sequence 85—90 in combination with a region close to L-methionine-105. Structural studies of the purified peptides by c.d. revealed that only 30% of the original α-helicity remained. It was concluded that long-range interactions stabilize the natural configuration of lysozyme and that structural differences are not responsible for the differences in antigenic specificities between related peptides.

The interaction of lysozyme with guanidinium chloride in aqueous solution has been investigated using equilibrium dialysis and differential refractometry; the denaturant was preferentially bound at all concentrations used.[328]

The dependence on pH of the free energies and enthalpies of the interactions of mono- and di-saccharides with lysozyme has been measured.[329]

The binding of 2-acetamido-2-deoxy-α- and -β-D-glucopyranose to hen egg-white lysozyme and to a form of the enzyme oxidized at L-tryptophan-62 has been

[321] A. Hindenburg, J. Spitznagel, and N. Arnheim, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1653.
[322] P. M. Conn and B. W. O'Malley, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 740.
[323] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 565.
[324] Y. Eshdat, A. Dunn, and N. Sharon, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1658.
[325] G. E. Matthyssens and L. Kanarek, *European J. Biochem.*, 1974, **43**, 363.
[326] S. Sugai, H. Yashiro, and K. Nitta, *Biochim. Biophys. Acta*, 1973, **328**, 35.
[327] G. E. Matthyssens, G. Giebens, and L. Kanarek, *European J. Biochem.*, 1974, **43**, 353.
[328] J. Špan, S. Lenarčič, and S. Lapanje, *Biochim. Biophys. Acta*, 1974, **359**, 311.
[329] S. K. Banerjee, G. E. Vandenhoff, and J. A. Rupley, *J. Biol. Chem.*, 1974, **249**, 1439.

measured by microcalorimetry.[330] The apparent enthalpies and dissociation constants at pH 5.0 indicated that the α-anomer binds in two, mutually exclusive, conformations at subsite C of the active cleft; one of the conformations corresponds to that adopted by the β-anomer.

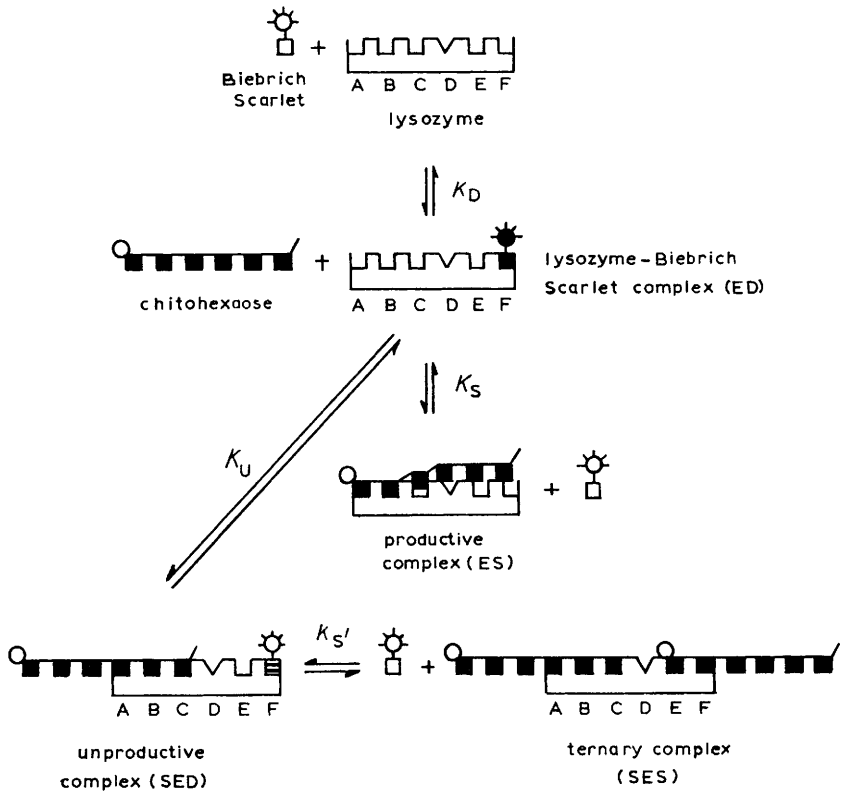
Although a method for determining productive and unproductive lysozyme–chito-oligosaccharide complexes has not been available hitherto, it has been found that Biebrich Scarlet forms a 1 : 1 complex with part of the substrate-binding site.[331] Complex formation perturbs the spectrum of the dye, so enabling its dissociation constant (K_D = 0.13 mmol l^{-1}) to be determined. Determination of the dissociation constants for three principal enzyme–chito-oligosaccharide complexes demonstrated that: (i) chito-oligosaccharides that bind only to sites A—C of lysozyme perturb the spectrum of the dye–lysozyme complex without affecting the dissociation constant (K_U = 0.01 mmol l^{-1}) of the dye; (ii) chito-oligosaccharides interact with sites D—F by displacing the dye ($K_{S'}$ = 5—15 mmol l^{-1}); (iii) hexa-*N*-acetylchitohexaose forms a complex that involves the entire binding site and, therefore, displaces the dye (see Scheme 1). This complex (K_S = 0.03 mmol l^{-1}) is considered to be the productive one. The binding mechanism proposed on this evidence differs from any considered previously.

Stable complexes were formed when [^{14}C]cello-oligosaccharides that contain three or more (1 → 4)-linked β-D-glucopyranosyl residues interacted with lysozyme.[332] Although complexation occurred over the pH range 3—9, it was favoured at high pH, and the extent of complexation was inversely proportional to the temperature. The complexes contained equimolar amounts of carbohydrate and protein, but no fragments containing radioactivity were isolated from a tryptic digest. Acceptors and competitive inhibitors of lysozyme inhibited complexation and also dissociated the complexes. The enzyme did not hydrolyse the oligosaccharides nor did it use them as D-glucosyl donors. Ultracentrifugation, molecular-sieve chromatography, and light-scattering studies indicated that the precipitated complexes are large, heterogeneous, aggregates composed of equal amounts of protein and oligosaccharides that are in equilibrium with the individual components.

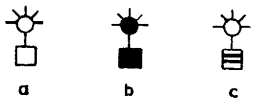
A fluorescence polarization method has been used to compare the association constants of the interactions between κ- and α_{S1}-caseins and between lysozyme and ovalbumin.[333] The latter interaction is electrostatic in nature.

The association constant for the binding of Gd^{3+} ions to lysozyme has been determined at pH 5.2 (by measuring the release of protons) and at pH 6.5 (by u.v. spectroscopy).[334] In agreement with *X*-ray data, it was found that Gd^{3+} ions bind to the carboxy-groups of L-glutamic acid-35 and L-aspartic acid-52 at the junction of sites D and E in the cleft of the enzyme. The association constants for the binding of several inhibitors and substrates to lysozyme and to the complex with Gd^{3+} were determined by fluorescence spectroscopy; the lactone derived from the tetra-*N*-acetylchitotetraose (a transition-state analogue) had a

[330] A. Cooper, *Biochemistry*, 1974, **13**, 2853.
[331] E. Holler, J. A. Rupley, and G. P. Hess, *Biochemistry*, 1975, **14**, 1088.
[332] J. A. Thoma, G. V. K. Rao, A. Bowanko, A. L. Jennings, and C. Crook, *Carbohydrate Res.*, 1974, **38**, 279.
[333] S. Nakai and C. M. Kason, *Biochim. Biophys. Acta*, 1974, **351**, 21.
[334] I. I. Secemski and G. E. Lienhard, *J. Biol. Chem.*, 1974, **249**, 2932.



Scheme 1. *A model for multiple binding of chitohexaose to the cleft of lysozyme, illustrating the principle of the method for determining the dissociation constants of the various complexes. The major chitohexaose–lysozyme complexes show nare the following.* (i) *A productive complex which competes with the binding of Biebrich Scarlet. This complex involves contacts of the non-reducing end of the saccharide with site A, good contacts with sites B and C, and protrusion of the rest of the saccharide into the D—F region.* (ii) *An unproductive complex which perturbs the absorption spectra of Biebrich Scarlet. This complex involves contact of the reducing end of the saccharide with site C, good contacts with sites B and A, and protrusion of the rest of the saccharide into solvent.* (iii) *A ternary complex in which another chitohexaose binds to the unproductive complex and displaces Biebrich Scarlet. The absorption spectra of the free inhibitor (a), of the inhibitor bound to the enzyme (b), and of the inhibitor bound to the unproductive lysozyme–chitohexaose complex (c) are different. The binding sites of lysozyme are*



indicated by open squares, except for site D which is triangular (to indicate that distortion of the pyranose ring is required for binding to this site). ES is considered to be the final reactive enzyme–substrate complex in which site D is filled. The dissociation constants of the various complexes are defined in the text.*

greater affinity for lysozyme than for the Gd^{3+} complex at pH 6.5, suggesting that it occupies sites A—D in the free enzyme. By contrast, the corresponding aldonic acid bound more tightly to the complex, probably due to interaction between its carboxy-group and the metal ion. The following conclusions were reached concerning the binding of lysozyme with oligosaccharides at low concentrations: (i) for trisaccharides, either tetra- or penta-saccharides, and hexasaccharides there are one, two, and three significant modes of binding, respectively; (ii) one of the modes is common to all the substrates; (iii) bound Gd^{3+} ions interfere with the binding of penta- and hexa-saccharides in the uncommon mode(s).[14]

Precise data on the kinetics of hydrogen-exchange for lysozyme have been obtained at pH 6.8 in the presence or absence of 2-acetamido-2-deoxy-D-glucose, which acts as an inhibitor.[335]

Derivatization with 7-chloro-4-nitrobenz-2,1,3-oxadiazole has been used in studies of the functions of the primary amino-groups of hen egg-white lysozyme.[336] A kinetically controlled intermediate, in which L-tyrosyl residues are modified, was fully active, and its formation resulted in an induction period in the derivatization of amino-groups. Differences between the c.d., fluorescence emission, and excitation spectra of derivatized and underivatized lysozymes indicated that limited structural changes occur in the helical segment on derivatization. The enhanced reactivity of the amino-groups that results from a conformational change was discussed. Lysozyme has also been derivatized with 7-chloro-4-nitrobenz-2,1,3-oxadiazole in order that a conformational change of the molecule in alkali could be studied.[337] The values for the thermodynamic parameters (pK_a 9.89; ΔH 4.74 kcal mol^{-1}; ΔS − 29.9 cal $°C^{-1}$ mol^{-1} at 300 K) suggested that this change depends on the ionization of an L-tyrosyl residue in the derivatized enzyme.

The susceptibility of egg-white to γ-irradiation has been examined.[338] Since inactivation of lysozyme in isolation increased exponentially, whereas that in egg-white was only slight, it appears that the enzyme is protected by other proteins from the radiation. This protective effect could be simulated by ethanol. Gel-filtration indicated that irradiation of lysozyme produced an inactive polymer of mol. wt. 7×10^5.

4-Methylumbelliferyl tri-*N*-acetyl-β-chitotrioside is a convenient substrate for the assay of lysozyme, since the 4-methylumbelliferone formed can be measured by the increased fluorescence at 442 nm.[339] The binding constant and rate of hydrolysis of this trioside were higher than those of 4-nitrophenyl tri-*N*-acetyl-β-chitotrioside. 4-Methylumbelliferyl glycosides of 2-acetamido-2-deoxy-β-D-glucopyranose and di-*N*-acetyl-β-chitobiose were also investigated as substrates.

A number of synthetic homologues of the loop-region (residues 64—82) of lysozyme that had amino-acid residues replaced singly or two at a time with L-alanine have been assessed in terms of their capacity to bind with anti-loop

[335] R. R. Wickett, G. J. Ide, and A. Rosenberg, *Biochemistry*, 1974, **13**, 3273.
[336] A. A. Aboderin, E. Boedefeld, and P. L. Luisi, *Biochim. Biophys. Acta*, 1973, **328**, 20.
[337] A. A. Aboderin, E. Boedefeld, and P. L. Luisi, *Biochim. Biophys. Acta*, 1973, **328**, 31.
[338] T. Kume, Y. Sato, and Y. Umemoto, *J. Agric. Chem. Soc. Japan*, 1973, **47**, 549.
[339] F. M. Delmotte, J. P. D. J. Privat, and M. L. P. Monsigny, *Carbohydrate Res.*, 1975, **40**, 353.

antibodies.[340] The results were correlated with the conformations of the homologues, and the involvement of hydrogen-bonding and the β-structure in antigenic activity was noted.

The identification and characterization of lysozyme from the haemolymph of the soft-shelled clam (*Mya arenaria*) have been reported.[341]

A lytic activity that degrades the cell walls of a *Rhizopus* species has been found in culture filtrates of a *Bacillus* species.[342] The lytic activity was separated (by ion-exchange chromatography) into two fractions: one of the fractions was identified as a protease, whereas the other liberated material containing 2-amino-2-deoxyhexose from *Rhizopus* cell walls and was also active on glycolated chitosan.

Bacillus subtilis has been shown to produce enzymes that degrade *Pseudomonas aeruginosa*.[343] One of the enzymes hydrolysed a peptidoglycan obtained from *P. aeruginosa* to disaccharides containing reducing muramic acid residues, and is presumably a type of lysozyme.[344] However, egg-white lysozyme was hardly active against the cellular substrate. Various physicochemical tests and the synergistic effect of the endogenous peptidase from *B. subtilis* were used to explain the superior lytic activity of the bacterial enzyme towards cells of *P. aeruginosa*. The enzymic properties, amino-acid composition, and physicochemical properties (mol. wt. 1.3×10^4, p*I* 9.8, pH optimum 6.2 towards cell walls of *Micrococcus lysodeikticus*) of this enzyme have been compared with those of hen egg-white lysozyme.[345] The bacterial enzyme is particularly rich in basic amino-acids (especially L-lysine) and is stable at pH 3.5—6.0; it is less active against *M. lysodeikticus* cell walls and yielded more high-molecular-weight products than did egg-white lysozyme, which appears to have a different substrate specificity.

The lysozyme from a *Chalaropsis* species attacked the murein present in cells and cell walls of *Staphylococcus aureus* in a random manner.[346] Lysozyme is one of the murein hydrolases present in the envelope of *Escherichia coli*.[347] Its properties *in situ* and its solubilization from the envelope were reported.

A lysozyme-like enzyme has been isolated from culture fluids of *S. aureus* by affinity chromatography on chitin.[259] The enzyme is a lipoprotein (80% lipid; mol. wt. 1.45×10^4). Defatting with ethanol–ether rendered the enzyme hydrophobic while its specific activity increased to that of egg-white lysozyme, which possesses a similar amino-acid composition.

The three-dimensional structure of lysozyme from bacteriophage T4 has been determined at 2.5 Å resolution.[348] The structure differs from that of hen egg-white lysozyme, although it is not clear whether or not the mechanisms of catalysis of the enzymes are related. Other physical properties, including the reversible unfolding, of bacteriophage T4 lysozyme have been reported.[349]

[340] R. Arnon, E. Teicher, and H. A. Scheraga, *J. Mol. Biol.*, 1975, **90**, 403.
[341] T. C. Cheng and G. E. Rodrick, *Biol. Bull.*, 1974, **147**, 311.
[342] Y. Tsujisaka, Y. Tominaga, and M. Iwai, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 145.
[343] S. Murao and Y. Takahara, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2305.
[344] Y. Takahara, E. Machigaki, and S. Murao, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2349.
[345] Y. Takahara, E. Machigaki, and S. Murao, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2357.
[346] J. P. Robinson, R. D. Robinson, and J. H. Hash, *J. Bacteriol.*, 1974, **117**, 900.
[347] R. Hartmann, S. B. Bock-Hennig, and U. Schwarz, *European J. Biochem.*, 1974, **41**, 203.
[348] B. W. Matthews and S. J. Remington, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4178.
[349] M. Elwell and J. Schellman, *Biochim. Biophys. Acta*, 1975, **386**, 309.

Hen egg-white lysozyme has been used as a model in investigations of the modification of proteins in non-aqueous media.[350] Cross-linking of lysozyme with *N,N'*-carbonyldi-imidazole in anhydrous DMF gave a water-insoluble derivative which could be used as an immobilized form of the enzyme.

D-Mannanases (Miscellaneous)

D-Mannanases occurring in *Actinomyces griseinus* are synthesized independently of other glycanases, and it was possible to stimulate the synthesis of D-mannanases while inhibiting that of D-glucanases.[288] A correlation exists between the total lytic activity and the D-mannanase activity.

Oligo-1,6-D-Glucosidases

The levels of oligo-1,6-D-glucosidase activity in jejunal biopsy specimens from controls and from patients with a deficiency of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase–isomaltase') have been determined.[172] Polyacrylamide gel electrophoresis of material from the brush-border membranes of two of the patients revealed the absence of a protein band that is normally associated with the sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase complex.

Polygalacturonases

The use of polygalacturonases in food processing has been reviewed.[21]

Polygalacturonase activity in polyacrylamide gels (particularly after isoelectric focusing) can be detected by incubation of the gel with an appropriate substrate, after which it is stained with Ruthenium Red.[351] Polygalacturonase isoenzymes were well separated from those of pectin lyase by isoelectric focusing.

The distribution of pectolytic enzymes among the Basidiomycetes has been investigated, but only a few species exhibited high activity towards potato tubers.[352] *Fomitopsis cytisina* and *Irpex lacteus* (Aphyllophorales) and *Lamptero-myces japonicus* (Agaricales) also exhibited activity. Only one strain of Aphyllo-phorales, *viz. Coriolus hirsutus*, exhibited high activity in the liquefaction of pectic acid when cultured in a pectin-free medium, although a number of species (*e.g.*, *C. hirsutus*, *Daedaleopsis styracina*, *I. lacteus*, and *Poria vaporaria*) showed high activity when cultured in the presence of pectin. Several species of Aphyllo-phorales brought about the liquefaction of pectin more easily than that of pectic acid, but species of Agaricales, with the exception of *L. japonicus*, that hydrolysed pectic acid did not hydrolyse pectin. Although there is no apparent correlation between the macerating and pectolytic activities, an enzyme preparation from *L. japonicus* possessed identical pH–activity profiles for both.

Polygalacturonases from an *Aspergillus* species and from *Saccharomyces fragilis* have been fractionated, and the products resulting from hydrolysis of pectate by the fractions were analysed by gel filtration at various stages of the reaction.[353] Oligosaccharides were released by *endo-* and *exo*-polygalacturonases

[350] G. J. Bartling, H. D. Brown, and S. K. Chattopadhyay, *Enzyme*, 1974, **18**, 310.
[351] N. Lisker and N. Retig, *J. Chromatog.*, 1974, **96**, 245.
[352] M. Kawai, *J. Agric. Chem. Soc. Japan*, 1973, **47**, 523.
[353] J. H. McLendon, *Phytochemistry*, 1975, **14**, 377.

and by an enzyme designated 'S-polygalacturonase'. The initial products of the *endo*-polygalacturonase were identified as mixed oligosaccharides of DP 5—30; mono- and di-saccharides appeared in the early stages of the reaction, but represented $<2\%$ of the products until later in the reaction.

A polygalacturonase obtained from culture fluids of *Botrytis cinerea* was shown to be homogeneous by disc gel electrophoresis, although it also exhibited pectinesterase activity.[354] The enzyme acted on sodium pectate and oligosaccharides of D-galacturonic acid to give D-galacturonic acid only; the viscosity of sodium pectate was reduced by half after 4.6% of the glycosidic linkages had been cleaved. The polygalacturonase (mol. wt. $\sim 6.9 \times 10^4$) was inactivated by 4-chloromercuribenzoic acid, tetranitromethane, and urea.

The synthesis of polygalacturonase by *Colletotrichum capsici* has been investigated.[355]

exo-Polygalacturonases

The use of *exo*-polygalacturonases in food processing has been discussed.[21]

exo-Polygalacturonases from an *Aspergillus* species and from *Saccharomyces fragilis* released D-galacturonic acid from D-galacturonans, thereby distinguishing them from polygalacturonases and 'S-polygalacturonase'.[353]

The distribution of pectolytic enzymes among the Basidiomycetes has been investigated.[352]

exo-Polygalacturonase has been extracted from green lemon peel after it had been inocculated with *Penicillium digitatum*.[351] The isoenzymes were separated from those of pectin lyase by isoelectric focusing in polyacrylamide gel; bands were located by incubating the gel with an appropriate substrate and staining with Ruthenium Red.

exo-Poly-α-D-Galacturonosidases

A *Pseudomonas* species has been found to synthesize an enzyme that liberated α-D-galactopyranosyluronic acid-(1 → 4)-D-galacturonic acid only from pectic acid.[356] The enzyme was therefore identified as an *exo*-poly-α-D-galacturonosidase.

Pectate Hydrolases (Miscellaneous)

Fractionation of extracts from an *Aspergillus* species and from *Saccharomyces fragilis* separated the 'S-polygalacturonase' (see p. 381) from the *endo*- and *exo*-polygalacturonases.[353] S-Polygalacturonase could also be distinguished from the other enzymes by means of the products of its action on poly(D-galacturonic acids); although the liberation of reducing groups could be reproduced by a mixture of *exo*- and *endo*-polygalacturonases, the distribution of products and the pH–activity profile could not. The initial products of S-polygalacturonase acting on poly(D-galacturonic acids) were mono-, di-, and penta-saccharides, and some tri- and tetra-saccharides, although the pentasaccharide was subsequently hydrolysed.

[354] H. Urbanek and J. Zalewska-Sobczak, *Biochim. Biophys. Acta*, 1975, **377**, 402.
[355] J. S. Solanki, J. P. Jain, and J. P. Agnihotri, *Mycopath. Mycologia Applicata*, 1974, **54**, 189.
[356] C. Hatanaka and T. Imamura, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2267.

The distribution of pectolytic enzymes among the Basidiomycetes[352] and the synthesis of a 'protopectinase' by *Colletotrichum capsici*[355] have been reported.

Pectate Lyases

Pectolytic enzymes used in food processing have been reviewed.[21]

The distribution of pectolytic enzymes among the Basidiomycetes has been examined.[352]

Pectin Lyases

Pectin lyases used in food processing have been reviewed.[21]

Pectin lyases are among the pectolytic enzymes found in various species of Basidiomycetes.[352]

Pectin lyase has been obtained from culture fluids of *Alternaria solani*[351] (see also p. 381).

Pullulanases

A viscosimetric assay of debranching enzymes of the pullulanase type has been reported; interference by α-D-glucosidase is avoided.[357]

A pullulanase in *E. coli* appears to be bound to the cell wall; its properties are very similar to those of a pullulanase from *Klebsiella* (*Aerobacter*) *aerogenes*.[358] However, no pullulanase activity was detected in cultures of *E. coli* grown on maltose.[359] Neither this nor forty other strains of *E. coli* grew on pullulan, whereas all the strains grew on maltose, and some on maltotriose. The synthesis of pullulanase by various strains of *Klebsiella* (*Aerobacter*) *aerogenes* using maltose both as an inducer and as a source of carbon has been studied.[150] Conditions leading to the synthesis of pullulanase were reported.

αα-Trehalases

The metabolism of αα-trehalase has been reviewed.[360]

The levels of αα-trehalase activity in jejunal biopsy specimens from normals and from patients with a deficiency of sucrose α-D-glucohydrolase–oligo-1,6-D-glucosidase ('sucrase–isomaltase') have been compared.[102]

Digestion of closed, right-side-out vesicles of porcine, rabbit, and rat brush-border jejunum with papain released αα-trehalase and other carbohydrate hydrolases that had been bound to the membrane without unduly affecting the lipid bilayer that limits the vesicles.[140] The enzymes appear to be surface components attached to the outside of the membrane.

Among the Basidiomycetes, *Trametes kusanoana* exhibited high αα-trehalase and amylolytic activities, whereas some species of Agaricales and *Poria placenta* exhibited high αα-trehalase activity but no amylolytic activity.[228]

The αα-trehalase from *Phycomyces blakesleeanus* occurs in two interconvertible forms: one form of low activity occurs in dormant spores, whereas one of high

[357] D. G. Hardie and D. J. Manners, *Carbohydrate Res.*, 1974, **36**, 207.
[358] T. N. Palmer, G. Wöber, and W. J. Whelan, *European J. Biochem.*, 1973, **39**, 601.
[359] A. Dessein and M. Schwartz, *European J. Biochem.*, 1974, **45**, 363.
[360] A. D. Elbein, *Adv. Carbohydrate Chem. Biochem.*, 1974, **30**, 227.

activity is found after dormancy ends.[361] The molecular weights ($\sim 2.1 \times 10^5$) and electrophoretic mobilities of these forms are indistinguishable.

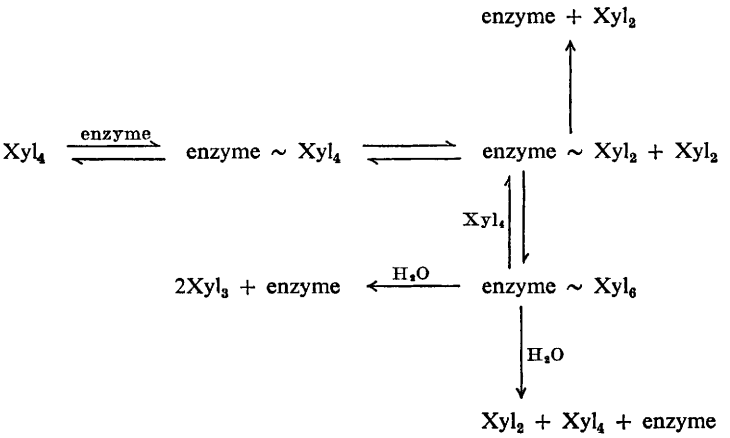
An inactive form of αα-trehalase and its activating protein have been partly purified from resting cells of baker's yeast (*Saccharomyces*) by a combination of fractional precipitation with ammonium sulphate and ion-exchange chromatography.[362] For activation by adenosine 3′,5′-cyclic phosphate, this system appeared to depend on the presence of ATP and Co^{2+}, or Mg^{2+}, or Mn^{2+} cations. The activating protein lost some of its dependence on adenosine 3′,5′-cyclic phosphate during purification, and it may be a protein kinase; activation of αα-trehalase would then be associated with phosphorylation of the kinase.

Xylanases (Miscellaneous)

A *Bacillus* species from soil was able to synthesize a xylanase in alkaline, but not neutral, media.[363] After purification by ion-exchange chromatography and gel filtration, the xylanase (3.5*S*, p*I* 6.3) exhibited activity in the pH range 5.5—9.0 and was most stable at pH 7. Since the enzyme hydrolysed xylan to xylobiose and higher oligosaccharides, it is a type of *endo*-xylanase. The xylanase was inhibited by Ag^+, Cd^{2+}, and Hg^{2+} ions.

Various species of Basidiomycetes have been screened for xylanase activity.[228] Nearly all strains of Aphyllophorales and Agaricales are able to synthesize a xylanase.

A culture of the plant pathogen *Ceratocystis paradoxa* produced at least five extracellular hemicellulases when grown on a medium containing a commercial hemicellulose.[244] Hemicellulase I, one of the enzymes, was purified by precipitation with ammonium sulphate, ion-exchange chromatography, and isoelectric focusing at pH 3—10 and 8—10; it degraded hemicellulose B (an arabino-4-*O*-methylglucuronoxylan) and arabinoxylan to arabinose, D-xylose, xylobiose, and



Scheme 2

[361] J. A. van Assche and A. R. Carlier, *Biochim. Biophys. Acta*, 1975, **391**, 154.
[362] P. van Solingen and J. B. van der Plaat, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 553.
[363] K. Horikoshi and Y. Atsukawa, *Agric. and Biol. Chem. (Japan)*, 1973, **37**, 2097.

a mixture of arabino-D-xylo- and D-xylo-oligosaccharides. Xylotetraose, the smallest xylo-oligosaccharide attacked, yielded xylobiose and xylotriose by the mechanism shown in Scheme 2. Arabinosylxylo-biose, -triose, -tetraose, and -pentaose were slowly hydrolysed to arabinose and, by way of the corresponding D-xylo-oligosaccharide, to xylo-biose and -triose. Hydrolysis of the arabinosyl linkages does not appear to occur at the same active site as that of the xylo-oligosaccharides. Hemicellulose B fractions from a variety of sources were degraded by the enzyme, which exhibited optimum activity at pH 5.5 and 40 °C (K_m 4.24 mg ml^{-1}).

Carbohydrate Isomerases

D-Glucose Isomerases.—Active immobilized forms of D-glucose isomerase have been obtained by reaction with cyanogen bromide-treated alumina, glass, or titania.[364]

D-Xylose Isomerases.—An enzymic method that employs a commercial preparation of L-iditol dehydrogenase containing D-xylulose reductase activity has been reported for the assay of D-xylose isomerase.[365]

Carbohydrate Oxidases

D-Galactose Oxidases.—The mechanism of the action of a D-galactose oxidase from *Polyporus circinatus* has been determined by examination of its substrate specificity, activation by peroxidase, and e.s.r. spectrum.[366] The results suggested that the copper atom is in a plane containing four nitrogen atoms that has axial symmetry (see Figure 1).

D-Glucose Oxidases.—Titanium compounds have been shown to affect the measurement of D-glucose oxidase, and therefore of D-glucose, by the D-glucose–D-glucose oxidase–peroxidase–2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay system.[367] The validity of measurement of the activity of D-glucose oxidase immobilized on supports based on titanium oxide is affected by complexation of the intermediate hydrogen peroxide with the supports, which may prove to be unsuitable for the immobilization of D-glucose oxidase. The formation of titanic peroxides may be responsible for the various reactions encountered. The use of the assay system for the determination of D-glucose oxidase contaminated with catalase and for the determination of hydrogen peroxide was also discussed.

Active immobilized forms of D-glucose oxidase have been prepared by covalent attachment of the enzyme to a diazoaryl derivative of porous glass[368] and various derivatives of nylon tubes,[369] and by covalent attachment of the periodate-oxidized enzyme to poly(4-aminostyrene).[370] Immobilized forms of D-glucose

[364] H. H. Weetall and C. C. Detar, *Biotechnol. and Bioeng.*, 1975, **17**, 295.
[365] K. Yamanaka, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2035.
[366] L. Cleveland, R. E. Coffman, P. Coon, and L. Davis, *Biochemistry*, 1975, **14**, 1108.
[367] J. F. Kennedy and I. Kay, *Carbohydrate Res.*, 1975, **44**, 291.
[368] W. Dritschilo and M. K. Weibel, *Biochem. Med.*, 1974, **9**, 32.
[369] J. Campbell, W. E. Hornby, and D. L. Morris, *Biochim. Biophys. Acta*, 1975, **384**, 307.
[370] O. R. Zaborsky and J. Ogletree, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 210.

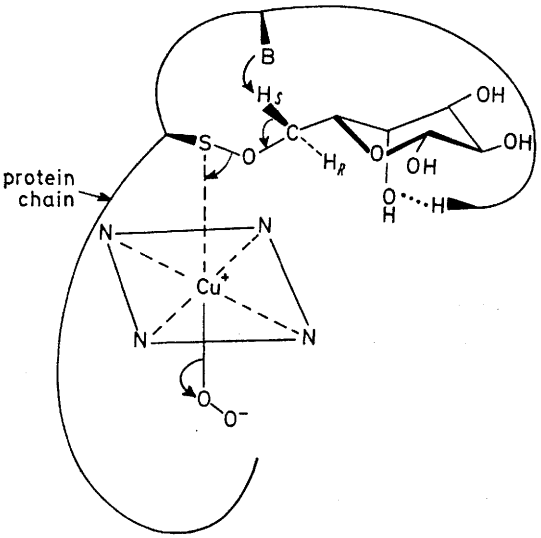


Figure 1 *The binding of* D-*galactose to the active site of* D-*galactose oxidase*

oxidase have also been obtained *via* glutaraldehyde cross-linking to albumin,[371] platinum screens,[372] silicon carbide, Kieselguhr, and silica–alumina,[373] and by entrapment within polyacrylamide gels.[374]

Carbohydrate Transferases

Cyclodextrin D-Glucanotransferase.—Cyclodextrin D-glucanotransferase has been prepared from culture fluids of a *Bacillus* species by adsorption onto corn starch, fractional precipitation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[375] Disc electrophoresis and isoelectric focusing separated the enzyme into two fractions (p*I* 6.07 and 6.80), which were able to synthesize cycloamyloses (predominantly cyclohepta-amylose) from starch. The enzymes differ from the cyclodextrin D-glucanotransferase from *B. macerans*, notably in their actions on starch.

Dextransucrases.—Dextransucrase has been obtained from culture fluids of *Streptococcus mutans* by fractional precipitation with ammonium sulphate, ion-exchange chromatography, and isoelectric focusing (p*I* 4.0).[376] The enzyme (mol. wt. 9.4×10^4) exhibited a broad temperature optimum (34—42 °C) and displayed maximum activity at pH 5.5. D-Fructose competitively inhibited the dextransucrase (K_I 27 mmol l^{-1}), which normally uses sucrose (K_m 3 mmol l^{-1}) as a substrate. Another report has also described the purification of dextransucrase from *S. mutans* by gel filtration and ion-exchange chromatography.[91]

[371] M. Nanjo and G. G. Guilbault, *Analyt. Chim. Acta*, 1974, **73**, 367.
[372] E. J. Lahoda, C. C. Liu, and L. B. Wingard, jun., *Biotechnol. and Bioeng.*, 1975, **17**, 413.
[373] P. E. Markey, P. F. Greenfield, and J. R. Kittrell, *Biotechnol. and Bioeng.*, 1975, **17**, 285.
[374] L. D. Mell and J. T. Maloy, *Analyt. Chem.*, 1975, **47**, 299.
[375] S. Kitahata, N. Tsuyama, and S. Okada, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 387.
[376] A. M. Chludzinski, G. R. Germaine, and C. F. Schachtele, *J. Bacteriol.*, 1974, **118**, 1.

This preparation was homogeneous on examination by polyacrylamide gel electrophoresis and immunodiffusion, but gave two bands (mol. wt. 1.6×10^5 and 1.7×10^5) on electrophoresis in the presence of sodium dodecyl sulphate. The enzyme displayed maximum activity at pH 5.25. Immunoglobulins IgA from human saliva and colostrum accelerated the enzymic action, whereas serum immunoglobulins IgA and IgG were without effect.[377] Two distinct forms of dextransucrase activity have been isolated from cells of *S. mutans* grown on D-glucose.[378]

Glycopeptide Linkage Hydrolases

N-Acetylmuramyl-L-alanine Amidases.—An *N*-acetylmuramyl-L-alanine amidase from *Bacillus subtilis* has been purified to homogeneity; this monomeric protein (mol. wt. 5.0×10^4) possesses a high affinity for the homologous cell walls, which it hydrolyses one at a time.[379]

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases.—The action of a 4-L-aspartyl-β-D-glucosylamine amidohydrolase from *Limnaea stagnalis* on acetylurea, D-glucosylurea, L-asparagine, 2-acetamido-1,2-dideoxy-1-*N*-(5-L-glutamoyl)- and -(4-L-aspartoyl)-β-D-glucopyranosylamine and an ovine albumin glycopeptide has been examined, but only the two last compounds were hydrolysed.[380] L-Aspartic acid, L-asparagine, 2-acetamido-1-amino-4,6-*O*-benzylidene-1,2-dideoxy-D-glucose, and, to a lesser degree, glycine inhibited the enzyme. These and other data indicated that substrates for this amidohydrolase must contain an amino-acid residue with free α-amino- and α-carboxy-groups and a chain of four carbon atoms. The presence of two substrate-binding sites was assumed; *viz.* the amino-group at C-1 of the glycone and the α-amino- and α-carboxy-groups of L-aspartic acid.

Miscellaneous.—An *endo*-β-D-acetamidodeoxyglucanase obtained from culture filtrates of *Streptomyces griseus* rapidly released oligosaccharides from *Saccharomyces cerevisiae* β-fructofuranosidase, bovine pancreatic ribonuclease I and deoxyribonuclease I, and sulphitolysed ovalbumin; these glycoproteins contain a D-mannopyranosyl residue attached to di-*N*-acetylchitobiose, which, in turn, is linked to an L-asparagyl residue.[82] Analysis of the hydrolysis products showed that the enzyme cleaved between the 2-acetamido-2-deoxy-D-glucopyranosyl residues.

Proteinases

Bacterial Proteinases.—C.d. studies have indicated that the glycoprotein protease synthesized by *Mucor miehei* possesses a somewhat distorted β-structure but little, if any, α-helix within the pH range 2.5—7.0.[381] Oxidation of the proteinase with periodate caused rapid loss of 40% of the carbohydrate, although no detectable changes in the structure or enzymic activity occurred. However, removal of the periodate-resistant carbohydrate resulted in loss of enzymic activity.

[377] A. K. Fukui, Y. Fukui, and T. Moriyama, *J. Bacteriol.*, 1974, **118**, 805.
[378] H. K. Kuramitsu, *Infection and Immunity*, 1974, **10**, 227.
[379] D. R. Herbold and L. Glaser, *J. Biol. Chem.*, 1975, **250**, 1676.
[380] A. I. Techukhrova, V. V. Kisseleva, and E. D. Kaverzneva, *Biokhimiya*, 1974, **39**, 176.
[381] P. A. McBride-Warren and W. S. Rickert, *Biochim. Biophys. Acta*, 1973, **328**, 52.

Ribonucleases and Deoxyribonucleases

The structure–activity relationships of bovine pancreatic ribonuclease I have been examined.[323] About 90% of the enzymic activity was lost on modification of two thirds of the L-arginine residues with cyclohexanedione.

The oligosaccharide chains of bovine pancreatic ribonuclease I and deoxyribonuclease I have been cleaved, using a specific *endo*-β-D-acetamidodeoxyglucanase, although one of the two 2-acetamido-2-deoxy-D-glucopyranosyl residues was not detached from the protein chain.[82]

Sulphatases

2-Acetamido-2-deoxy-D-galactose 4-Sulphate Sulphatases.—Extracts of skin fibroblasts obtained from patients with Maroteaux–Lamy syndrome exhibited a marked reduction in 2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase activity towards chondroitin 4-[^{35}S]sulphate and a heptasaccharide derived therefrom.[48]

A purified 2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase from human placenta has been shown to be identical with an arylsulphatase B from the same source.[382] The levels of the two enzymes were compared in cases of Maroteaux–Lamy syndrome. UDP-2-Acetamido-2-deoxy-D-galactose 4-sulphate is the natural substrate for the former sulphatase.

2-Acetamido-2-deoxy-D-glucose 6-Sulphate Sulphatases.—Extracts of an *Actinobacillus* species and a *Pseudomonas* species have been found to contain a sulphatase which, together with other endogenous enzymes, catalysed extensive degradation of corneal keratan sulphate.[74] The *Actinobacillus* sulphatase had little activity towards keratan sulphate, but acted on 2-acetamido-2-deoxy-D-glucose 6-sulphate, 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl 6-sulphate)-D-galactose, and a trisulphated tetrasaccharide (derived from keratan sulphate) containing 2-acetamido-2-deoxy-D-glucose 6-sulphate as the non-reducing residue; the sulphatase removed one sulphate group from the tetrasaccharide, which was then susceptible to the action of an *exo*-β-D-acetamidodeoxyglucosidase. Desulphation of sulphated oligosaccharides by the sulphatase appears to take place only at the non-reducing residue.

Arylsulphatases.—A mutant arylsulphatase B has been shown to be present in the skin fibroblasts obtained from a patient with Maroteaux–Lamy syndrome.[383] The electrophoretic mobility and antigenic determinants of the mutant form are identical to those of arylsulphatase B from normal fibroblasts, but the mutant possesses only 15% of the normal enzymic activity. It was suggested that the reduction in activity of the mutant arylsulphatase is responsible for the biochemical aberrations of at least one form of Maroteaux–Lamy syndrome. Another report on the arylsulphatases present in skin fibroblasts obtained from patients with Maroteaux–Lamy syndrome has noted that the levels of the A and B enzymes are equal to, and >15% of, respectively, those of normal fibroblasts.[48]

[382] A. L. Fluharty, R. L. Stevens, D. Fung, S. Peak, and H. Kihara, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 955.

[383] E. Shapira, R. R. DeGregorio, R. Matalon, and H. L. Nadler, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 448.

The natural substrate of arylsulphatase B was shown to be 2-acetamido-2-deoxy-D-galactose 4-sulphate.

Arylsulphatase A from human liver and kidney has been shown to possess endogenous cerebroside sulphatase activity, and both activities appear to be manifest by a single active site.[384] The arylsulphatase was inhibited by cerebroside sulphate in a way that depends on the ionic strength of the solvent. Differences in the two types of reaction catalysed were ascribed to differences in the physicochemical states of the substrates.

The requirement for bile salts and manganese chloride, and the pH optimum and K_m value of a purified human arylsulphatase A have been reported.[385] Aryl sulphate and 2-*O*-acyl-1-*O*-alkyl-3-*O*-(β-D-galactopyranosyl 3-sulphate)glycerol were hydrolysed by the enzyme under identical conditions. The glycolipid was not hydrolysed by arylsulphatase B and appears to be the physiological substrate for arylsulphatase A. Human arylsulphatase B was not active towards cerebroside sulphate.

A number of the properties of a highly purified arylsulphatase A from human placenta have been reported.[386] Human placental arylsulphatase B hydrolysed UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate, indicating that it is a 2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase.[382] No related activity was exhibited by arylsulphatase A. Cultures of fibroblasts obtained from patients with Maroteaux–Lamy syndrome were equally deficient in arylsulphatase B and 2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase.

Arylsulphatase A (pH optimum 4.8) from bovine liver hydrolysed L-ascorbic acid 2-sulphate; Michaelis kinetics (K_m 21 mmol l^{-1}) were obeyed at pH 5.6, but not at pH 4.8.[387] Potassium sulphate is a competitive inhibitor of the enzyme (K_i 0.2 and 0.6 mmol l^{-1} at pH 4.8 and 5.6, respectively), which is converted into a substrate-modified form during the reaction. The corresponding arylsulphatase B also hydrolysed L-ascorbic acid 2-sulphate (K_m 8 μmol l^{-1}), but the activity was appreciably decreased in the absence of sodium chloride; the enzyme was also competitively inhibited by potassium sulphate (K_i 0.1 mmol l^{-1}). Arylsulphatases from the mollusc *Dicathais orbita* and from *Aerobacter aerogenes* did not hydrolyse L-ascorbic acid 2-sulphate.

The arylsulphatase activity of rat spleen has been separated from the endogenous sulphamidase activity by isoelectric focusing.[388] Mucosal scrapings from rats' stomachs also contain arylsulphatase activity, although other sulphatases and chondroitin 4- and 6-sulphate lyases are also present.[262] The stability of the arylsulphatase to storage was investigated.

Rat-kidney lysosomal arylsulphatase underwent autolysis at mildly acid pHs.[163] Autolysis may be responsible for the heterogeneity of lysosomal arylsulphatase.

Multiple forms of the arylsulphatases from mussels and snails (Mollusca), earthworms (Annelida), insects (Arthropoda), starfish (Echinodermata), seasquibs (Tunicata), sponges (Porifera), and hydroids (Cnidaria) have been

[384] K. Stinshoff and H. Jatzkewitz, *Biochim. Biophys. Acta*, 1975, **377**, 126.
[385] A. L. Fluharty, R. L. Stevens, R. T. Miller, and H. Kihara, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 348.
[386] J. Gnidt-Szulzycka, *Acta Biochim. Polonica*, 1974, **21**, 247.
[387] A. B. Roy, *Biochim. Biophys. Acta*, 1975, **377**, 356.
[388] Y. Friedman and C. Arsenis, *Biochem. J.*, 1974, **139**, 699.

separated by isoelectric focusing.[389] Acidic forms of the arylsulphatases possess a cerebroside sulphatase activity akin to that of the lysosomal arylsulphatase A from vertebrates. Cerebroside 3-sulphate inhibited the arylsulphatase activities.

Purification of the arylsulphatase from the liver of a marine gastropod, *Charonia lampas*, failed to remove the associated L-ascorbic acid 2-sulphate sulphatase activity.[390] The effects of various compounds on the arylsulphatase activity have been investigated.[391]

L-Ascorbic Acid 2-Sulphate Sulphatases.—L-Ascorbic acid 2-sulphate served as a substrate for the arylsulphatase from bovine liver, and it was concluded that arylsulphatase and L-ascorbic acid 2-sulphate sulphatase activities are manifest by the same molecule.[387] A similar conclusion was reached in an investigation of the enzymes in the liver of a marine gastropod, *Charonia lampas*.[390] L-Ascorbic acid 2-sulphate sulphatase was inhibited by ADP and related compounds.[392]

Cerebroside Sulphatases.—An enzyme present in human-skin fibroblasts is active towards (D-galactosyl sulphate)sphingosine.[393] The rate of this reaction is linear for short periods and proportional to the amount of enzyme (pH optimum 4.5, K_m 3.32×10^{-4} mol l^{-1}). These and other findings suggested that a single enzyme is able to degrade (D-galactosyl sulphate)-sphingosine and -ceramide.

An assay for cerebroside sulphatase is based on t.l.c. and the use of tritium-labelled cerebroside sulphate as a substrate.[384] The cerebroside sulphatase activity of the arylsulphatase A in human liver and kidney was investigated using this assay. Kinetic and other evidence suggested that cerebroside sulphatase also possesses arylsulphatase activity, although both activities are manifest by the same active site. Pure human cerebroside sulphatase has been shown to require the presence of bile salts and to be stimulated by manganese chloride.[385]

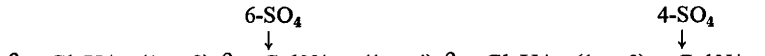
Cerebroside sulphatases isolated from a number of invertebrates are composed of multiple forms that were separated by isoelectric focusing.[389] These enzymes have activities comparable to those from vertebrates, and they also exhibit arylsulphatase activity. Kinetic data (pH optima and K_m values *etc.*) were reported for the enzymes, which were inhibited by nitrocatechol sulphate.

Chondroitin 4-Sulphate Sulphatases.—The chondroitin 4-sulphate sulphatase from *Proteus vulgaris* preferentially attacked the sulphate group at O-4 of the reducing 2-acetamido-2-deoxy-D-galactopyranosyl residues in the tetrasaccharides (10)—(13).[394] The sulphate groups at O-4 of the internal 2-acetamido-2-deoxy-D-galactopyranosyl residues in the tetrasaccharides (12) and (13) were also hydrolysed, but much more slowly.

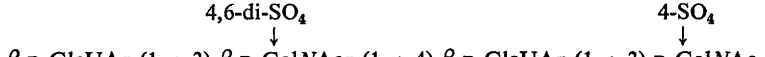
Chondroitin 4-sulphate sulphatase activity has been detected in mucosal scrapings from rats' stomachs.[262]

Chondroitin 6-Sulphate Sulphatases.—A chondroitin 6-sulphate sulphatase from *Proteus vulgaris* did not hydrolyse the 6-sulphate groups of the tetrasaccharides (10), (11), and (14).[394]

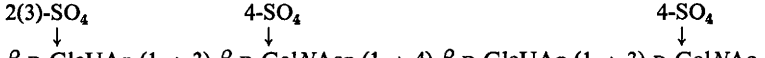
[389] W. Mraz and H. Jatzkewitz, *Z. physiol. Chem.*, 1974, **355**, 33.
[390] H. Hatanaka, Y. Ogawa, and F. Egami, *J. Biochem.* (*Japan*), 1975, **77**, 353.
[391] H. Hatanaka, Y. Ogawa, and F. Egami, *J. Biochem.* (*Japan*), 1975, **77**, 801.
[392] H. Hatanaka, Y. Ogawa, and F. Egami, *J. Biochem.* (*Japan*), 1975, **77**, 807.
[393] Y. Eto, U. Wiesman, and N. N. Herschkowitz, *J. Biol. Chem.*, 1974, **249**, 4955.
[394] N. Seno, F. Akiyama, and K. Anno, *Biochim. Biophys. Acta*, 1974, **362**, 290.



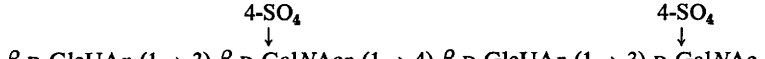
(10)



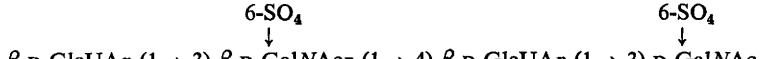
(11)



(12)



(13)



(14)

Mucosal scrapings from rats' stomachs contain chondroitin 6-sulphate sulphatase.[262]

Glycolipid Sulphate Sulphatases.—The use of 2-*O*-acyl-1-*O*-alkyl-(β-D-galactopyranosyl 3-[^{35}S]sulphate)glycerol as a substrate has shown that human arylsulphatase A also exhibits glycolipid sulphate sulphatase activity.[385] The requirement for bile salts and manganese chloride, and the pH optima and K_m values for the two activities, are similar. The enzyme also exhibits cerebroside sulphatase activity, although cerebroside sulphate competitively inhibited the hydrolysis of the foregoing glycolipid (K_i 0.2 mmol l^{-1}).

Heparin Sulphatases.—Mucosal scrapings from rats, stomachs contain heparin sulphatase activity.[262]

L-Iduronic Acid 2-Sulphate Sulphatases.—2,5-Anhydro-4-*O*-(α-L-idopyranuronosyl 2-sulphate)-D-[1-^{3}H]mannitol 6-sulphate has been recommended as a substrate for the measurement of L-iduronic acid 2-sulphate sulphatase activity, since it furnishes a radioactive monosulphate that is readily separated by paper chromatography or electrophoresis.[395] The enzymic reaction followed Michaelis–Menten kinetics (K_m 3 mmol l^{-1}) and was strongly inhibited by phosphate ions.

Lactose Sulphate Sulphatases.—A deficiency of lactose sulphate sulphatase has been demonstrated in cases of metachromatic leukodystrophy (sulphatidosis).[396] The enzyme also hydrolysed D-galactosyl sulphatide.

[395] T. W. Lim, I. G. Leder, G. Bach, and E. F. Neufeld, *Carbohydrate Res.*, 1974, **37**, 103.
[396] K. Harzer and H. U. Benz, *Z. physiol. Chem.*, 1974, **355**, 744.

Sulphatases (Miscellaneous).—The sulphate groups of polyanions isolated from gastric and duodenal mucosa were hydrolysed by enzymes present in mucosal scrapings from rats' stomachs.[262]

Miscellaneous Enzymes

Glyco-α-lactalbumins.—The conformational change that occurs on immobilization of bovine glyco-α-lactalbumin on agarose cyclic imidocarbonate has been studied fluorometrically.[397]

α-Lactalbumins.—The isolation, compositions, and functions of α-lactalbumins from several species have been reported.[398]

The reversible unfolding of α-lactalbumin in the presence of guanidinium hydrochloride has been examined at 25 °C by using difference spectra and optical data.[326] At pH 5.5, two L-tryptophanyl residues, which are normally buried in the interior of the molecule, become exposed on the surface as part of the denaturation process. The unfolding depends on the concentration of guanidinium hydrochloride, and the dependence of the equilibrium constant for the unfolding reaction on pH is considered to be due to the abnormal behaviour of L-histidyl residues. The intrinsic binding constant of the denaturant with α-lactalbumin is smaller than that for lysozyme, and the difference between the number of binding sites on the denatured and native molecules is also smaller.

Conformational changes that occur on immobilization of α-lactalbumins on agarose cyclic imidocarbonate have been studied by fluorometric methods.[397] Immobilized bovine α-lactalbumin has been employed in a study of acceptor–ligand interactions in the lactose synthase system.[399]

Lactose Synthases.—Lactose synthases and related systems have been reviewed.[400]

Affinity chromatography has been used in a quantitative study of acceptor–ligand interactions in the lactose synthase system.[399] The effects of D-glucose and 2-acetamido-2-deoxy-D-glucose on the elution of the A-protein of human lactose synthase from a column of α-lactalbumin immobilized on agarose cyclic imidocarbonate gave values of 200 and 0.57 l mol^{-1}, respectively, for the association constants representing the interaction between the enzyme and the monosaccharides.

Pectinesterases.—An electrophoretically homogeneous preparation of polygalacturonase obtained from *Botrytis cinerea* also exhibited pectinesterase activity.[354]

The synthesis of pectinesterase and pectinolytic enzymes by *Colletotrichum capsici* has been investigated.[355]

Polysaccharide Deacetylases.—The presence of a polysaccharide deacetylase in Vi-phages I and III has been demonstrated.[401]

Sulphamidases.—A study has been made of rat-spleen sulphamidase, which is able to hydrolyse sulphate from *N*-[^{35}S]sulphated heparin; the enzyme is associated

[397] J.-P. Prieels, *European J. Biochem.*, 1975, **50**, 463.
[398] G. J. Quarfoth and R. Jenness, *Biochim. Biophys. Acta*, 1975, **379**, 476.
[399] P. Andrews, B. J. Kitchen, and D. J. Winzor, *Biochem. J.*, 1973, **135**, 897.
[400] 'Advances in Enzymology and Related Areas of Molecular Biology', Vol. 43, ed. A. Meister, Wiley, Chichester, 1975.
[401] H. Szczeklik, B. Kwiatkowski, and A. Taylor, *Acta Biochim. Polonica*, 1974, **21**, 33.

with a lysosomal fraction that also contains heparin.[388] The sulphamidase was separated from other arylsulphatases by isoelectric focusing.

D-Thioglucosidases.—Of many micro-organisms screened, *Enterobacter cloacae* exhibited a strong capacity to synthesize D-thioglucosidase when treated with either sinigrin or a mustard extract.[402] A homogeneous D-thioglucosidase (mol. wt. 6.1×10^4) has been obtained from a cell-free extract of *Enterobacter cloacae* by fractional precipitation, ion-exchange chromatography, and gel filtration; it exhibited maximum activity at pH 6.8 and was stable below 40 °C at pH 5.0—7.0.[145] Whereas the enzyme was largely unaffected by sulphydryl reagents, it was strongly inhibited by Cu^+, Cu^{2+}, Fe^{2+}, and Hg^{2+} ions, and by H_4edta. Many carbohydrates, including monosaccharides and glycosides thereof, inhibited the enzyme, which, unlike plant D-thioglucosidase, was also inhibited by L-ascorbic acid. The properties of plant, fungal, and bacterial D-thioglucosidases were compared, and the relation between bacterial β-D-glucosidase and D-thioglucosidase was discussed.

Index of Enzymes Referred to in Chapter 6*

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
α-D-Acetamidodeoxygalactosidase	2-Acetamido-2-deoxy-α-D-galactoside acetamidodeoxygalactohydrolase	3.2.1.49	330
2-Acetamido-2-deoxy-D-galactose 4-sulphate sulphatase			388
β-D-Acetamidodeoxygalactosidase	2-Acetamido-2-deoxy-β-D-galactoside acetamidodeoxygalactohydrolase	3.2.1.53	330
exo-β-D-Acetamidodeoxyglucanase			354
2-Acetamido-2-deoxy-D-glucose 6-sulphate sulphatase			388
α-D-Acetamidodeoxyglucosidase	2-Acetamido-2-deoxy-α-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.50	330
β-D-Acetamidodeoxyglucosidase	2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	330
αβ-D-Acetamidodeoxyglucosidase			330
endo-β-D-Acetamidodeoxyglucanase	*see* Chitinase	3.2.1.14	363
exo-β-D-Acetamidodeoxyglucosidase			330
exo-β-D-Acetamidodeoxyglycanase			354
β-D-Acetamidodeoxyhexosidase	2-Acetamido-2-deoxy-β-hexoside acetamidodeoxyhexohydrolase	3.2.1.52	330
exo-β-D-Acetamidodeoxyhexosidase			330
N-Acetylmuramyl-L-alanine amidase	Mucopeptide amidohydrolase	3.5.1.28	387

[402] N. Tani, M. Ohtsuru, and T. Hata, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 1617.

* See Introduction (Part II, Chapter 1, p. 207).

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
α-Amylase	1,4-α-D-Glucan glucanohydrolase	3.2.1.1	355
β-Amylase	1,4-α-D-Glucan maltohydrolase	3.2.1.2	360
Arabinanase			361
α-L-Arabinofuranosidase	α-L-Arabinofuranoside arabinohydrolase	3.2.1.55	336
Arylsulphatase	Aryl sulphate sulphohydrolase	3.1.6.1	388
L-Ascorbic acid 2-sulphate sulphatase			390
4-L-Aspartyl-β-D-glucosylamine amidohydrolase	2-Acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucosylamine	3.5.1.37	387
Bacterial proteinase			387
Carbohydrate isomerases			385
Carbohydrate oxidases			385
Carbohydrate transferases			386
Cellulase	1,4-(1,3;1,4)-β-D-Glucan 4-glucanohydrolase	3.2.1.4	362
Cerebroside sulphatase	Cerebroside-3-sulphate 3-sulphohydrolase	3.1.6.8	390
Chitinase	Poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)]glycanohydrolase	3.2.1.14	363
Chitosanase			364
Chondroitin sulphate lyase ABC	Chondroitin ABC lyase	4.2.2.4	365
Chondroitin sulphate lyase AC	Chondroitin AC lyase	4.2.2.5	365
Chondroitin 4-sulphate lyase			365
Chondroitin 6-sulphate lyase			365
Chondroitin 4-sulphate sulphatase	$\Delta^{4,5}$-β-D-Glucuronosyl-(1,4)-2-acetamido-2-deoxy-D-galactose-4-sulphate 4-sulphohydrolase	3.1.6.9	390
Chondroitin 6-sulphate sulphatase	$\Delta^{4,5}$-β-D-Glucuronosyl-(1,4)-2-acetamido-2-deoxy-D-galactose-6-sulphate 6-sulphohydrolase	3.1.6.10	390
Cyclodextrin D-glucanotransferase	1,4-α-D-Glucan 4α-(1,4-α-glucano)-transferase (cyclizing)	2.4.1.19	386
Deoxyribonuclease			388
Deoxyribonuclease I	Deoxyribonucleate 3′-oligonucleotidohydrolase	3.1.4.5	388
Dextranase	1,6-α-D-Glucan 6-glucanohydrolase	3.2.1.11	365
Dextransucrase	Sucrose:1,4-α-D-glucan 4-α-glucosyltransferase	2.4.1.5	386
β-D-Fructofuranosidase	β-D-Fructofuranoside fructohydrolase	3.2.1.26	336
α-L-Fucosidase	α-L-Fucoside fucohydrolase	3.2.1.51	337
α-1,2-L-Fucosidase			337
β-D-Fucosidase *	β-D-Fucoside fucohydrolase	3.2.1.38	337
endo-Galactanase			367
endo-β-D-Galactanase			367
exo-β-D-Galactanase			367
D-Galactose oxidase	D-Galactose: oxygen 6-oxidoreductase	1.1.3.9	385
α-D-Galactosidase	α-D-Galactoside galactohydrolase	3.2.1.22	338
β-D-Galactosidase	β-D-Galactoside galactohydrolase	3.2.1.23	338

* This entry is now deleted from tables of Enzyme Nomenclature, but is retained in this Index and Report since it is still used by some workers; see Vol. 7, p. 201 of this series.

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
β-D-Galactosidases(glycosphingolipid-specific)			338
β-D-Galactosphingosidase			338
β-D-Galactosylceramidase	D-Galactosyl-*N*-acylsphingosine galactohydrolase	3.2.1.46	338
β-D-Galactosyl-D-glucosylceramidase			338
endo-β-1,3-D-Glucanase	1,3-β-D-Glucan glucanohydrolase	3.2.1.39	368
endo-β-1,6-D-Glucanase	1,6-β-D-Glucan glucanohydrolase	3.2.1.75	369
exo-D-Glucanase			369
exo-β-1,3-D-Glucanase	1,3-β-D-Glucan glucohydrolase	3.2.1.58	369
exo-β-1,4-D-Glucanase	1,4-β-D-Glucan glucohydrolase	3.2.1.74	370
exo-α-1,6-D-Glucanase	1,6-α-D-Glucan glucohydrolase	3.2.1.70	369
D-Glucanases (miscellaneous)			370
Glucoamylase	1,4-α-D-Glucan glucohydrolase	3.2.1.3	371
D-Glucose isomerase	D-Glucose ketol-isomerase	5.3.1.18	385
D-Glucose oxidase	β-D-Glucose:oxygen 1-oxidoreductase	1.1.3.4	385
α-D-Glucosidase	α-D-Glucoside glucohydrolase	3.2.1.20	344
β-D-Glucosidase	β-D-Glucoside glucohydrolase	3.2.1.21	344
exo-β-1,3-D-Glucosidase	1,3-β-D-Glucan glucohydrolase	3.2.1.58	369
exo-α-1,6-D-Glucosidase	1,6-α-D-Glucan glucohydrolase	3.2.1.70	369
exo-β-1,4-D-Glucosidase	1,4-β-D-Glucan glucohydrolase	3.2.1.74	370
β-D-Glucosylcerebrosidase			344
β-D-Glucuronidase	β-D-Glucuronide glucuronohydrolase	3.2.1.31	347
Glycanase			372
Glyco-α-lactalbumin	*see*	2.4.1.22	392
Glycolipid sulphate sulphatase			391
Glycopeptidases			387
Glycopeptide-linkage hydrolases			387
Glycopeptide-linkage hydrolases (miscellaneous)			387
β-D-Glycylamidodeoxyglucosidase			350
Heparin sulphatase			391
Hyaluronidase	Hyaluronate 4-glycanohydrolase	3.2.1.35	373
α-L-Iduronidase	α-L-Iduronide iduronohydrolase	3.2.1.76	350
L-Iduronic acid 2-sulphate sulphatase			391
Isoamylase	Glycogen 6-glucanohydrolase	3.2.1.68	374
Isomaltase	*see* α-D-Glucosidase		344
Keratan sulphate hydrolase			374
α-Lactalbumin	*see*	2.4.1.22	392
Lactose sulphate sulphatase			391
Lactose synthase	UDP-Galactose:D-glucose 4-β-galactosyltransferase	2.4.1.22	392
Laminarinase	1,3-(1,3;1,4)-β-D-Glucan 3(4)-glucanohydrolase	3.2.1.6	374
Lichenase	1,3-1,4-β-D-Glucan 4-glucanohydrolase	3.2.1.73	374
Lysozyme	Mucopeptide *N*-acetylmuramylhydrolase	3.2.1.17	374
'Maltase'	*see* α-D-Glucosidase		344
D-Mannanases (miscellaneous)			381
α-D-Mannosidase	α-D-Mannoside mannohydrolase	3.2.1.24	350

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
β-D-Mannosidase	β-D-Mannoside mannohydrolase	3.2.1.25	350
Neuraminidase	Acylneuraminyl hydrolase	3.2.1.18	351
Oligo-1,6-D-glucosidase	Dextrin 6-α-glucanohydrolase	3.2.1.10	381
Pectate hydrolases (miscellaneous)			382
Pectate lyase	Poly(1,4-α-D-galacturonide) lyase	4.2.2.2	383
Pectinesterase	Pectin pectylhydrolase	3.1.1.11	392
Pectin lyase	Poly(methoxygalacturonide) lyase	4.2.2.10	383
β-D-Phosphogalactosidase			353
β-D-Phosphoglucosidase			353
Polygalacturonase	Poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15	381
exo-Polygalacturonase	Poly(1,4-α-D-galacturonide) galacturonohydrolase	3.2.1.67	382
exo-Poly-α-galacturonosidase	Poly(1,4-α-D-galactosiduronate) digalacturonohydrolase	3.2.1.82	382
Polysaccharide deacetylase			392
Proteinases			387
Pullulanase	Pullulan 6-glucanohydrolase	3.2.1.41	383
Ribonuclease			388
Ribonuclease I	Ribonucleate 3′-pyrimidino-oligonucleotidohydrolase	3.1.4.22	388
Sialidase *			351
Sucrose α-D-glucohydrolase	Sucrose α-D-glucohydrolase	3.2.1.48	354
Sulphamidase			392
Sulphatase			388
Sulphatases (miscellaneous)			392
D-Thioglucosidase	Thioglucoside glucohydrolase	3.2.3.1	393
αα-Trehalase	αα-Trehalose glucohydrolase	3.2.1.28	383
Xylanase (miscellaneous)			384
D-Xylose isomerase	D-Xylose ketol-isomerase	5.3.1.5	385
β-D-Xylosidase	β-D-Xyloside xylohydrolase	3.2.1.37	354

* Sialidase is a term generally used to include neuraminidase and unspecified acylneuraminyl hydrolases. However, since there is little evidence for the existence of acylneuraminyl hydrolases that do not cleave 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonyl residues, use of the term neuraminidase is preferred.

7
Glycolipids and Gangliosides

BY R. J. STURGEON

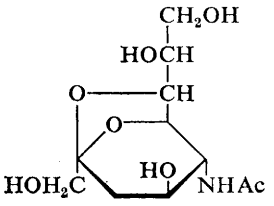
Introduction

Reviews covering the isolation and characterization of membrane glycosphingolipids,[1] and the normal and abnormal biosynthesis of gangliosides have appeared.[2] A review dealing with the structures and the organization of cell-surface glycolipids and their dependence on cell growth and on malignant transformations has discussed changes occurring in the glycolipids in transformed cells and in the biosynthesis of glycolipids.[3] A critical evaluation of the evidence for the localization of glycosyltransferases concerned with the biosynthesis of glycolipids in eukaryotic cells has been published.[4] The glycolipid and glycoprotein components of plasma membranes have been discussed with regard to the structures of the oligosaccharide chains and their role in such intracellular processes as recognition and adhesion.[5] Glycosphingolipid antigens possessing ABH and Lewis blood-group specificities, and the distribution of blood-group glycolipids have been dealt with in the second volume of 'The Antigens'.[6] Reviews have also appeared on the chemistry and control of hereditary lipid diseases[7] and on the multiple forms of glycosidases in normal and pathological conditions.[8] Possible roles of glycolipids and glycoproteins in the brain, and the distribution and metabolism of glycolipids and glycoproteins during development of the brain have been discussed.[9] An examination of the catalytic, immunological, physical, and genetic characteristics of both forms of hexosaminidase has resulted in several models being proposed to explain the relationship between these enzymes and their involvement in G_{M_2}-gangliosidoses.[10] The isolation, occurrence, distribution, and metabolism of glycosyl glycerides in animals, plants, and bacteria have been reviewed.[11] The purification and structures of bacterial glycophospholipids, fish-brain polysialogangliosides, and glycerosulpholipids

[1] R. A. Laine, K. Stellner, and S.-I. Hakomori, *Methods Membrane Biol.*, 1974, **2**, 205.
[2] P. H. Fishman, *Chem. and Phys. Lipids*, 1974, **13**, 305.
[3] S.-I. Hakomori, *Biochim. Biophys. Acta*, 1975, **417**, 55.
[4] T. W. Keenan and D. J. Morré, *F.E.B.S. Letters*, 1975, **55**, 8.
[5] S. Roseman, *Hospital Pract.*, 1975, **10**, 61.
[6] S.-I. Hakomori and A. Kobata, in 'The Antigens', Vol. 2, ed. M. Sela, Academic Press, New York, 1974, p. 80.
[7] R. O. Brady, *Chem. and Phys. Lipids*, 1974, **13**, 271.
[8] D. Robinson, *Enzyme*, 1974, **18**, 114.
[9] L. N. Irwin, in 'Reviews of Neuroscience', Vol. 1, ed. S. Ehrenpreis and I. J. Kopin, Raven Press, New York, 1974, p. 137.
[10] J. F. Tallman, *Chem. and Phys. Lipids*, 1974, **13**, 292.
[11] P. S. Sastry, *Adv. Lipid Res.*, 1974, **12**, 251.

have been reported,[12] and the structures and functions of sulphated glycolipids [13] have been discussed.

An improved procedure for the *in vitro* labelling of gangliosides, by means of D-galactose oxidase and sodium borotritide, has made use of a non-ionic detergent.[14] The anomeric configuration of sugars in glyceroglycolipids and glycosphingolipids has been determined by oxidation with chromium trioxide.[15] The site of substitution on 2-acetamido-2-deoxy-D-hexosyl residues in oligosaccharide chains of glycolipids has been determined by permethylation of the glycolipid, methanolysis under mild conditions, acetylation, and identification of the resulting substituted methyl glycosides of 2-deoxy-2-(*N*-methylacetamido)-D-hexoses by g.l.c.[16, 17] Addition of tritium gas to the unsaturated linkages has been used to prepare labelled glycosphingolipids.[18] The formation of water-insoluble, ganglioside–calcium ion complexes has been used to separate gangliosides from water-soluble materials.[19] A study of the interaction between a D-galactose-specific lectin from *Ricinus communis* and liposomes containing differing amounts of the monosialoganglioside G_{M_1} has shown that the liposomes can be used as a model system to investigate the agglutination of cells by lectins; the association constant K_a was found to be 100—1000-times greater than that of D-galactose or 4-nitrophenyl β-D-galactopyranoside.[20] The mass spectra of some permethylated derivatives of glycosphingolipids have been reported.[21] Fragments containing one, two, and three sugar moieties were used to establish the positions of the 2-amino-2-deoxy-D-hexosyl residues in the oligosaccharide chain. Acetyl derivatives of monoglycosyldiglycerides have also been examined by m.s.[22] The c.d. spectra of neuraminic acid and several of its derivatives have been examined with a view to evaluating the usefulness of this technique in stereochemical and structural studies of the oligosaccharides of glycoproteins and glycolipids.[23] Interpretation of the c.d. spectra allowed the transition due to the carboxylate



(I)

[12] I. Ishizuka, *Seikagaku*, 1974, **46**, 771.
[13] B. L. Slomiany, A. Slomiany, and J. Badurski, *Postepy Biochem.*, 1975, **21**, 319.
[14] R. Ghidoni, S. Tettamanti, and V. Zambotti, *Ital. J. Biochem.*, 1974, **23**, 320.
[15] R. A. Laine and O. Renkonen, *J. Lipid Res.*, 1975, **16**, 102.
[16] S. K. Kundu, R. W. Ledeen, and P. A. J. Gorin, *Carbohydrate Res.*, 1975, **39**, 179.
[17] S. K. Kundu, R. W. Ledeen, and P. A. J. Gorin, *Carbohydrate Res.*, 1975, **39**, 329.
[18] J. L. Di Cesare and M. M. Rapport, *Chem. and Phys. Lipids*, 1974, **13**, 447.
[19] J. P. Carter and J. N. Kanfer, *Methods Enzymol.*, 1975, **35**, 549.
[20] A. Surolia, B. K. Bachhawat, and S. K. Podder, *Nature*, 1975, **257**, 802.
[21] R. W. Ledeen, S. K. Kundu, H. C. Price, and J. W. Fong, *Chem. and Phys. Lipids*, 1974, **13**, 429.
[22] S. G. Batrakov, E. F. Il'ina, B. V. Rozynov, and L. D. Bergel'son, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2229.
[23] H. R. Dickinson and C. A. Bush, *Biochemistry*, 1975, **14**, 2299.

chromophore to be distinguished from that due to the amide chromophore. Reduction of ganglioside esters with sodium borohydride, followed by mild hydrolysis with acid, yielded a nonulosamine and a less polar product, both of which gave the same characteristic colour with resorcinol.[24] The latter product is probably 5-acetamido-2,7-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulopyranose (1).

Animal Glycolipids and Gangliosides

The precipitation of galactocerebrosides with anti-cerebroside sera has been found to require the presence of cholesterol and lecithin when the reaction is conducted in isotonic media.[25] The galactocerebroside isolated from human white matter behaves in the same way as conventional covalently bound, non-lipid haptens in eliciting an immune response, since antibodies were induced only when the hapten was bound to an immunogenic carrier.[26] Since the galactocerebroside is a normal body constituent, the findings also define some of the conditions under which the central nervous system fails to tolerate endogenous glycolipids. Changes in the contents of glycolipids and in the activities of lysosomal enzymes involved in the metabolism of glycolipids have been studied in the human brain at various stages of development.[27] The first stage, which is characterized by multiplication of the neuronal and glial cells, showed an increase in the ganglioside content, whereas the rate of formation of neurons slowed down, with a concomitant decrease in the ganglioside content and an increase in the β-galactosidase and β-hexosaminidase activities, in the second stage. The increase in the content of gangliosides at the later stages of development correlated with decreases in the activities of these enzymes. D-[6,6-$^2H_2$]Glucose has been used as a tracer in a comparative study of the metabolism of neutral glycosylceramides in the plasmas of a control subject and a patient with Fabry's disease; the incorporation of tracer into the D-glucosyl and D-galactosyl residues of the glycosphingolipids was measured by g.l.c.–m.s. of the methyl glycoside tetra-acetates.[28] Maximum incorporation of the tracer into lactosylceramide [β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide] was twice as great in the Fabry patient as in the control, whereas a trihexosylceramide [α-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide] was not labelled significantly in the Fabry patient although it was labelled in the control. An unusual lipid-storage disease has been identified by the accumulation of the haematoside G_{M_3} in liver and brain tissues.[29] In contrast to other sphingolipidoses, accumulation of this glycolipid is not a consequence of defective catabolism but is caused by a deficiency of the enzyme UDP-2-acetamido-2-deoxy-D-galactose:G_{M_3}-2-acetamido-2-deoxy-D-galactosyltransferase, which is involved in the biosynthesis of gangliosides. Mass spectrometry of methylated

[24] S. K. Gross, J. E. Evans, V. N. Reinhold, and R. H. McCluer, *Carbohydrate Res.*, 1975, **41**, 344.
[25] B. Niedieck, *Immunochemistry*, 1975, **12**, 807.
[26] A. Czlonkowska and S. Leibowitz, *Immunology*, 1974, **27**, 1117.
[27] R. M. Aruna and D. Basu, *Indian J. Biochem. Biophys.*, 1974, **11**, 144.
[28] D. E. Vance, W. Krivit, and C. C. Sweeley, *J. Biol. Chem.*, 1975, **250**, 8119.
[29] P. H. Fishman, S. R. Max, J. F. Tallman, R. O. Brady, N. K. Maclaren, and M. Cornblath, *Science*, 1975, **187**, 68.

and of methylated-and-reduced derivatives has been used to characterize the blood-group A active glycolipids in human and dog small intestines.[30] Thus, the glycolipids were shown to be hexaglycosylceramides containing phytosphingosine as the principal long-chain base and hydroxypalmitic acid as the principal fatty acid. The sequence of sugar residues was established as hexosamine → [L-fucose] → hexose → hexosamine → hexose → hexose → ceramide. A penta-glycosylfucolipid (2) exhibiting blood-group Le^a activity has been isolated from

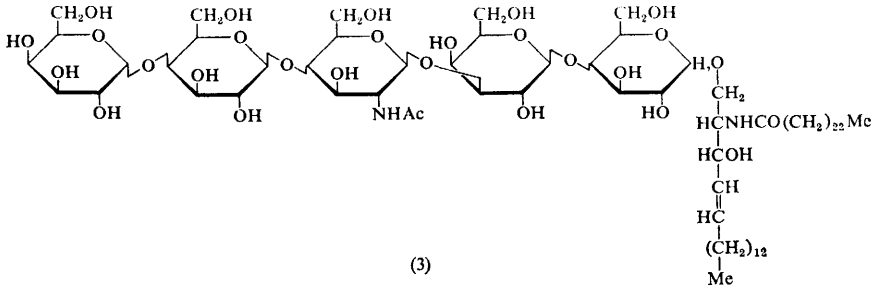
$$\begin{array}{l}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-D-Glc}p\text{-ceramide} \\ \qquad\qquad\qquad\quad 4 \\ \qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\quad 1 \\ \qquad\qquad\quad \alpha\text{-L-Fuc}p\end{array}$$

(2)

human small intestines; a combination of periodate oxidation, methylation, m.s., and enzymic studies was used to establish its structure.[31]

Differences in ganglioside (G_{M_1}) contents of the small intestinal mucosa of man, pigs, and cattle have been related to different abilities of the mucosal cells to bind cholera toxin.[32] Although *Vibrio cholerae* sialidase hydrolysed isolated intestinal di- and tri-sialogangliosides to G_{M_1}, no changes were observed in the ganglioside patterns of intestinal mucosa, the number of toxin-binding sites on intestinal cells, and the sensitivity of the small bowel to the diarrheogenic action of the toxin. The results suggest that G_{M_1} ganglioside is the receptor for cholera toxin in the intestines. An examination of the metabolism of D-glucosyl-[^{3}H]-ceramide by human-skin fibroblasts from normal and glucosylceramidotic patients indicated that the glycolipid is hydrolysed in normal skin fibroblasts to [^{3}H]ceramide and then to [^{3}H]dihydrosphingosine and [^{3}H]-labelled fatty acids; these compounds are subsequently incorporated into cellular phospholipids.[33] The utilization of D-glucosyl-[^{3}H]ceramide in this catabolic sequence and the incorporation of the breakdown products into phospholipids predominate in normal skin fibroblasts. These processes are greatly reduced in glucosylceramidotic skin fibroblasts, where a greater amount of the D-glucosyl-[^{3}H]ceramide is transformed into highly glycosylated glycosphingolipids. The glycosphingolipid (3) possessing blood-group P_1 activity has been isolated from human erythrocyte stroma; the purified glycolipid contains D-galactose, 2-acetamido-2-deoxy-D-glucose, and D-glucose (molar ratio 3 : 1 : 1).[34] Treatment of (3) with α-galactosidase released a single D-galactosyl residue, together with a product devoid of blood-group activity that was identified as paragloboside. The substitution pattern of the sugar residues was established by m.s. of the methylated alditol acetates.

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[34] M. Naiki, J. Fong, R. Ledeen, and D. M. Marcus, *Biochemistry*, 1975, **14**, 4831.



(3)

Comprehensive data on the immunochemistry of human blood-group P_1, P, and p^K glycosphingolipid antigens have been presented.[35] Two schemes for the biosynthesis of these antigens, which could be tested by characterization of the transferases involved, were proposed. Rationalization of the immunochemical data did not support an earlier suggestion that the P antigen is the biosynthetic precursor of p^K specificity.[36] H-Active ceramide heptasaccharide (H_2-glycolipid) and ceramide decasaccharide (H_3-glycolipid) have been isolated from erythrocyte membranes of individuals in blood-group O.[37] Their structures were investigated by conventional methylation analysis, enzymic degradation, and direct mass spectrometric examination of the products of enzymic degradation following methylation and reduction [with sodium bis(2-methoxyethoxy)aluminium hydride]. A ceramide hexasaccharide composed of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and L-fucose (molar ratio 1 : 3 : 1 : 1) and a ceramide octasaccharide containing the same sugars in the molar ratio 1 : 4 : 2 : 1 have been isolated from human blood-group B erythrocytes.[38] Ceramide trihexosides that accumulate in the erythrocyte stroma of patients with congenital dyserythropoietic anaemia have been purified and shown to consist of approximately equimolar amounts of α-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide; part of the ceramide tetrahexoside was identified as paragloboside [β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide].[39] Human-erythrocyte ghosts have been cross-linked with a group of difunctional cross-linking reagents; the data suggested that the glycolipids and proteins may be cross-linked in the complex.[40]

A blood-group H-related glycosphingolipid has been obtained with the aid of an α-fucosyltransferase present in bovine spleen.[41] Thus, L-[^{14}C]fucose was transferred from GDP-L-[^{14}C]fucose to a tetraglycosylceramide [β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide] to form the glycosphingolipid. Five ganglio-

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[40] T. H. Ji, *J. Biol. Chem.*, 1974, **249**, 7841.
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sides have been isolated from extracts of bovine adrenal medulla by preparative t.l.c.; a combination of periodate oxidation and methylation analysis demonstrated the absence of (2 → 8)-linkages between sialic acid residues.[42] The transfer of D-mannose from GDP-D-[U-^{14}C]mannose into endogenous acceptors of bovine adrenal medulla and rat parotid has been studied.[43] One of the glycolipids was found to be labelled rapidly and it was subsequently purified and characterized as dolichyl α-D-mannosyl phosphate. D-Mannose from GDP-D-mannose was also incorporated into another acceptor, and it was suggested that the transfer of D-mannose from GDP-D-mannose to mannosylated protein occurred *via* this acceptor and dolichyl α-D-mannosyl phosphate. Semen sialoglycolipids isolated from bulls, buffalos, and goats have been tentatively identified as gangliosides on the bases of their chromatographic behaviour.[44]

Three new fucolipids (4)—(6), two of which exhibited blood-group A activity, have been isolated from the water-soluble glycolipids of hog gastric mucosa.[45]

α-D-Gal*N*Ac*p*-(1 → 3)-D-Gal*p*-(1 → 3 and 1 → 4)-D-Glc*N*Ac*p*-(1 → 3)-D-Gal*p*-
2 (1 → 4)-D-Glc*p*-(1 → 1)-ceramide
↑
1
α-L-Fuc

(4)

D-Gal*p*-(1 → 4)-D-Glc*N*Ac*p*-(1 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide
3
↑
1
L-Fuc*p*

(5)

α-D-Gal*N*Ac*p*-(1 → 3)-D-Gal*p*-(1 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide
2
↑
1
α-L-Fuc

(6)

Partial hydrolysis with acid and methylation analysis were used in elucidating their structures. A difucosylglycolipid that exhibited blood-group A activity was also isolated from the same source; the structure (7) of this glycolipid was established by means of partial hydrolysis with acid, sequential degradation with specific glycosidases, and methylation studies.[46]

α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-
2 3 (1 → 4)-D-Glc*p*-(1 → 1)-ceramide
↑ ↑
1 1
α-L-Fuc*p* α-L-Fuc*p*

(7)

[42] H. Price, S. Kundu, and R. Ledeen, *Biochemistry*, 1975, **14**, 1512.
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[45] B. L. Slomiany, A. Slomiany, and M. I. Horowitz, *European J. Biochem.*, 1975, **56**, 353.
[46] A. Slomiany and B. L. Slomiany, *Biochim. Biophys. Acta*, 1975, **388**, 135.

The principal structural features of the Forssman glycolipid hapten and of an Le^b-like glycolipid isolated from dog small intestines have been identified by m.s. of the methylated and reduced derivatives.[47] The glycolipid exhibiting Forssman activity was shown to be a pentaglycosylceramide possessing hexosamine → hexosamine → hexose → hexose → hexose → ceramide as the carbohydrate sequence, dihydroxysphingosine as the major long-chain base, and normal fatty acids. The glycolipid having Le^b-like activity was shown to be a hexaglycosylceramide consisting of L-fucose → hexose → [L-fucose] → hexosamine → hexose → hexose, phytosphingosine (as the principal long-chain base), and only 2-hydroxylated fatty acids. Human blood-group H activity was exhibited by the fucolipid (8) isolated from dog small intestines; the structure (8) was based on immunochemical and chemical studies.[48]

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*NAcp*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

(8)

A polar, glycosphingolipid fraction isolated from dog small intestines has been shown to contain two components, which were characterized by m.s. of the methylated and the methylated-and-reduced glycolipids.[49] One of the components was identified as a hexaglycosylceramide [L-fucose → hexose → (L-fucose) → hexosamine → hexose → hexose → ceramide] containing a terminal oligosaccharide structure similar to that of the blood-group Le^b determinants, and the other as a heptaglycosylceramide containing the sequence hexosamine → (L-fucose) → hexose → (L-fucose) → hexosamine → hexose → hexose → ceramide. The binding of the gangliosides G_{Gtet_1}, $G_{Gtet_{2a}}$, and G_{Lac_1} all of which contain neuraminic acid, to dog erythrocytes and to SV 40-transformed murine fibroblasts has been discussed.[50] That the bound gangliosides were oriented on the cell surface in a way different from the native gangliosides present in the plasma membrane was indicated by the levels of neuraminic acid resistant to the action of neuraminidase.

Cerebroside, diglycosylceramide, triglycosylceramide, and globoside have been identified in rabbit aorta, plasma, and red-blood cells.[51] The levels of incorporation of 2-[^{3}H]acetamido-2-deoxy-D-mannose into the sialic acids of the principal gangliosides of rabbit retina have been investigated.[52]

Increased levels of gangliosides, sulphatides, and neutral glycosylceramides have been found in the skeletal muscles of rabbits with experimental dystrophy when compared to controls.[53] Data obtained from the *in vitro* synthesis of glycolipids in rabbit platelets have indicated that D-glucose is used mainly for the production of D-glucosylceramide; since this glycolipid is common to all

[47] E. L. Smith, J. M. McKibbin, K.-A. Karlsson, I. Pascher, and B. E. Samuelsson, *Biochim. Biophys. Acta*, 1975, **388**, 171.
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gangliosides, it may serve as a precursor for the synthesis of gangliosides in platelets.[54]

A β-galactosidase isolated from rat brain, which is capable of hydrolysing glycosphingolipids, has been characterized.[55] Inhibition studies using simple sugars, oligosaccharides prepared from glycosphingolipids, and intact sphingolipids have indicated the importance of the lipophilic groups and, possibly, the molecular configuration of glycosphingolipids in determining the specificity of this glycosphingolipid β-galactosidase. The structure has been established of a neuraminidase-labile monosialoganglioside that is formed *in vitro* from an asialoganglioside [β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 1)-ceramide] and cytidine 5′-(*N*-acetylneuraminyl monophosphate) in the presence of a rat-brain sialyltransferase.[56] The added sialic acid residue was shown to be linked to C-3 of the terminal D-galactosyl residue of the asialoganglioside. Gangliosides extracted from rat brain have been shown to occur in the free form and as a ganglioside–protein complex.[57] Extraction of whole-brain tissue yielded only the ganglioside–protein complex, whereas mitochondrial, microsomal, and synaptosome-enriched fractions yielded both free and protein-bound gangliosides. A sulphogalactosylceramide and two minor sulpholipids, one of which was characterized as a 2-*O*-acyl-1-*O*-alkyl-3-*O*-(D-galactopyranosyl 3-sulphate)-glycerol, have been isolated from rat brain.[58] Ceramide mono-, di-, and trihexosides, two globosides, and haematoside have been isolated from metastatic rat-mammary tumours.[59] The fatty acid, base, and sugar compositions of each glycolipid were determined, and the arrangement of sugars and the anomeric configurations and positions of the linkages were established by a combination of periodate oxidation, enzymic hydrolysis, and methylation analysis. The two globosides were shown to have structures identical to cytolipin R [β-D-Gal*NAcp*-(1 → 3)-α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide], excepting that their fatty-acid components are different. The structure of the ceramide trisaccharide was established as α-D-galactopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 3)-D-glucopyranosyl-(1 → 1)-ceramide, and the ceramide trisaccharide is considered to be a possible precursor of cytolipin R. The levels of gangliosides in plasma membranes isolated from normal rat liver and from Morris hepatoma have been compared.[60] An eight-fold increase in the total gangliosides in the plasma membrane from the hepatoma compared with those present in normal liver microsomes resulted from a five-fold increase in the haematosides, an eight-fold increase in the monosialogangliosides, and a 22-fold increase in the disialogangliosides. Trisialoganglioside is present in normal liver, but not in the hepatoma. Sphingomyelin and *N*-acyl-4-*t*-sphingenine have been shown to serve preferentially as acceptors in the biosynthesis of ceramide

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monohexoside, ceramide lactoside, and haematoside.[61] Most of the sulphoglycerogalactolipid 2-*O*-acyl-1-*O*-alkylglycerylmonogalactoside sulphate has been located in the germinal cells of rat testis, and the primary spermatocytes are the earliest spermatogenic cells to contain high levels of this lipid.[62] The gangliosides of embryonic rat cells transformed chemically and/or virally have been examined after the uptake of 2-amino-2-deoxy-D-[^{14}C]glucose.[63] Hydrolysis of the gangliosides indicated that 2-amino-2-deoxy-D-galactose and *N*-acetyl- and *N*-glycolylneuraminic acids were labelled in all of the cell lines. The principal ganglioside of the three transformants was identified as *N*-acetylneuraminyl-D-galactopyranosyl-D-glucopyranosylceramide, whereas that of the control was shown to be 2-acetamido-2-deoxy-D-galactopyranosyl-[*N*-acetylneuraminyl]-D-galactopyranosyl-D-glucopyranosylceramide.

Hamster-embryo fibroblast cells transformed by polyoma virus have been shown to contain an additional ceramide tetrasaccharide, which is absent from normal cells; this glycolipid was identified as lacto-*N*-neotetraosylceramide [β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 3)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosyl-(1 → 1)-ceramide].[64] The component gangliosides of normal astroblasts in tissue culture and of glial cells obtained from hamster brain by centrifugation on sucrose–ficoll gradients have been compared.[65] In view of the simple ganglioside pattern found in glial cells in culture, the complex pattern shown by glial fractions prepared by centrifugation was indicated to result from contamination by a material rich in polysialogangliosides (*e.g.* synaptic plasma membranes). The ganglioside patterns of normal cell lines and two variants of an SV 40-induced hamster cell line suggested that all the cell lines contain *N*-acetylneuraminyl-D-galactopyranosyl-D-glucopyranosylceramide (haematoside, G_{M_3}), 2-acetamido-2-deoxy-D-galactopyranosyl-*N*-acetylneuraminyl-D-galactopyranosyl-D-glucopyranosylceramide (G_{M_2}), and a higher ganglioside, tentatively identified as disialohaematoside.[66] Hamster cells transformed by either polyoma virus or the carcinogen dimethylnitrosamine and revertants from the transformed cells have been examined for UDP-2-acetamido-2-deoxy-D-glucose: haematoside 2-acetamido-2-deoxy-D-galactosyltransferase activity and for the types of gangliosides present.[67] The transformed cells contained a lower level of enzyme activity and a simplified pattern of gangliosides. The principal glycosphingolipids of wild-type, Chinese-hamster ovarian cells have been reported to be G_{M_2}, G_{M_3}, and monoglucosylceramides.[68] Mutant cells, which were resistant to the cytotoxic effects of concanavalin A and phytohaemagglutinin P, revealed no alterations in the glycosphingolipid profile when compared with the wild-type cells. It was concluded that the glycosphingolipids

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present in these hamster cells are unlikely to be the primary sites of interaction with the lectins.

The addition of dibutyryl cyclic-AMP and theophylline to either normal or virus-transformed mouse fibroblasts in concentrations sufficient to affect their growth characteristics produced no qualitative alteration in the patterns of D-[1-^{14}C]galactose incorporated into the principal glycosphingolipids of the cells.[69] Exogenous monosialogangliosides have been shown to be much more effective than disialogangliosides in inhibiting the rate of growth of both transformed and untransformed 3T3 mouse cells; the isolated carbohydrate moiety of G_{Gtet_1}, the sialo-oligosaccharide D-galactopyranosyl-2-acetamido-2-deoxy-D-galactopyranosyl-[*N*-acetylneuraminyl]-D-galactopyranosyl-D-glucose, and ceramide produced no inhibition.[70] Marked variations have been observed in the ganglioside patterns, but not in the neutral glycosphingolipid patterns, in clonal lines of mouse neuroblastoma and neuroblastoma cell hybrids.[71] Despite widely differing membrane electrogenic activities, the glycosphingolipid profiles of the hybrid lines were generally similar. The metabolism of 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose, and *N*-acetylneuraminic acid in extracts of mouse cells with or without added 2-deoxy-2-(2-fluoroacetamido)-α-D-glucose has been studied.[72] The accumulation of uridine 5′-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate) and cytidine 5′-(*N*-acetylneuraminyl monophosphate) was inhibited by the fluoro-sugar. Since this fluoro-sugar can modify amino-sugar metabolism, it could be used at non-toxic levels in mouse cells to explore changes in glycolipid biosynthesis. Chemically and *X*-ray-transformed subclones of mouse-embryo cells were found to contain reduced levels of mono- and di-sialogangliosides, *viz.* D-galactopyranosyl-2-acetamido-2-deoxy-D-galactopyranosyl-(*N*-acetylneuraminyl)-D-galactopyranosyl-D-glucopyranosylceramide (G_{M_1}) and *N*-acetylneuraminyl-D-galactopyranosyl-2-acetamido-2-deoxy-D-galactopyranosyl-(*N*-acetylneuraminyl)-D-galactopyranosyl-D-glucopyranosylceramide ($G_{D_{1a}}$), but increased levels of 2-acetamido-2-deoxy-D-galactopyranosyl-(*N*-acetylneuraminyl)-D-galactopyranosyl-D-glucopyranosylceramide (G_{M_2}).[73] The activity of UDP-D-galactose: G_{M_2}-galactosyltransferase in the transformed clones was reduced to between 2.7 and 14.3% of the normal value, but other ganglioside glycosyltransferase activities were unaffected. This enzymic change is consistent with the observed alteration in the ganglioside pattern in the transformed cells. The residual galactosyltransferase activity in the transformed cells was kinetically similar to the normal enzyme, suggesting that transformation alters the biosynthesis of gangliosides by blocking the synthesis of the glycosyltransferase at the translational or transcriptional level. A particulate fraction obtained from mouse kidney was able to synthesize D-galactosylceramide from UDP-D-[^{14}C]galactose and ceramide; when UDP-D-glucose was used instead of UDP-D-galactose, the product was D-glucosylceramide.[74]

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A particulate preparation from embryonic chicken brain has been used to transfer 2-acetamido-2-deoxy-D-galactose from UDP-2-acetamido-2-deoxy-D-galactose to the ganglioside G_{M_3} (haematoside, sialyl-lactosylceramide) to give the Tay–Sachs ganglioside (G_{M_2}).[75] The preparation was also able to use 2-acetamido-2-deoxy-D-glucose, but kinetic experiments indicated that the acceptors (haematoside and 2-acetamido-2-deoxy-D-glucose) are substrates for different transferases. The activity of UDP-D-glucose:ceramide glucosyltransferase during chicken-retina ontogenesis reached a maximum in 8-day-old embryos; the level of activity then decreased, but reached a second maximum by the eleventh day of post-natal life.[76] The enzymic activity was stimulated by phosphatidylcholine, and the product of the enzymic reaction was identified as D-glucosylcerebroside.

Two monoglucosylceramides and a monogalactosylceramide have been isolated from the skin of the Japanese pond frog (*Rana nigromaculata*).[77] The brain-ganglioside patterns of taxonomically related fish species living at different temperatures have been compared with those of mammals.[78] The results demonstrated a correlation between the body temperatures and the brain-ganglioside patterns: the higher the body temperature of the species, the lower is the relative proportion of polysialoganglioside in the brain. The principal glycosphingolipids from the salt (rectal) gland of the spiny dogfish have been shown to contain sulphatides, having D-galactose 3-sulphate as the polar moiety, and *N*-acetylneuraminyl-D-galactosyl-D-glucosylceramides, in addition to minor proportions of D-glucosyl- and D-galactosyl-ceramides.[79] A number of glycolipids have been isolated and identified from puffer fish (*Fugu rubripes rubripes*) testis.[80] Although seminolipid, a sulphogalactolipid specific to mammalian testis, was absent, a sulphatide and two other gangliosides (9) and (10) were detected.

D-Gal*N*Ac*p*-(1 → 4)-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide
3
↑
2
Neu*N*Ac

(9)

Neu*N*Ac-(2 → 3)-D-Gal*p*-(1 → 4)-D-Glc*N*Ac*p*-(1 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-ceramide

(10)

Neutral glycosphingolipids containing D-mannose have been extracted from whole tissues of the fresh-water bivalve *Corbicula sandai*.[81] Chemical, enzymic, and physical methods were used to characterize two novel glycolipids, *viz.* β-D-mannopyranosyl-(1 → 4)-D-glucopyranosylceramide and α-D-mannopyranosyl-(1 → 4)-β-D-mannopyranosyl-(1 → 4)-D-glucopyranosylceramide. The compositions of the sialosphingolipids of sea-urchin eggs and spermatozoa have been

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shown to be characteristic for the species and gamete; the sialosphingolipids may have a common, carbohydrate backbone composed of hexoses and sialic acid.[82] A monosialoglucosyl- and a disialoglucosyl-ceramide were identified following separation by t.l.c.[83]

Plant and Algal Glycolipids

Analytical, structural, physiological, and biosynthetic investigations of galactosyldiglycerides, sulpholipids, and sphingolipids have been reviewed.[84] The products of deacylation and methanolysis of some plant glycolipids have been examined by g.l.c.; the sugar components of a monogalactosyldiglyceride and a digalactosyldiglyceride were determined quantitatively by this procedure.[85] A glycolipid fraction isolated from wheat gluten has been shown to contain a glucolipid and a galactolipid as components.[86] Two of the principal glycolipids isolated from tobacco (*Nicotiana tabacum*) leaves have been partially characterized as 2-acetamido-2-deoxy-D-glucosyl- and 2-amino-2-deoxy-D-glucosyl-D-glucuronosylinositol phosphorylceramides.[87] Chloramphenicol and cycloheximide have been used to inhibit the biosynthesis of linoleic acid and galactolipids in pea leaves.[88] The isolation and chemical characterization of a sulphoquinovosyldiglyceride and a triglycosyldiglyceride from rice grains have been reported.[89] The biosynthesis of sulphoquinovosyldiacylglycerol in germinating alfalfa (*Medicago sativa*) seeds probably proceeds by a pathway similar to that in *Euglena*, since adenosine 3′-phosphate 5′-diphosphosulphate, cysteic acid, sulphite, and sulpholactic acid are involved.[90] Two phytoglycolipids present in the seeds of *Pistacia vera* have been reported to be composed of inositol, D-glucuronic acid, 2-amino-2-deoxy-D-glucose, D-mannose, D-galactose, and L-arabinose.[91] The ratio of monogalactosyl- and digalactosyl-diglycerides in bracken (*Pteridium aqualinum*) has been shown to vary from 1.8 in pinnae, 1.0 in the lower petioles, and 0.3 in the storage rhizomes.[92] Zones highly active in galactosyltransferase have been found in mitochondria, chloroplast lamellae, and osmotically shocked chloroplasts of *Spinacia oleracea*.[93] The highly active fractions are considered to originate from the chloroplast envelopes, in agreement with the suggestion that galactosyldiglycerides are synthesized in the chloroplast envelope, and are then transported to the thylakoid system for incorporation as mono- or di-galactosyldiglycerides. The principal lipids of the chloroplast-envelope membrane of

[82] Y. Nagai and M. Hoshi, *Biochim. Biophys. Acta*, 1975, **388**, 146.
[83] M. Hoshi and Y. Nagai, *Biochim. Biophys. Acta*, 1975, **388**, 152.
[84] J. B. Mudd and R. Garcia, in 'Recent Advances in the Chemistry and Biochemistry of Plant Lipids', Phytochemical Society Symposia, Series No. 12, Academic Press, London, 1975, p. 162.
[85] J. P. Williams, G. R. Watson, M. U. Khan, S. Leung, A. Kuksis, O. Stachnyk, and J. J. Myher, *Analyt. Biochem.*, 1975, **66**, 110.
[86] Y. Inouye, T. Ito, T. Nakahara, A. Fukuda, S. Hirana, and I. Morishima, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1415.
[87] K. Kaul and R. L. Lester, *Plant Physiol.*, 1975, **55**, 120.
[88] J. Vichanska, A. Trémolières, and P. Mazliak, *Compt. rend.*, 1973, **277**, *D*, 501.
[89] Y. Kondo, S. Ito, and Y. Fujino, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 2549.
[90] J. L. Harwood, *Biochim. Biophys. Acta*, 1975, **398**, 224.
[91] Z. Imre, *Z. Naturforsch.*, 1974, **29c**, 195.
[92] M. C. Jarvis and H. J. Duncan, *Phytochemistry*, 1974, **13**, 979.
[93] H. C. Van Hummel, T. J. M. Hulsebos, and J. F. G. M. Wintermans, *Biochim. Biophys. Acta*, 1975, **380**, 219.

Vicia faba have been reported to be monogalactosyldiglyceride (29%) and digalactosyldiglyceride (32%).[94] The formation of mono- and di-galactosyldiglycerides following the incorporation of $^{14}CO_2$ into leaf discs of *Vicia faba* is consistent with the view that galactosylation of a diglyceride affords a monogalactosyldiglyceride, which adds a second D-galactosyl residue to form the digalactosyldiglyceride.[95, 96] Monogalactosyl- and digalactosyl-diacylglycerols have been shown to be necessary for the photosystem I-activity in lyophilized spinach chloroplasts, where they may be involved in restoration of the thylakoid membranes.[97]

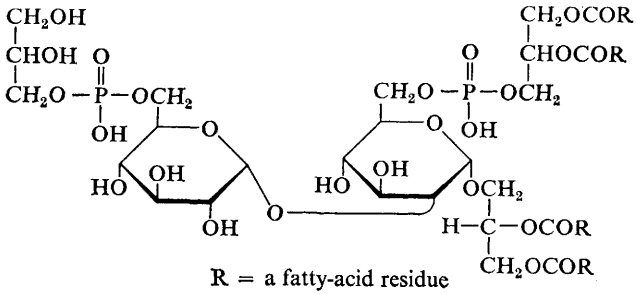
The effect of the pesticide *N'*-(3,4-dichlorophenyl)-*NN*-dimethylurea (DCMU) on the metabolism of galactolipids in *Chlorella* has been investigated.[98] Although treatment of *Chlorella* with DCMU in daylight led to preferential, though not exclusive, inhibition of galactolipid synthesis, resulting in increased levels of phosphatidyl-ethanolamine and -glycerol, it had no effect on lipid synthesis in the dark. Cell-free preparations of *Tetrahymena pyriformis* catalysed the incorporation of D-glucose from UDP-D-glucose into a glucolipid possessing properties identical to those of other sugar derivatives of dolichyl phosphate.[99] Kinetic and other evidence indicated that this glucolipid donates a D-glucosyl residue to an oligosaccharyl-lipid and glycoprotein(s).

Microbial Glycolipids

A strain of *Bacillus stearothermophilus* has been found to contain glycosyldiglycerides but no phosphatidylethanolamine, whereas another strain contains phosphatidylethanolamine but no glycolipids.[100] Most of the phospholipids detected in stationary phase cultures of *Bifidobacterium bifidum* have been characterized as phosphogalactolipids; the levels of glycerophosphorylgalactosyldiglyceride and of a related monoglyceride were found to vary with pH.[101] Small amounts of oligomannosylphosphoinositides have been identified in stationary phase cells of *Corynebacterium aquaticum*; pulse-chase experiments suggested that the first step in the biosynthesis of the oligomannosylphosphoinositides involves the conversion of phosphatidylinositol into a monomannosylphosphoinositide.[102] Di- and tri-mannosylphosphoinositides appear to be the main phosphoglycolipids synthesized during exponential growth of the cells. A particulate enzyme preparation from *Mycobacterium smegmatis* has been found to catalyse the transfer of D-[^{14}C]glucose from UDP-D-[^{14}C]glucose to neutral glycolipids, which were identified as monoglucosyl- and diglucosyldiglycerides.[103] A series of serologically active oligomannosylphosphatidylinositides occurring in *M. tuberculosis* has been isolated and characterized.[104]

[94] L. R. O. Mackender and R. M. Leech, *Plant Physiol.*, 1974, **53**, 496.
[95] J. P. Williams, G. R. Watson, M. U. Khan, and S. Leung, *Plant Physiol.*, 1975, **55**, 1038.
[96] J. P. Williams, M. U. Khan, and S. Leung, *J. Lipid Res.*, 1975, **16**, 61.
[97] Z. Krupa and T. Baszynski, *Biochim. Biophys. Acta*, 1975, **408**, 26.
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[99] R. W. Keenan, M. Kruczek, and L. Fusinato, *Arch. Biochem. Biophys.*, 1975, **167**, 697.
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[101] F. W. Van Schaik and J. H. Veerkamp, *Biochim. Biophys. Acta*, 1975, **388**, 213.
[102] J. A. Hackett and P. J. Brennan, *Biochem. J.*, 1975, **148**, 253.
[103] A. D. Elbein, W. T. Forsee, J. C. Schultz, and R. A. Laine, *Lipids*, 1975, **10**, 427.
[104] A. Sasaki, *J. Biochem.* (*Japan*), 1975, **78**, 547.

The extracellular slime layer of *Pseudomonas aeruginosa* has been shown to be a glycolipoprotein (mol. wt. 1×10^5) containing residues of L-rhamnose, D-mannose, D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose.[105] The carbohydrate moiety of this glycoprotein is responsible for the antigenic specificity and for the inhibition of phagocytosis, whereas the lipid moiety is associated with the leucopenic and lethal effects. A phosphoglucolipid isolated from *Streptococcus faecalis* is composed of D-glucose, glycerol, fatty acids, and phosphate (molar ratio 2 : 3 : 4 : 2).[106] Structural studies indicated that the glycolipid is 3(1)-*O*-[6″-(*sn*-glycerol 1-phosphoryl)-6′-(1,2-diacyl-*sn*-glycerol 3-phosphoryl)-2′-*O*-α-D-glucopyranosyl-α-D-glucopyranosyl]-1(3),2-diacylglycerol (11); thus, it is related to the phosphatidyl- and glycerophosphoryl-kojibiosyldiacylglycerols previously isolated from *S. faecalis*. The glycolipid (11)



R = a fatty-acid residue

(11)

may be involved in the biosynthesis of the lipoteichoic acid or it may be derived from enzymic degradation of the lipoteichoic acid. A water-soluble [^{14}C]glycerol-labelled lipoteichoic acid isolated from membrane preparations of *S. faecalis* released phosphatidylkojibiosyldiglyceride in almost quantitative yield on mild hydrolysis with acid, whereas more vigorous hydrolysis gave kojibiosyldiglyceride, monoglucosyldiglyceride, monoglyceride, and phosphatidylmonoglucosyldiglyceride.[107] These and other results demonstrate that the membrane teichoic acid is linked covalently through a phosphodiester bond to phosphatidylkojibiosyldiglyceride. Evidence has been provided for the non-proteinaceous nature of the receptor for the enterotoxin of *Vibrio cholerae* on murine lymphoid cells.[108] The results suggest that G_{M_1} is the most likely receptor for choleragen.

A new type of phosphorylated glycosphingolipid has been isolated from cultures of *Aspergillus niger*.[109] After purification by ion-exchange chromatography and preparative t.l.c., this water-soluble glycosphingolipid was shown to be composed of inositol, D-galactose, D-mannose, 2-amino-2-deoxy-D-glucose, ceramide, and phosphate. Sindbis virus grown in chicken-embryo fibroblasts has been found to contain the same four glycolipids as in the host cell.[110] In a study

[105] J. W. Sensakovic and P. F. Bartell, *Infection and Immunity*, 1975, **12**, 808.
[106] W. Fischer and H. R. Landgraf, *Biochim. Biophys. Acta*, 1975, **380**, 227.
[107] M. C. W. Ganfield and R. A. Pieringer, *J. Biol. Chem.*, 1975, **250**, 702.
[108] D. A. Hart, *Infection and Immunity*, 1975, **11**, 742.
[109] P. J. Brennan and J. Roe, *Biochem. J.*, 1975, **147**, 179.
[110] C. B. Hirschberg and P. W. Robbins, *Virology*, 1974, **61**, 602.

of the immunological properties of glycolipids from membranes of *Acholeplasma laidlawii*, the predominant complement-fixing activity of the membrane lipids was shown to be associated with monoglucosylglyceride, diglucosyldiglyceride, and glycerylphosphoryldiglucosyldiglyceride.[111]

[111] M. D. Ryan, P. Noker, and L. L. Matz, *Infection and Immunity*, 1975, **12**, 799.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

BY J. F. KENNEDY

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Enzymes, and Glycolipids

Polysaccharides.—Amylosucrase, a bacterial α-D-glucosylase, has been shown to convert sucrose into an amylaceous α-glucan and small amounts of malto-oligosaccharides; only α-D-glucosyl residues were transferred from amylopectin, maltoheptaose, and sucrose to D-[^{14}C]fructose to form labelled sucrose.[1] The terminal 4-linked α-maltosyl, α-maltotriosyl, *etc.* segments of amylopectin and maltoheptaose, and the α-maltosyl residue of 4-*O*-α-D-glucopyranosylsucrose were not transferred. The α-glucan formed from sucrose resembles glycogen, and was hydrolysed to the extent of 48% by β-amylase. Branches are considered to be produced either by a contaminating dextrinyl-transferring (branching) enzyme or by monomer transfer by amylosucrase itself. Synthesis required a primer (*e.g.* amylopectin or glycogen), and amylosucrase produced an α-glucan composed of D-[^{14}C]glucose (99.6%) and primer (0.4%) from D-[^{14}C]fructose.

In a new approach to the direct synthesis of polysaccharides, melibiose 1,2,3,4,2′,3′,4′-hepta-acetate was treated with 2-methylpropene in the presence of acid to give the t-butyl ether at O-6′; the glycosyl chloride was then formed by reaction with titanium(IV) chloride.[2] Polymerization of the glycosyl chloride in nitromethane with mercury(II) cyanide, followed by deacetylation, yielded a high-molecular-weight product, which on reduction (sodium borohydride) and methanolysis gave a linear, regular polysaccharide. The polysaccharide (mol. wt. $\sim 4 \times 10^3$) possessed a galactoglucan structure having alternate (1 → 6)-linked D-galactosyl and D-glucosyl residues.

Oligosaccharides.—An edited series on the methods and the applications of solid-phase syntheses includes a section on the solid-phase synthesis of oligosaccharides.[3]

Attempts have been made to use an inorganic support in the solid-phase synthesis of oligosaccharides; the saccharide unit was coupled to zirconium-hardened glass carrying olefinic chains.[4] Several D-glucosyl derivatives of 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranose, *viz.* the D-glucosyl quaternary ammonium salt, the D-glucosyl bromide, and the D-glucosyl 1-toluene-

[1] G. Okado and E. J. Hehre, *J. Biol. Chem.*, 1974, **249**, 126.
[2] N. K. Kochetkov, E. M. Klimov, and M. B. Kislov, *Izvest. Akad. Nauk S.S.S.R.*, 1973, 2400.
[3] 'Solid-Phase Synthesis', ed. E. C. Blossey and D. C. Neckers, Wiley, Chichester, 1975.
[4] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1975, **39**, 151.

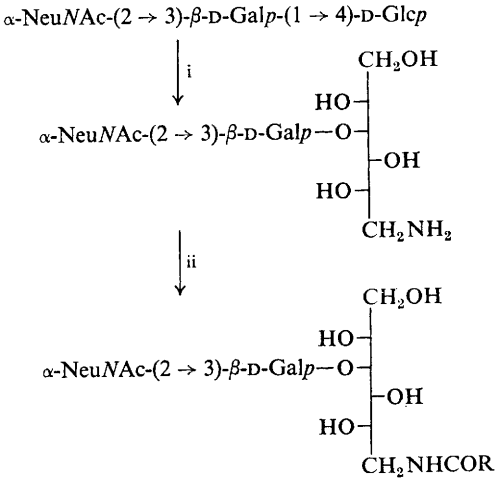
p-sulphonate were used. However, unless the cause for incomplete coupling can be identified and corrected, it is unlikely that porous glasses will be used in the solid-phase synthesis of oligosaccharides.

In the biological synthesis of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses by the action of enzymes from *Bacillus megaterium* and *B. macerans* on [^{14}C]-starch, the amounts of each cycloamylose formed were determined by measurement of the radioactivity.[5] The cycloamyloses were produced by the respective enzymes in the ratios 1 : 2.4 : 1 and 2.7 : 1 : 1 and the ratios were largely independent of the pH of the media. Cyclohexa- and cyclo-octa-amyloses were produced directly from starch and not by way of cyclohepta-amylose.

Glycoproteins.—In the preparation of carbohydrate-modified proteins, the α-D-galactopyranosylamine–ammonia complex, lactosylamine, and 2-amino-2-deoxy-D-glucose have been attached covalently to lysozyme with the aid of a water-soluble carbodi-imide.[6] The carbohydrate moieties introduced were shown to be bound to the carboxy-groups of L-glutamic and L-aspartic acids by carboxymethylation of the pseudoglycoproteins, conversion into glycopeptides, and peptide mapping.

A pseudoglycoprotein has also been obtained from lysozyme by specific esterification of L-aspartic acid-52 with the affinity label 2,3-epoxypropyl β-*NN'*-diacetylchitobioside.[7]

A semi-synthetic agglutinin that simulates the action of phytohaemagglutinins has been prepared by polymerization of lysozyme with glutaraldehyde.[8] The



R = stearoyl

Reagents: i, $NaCNBH_3$–NH_4OAc; ii, 4-nitrophenyl stearate

Scheme 1

[5] S. Kitahata and S. Okada, *Agric. and Biol. Chem.* (*Japan*), 1974, **38**, 2413.
[6] E. Moczar, *Experientia*, 1973, **29**, 1576.
[7] Y. Eshdat, A. Dunn, and N. Sharon, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1658.
[8] V. Hořejší and H. Kocourek, *Experientia*, 1974, **30**, 1348.

products, particularly higher oligomers of lysozyme, were able to haemagglutinate erythrocytes.

Enzymes.—Synthetic homologues of the loop region (residues 64—82) of lysozyme having one or two amino-acid residues replaced by L-alanine have been prepared, and their antigenic activities have been assessed by the ability to bind anti-loop antibodies.[9] The activities observed were compared with the probability of a β-bend occurring, as computed from statistical analysis of conformations based on the amino-acid sequence. The antigenic activity of most of the peptides tested correlated well with the relative probability of a β-bend forming. It appears that a β-bend and hydrogen bonding are involved in the antigenic activity of the conformation-dependent lysozyme loop.

Glycolipids.—Synthetic glycolipids have been obtained from oligosaccharides *via* reductive amination and amidation processes, as shown in Scheme 1.[10]

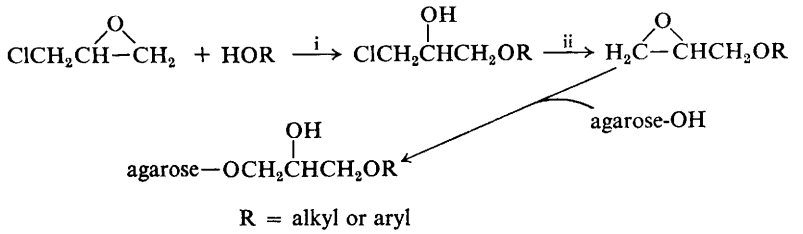
Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

A review on the solubility of polysaccharides and the behaviour of polysaccharides in solution refers to derivatives of polysaccharides and the modification of polysaccharides to render them insoluble.[11]

Derivatives of polysaccharides continue to be widely used as matrices for the immobilization of enzymes. Reviews have dealt with the uses of immobilized enzymes in food and microbial processes,[12] in enzyme engineering,[13] as stationary phases in chromatography,[14] and as immobilized biochemicals.[15] Articles on the principles of the technology of immobilized enzymes [16] and on techniques for the immobilization of enzymes [17] also refer to active enzyme derivatives of water-insoluble polysaccharides. Derivatives of polysaccharides also continue to be used extensively for the production of materials for affinity chromatography; such uses are mentioned in reviews on bonded stationary phases in chromatography,[14] immobilized biochemicals and affinity chromatography,[15] biospecific affinity chromatography,[18] and affinity-chromatographic techniques for the purification of enzymes.[19]

Agaroses.—Various aliphatic and aromatic groups have been introduced into agarose as shown in Scheme 2; the coupling-yields were determined in each

[9] R. Arnon, E. Teicher, and H. A. Scheraga, *J. Mol. Biol.*, 1975, **90**, 403.
[10] H. Wiegandt and W. Ziegler, *Z. physiol. Chem.*, 1974, **355**, 11.
[11] R. L. Whistler, in 'Carbohydrates in Solution', ed. R. F. Gould, Adv. Chem. Ser. 117, 1973, p. 242.
[12] 'Immobilized Enzymes in Food and Microbial Processes', ed. A. C. Olson and C. L. Cooney, Plenum Press, New York, 1974.
[13] 'Enzyme Engineering', Vol. 2, ed. E. Kendall Pye and L. B. Wingard, jun., Plenum Press, London, 1974.
[14] 'Bonded Stationary Phases in Chromatography', ed. E. Grushka, Wiley, Chichester, 1974.
[15] 'Immobilized Biochemicals and Affinity Chromatography', ed. R. B. Dunlop, *Adv. Exptl. Med. Biol.*, Vol. 42, Plenum Press, New York, 1974.
[16] S. A. Barker and I. Kay, in 'Handbook of Enzyme Biotechnology', ed. A. Wiseman, Horwood, Chichester, 1975, p. 89.
[17] S. A. Barker and J. F. Kennedy, in ref. 16, p. 203.
[18] J. Porath and T. Kristiansen, in 'The Proteins', 3rd edn., ed. H. Neurath and R. L. Hill, Academic Press, New York, 1975, p. 95.
[19] 'Methods in Enzymology', Vol. 34, ed. W. B. Jakoby and M. Wilchek, Academic Press, New York, 1974.



Reagents: i, BF_3–Et_2O; ii, OH^-

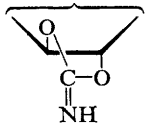
Scheme 2

case.[20] The products are suitable for hydrophobic-interaction chromatography used in the purification of cellulase, β-D-glucosidase, and β-D-galactosidase. The hydrophobicity of the agarose derivatives could be varied by altering the length of the hydrocarbon chains, thereby increasing the versatility of the matrix. Other proteins, viruses, and whole cells could also be adsorbed. By contrast to ion-exchange chromatography, adsorption in hydrophobic-interaction chromatography decreases with decreasing ionic strength and with decreasing temperature.

A new, simple method for the covalent attachment of proteins to polysaccharides has been described.[21] An agarose carrier was derivatized by treatment with 4-benzoquinone in ethanol, and the proteinaceous material was then coupled directly; stable immobilized derivatives of serum albumin, ribonuclease, and chymotrypsin were obtained by this technique. Another advantage of the method is that coupling occurs over a wide range of pH.

Macroporous agaroses in bead form (*e.g.* Sepharose and Bio-Rad Agarose) can be converted into cyclic imidocarbonate derivatives by reaction with cyanogen bromide. The high solubility of cyanogen bromide in *N*-methyl-2-pyrrolidone affords an efficient way of controlling the amount of reagent used to activate the agaroses, so that the final amount of the ligand, *etc.*, attached can also be controlled.[22]

Cyclic imidocarbonate derivatives of agarose continue to be used extensively for the immobilization of biologically active macromolecules and for affinity chromatography. The reaction of a cyclic imidocarbonate group with a protein presumably occurs by nucleophilic attack of an amino-group on the *trans*-imidocarbonate ring (1) to give either an isourea, or an *N*-substituted imidocarbonate, or an *N*-substituted carbonate (see Vol. 7, p. 511). Recent references

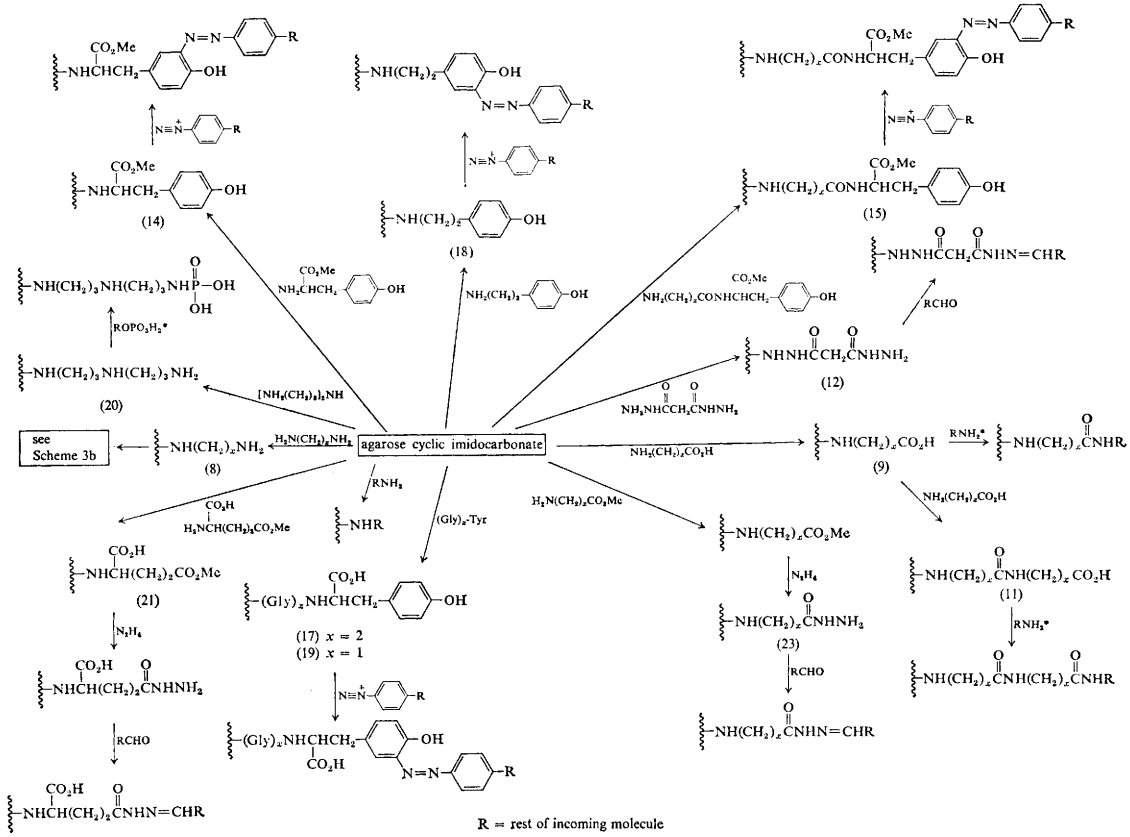


(1)

[20] S. Hjertén, J. Rosengren, and S. Pahlman, *J. Chromatog.*, 1974, **101**, 281.
[21] J. Brandt, L.-O. Andersson, and J. Porath, *Biochim. Biophys. Acta*, 1975, **386**, 196.
[22] A. H. Nishikawa and P. Bailon, *Analyt. Biochem.*, 1975, **64**, 268.

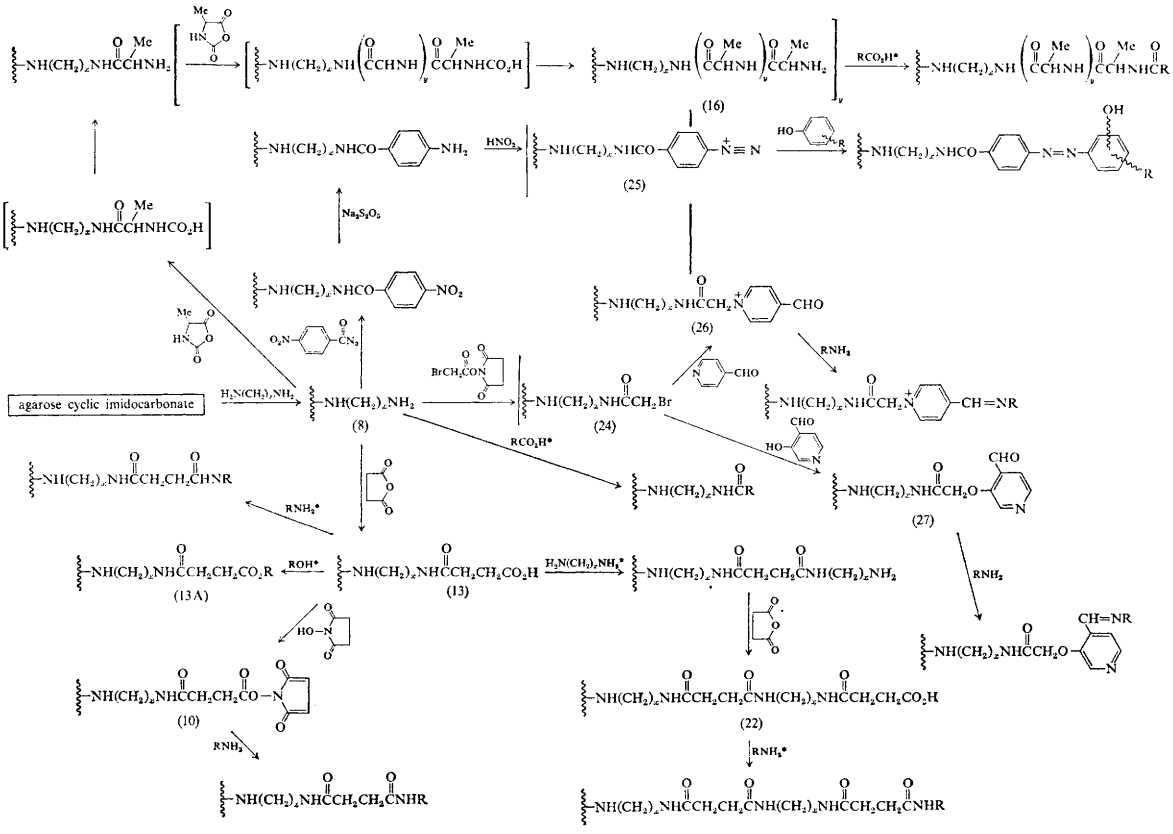
to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes) and immunologically active macromolecules (for use as immunoadsorbents) and of various affinants (for use as affinity-chromatography matrices) by such methods are summarized in Tables 1,[23–30] 2,[31–40] and 3,[41–70] respectively.

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[41] P. H. Plotz, P. D. Berk, B. F. Scharschmidt, J. K. Gordon, and J. Vergella, *J. Clin. Invest.*, 1974, **53**, 778.
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Scheme 3a

* = reaction involving carbodi-imide. In subsequent references to this scheme, the suffix A denotes the use of $H_2N(CH_2)_3NH(CH_2)_3NH_2$ instead of $NH_2(CH_2)_xNH_2$ for the production of (8), *etc.*



Scheme 3b

* = (see Scheme 3a)

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme coupled to agarose cyclic imidocarbonate	*E.C. No.*	*Agarose intermediate* [a]	*Ref.*
Aminopeptidase (microsomal)	3.4.11.2	—	23
β-Amylase	3.2.1.2	—	24
		(8)	24
		(9)	24
Aspartate 4-decarboxylase (subunits)	4.1.1.12	—	25
Catalase	1.11.1.6	—	26
Dextranase	3.2.1.11	—	27
Lactoperoxidase	—	—	28
Microbial metalloenzyme	3.4.24.4	—	23
Proline dipeptidase	3.4.13.9	—	23
Pronase	(3.4.24.4)	—	23
Protease, *ex Aspergillus* sp.	—	—	23
Tryptophanase	4.1.99.1	—	29
		pyridoxal 5′-phosphate (see p. 427)	30

[a] See text p. 414 and Scheme 3a, p. 417.

Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Agarose intermediate*	*Use of product*	*Ref.*
Anti-(β-D-acetamidodeoxyhexosidases 'A' + 'B') antibodies	—	Removal of β-D-acetamidodeoxyhexosidases 'A' and 'B' in the purification of human-brain β-D-acetamidodeoxyhexosidase 'C'	31
Anti-(serum albumin) antibody	—	Immunoaffinoelectrophoresis; a study of the interactions occurring during immunoadsorption in a simple system	32
Anti-(alkaline phosphatase) antibody	—	Purification of alkaline phosphatase	33
Anti-allotype serum	(10) [a]	Quantitative determination of allotypic markers by radioimmunoassay	34
Anti-(bovine prothrombin) antibody	—	Purification of prothrombin	35
Anti-(hamster Tamm–Horsfall glycoprotein) antibody	—	Isolation of a pseudo-Tamm–Horsfall glycoprotein	36
Anti-idiotype serum	(10A) [a]	Quantitative determination of idiotypic markers by radioimmunoassay	34
Anti-interferon antibody	—	Purification and concentration of interferon	37
Anti-prolactin antibody	—	Isolation of prolactin	38
Anti-(Tamm–Horsfall glycoprotein) serum	—	Isolation of a pseudo-Tamm–Horsfall glycoprotein	36

Table 2 (*cont.*)

Immunologically active component coupled to agarose cyclic imidocarbonate	*Agarose intermediate*	*Use of product*	*Ref.*
Blood-group substance A	—	Immunoadsorption of anti-(blood-group substance A) antibody	39 [b]
Cell material	—	Purification of anti-interferon	37
Cortisol derivatives of succinylated albumin	—	Isolation of cortisol-binding immunoglobulin	40
Goat immunoglobulin anti-(bovine prothrombin)	—	Purification of prothrombin	35
Prolactin	—	Isolation of anti-prolactin	38
Prothrombin	—	Purification of goat anti-(bovine prothrombin)	35
Rabbit immunoglobulin IgG anti-(hamster Tamm–Horsfall glycoprotein) antibody	—	Isolation of a pseudo-Tamm–Horsfall glycoprotein	36

[a] See text p. 414 and Scheme 3b, p. 418; [b] Agarose cross-linked with 2,3-dibromopropanol prior to reaction with cyanogen bromide.

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials without linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product* [a]	*Ref.*
Albumins (serum)	Removal of bilirubin and other albumin-bound substances from blood and plasma	41
	Selective binding of human and other interferons to immobilized albumins	42
Alkylamines	Purification of phosphorylase	43
2-Aminoethylamide of 2-hydroxyethyl α-ketoside of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid [b]	Affinity chromatography of neuraminidase	44 [c]
2-Aminoethylaminocarbonylmethyl α-ketoside of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid [d]		
2-Aminoethyl α-ketoside of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid [e]		
N-(6-Aminohexanoyl)-*N*-(6-aminohexanoyl)-α-D-galactopyranosylamine	Isolation of α-D-galactosidase	45 [f]
N-(6-Aminohexanoyl)-1-aminophenyltrimethylammonium bromide hydrobromide (3 and 4 isomers)	Purification of acetylcholinesterase	46 [g]
α-*N*-(6-Aminohexanoyl)-L-tryptophan methyl ester	Affinity chromatography of chymotrypsin; separation of chymotrypsin from trypsin	47 [h]

Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product* [a]	*Ref.*
N-(6-Aminohexyl)-L-aspartic acid	Affinity chromatography of asparaginase, aspartate ammonia-lyase, aspartate decarboxylase; isolation of asparaginase	48 [i]
4-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	Affinity chromatography of β-D-galactosidase and β-D-acetamidodeoxyhexosidase; removal of these enzymes in the purification of neuraminidase	49
Blood-group substance A (partially deacetylated)	Purification of anti-(blood-group A substance) antibody	39
Blue Dextran 2000	Affinity column for the dinucleotide fold in proteins	50
Bovine factor-X	Affinity chromatography of the venom coagulant protein of *Vipera russelli*	51
n-Butyl 4-aminobenzoate	Possible affinity-chromatography matrix for the isolation of plasminogen	52
(t-Butyloxycarbonyl-L-threonyl)-L-phenylalanyl-L-prolyl-L-tyrosine	Affinity chromatography of neuraminidases	53
Catalase	Purification of catalase inhibitor from maize	26
Concanavalin A	Investigation of the interaction of interferon with immobilized concanavalin A; demonstration of carbohydrate recognition and hydrophobic interactions	54
	Purification of β-D-acetamidodeoxyhexosidase 'A'	55
Deoxyribonuclease I	Isolation of the naturally occurring inhibitor of deoxyribonuclease I and its identification as actin	56
α,ω-Diaminohexanes	Affinity chromatography and purification of histidinol phosphate aminotransferase	57
	Fractionation of tRNA	58
'2'-Fucosyl-lactose', *N*-alkyl glycoside [j]	Affinity chromatography and purification of carbohydrate-specific immunoglobulins	59
Glycylglycyl-L-tyrosine	Affinity chromatography of neuraminidases	53
Glycyl-L-tyrosine	Affinity chromatography of neuraminidases	60
Haemoglobin	Isolation of cathepsin D	61
L-Histidinol phosphate	Investigation of specific ion effects in the affinity chromatography and purification of histidinol phosphate aminotransferase	62
11α-Hydroxyprogesterone 11-hemisuccinyl-bis-β-aminoethyldisulphide monoamide	Affinity-chromatography matrix for the recovery of proteins labile to conventional desorption conditions; purification of 20β-hydroxy-steroid dehydrogenase	63

Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product* [a]	*Ref.*
α-Lactalbumin	Quantitative studies of acceptor-ligand interactions in the lactose synthetase system; affinity chromatography of lactose synthetase A-protein	64
'Lacto-*N*-difucohexaose I', *N*-alkyl glycoside [k] 'Lacto-*N*-fucopentaose I', *N*-alkyl glycoside [l] 'Lacto-*N*-tetraose', *N*-alkyl glycoside [m]	Affinity chromatography and purification of carbohydrate-specific immunoglobulins	59
Lens culinaris phytohaemagglutinin	Fractionation of synaptic plasma-membrane glycoproteins	65
Lipoprotein (egg yolk)	Isolation of phospholipase C	66
L-Lysine L-Lysine-like compounds	Investigation of the relevance of L-lysine bound to agarose on the affinity of rabbit plasminogen for the matrix	52
8-L-Lysylvasopressin	Affinity chromatography of neurophysins	67
β-D-Mannopyranosylamine	Affinity chromatography of α-mannosidase	68
Poly(uridylic acid)	Isolation of poly(adenylic acid)-rich RNAs	69
Tyramine	Affinity chromatography of neuraminidases	53
Vicia cracca phytohaemagglutinin	Affinity chromatography of blood-group A substance	70
Wheat-germ phytohaemagglutinin	Fractionation of synaptic plasma-membrane glycoproteins	65

[a] 'Affinity chromatography' indicates that the chromatographic behaviour and mobility have been investigated; 'purification' indicates partial isolation; 'isolation' indicates complete purification; [b] See (2), p. 423; [c] Product also modified by periodate oxidation to convert 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid into 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid [see p. 423, and (5)—(7)]; [d] See (3), p. 423; [e] See, (4) p. 423; [f] The product is identical with that obtained by attachment of α-D-galactopyranosylamine *via* linkage extension as in (11) (see Scheme 3a, p. 417); [g] Agarose cross-linked with epichlorohydrin and reduced prior to activation with cyanogen bromide; [h] The product is identical with that obtained by attachment of L-tryptophan methyl ester *via* linkage extension as in (9) (see Scheme 3a, p. 417); [i] The product is identical with that obtained by the attachment of L-aspartic acid *via* linkage extension as in (8) (see Scheme 3b, p. 418); [j] See (38), p. 441; [k] See (40), p. 441; [l] See (39), p. 441; [m] See (41), p. 441.

These derivatives are listed according to the uses prescribed, since, for example, the insoluble form of an enzyme used for affinity chromatography may not be enzymically active.

Agarose cyclic imidocarbonate is unsuitable for the immobilization of carboxypeptidase, used as a probe for studying the thermally induced unfolding of bovine pancreatic ribonuclease, since agarose undergoes conformational transitions at *ca.* 50 °C.[71]

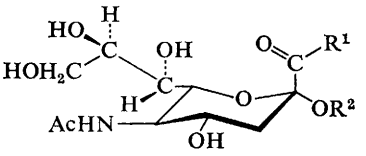
Parameters relevant to the activation and to the coupling reactions have been investigated in the covalent binding of macromolecules to agarose cyclic imido-

[71] A. W. Burgess, L. I. Weinstein, D. Gabel, and H. A. Scheraga, *Biochemistry*, 1975, **14**, 197.

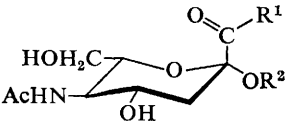
carbonate.[72] Immunofluorescent studies on the products obtained by coupling immunoglobulins IgA, IgG, and IgM to agarose cyclic imidocarbonate demonstrated that the bound macromolecules are distributed uniformly throughout the beads.

The technique of immunoaffinoelectrophoresis has been extended by using antibodies coupled to agarose cyclic imidocarbonate; it was used in an investigation of the interactions occurring during immunoadsorption in a simple system.[32]

Matrices containing neuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid) residues have been prepared by reaction of 2-aminoethylamides of the 2-hydroxyethyl, 2-aminoethylaminocarbonylmethyl, and 2-aminoethyl α-ketosides of the acid with agarose cyclic imidocarbonate to give (2)—(4), respectively.[44] The amount of bound neuraminic acid was estimated after its release by hydrolysis with neuraminidase or with acid, the latter being the more effective. The agarose derivatives were modified further by oxidation with periodate to give residues of 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid bound to the matrix [*e.g.* (5)—(7)].



(2) R^1 = $NHCH_2CH_2NH$-agarose; R^2 = CH_2CH_2OH
(3) R^1 = OH; R^2 = $CH_2CONHCH_2CH_2NH$-agarose
(4) R^1 = OH; R^2 = CH_2CH_2NH-agarose



(5) R^1 = $NHCH_2CH_2NH$-agarose, R^2 = CH_2CH_2OH
(6) R^1 = OH, R^2 = $CH_2CONHCH_2CH_2NH$-agarose
(7) R^1 = OH, R^2 = CH_2CH_2NH-agarose

The coupling of 11α-hydroxyprogesterone 11-hemisuccinyl-bis-β-aminoethyl-disulphide monoamide to agarose cyclic imidocarbonate produces a novel derivative, since coupling is effected *via* a disulphide bridge.[63] The affinity material obtained was designed to see if it was possible to recover adsorbed proteins that are unstable under conventional recovery conditions, but that are stabilized by 2-thioethanol.

In the coupling of L-histidinol phosphate to agarose cyclic imidocarbonate, it was shown that specific ion effects provide an additional parameter that could be adjusted in order to optimize the purification of a protein by affinity chromatography.[62]

Direct attachment of an affinity ligand or enzyme to agarose cyclic imidocarbonate has frequently proved to be unrewarding, since the approach to the

[72] D. E. Stage and M. Mannik, *Biochim. Biophys. Acta*, 1974, **343**, 382.

ligand is sterically impeded; this difficulty can be overcome by inserting a covalent bridge between the matrix and the ligand. Bridging is also used when the functional groups on the ligand do not react with those on the matrix, since it allows a new type of functionality to be introduced. Modifications recently used to provide bridges in the preparation of affinity-chromatography matrices, *etc.*, from agarose cyclic imidocarbonates are depicted in Schemes 3a and 3b. The materials produced for affinity chromatography are listed in Table 4.[73–91] Where bridging has been used to immobilize enzymes and to prepare immunoadsorbents, appropriate details are incorporated into Tables 1 and 2.

A 4-diazophenyloxamic acid derivative of agarose cyclic imidocarbonate, obtained *via* (17) or (19), has been widely used over the past year or so in the isolation and purification of neuraminidases; however, the derivative produced *via* (19) was also found to bind haemoglobin, haemolysin, and phospholipase C at pH 5.5.[60] Although none of these proteinaceous molecules is absorbed onto macroporous agarose, they can bind to agarose cyclic imidocarbonate treated with glycyl-L-tyrosine. In this case, the agarose derivative appears to act as a cation-exchange resin, since non-specific adsorption of haemoglobin, haemolysin, and phospholipase C did not occur at pH 7.5, although neuraminidase was adsorbed at this pH. It is uncertain whether this adsorption is due to ion-exchange or to specific affinity effects, but these materials are unsuitable for use in the purification of neuraminidases owing to their failure to reject non-specific proteins, *etc.*

Affinity-chromatography materials produced by the reaction of tyramine, glycylglycyl-L-tyrosine, and (t-butyl-L-threonyl)-L-phenylalanyl-L-prolyl-L-tyrosine with agarose cyclic imidocarbonate adsorbed neuraminidase; the adsorption and elution of the enzyme are independent of whether 4-diazophenyloxamic acid groups [produced *via* (17) and (18)] are present.[53] It was recommended that earlier biological data (see Vol. 5, p. 356) obtained for neuraminidase should be re-evaluated in the light of these findings and the observation that other glycoside hydrolases from the same source are adsorbed and eluted under conditions identical with those for neuraminidase. The mechanism earlier suggested to operate during the affinity chromatography of neuraminidase is no longer tenable, although

[73] A. Oplatka, R. Lamed, and A. Muhlrad, *Biochim. Biophys. Acta*, 1975, **385**, 20.
[74] P. H. Chan and W. Z. Hassid, *Analyt. Biochem.*, 1975, **64**, 372.
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[76] C. Brandolin, C. Meyer, G. Defaye, P. M. Vignais, and P. V. Vignais, *F.E.B.S. Letters*, 1974, **46**, 149.
[77] M. Hierowski and R. Brodersen, *Biochim. Biophys. Acta*, 1974, **354**, 121.
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[79] R. J. Boegman, *F.E.B.S. Letters*, 1975, **53**, 99.
[80] R. Jost and A. Yaron, *European J. Biochem.*, 1974, **48**, 119.
[81] L. J. Lin and J. F. Foster, *Analyt. Biochem.*, 1975, **63**, 485.
[82] R. A. Laine, G. Yogeeswaran, and S.-I. Hakomori, *J. Biol. Chem.*, 1974, **249**, 4460.
[83] J. F. McGinnis and J. de Vellis, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 186.
[84] C. Godinot, J. H. Julliard, and D. C. Gautheron, *Analyt. Biochem.*, 1974, **61**, 264.
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[86] C. R. Shobe and J. D. Brosseau, *Canad. J. Microbiol.*, 1974, **20**, 1147.
[87] F. Pittner, W. Fried, and O. Hoffmann-Ostenhof, *Z. physiol. Chem.*, 1974, **355**, 222.
[88] B. Tabakoff and J. P. von Wartburg, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 957.
[89] M. Don and C. J. Masters, *Biochim. Biophys. Acta*, 1975, **384**, 25.
[90] W. Eventoff, K. W. Olsen, and M. L. Hackert, *Biochim. Biophys. Acta*, 1974, **341**, 327.
[91] L. Bachner, E. de Clercq, and M. N. Thang, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 476.

Table 4 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials* via *linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Adenosine 5′-(β,γ-imino)triphosphate (periodate oxidized) Adenosine 5′-pyrophosphate (periodate oxidized) Adenosine 5′-triphosphate (periodate oxidized)	(12)	Affinity chromatography of meromyosin fragments and derivatives thereof	73
8-β-Aminoethylthioadenosine triphosphate	(10)	Isolation of L-arabinokinase and D-galactokinase	74
4-Aminophenyl 1-β-D-thiogalactopyranoside	(13)	Purification of β-D-galactosidase-specific polysomes	75
Atractyloside (succinylated)	(8A)	Purification of atractyloside-binding protein	76 [b]
Bilirubin	(8)	Affinity chromatography of serum albumin	77
4-Chloromercuribenzoate	(8)	Isolation of creatine kinase	78
		Purification of creatine kinase on an organomercury matrix	79
Diazo derivatives of peptides	(14), (15)	Affinity chromatography of aminopeptidase (cytosol)	80
	(14)—(16)	Inhibition studies of aminopeptidase (cytosol)	80
N-(4-Diazophenyl)oxamic acid	(17), (18)	Affinity chromatography of α-D-acetamidodeoxy-galactosidase, β-D-acetamidodeoxyglucosidase, α-1,2-L-fucosidase, β-D-galactosidase, and neuraminidases	53
	(19)	Affinity chromatography of β-D-acetamidodeoxy-hexosidase, β-D-galactosidase, and neuraminidases; purification of neuraminidase	49
		Affinity chromatography of neuraminidases and the demonstration of non-specific adsorption by the affinant	60
5,5′-Dithiobis(2-nitrobenzoic acid)	(8)	Separation of non-thiol proteins and peptides from those containing a free thiol group (*e.g.* albumin and mercaptalbumin)	81
Globoside glycosphingolipid (modified by oxidative ozonolysis)	(8)	Purification of anti-(globoside glycosphingolipid) antibodies	82

Table 4 (*cont.*)

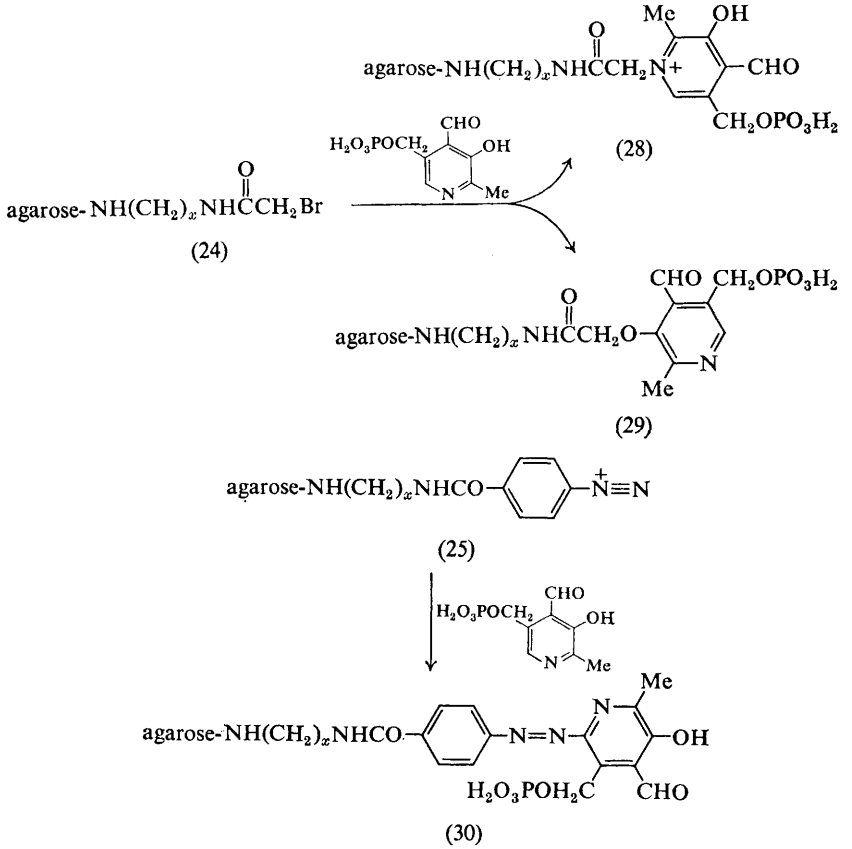
Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
D-Gluconic acid 6-phosphate	(8), (20)	Purification of glycerol 3-phosphate dehydrogenase	83
Guanosine 5′-triphosphate (periodate oxidized)	(21)	Purification of glutamate dehydrogenase	84
Haematoside glycosphingolipid (modified by oxidative ozonolysis)	(8)	Purification of anti-(globoside glycosphingolipid) antibodies	82
Hydroxocobalamin	(8A)	Purification of cobalamin-binding proteins	85 [c]
Hydroxylamine	(13), (22)	Purification of urease	86
Hydroxylurea	(9), (13), (13A), (22)	Purification of urease	86
β-D-Mannopyranosylamine	(9)	Affinity chromatography of α-mannosidase	68
Nicotinamide adenine dinucleotide	(9)	Purification of *myo*-inositol-1-phosphate synthase	87
Nicotinamide adenine dinucleotide phosphate	(9)	Separation of aldehyde reductases and alcohol dehydrogenase	88
Oxalic acid	(8)	Application of affinity chromatography to turnover studies of the lactate dehydrogenase isoenzymes	89
		Purification of lactate dehydrogenase	90
Peptides	(16)	Affinity chromatography of aminopeptidase (cytosol)	80
Poly(inosinic acid) (periodate oxidized)	(23)	Hybridization with poly(cytidylic acid) for the interaction with cells and the production of interferon	91
Pyridoxal 5′-phosphate	(24), (25)	Preparation, *via* affinity coupling, of active immobilized tryptophanase by treatment with apotryptophanase	30
tRNA (periodate oxidized)	(23)	Isolation of aminoacyl-tRNA synthetases	62

[a] See Schemes 3a and 3b, pp. 417 and 418; [b] The product is identical with that obtained by attachment of atractyloside *via* linkage extension as in (12) (see Scheme 3a, p. 417); [c] Non-covalently linked.

the technique may still be useful. A new mechanism for the affinity action was proposed.

Affinity-chromatography materials obtained by carbodi-imide-assisted coupling of bilirubin to the agarose cyclic imidocarbonate derivative (8) have been shown to bind albumin.[77] Lengthening of the aminoalkyl bridge, although increasing the ability of the material to bind albumin, also increased the extent to which other proteins were bound.

In the reaction of pyridoxal 5′-phosphate with the agarose cyclic imidocarbonate derivatives (24) and (25) to prepare an affinant specific for binding tryptophanase, the ligand can react *via* the ring nitrogen atom, or the hydroxy-group, or the methyne-group to give (28)—(30), respectively.[30] However, the solvent and pH can be chosen to suppress the protonation or the ionization of various groups.



A new principle of biospecific affinity chromatography was demonstrated following the addition of a solution of hydroxocobalamin to the agarose cyclic imidocarbonate derivative (8A).[85] The bond formed is non-covalent and temperature sensitive.

Products obtained by various couplings to agarose cyclic imidocarbonates for miscellaneous uses are listed in Table 5.[92–95]

The chemical stability and the release of isoproterenol bound to a diazo derivative of agarose cyclic imidocarbonate (25) have been investigated by a double-labelling procedure.[95] By using [^{3}H]isoproterenol and 1,2-diamino-[^{14}C]ethane [the first part of the bridge in (8)], it was demonstrated that the bound ligand is readily released in a soluble form, primarily by hydrolysis of the bridge-linked ligand from agarose cyclic imidocarbonate. Hydrolysis occurred with extensive washing and on incubation under physiological conditions. These observations should lead to a critical analysis of the physiological effects produced by agarose-bound hormones *in vitro*.

Divinylsulphonyl-agarose reacted with luteinizing and growth hormones to give immunoadsorbents suitable for use in the isolation of the corresponding hormonal antibodies.[96]

Macroporous agarose has been cross-linked with 2,3-dibromopropanol, divinylsulphone, and epichlorohydrin; the merits in using these reagents to increase the chemical stability of agarose as an immunoadsorbent were discussed.[39] The amount of carbohydrate released from the three cross-linked derivatives by 3M-thiocyanate ion varied in the ratio 2.1 : 2.9 : 4.5, respectively. After conversion of 2,3-dibromopropanol-agarose into the cyclic imidocarbonate derivative, it was shown that 3M-trifluoroacetate liberated only half as much carbohydrate as released from the non-cross-linked cyclic imidocarbonate. Trials were also conducted on the purification of anti-(blood-group A substance) antibodies by immunoadsorption using a partially deacetylated blood-group A substance as the ligand.

Table 5 *Use of agarose cyclic imidocarbonates for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonamide	—	Experimental control in the affinity chromatography of neuraminidase	44
6-Aminohexanoic acid	—	Demonstration of an improved and controlled procedure in the formation of agarose cyclic imidocarbonate	22
Aspartate 4-decarboxylase subunits	—	Studies of the structural relationship between the subunits of aspartate 4-decarboxylase and of their activities in immobilized form	25
Bovine factor-X	—	Investigation of the interaction of lanthanide ions with bovine factor-X	51

[92] J. P. Prieels, *European J. Biochem.*, 1975, **50**, 463.
[93] M.-C. Postel-Vinay, N. I. Swislocki, and M. Sonenberg, *Endocrinology*, 1974, **95**, 1554.
[94] H. J. Kolb, R. Renner, K. D. Hepp, L. Weiss, and O. H. Wieland, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 248.
[95] G. Vauquelin, M.-L. Lacombe, J. Hanoune, and A. D. Strosberg, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1076.
[96] M. S. Sairam, W. C. Clarke, D. Chung, J. Porath, and C. H. Li, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 355.

Table 5 (*cont.*)

Species coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Ethanolamine	—	Experimental control in the affinity chromatography of neuraminidases	53
[2-^{14}C]Glycine	—	Investigation of the reaction of coupling molecules with agarose cyclic imidocarbonate	72
Glyco-α-lactalbumins	—	Fluorometric studies of conformation changes of glyco-α-lactalbumin on agarose carriers	92
Growth hormone	—	Examination of the biological activities of immobilized growth hormone *in vivo* and *in vitro*	93
Human immunoglobulin IgA [^{125}I]-Human immunoglobulin IgG Human immunoglobulin IgM	—	Investigation of the reaction of coupling molecules to agarose cyclic imidocarbonate	72
Insulin	—	Re-evaluation of agarose-insulin in the study of insulin action	94
Isoproterenol (tritium labelled)	(25) [a]	Examination of the stability of the linkage between the ligand and agarose	95
α-Lactalbumins	—	Fluorometric studies of conformation changes of various α-lactalbumins on agarose carriers	92
α-Lactalbumin (nitrated)	—	Fluorometric study of conformation changes of nitrated α-lactalbumin on agarose carriers	92
Pyridoxal 5′-phosphate	(24), (25) [a] (see p. 427)	Investigation of the properties of immobilized pyridoxal 5′-phosphates, including their catalytic activities in the non-enzymic cleavage of tryptophan	30
L-Tryptophan	(29), (30) [a]	Demonstration of the availability of the 4-formyl group of immobilized pyridoxal 5′-phosphate and its associated chemistry	30

[a] See scheme 3b, p. 418.

Agarose cross-linked with divinylsulphone has been used as a matrix for electrophoretic chromatography and for the immobilization of enzymes.[97] Advantages of this cross-linked material over other agarose gels include greater mechanical stability, greater flow rates in columns (allowed by the rigid form of beads), and higher stability in acidic and neutral media. There is slow destruction of the sulphone-containing bridges above pH 8, but without noticeable dissolution of moderately or highly cross-linked gels below pH 12. The divinylsulphone-linked matrix is sufficiently thermostable to allow it to be heated in an autoclave. These properties are attributed to the presence of different types of cross-linkages, depicted schematically in Figure 1.

[97] J. Porath, T. Låås, and J. C. Janson, *J. Chromatog.*, 1975, **103**, 49.

Cross-linking of macroporous agarose with epichlorohydrin produced hydrophobic matrices used in an enzyme-reactor.[98] The cross-linked material was converted into alkylated derivatives, which were then sulphated; sulphur analyses gave an indication of the extent of substitution by alkyl groups. An enzyme derivative was formed by adsorption of β-amylase.

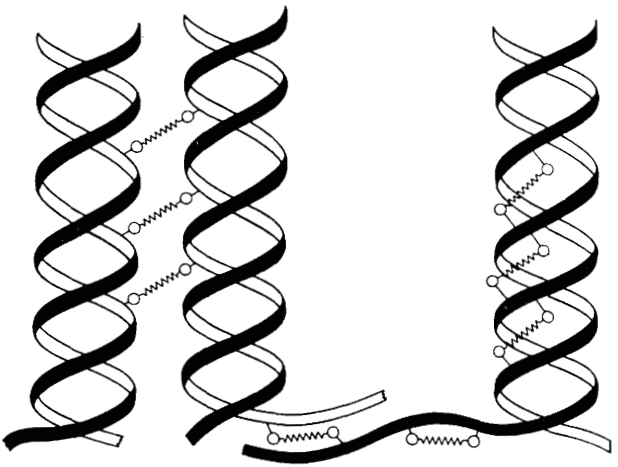


Figure 1 *Cross-linked agarose gels: the cross-linkages (symbolized by zig-zag lines) can occur as intra- or inter-molecular bridges in helical or non-helical regions of the gel*

Macroporous agarose cross-linked with epichlorohydrin has been reduced and then activated with cyanogen bromide to introduce cyclic imidocarbonate groups; this material reacted with the 3- and 4-isomers of *N*-(6-aminohexanoyl)-1-aminophenyltrimethylammonium bromide hydrobromide to give affinants that were used in the purification of acetylcholinesterase.[46]

The uptake of D-glucose, D-xylose, lactose, and dextran by agar and polyacrylamide gels has been compared; ionic additives reduced the uptake of sugars by agar, whereas that by polyacrylamide was only slightly affected.[99]

Spherical pellets of agar have been used to immobilize β-fructofuranosidase.[100]

Amylopectins.—A TMS derivative of amylopectin has been prepared using hexamethyldisilazane in DMF as the silylating reagent.[101] The extent of trimethylsilylation, which ranged from d.s. 0.9 to 2.2, depends on the structure and solubility of the polysaccharide. Trimethylsilylated amylopectin was found to be completely soluble in such organic solvents as toluene, benzene, and chloroform.

Amyloses.—Amylose has been labelled at the reducing end by treatment with D-[^{14}C]glucose 1-phosphate and phosphorylase; the labelled polysaccharide was used as a substrate in a study of the kinetics of amylolysis.[102] Malto-oligosac-

[98] K. D. Caldwell, R. Axén, and J. Porath, *Biotechnol. and Bioeng.*, 1975, **17**, 613.
[99] K. Sigler, *Biopolymers*, 1974, **13**, 2553.
[100] K. Toda and M. Shoda, *Biotechnol. and Bioeng.*, 1975, **17**, 481.
[101] R. E. Harmon, K. K. De, and S. K. Gupta, *Stärke*, 1973, **25**, 429.
[102] E. László, J. Holló, and B. Bánky, *Stärke*, 1973, **25**, 417.

charides terminated at the reducing end by D-[^{14}C]glucosyl residues have been used as substrates in comparative studies of the actions of α-amylases, including those that produce cycloamyloses.[5]

The effects of γ-radiation on the depolymerization and selected physical properties of amylose in the solid state and in solution have been investigated under conditions of graft copolymerization.[103] Irradiation furnished an alkali-sensitive material (*G*-value for scission 2.8), most likely owing to the presence of a β-alkoxycarbonyl structure. The *G*-value (1.3) obtained on irradiation of solid amylose at 0 °C under nitrogen is lower than values previously reported for amylose and other polysaccharides. A solution of amylose in water was protected against γ-radiation by the addition of DMSO (*G*-value of 2.3 *vs.* 30). A solvent system of DMSO–acetic acid–water degraded γ-irradiated amylose less than did aqueous DMSO.

The susceptibility of 6-amino-6-deoxyamyloses to α-amylases has been investigated.[104] Replacement of the primary hydroxy-groups of the D-glucopyranosyl residues evidently caused changes in the nature of the enzyme–substrate interaction, but such substitution did not prevent the hydrolysis of unsubstituted regions of the polysaccharide by the enzyme, provided the degree of substitution overall is low.

Chlorite-oxidized amylose (oxyamylose) has been found to possess a leukaemo-suppressive activity against established Friend virus leukaemia.[105]

Amylose dyed with Cibacron Blue F-3GA has been used as a substrate in measurements of the levels of α-amylase in blood, *etc.*[106]

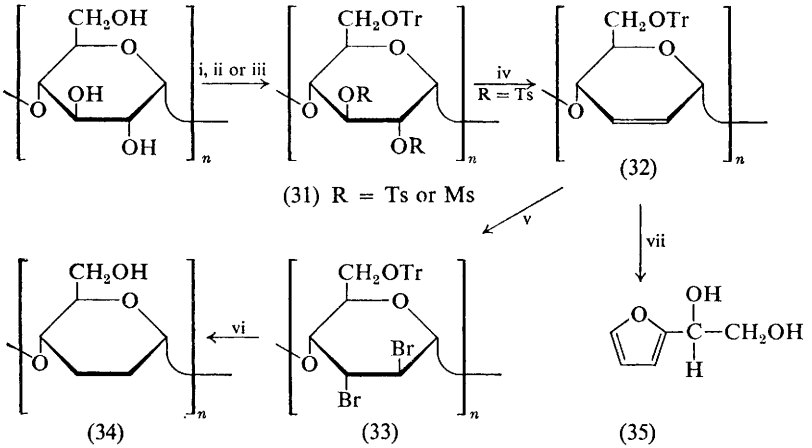
A convenient procedure for the synthesis of 6-deoxyamylose has been developed.[107] Selective bromination of the primary hydroxy-groups of amylose and subsequent reductive debromination gave the 6-deoxy derivative. The conformations of acetylated 6-bromo-6-deoxy- and 6-deoxy-amyloses were investigated by ^{1}H n.m.r. spectroscopy.

TMS derivatives (d.s. 0.9—2.2) of amylose have been prepared using hexamethyldisilazane in DMF as the silylating agent.[101] The trimethylsilylated polysaccharides are completely soluble in such organic solvents as benzene, toluene, and chloroform.

Amylose has been converted into the disulphonate derivative (31) and thence into 2,3-dideoxy-6-*O*-trityl-α-D-*erythro*-hex-2-enopyranoglycan (32) (Scheme 4).[108] The 2,3-unsaturated polysaccharide could be converted into a 2,3-dibromo-derivative (33) and hydrogenated with concomitant detritylation to the saturated analogue (34). On treatment with aqueous acetic acid, the unsaturated polysaccharide gave 2-(D-*glycero*-1,2-dihydroxyethyl)furan (35).

Celluloses.—The current volume in the series 'Macromolecular Syntheses' includes an article on the modification of such natural polymers as cellulose.[109]

[103] B. T. Hofreiter, *J. Polymer Sci., Polymer Chem.*, 1974, **12**, 2755.
[104] C. E. Weill, J. B. Nickel, and J. Guerrera, *Carbohydrate Res.*, 1975, **40**, 396.
[105] O. S. Weislow and E. F. Wheelock, *Infection and Immunity*, 1975, **11**, 129.
[106] O. H. Pivetta and N. Kaliss, *Proc. Soc. Exp. Biol. Med.*, 1974, **147**, 775.
[107] K. Takeo, T. Sumimoto, and T. Kuge, *Stärke*, 1974, **26**, 111.
[108] D. Horton and M. H. Meshreki, *Carbohydrate Res.*, 1975, **40**, 345.
[109] 'Macromolecular Synthesis', Vol. 5, ed. E. L. Wittbecker, Wiley, Chichester, 1974.



Reagents: i, TrCl–py; ii, TsCl–py; iii, MsCl–py; iv, NaI–Zn–DMF; v, Br_2–MeOH–AgOAc; vi, H_2; vii, AcOH–H_2O

Scheme 4

The effect of the wavelength of light used on the formation of free radicals from cellulose has been studied.[110] The moisture content of cellulose has also been shown to affect the formation of free radicals during irradiation.[111] The physical state of the sensitizer is a vital factor in the photosensitized degradation of cellulose; C.I. Vat Orange 9 acted as a desensitizer on irradiation of cotton cellulose and its acetylated, benzoylated, and cyanoethylated derivatives with short-wavelength u.v. light.[112] A mechanism was suggested for photosensitization and desensitization in which excitation of the sensitizer (n–π^* or π–π^* transitions) is followed by hydrogen abstraction from the substrate.

[^{14}C]Cello-oligosaccharides have been prepared by degradation of labelled cellulose.[113] Complexes were formed when these oligosaccharides were incubated with lysozyme at pH 3—9, particularly at higher pH values; the extent of complexation was greater at low temperatures. The complexes contained carbohydrate and protein (1 : 1), but no radio-labelled peptides were detected in tryptic digests. Acceptors and competitive inhibitors of lysozyme inhibited complexation and caused dissociation of the complexes. Ultracentrifugation, molecular-sieve chromatography, and light-scattering studies indicated that the complexes are large heterogeneous aggregates of protein and cello-oligosaccharides formed in the following equilibrium:

$$n \text{ lysozyme} + n \text{ cello-oligosaccharide} \rightleftharpoons (\text{lysozyme-cello-oligosaccharide})_n.$$

The comparative reactivities of cellulose and other polysaccharides towards acetylation, nitration, hydrolysis, and etherification have been investigated.[114]

[110] N. S. Hon, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 1347.
[111] N. S. Hon, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 955.
[112] E. H. Daruwalla, S. M. Moonim, and P. K. Tamboskar, *Cellulose Chem. Technol.*, 1972, **6**, 695.
[113] J. A. Thoma, G. V. K. Rao, A. Bowanko, A. L. Jennings, and C. Crook, *Carbohydrate Res.*, 1974, **38**, 279.
[114] L. S. Galbraich, I. L. Voytenko, and Z. A. Rogovin, *Cellulose Chem. Technol.*, 1972, **6**, 627.

The rates of acetylation and methylation were influenced by the configuration of the secondary hydroxy-groups and by the conformation of the hexosyl unit. Physicochemical measurements (sorption, heat of solvation, *etc.*) were used to establish the relation between changes of conformation of the hexosyl units and the polysaccharide's structure. The influence of additives on the activation or acetylation of cellulose has been studied; only alcohols accelerated the acetylation of cellulose, depending on the solvent used.[115] This effect is possibly due to the catalytic action of an alkyl sulphuric acid formed from the alcohol and adventitious sulphuric acid in the medium.

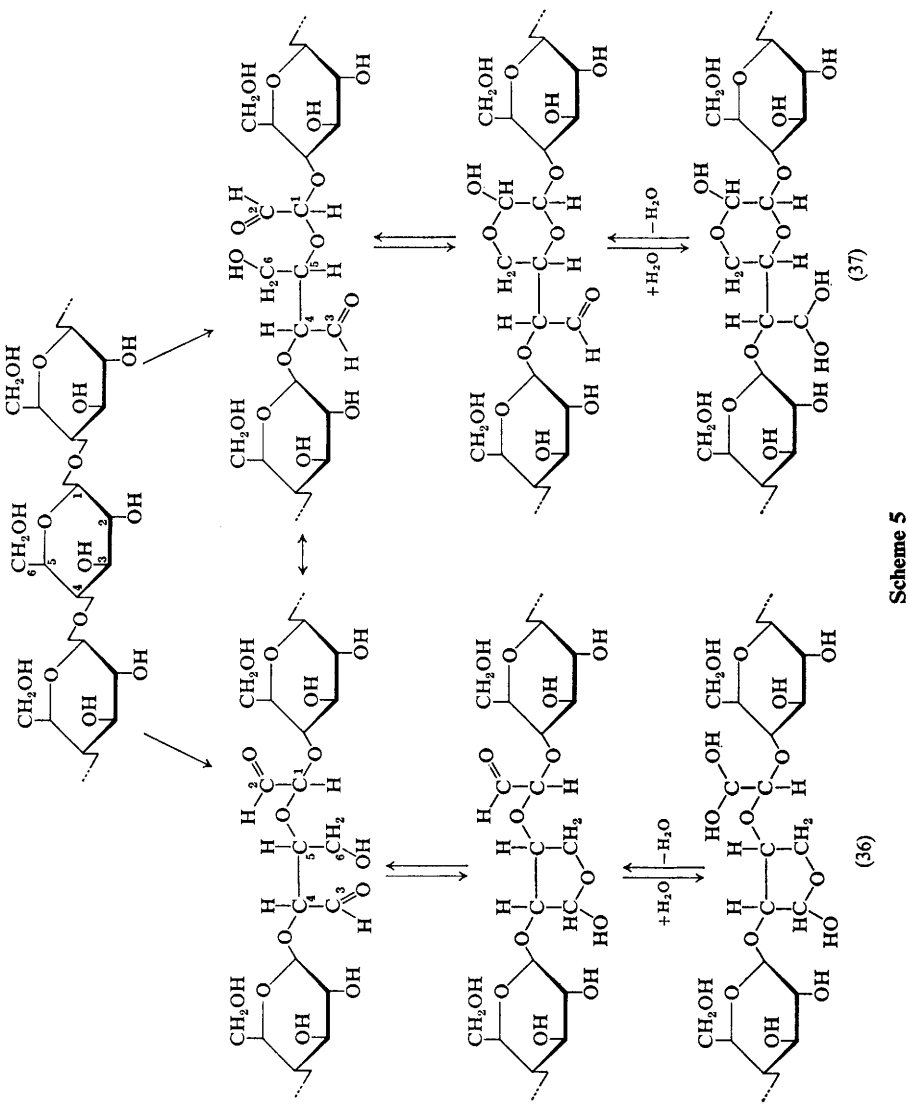
Crystallization of cellulose acetates (d.s. 2.2—3.0) has been achieved by annealation in the presence of either nitromethane or nitromethane–n-butanol in overcooled dilute solutions.[116] Cellulose acetate I crystallized over the whole range of compositions when annealed above the glass temperature; thermal treatment of cellulose acetate II brought about crystallization of samples of d.s. 2.6, whereas heating in the presence of polar liquids resulted in crystallization over the whole range of compositions. Cellulose acetate I was converted into cellulose acetate II on heating in ethanol, and cellulose acetate II crystallized spontaneously from acetone at room temperature. A comparison of the crystalline forms obtained from cellulose acetates of different d.s. was also made.

It has been possible to estimate the melting temperatures of a series of partly acetylated celluloses by making use of the fact that their melting temperatures decrease after swelling in trichloroethyl phosphate.[117] Cellulose acetate prepared by heterogeneous acylation or obtained by partial deacetylation melted at higher temperatures than the homogeneous counterpart: this was explained using Flory's theory of the block distribution of substituents in the acetylated polysaccharides. Comparison of the experimental melting temperatures of various cellulose acetates with theoretical values based on Flory's theory indicated that co-crystallization of fully and partially substituted D-glucosyl residues occurs in the polymers.

Monocrystals in films obtained from solutions of homogeneous or heterogeneous cellulose acetates in methylene chloride have been examined by electron microscopy.[118] A comparison was made of the crystalline forms of celluloses acetylated either directly or indirectly.

The molecular-weight distribution of polystyrene grafted onto pre-irradiated cellulose acetate has been calculated by simulating the reaction with several models.[119] A model in which the probability of the graft radicals coupling is proportional to the inverse square-root of the chain length, and in which the rate of growth is widely dispersed about the average value, gave a molecular-weight distribution in agreement with that observed. Factors affecting the molecular-weight distribution of graft chains were also discussed on the basis of results obtained in the simulated reactions.

[115] Z. N. Medved, N. A. Kozlov, and O. G. Tarakanov, *Cellulose Chem. Technol.*, 1972, **6**, 571.
[116] P. V. Kozlov, E. V. Gert, and Z. S. Belokon, *Cellulose Chem. Technol.*, 1972, **6**, 531.
[117] E. V. Gert, M. K. Umarova, N. F. Bakeev, and P. V. Kozlov, *Cellulose Chem. Technol.*, 1972, **6**, 547.
[118] W. Sarmadjiewa, P. V. Kozlov, and K. Dimov, *Cellulose Chem. Technol.*, 1972, **6**, 671.
[119] T. Yasukawa, Y. Sasaki, and K. Murakami, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 17.



(36) (37)

Scheme 5

Activation of the pore surfaces of cellulose-acetate membranes by pressure-driven reactions that permit the coupling of enzymes has been applied to the production of chymotrypsin-coupled ultrafiltration membranes.[120]

The introduction of 5,6-unsaturated linkages into cellulose has been achieved by thermal degradation of *S*-allyl and *S*-benzyl dithiocarbonates.[121] Additions to the unsaturated linkages afforded new polymers possessing useful physical and technological properties.

Carboxymethylcellulose has been activated with cyanogen bromide, yielding a material containing cyclic imidocarbonate groups through which α-amylase was attached to give an active immobilized enzyme.[122]

Dialdehydocellulose, prepared by oxidation of cellulose with periodate ion (Scheme 5), has been shown to exist in two forms [(36) and (37)].[123]

Carboxymethylcellulose has been cross-linked with epichlorohydrin; the retention of water by the cross-linked material was enhanced by γ-ray-induced grafting of polystyrene.[124]

Cellulose has been converted into more reactive forms by chelation with titanium(III), iron(III), tin(IV), vanadium(III), and zirconium(IV) salts.[125] The unsubstituted ligands of the bound metals could be replaced by electron-donating groups of antibiotics (*e.g.* ampicillin, gentamicin, kanamycin, neomycin, paromycin, *etc.*) to yield materials possessing antimicrobial activity; the original chelated celluloses were inactive. The chelation procedure offers an easy way of rendering cellulose more resistant to microbial attack than that afforded by non-covalent adsorption of the antibiotic.

4-Diazobenzylcellulose has been used as a reactive matrix for the covalent immobilization of chymotrypsin and trypsin.[126]

DEAE-cellulose has been reported to undergo irreversible binding, *via* a base-pairing mechanism, with poly(vinyl alcohol) substituted with oligo(deoxythymidine 5′-phosphate).[127] It was possible to separate mixtures of synthetic oligonucleotides using the complexed cellulose derivative as an affinity-chromatography matrix.

An active immobilized form of β-D-fructofuranosidase has been prepared by adsorption onto DEAE-cellulose.[100] The product was used in a continuous fluidized bed reactor.

Hydroxypropylcellulose has been used to produce semi-solid bases in the manufacture of pharmaceuticals.[128] Cellulose nitrate (in the form of collodion microcapsules) has been used to encapsulate β-D-galactosidase as an active immobilized form.[129]

[120] H. P. Gregor and P. W. Rauf, *Biotechnol. and Bioeng.*, 1975, **17**, 445.
[121] P. G. Descotes, A. Faure, and J. C. Martin, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 189.
[122] Y.-Y. Linko, P. Saarinen, and M. Linko, *Biotechnol. and Bioeng.*, 1975, **17**, 153.
[123] S. H. Nadzhimutdinov, A. A. Sarymsakov, and K. H. V. Usmanov, *Doklady Akad. Nauk S.S.S.R.*, 1974, **219**, 1371.
[124] S. S. Bhattacharjee and A. S. Perlin, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 113.
[125] J. F. Kennedy, S. A. Barker, and A. Zamir, *Antimicrob. Agents Chemotherapy*, 1974, **6**, 777.
[126] K. Martinel, V. S. Goldmacher, A. M. Klibanov, and I. V. Berezin, *F.E.B.S. Letters*, 1975, **51**, 152.
[127] H. Schott, *J. Chromatog.*, 1974, **96**, 79.
[128] Y. Machida and T. Nagai, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1003.
[129] M. A. Paine and R. G. Carbonell, *Biotechnol. and Bioeng.*, 1975, **17**, 619.

RNA and DNA have been coupled to cellulose with the aid of a water-soluble carbodi-imide and to cellulose phosphate activated by 1,1′-carbonyldi-imidazole; the products were used in the isolation of specific nucleotide sequences by affinity chromatography.[130]

Toluene-*p*-sulphonylation of cellulose resulted in a breakdown of the original cellulose I lattice, producing a stable, amorphous lattice.[131] 6-*O*-Toluene-*p*-sulphonylcellulose reacted with potassium fluoride in ethylene glycol to yield a material containing residues of D-*xylo*-hex-5-enopyranose (5,6-D-glucoseen), 6-deoxy-6-fluoro-D-glucopyranose, and 3,6-anhydro-D-glucopyranose. The unsaturated material was examined by *X*-ray diffraction and i.r. spectroscopy.

Cellulose, carboxymethylcellulose, and DEAE-cellulose have been activated with *sym*-triazine trichloride in order to provide matrices for the immobilization of molecules containing free amino-sugars.[132] Amino-acids, [^{14}C]carboxymethyl-amidoalbumin, [^{14}C]acetylalbumin, [^{14}C]acetylmyoglobin, [^{14}C]acetylcytochrome *c*, [^{14}C]carboxymethylamidomyoglobin, hexokinase, pronase, phosphorylase, and D-glucose 6-phosphate dehydrogenase were coupled to activated cellulose to test the binding capabilities of the matrix, and, in some cases, to provide active immobilized enzymes. [^{14}C]Carboxymethylamido-albumin and -myoglobin were also coupled to the activated cellulose derivatives.

The thiol groups of cellulose xanthate can form disulphide bridges reversibly with L-cysteine, a property that has been utilized in the immobilization of proteins and enzymes.[133] Proteinaceous macromolecules, as polythiol derivatives, formed disulphide linkages with the xanthated matrix without serious disturbance of the active sites, and were not eluted by normal washing procedures. Trypsin and albumin derivatives of cellulose xanthate were prepared by this inexpensive procedure, which was found to be selectively reversible.

The decay of radicals produced by photo-irradiation of cellulose at room temperature, and the characteristics of photo-irradiated cellulose for the initiation of graft copolymerization with methyl methacrylate have been investigated.[134] The e.s.r. spectra of irradiated samples of untreated, swollen, oximated, or ferric-ion-sensitized celluloses were examined. The decay of radicals was accelerated by solvents (water $\doteq$ methanol $\gg$ acetone $>$ *p*-dioxan) and was retarded by methacrylic acid $>$ methyl methacrylate $\doteq$ styrene. Graft copolymerization of methyl methacrylate with photo-irradiated cellulose was effectively initiated by water or methanol, but not by either acetone or *p*-dioxan. It appears that initiation of the graft copolymerization onto pre-irradiated cellulose is promoted by radicals exhibiting a singlet in the e.s.r. spectrum.

Copolymerization of methyl methacrylate with cellulose has also been initiated by the decomposition of peroxide groups formed on ozonolysis of cellulose.[135] An examination of the polydispersity of poly(methyl methacrylate) chains

[130] T. Y. Shih and M. A. Martin, *Biochemistry*, 1974, **13**, 3411.
[131] H. C. Srivastava, A. K. Kulshreshtha, and V. K. Srivastava, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 65.
[132] N. L. Smith and H. M. Lenhoff, *Analyt. Biochem.*, 1974, **61**, 392.
[133] J. F. Kennedy and A. Zamir, *Carbohydrate Res.*, 1975, **41**, 227.
[134] H. Kubota, Y. Ogiwara, and K. Matsuzaki, *J. Polymer Sci., Polymer Chem.*, 1974, **12**, 2809.
[135] C. Simionescu, S. Oprea, and R. Butnaru, *Cellulose Chem. Technol.*, 1972, **6**, 365.

isolated by hydrolysis of the grafted copolymers yielded information on the grafting frequency onto the supporting matrix.

Elastin absorbed onto cellulose has been used as an affinity-chromatography matrix for the separation of elastase and proelastase.[136] The surface structures of cellulose-based and poly(acrylonitrile)-based graphite fibres have been compared.[137]

Chitosans.—Columns of chitosan (obtained from chitin by treatment with alkali) effectively removed mercury salts from aqueous solution.[138]

Conditions for the formation of insoluble polyelectrolyte complexes between chitosan and sodium dextran sulphate have been investigated.[139]

Chitins.—Chitin has been converted into a carboxymethyl derivative; the physicochemical characteristics of this derivative have been determined by light scattering and i.r. spectroscopy.[140] Glycolchitin (2-hydroxyethylchitin), obtained when chitin reacts with ethylene oxide, was similarly studied. In 0.5M-sodium chloride solution, these chitin derivatives exhibited weight-average molecular weights of 1.896×10^6 and 1.819×10^6, intrinsic dissymmetries of 3.25 and 4.31, and *Z*-average radii of gyration of 143.49 and 251.57 nm, respectively. Carboxymethyl- and glycol-chitins possessed similar degrees of polymerization, underwent dissociation as the concentration of sodium chloride was increased, and exhibited essentially side-by-side packing of the chains. Conditions under which the modified chitins resemble chitin were also investigated, and it was concluded that there are a small, but significant, number of unacetylated amino-groups in native chitin. Carboxymethylchitin has been used as an affinity matrix for the purification of lysozymes.[141]

Chitin has been used as a matrix for the covalent immobilization of enzymes; for example, β-D-galactosidases, chymotrypsin, and acid phosphatase were attached to chitin *via* glutaraldehyde.[142] Pretreatment of chitin with acid, base, ammonia, or pronase was compared with regard to the release of titratable amino-groups and the ability of the polysaccharide to retain β-D-galactosidase activity.

Cycloamyloses.—To overcome some of the problems associated with the use of cycloamyloses as model compounds, groups expected to cluster in the form of a hydrophobic 'floor' in the cavity have been attached to the bottom or primary side of cyclohepta-amylose; for example, *N*-formyl derivatives of cyclohepta-(*N*-methylamylose-6-amine) and -(*N*-ethylamylose-6-amine) were prepared for this purpose.[143] The rate and dissociation constants for the reactions of these cyclohepta-amylose derivatives with 3-nitro- and 3-tert-butyl-phenyl acetates were

[136] Y. Legrand, G. Pignaud, J. P. Caen, B. Robert, and L. Robert, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 224.
[137] P. Bless and J. B. Lando, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 153.
[138] R. A. A. Muzzarelli and R. Rocchetti, *J. Chromatog.*, 1974, **96**, 115.
[139] Y. Kikuchi and H. Fukuda, *Makromol. Chem.*, 1974, **175**, 3593.
[140] R. H. Hackman and M. Goldberg, *Carbohydrate Res.*, 1974, **38**, 35.
[141] H. L. Guenther, N. Sorgentz, H. E. Guenther, R. Eisenstein, and K. E. Kuettner, *Biochim. Biophys. Acta*, 1974, **372**, 321.
[142] W. L. Stanley, G. G. Watters, B. Chan, and J. M. Mercer, *Biotechnol. and Bioeng.*, 1975, **17**, 315.
[143] J. Emert and R. Breslow, *J. Amer. Chem. Soc.*, 1975, **97**, 670.

determined. Competitive inhibitors of the formation of complexes by the cyclohepta-amylose derivatives were also investigated.

The stereospecificity of the interactions of several spin-labelled substrates with cyclohexa- and cyclohepta-amyloses, as models for chymotrypsin, has been studied.[144] Complexes of the cycloamyloses with 2,2,6,6-tetramethyl-4-oxy-pyridyl-1-oxide in aqueous solution were examined by e.s.r. spectroscopy; the nitroxide function moved to a relatively hydrophobic environment on binding to cyclohepta-amylose, and lost some freedom of rotation on binding to both cycloamyloses. The dissociation constant for the cyclohexa-amylose complex of the nitroxide is greater than that for the cyclohepta-amylose complex, consistent with measurements made on molecular models. In the hydrolysis of the asymmetric compound 3-carboxy-2,2,5,5-tetramethylpyrrolidyl-1-oxide 3-nitrophenyl ester, catalysed by cyclohexa-amylose, enantiomeric specificity was observed in the acylation step but not in formation of the 'Michaelis complex', or on hydrolysis of the acylated cycloamylose intermediate. No differences were found in the e.s.r. spectra of solutions of the trapped acylcyclohexa-amylose intermediates derived from (+)- and (±)-forms of the asymmetric nitroxide. The nitroxide function is less free to rotate in the acylcycloamylose intermediate than in the 'Michaelis complex' and is not included in the cycloamylose cavity.

In a study of the inclusion complexes of cyclohepta-amylose with tranquillizing drugs, the complexation of phenothiazines in aqueous solution was investigated by c.d., u.v., and ^{1}H n.m.r. spectroscopy.[145] Quantitative measurements revealed a 1 : 1 stoicheiometry, permitting the formation constants to be calculated. Protons located in the cavity of the complex with cyclohepta-amylose were anisotropically shielded, whereas those of phenyl- and N-substituents of the drugs shifted to lower field. The spectral changes produced on complexation indicated that the aromatic group of the phenothiazines is included in the cavity, whereas N-substituents interact with groups outside the cavity.

The inclusion of fenamates (antiflammatory drugs) into the cavity of cyclohepta-amylose was confirmed by c.d., u.v., and ^{1}H n.m.r. spectroscopy.[146] Changes occurring in the spectra and in the solubility of the drugs on complexation were measured, and values for the thermodynamic parameters, the stoichiometry (1 : 1), and the formation constants were obtained.

Cyclohepta-amylose has been used to enhance the fluorescence of dansylated amino-acids on silica-gel plates.[147]

Dextran.—Tritium–protium exchange in dextran at 25 °C has been studied.[148]

Oxidation of dextran with periodate gave the dialdehydo-derivative, which was used to label antibodies for examination by electron microscopy.[149] Covalent linkages were formed when dialdehydodextran reacted with a solution of rabbit immunoglobulin IgG anti-(human type-O) antibodies and with rabbit anti-(human type-O) red-blood cells.

[144] K. Flohr, R. M. Paton, and E. T. Kaiser, *J. Amer. Chem. Soc.*, 1975, **97**, 1209.
[145] M. Otagiri, K. Uekama, and K. Ikeda, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 188.
[146] K. Ikeda, K. Uekama, and M. Otagiri, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 201.
[147] T. Kinoshita, F. Iinuma, K. Atsumi, Y. Kanada, and A. Tsuji, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1166.
[148] N. V. B. Marsden, *Acta Chem. Scand. (B)*, 1974, **28**, 1100.
[149] S. P. Kent and D. V. Wilson, *J. Histochem. Cytochem.*, 1975, **23**, 169.

Blue Dextran 2000 (a dyed derivative of dextran) incorporated into agar gel has been used to detect dextranase-producing micro-organisms.[150] The action of dextranases altered the intensity of the blue colour in the medium. Blue Dextran 2000 has also been immobilized by reaction with agarose cyclic imidocarbonate; the product was used as an affinant for dinucleotide folds in proteins.[50]

Oxidation of dextran 2,4-benzeneboronate with DMSO–acetic anhydride has been used to prepare a ketodextran.[151] Enzymic hydrolysis of the ketodextran indicated that a carbonyl group at C-3 prevents cleavage of an adjacent D-glucosidic linkage and also strongly inhibits cleavage of the D-glucosidic linkage of the adjacent residue nearer the reducing end of the chain. However, hydrolysis occurred if the carbonyl group is located in the aglycone. Although the results demonstrated that oxidation at C-3 affects the hydrolysis of neighbouring glycosidic linkages, they do not explain why even a low degree of oxidation produced an appreciable decrease in the rate of hydrolysis of the polysaccharide.

Sodium dextran sulphate has been shown to form an insoluble complex with chitosan; factors affecting the yield of this insoluble complex have been investigated.[139] Polyelectrolyte complexes were also formed when gelatin was treated with sodium dextran sulphate.[152] The conditions for, and yields of, the reaction have been determined. The effects of dextran sulphate on cell-mediated resistance in mice to infection with *Listeria monocytogenes* have been investigated.[153] The loss of antibacterial resistance brought about by dextran sulphate was also studied. Morphological changes induced by dextran sulphate 500 in mononuclear phagocytes of *Listeria*-infected mice have been investigated.[154]

Sephadex (a dextran cross-linked with epichlorohydrin) has been used as an affinity matrix for the separation of [^{125}I]-concanavalin A from the unlabelled lectin.[155] If this technique is successful with other ^{125}I-labelled proteins, it would help to overcome the uncertainty of whether or not biological properties displayed by labelled materials arise from contamination by the unlabelled material.

The covalent coupling of proteins to Sephadex has been achieved by a new method that involves derivatization of the polysaccharide with 4-benzoquinone; derivatives of ribonuclease, chymotrypsin, and serum albumin were prepared by this method.[21]

Carboxymethyl-Sephadex has been condensed with 4-[(2,4-diaminophenyl)azo]- and 4-(aminomethyl)-benzenesulphonamides with the aid of a water-soluble carbodi-imide.[156] The products were used in an improved method for the purification of isoenzymes of carbonate dehydratase by affinity chromatography.

Sephadex has been converted into a cyclic imidocarbonate derivative by reaction with cyanogen bromide.[71] The cyclic imidocarbonate was used for the immobilization of carboxypeptidase A, yielding an enzyme derivative that is superior to that obtained with agarose cyclic imidocarbonate. The active enzyme

[150] R. H. Staat, T. H. Gawronski, and C. F. Schachtele, *Infection and Immunity*, 1973, **8**, 1009.
[151] R. G. Brown and B. Lindberg, *Carbohydrate Res.*, 1974, **38**, 369.
[152] Y. Kikuchi and U. Uemori, *Makromol. Chem.*, 1975, **176**, 821.
[153] H. Hahn, *Infection and Immunity*, 1974, **10**, 1105.
[154] H. Hahn and M. Bierther, *Infection and Immunity*, 1974, **10**, 1110.
[155] M. P. Czech, J. C. Lawrence, jun., and W. S. Lynn, *J. Biol. Chem.*, 1974, **249**, 7499.
[156] W. R. A. Osborne and R. E. Tashian, *Analyt. Biochem.*, 1975, **64**, 297.

derivative of Sephadex was used to probe the thermally-induced unfolding of ribonuclease.

Sephadex LH 20 (a lipophilic derivative of Sephadex) has been treated with 3,3′-diaminodipropylamine and a 4-phenyltrimethylammonium salt exhibiting cholinergic activity.[157] The products were used in the purification and separation of nicotinic and muscarinic hydrophobic proteins by affinity chromatography.

Glycogen.—TMS derivatives of glycogen have been prepared using hexamethyldisilazane in DMF as the silylating agent.[101] The derivatives (d.s. 0.9—2.2) were found to be soluble in common organic solvents.

Glycosaminoglycans.—A dodecasaccharide has been obtained from dermatan sulphate following treatments with hyaluronidase, chondroitin lyase AC, and β-D-glucuronidase; the oligosaccharide was used as a substrate for *exo*-β-D-acetamidodeoxyhexosidase.[158] The non-sulphated residues present in dermatan sulphate have been selectively oxidized with periodate ion.[159] Degradation of the resulting polysaccharide with alkali gave a mixture of products from which oxalacetic acid was isolated. Conditions giving optimal yields of oligosaccharides and minimal destruction of periodate-resistant structures were reported for Smith degradation of dermatan sulphate; one of the products of Smith degradation was tentatively identified as L-threonic acid. Alkaline and Smith degradations gave similar mixtures of oligosaccharides.

The solvolytic desulphation of the pyridinium salts of 2-deoxy-2-sulphoamido-D-glucose and D-glucose 6-sulphate in DMSO containing water or methanol has been examined in connection with the selective *N*-desulphation of heparin.[160]

Tritium-labelled oligomers of hyalobiouronic acid, prepared from hyaluronic acid by the Wilzbach technique, have been used in studies of complex enzyme kinetics.[161] The technique appears to be generally applicable to the preparation of labelled oligosaccharides.

Miscellaneous Polysaccharides and Oligosaccharides.—Various oligosaccharides have been transformed into synthetic glycolipids (see Scheme 1).[10]

Blood-group A substance has been partially deacetylated with toluene-*p*-sulphonic acid.[39] The deacetylated product reacted with a cyclic imidocarbonate derivative of agarose cross-linked with 2,3-dibromopropanol to give a material used as an immunoadsorbent for anti-(blood-group A) antibodies.

The milk oligosaccharides (38)—(41), sometimes referred to as 2′-fucosyllactose, lacto-*N*-fucopentaose I, lacto-*N*-difucohexaose I, and lacto-*N*-tetraose, respectively, have been labelled in the terminal, reducing D-glucosyl residue by reduction with sodium borotritide.[59] The binding of the reduced oligosaccharides to related antibodies was investigated. The molecular size of the oligosaccharide (40) has been increased by covalent coupling to poly(L-lysine) for the purpose of raising antibodies. The oligosaccharides (38)—(41) were treated with 2-(4-aminophenyl)ethylamine to form *N*-alkylglycosylamines as shown in Scheme 6. Reduction

[157] F. J. Barrantes, S. Arbilla, M. C. L. de Carlin, and E. de Roberts, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 194.
[158] J. Singh, G. V. Coppa, and N. DiFerrante, *Enzyme*, 1975, **19**, 15.
[159] L. A. Fransson and I. Carlstedt, *Carbohydrate Res.*, 1974, **36**, 349.
[160] K. Nagasawa and Y. Inoue, *Carbohydrate Res.*, 1974, **36**, 265.
[161] S. Highsmith and D. M. Chipman, *Analyt. Biochem.*, 1974, **61**, 557.

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-D-Glc*p*

(38)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*

(39)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*

4

↑

1

α-L-Fuc*p*

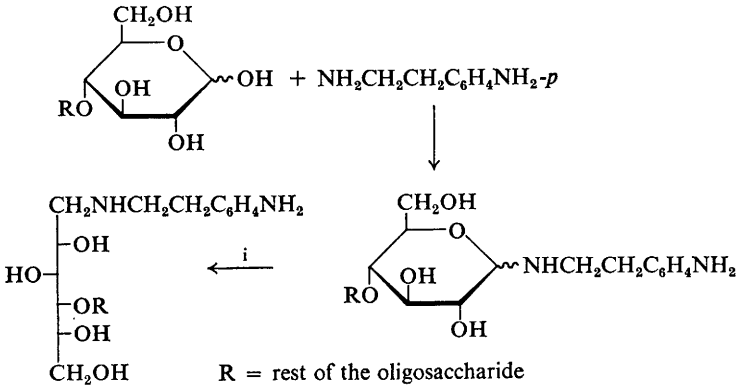
(40)

β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*

(41)

of the *N*-glycosides yielded stable secondary amines, which reacted with agarose cyclic imidocarbonate to give affinity materials suitable for the purification of antibodies to the oligosaccharides by immunoadsorption. The bound antibody could be eluted with a solution of the corresponding oligosaccharide.

Milk oligosaccharides coupled to poly(L-lysine) by a mixed anhydride procedure were shown to be effective precipitating antigens.[162] The conjugate



R = rest of the oligosaccharide

Reagent: i, $NaBH_4$–EtOH

Scheme 6

β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc

4

↑

1

α-L-Fuc*p*

(42)

obtained from oligosaccharide (42) (lacto-*N*-fucopentaose II) specifically precipitated antibodies directed against human Le^a blood-group antigen, whereas that prepared from the oligosaccharide (40) specifically precipitated antibodies

[162] D. A. Zopf and V. Ginsburg, *Arch. Biochem. Biophys.*, 1975, **167**, 345.

directed against human Le^b blood-group antigen. The conjugates of these oligosaccharides were used to determine the specificity of a human anti-I cold agglutinin.

The reactivity of a polysaccharide containing D-glucopyranosyl and D-altropyranosyl residues to acetylation, etherification, hydrolysis, and nitration has been investigated.[114] The way in which the structure and conformation of the polysaccharide affected the rates of derivatization was discussed.

Pneumococcal SIII polysaccharide has been treated with a water-soluble carbodi-imide and then reduced with sodium borohydride; the i.r. spectrum of the reduced polysaccharide showed negligible absorption attributable to the presence of carboxy-groups.[163] The reduced material furnished 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl- and 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl-D-glucitol following methylation, hydrolysis, further reduction, and acetylation, compatible with the presence of equal proportions of (1 → 3)- and (1 → 4)-linkages. The reduced polysaccharide was used as a substrate for various D-glucanases.

Starch.—Changes induced in the physical properties and DP of starch by γ-radiation have been investigated under conditions used for graft copolymerization.[103] The alkali-sensitive material produced on irradiation was considered to contain β-alkoxycarbonyl functions.

The enzymic degradation of starch to produce oligo- and mono-saccharides has been described.[164]

The dispersion of low-substituted, acetylated starches in water increased as the number of acetate groups was increased and as the temperature was raised.[165] The viscosity of starch-acetate pastes was lower than that of native starch pastes at all temperatures. The viscosity decreased until an acetyl content of 2.36% was reached, after which it changed only slightly. The reduced ability of starch acetates to form gels could be expressed in terms of the ratio of the viscosities at low and high temperatures. A rotation viscosimeter Rheotest was used to study the viscosities and the rheological properties of starch acetates.

Carboxymethyl-starches (d.s. 0.3—2.17) have been prepared; complexation with iodine and paper chromatography in perchloric acid of the substituted starches indicated that partial degradation occurred during carboxymethylation.[166] Carboxymethylation also inhibited the formation of iodine complexes by the starches. Chromatographic separation of the products of carboxymethylation was used to assess the degree of substitution.

Phadebas-starch, obtained by reaction of starch with a triazine dye (Cibacron Blue F8GA), continues to be used as a substrate for the assay of α-amylase.[28] Comparison of assays using Phadebas-starch with saccharogenic and amyloclastic procedures showed that the method is reliable and specific for α-amylase, since Phadebas-starch is not hydrolysed by glucoamylase.[167] Further work on Phadebas-starch showed that groups substituted on the helices inhibit the *exo*-action of β-amylase, but function only as accidental barriers to the *endo*-action of α-amylase.[168]

[163] M. A. Anderson and B. A. Stone, *F.E.B.S. Letters*, 1975, **52**, 202.
[164] T. Komaki, *J. Jap. Soc. Starch Sci.*, 1974, **21**, 34.
[165] I. Koubek, *Stärke*, 1974, **26**, 81.
[166] M. Čeh and Č. Stropnik, *Stärke*, 1975, **27**, 72.
[167] M. D. O'Donnell and K. F. McGeeney, *Comp. Biochem. Physiol.*, 1975, **50B**, 269.
[168] S. Scharpé, A. Lauwers, and W. Cooreman, *Experientia*, 1973, **29**, 1572.

Since the diameter of the dye molecule is greater than that of the amylose helix (13 Å) and the distance (8 Å) between adjacent spirals of the helix, the chromogenic groups of the dyed starch are assumed to be located on the exterior of the helix.

A dyed derivative of starch (Remazol Brilliant Blue starch), phosphate-derivatized starch, and cross-linked starch have been compared as substrates for α-amylases.[169] The different susceptibilities of the substrates to hydrolysis was used to ascertain the configurational requirements of the enzymes.

Starch has been trimethylsilylated with hexamethyldisilazane in DMF.[101] The trimethylsilylated starches (d.s. 0.9—2.2) are soluble in benzene, toluene, and chloroform.

Starch xanthate and alkyl diepoxides formed gels in concentrated (6—8%) solutions or in turbid suspensions in dilute (0.1—1.0%) solutions, the degree of gelling varying with the nature of the epoxide.[170] When wood pulp was added to dispersions or suspensions of the gel, the paper produced therefrom possessed improved strength. These improvements were unrelated to the degree of xanthation, but depended on the reaction times and on the order in which the diepoxide and xanthate were added.

The chemical nature of starch xanthate-poly(ethylenimine) has been studied in view of its efficacy as a wet-end additive for improving the mechanical strength of paper.[171] Model systems suggested that small amounts of starch xanthide and poly(ethylenimine)thiuram disulphide, possible products of atmospheric oxidation, might be present: these compounds would react rapidly with poly(ethylenimine) to yield thionocarbamates and thioureas, respectively.

Starch has been cross-linked with epichlorohydrin (65—5.4 D-glucosyl residues per cross-linkage) by an iterative method that preserves the crystalline structure of the starch granule.[172] The stability of the modified granules to digestion by α-amylase (measured by the loss of weight on incubation) increased with the extent of cross-linking, whereas the susceptibility to hydrolysis with acid decreased.

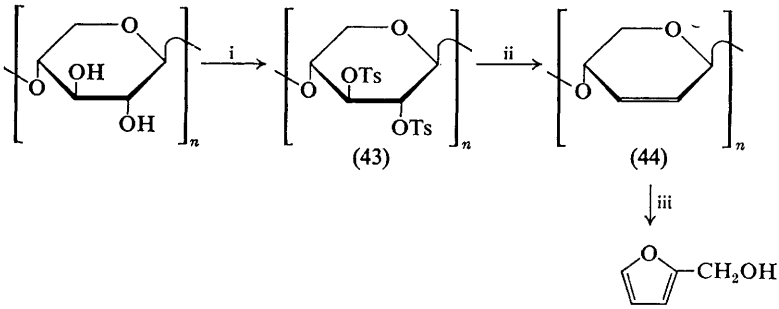
Graft copolymers of poly(acrylic acid) and poly(methacrylic acid) have been grafted onto starch by a Scott-xanthation process.[173] A combination of optical methods afforded information on the inter-chain linkages, and it was concluded that the carboxylic acid groups of the side-chain polymer are hydrogen-bonded to the hydroxy-groups of the polysaccharide. Such bonding leads to a crystalline phase of the copolymer that differs from that of starch, and it also influences the configuration of the polysaccharide chains in solution.

Graft co- and ter-polymers of starch have been prepared by free-radical polymerizations of vinyl monomers onto γ-irradiated starch in a prototype commercial process.[174] Products with graft contents of up to 46% were obtained by grafting acrylamide and quaternary amine-containing esters of methacrylic acid onto starch. The graft copolymers possessed retention and drainage properties superior to commercial flocculants.

[169] D. J. Stiefel and P. J. Keller, *Clinical Chem.*, 1975, **21**, 343.
[170] G. G. Maher, C. R. Russell, and C. E. Rist, *Stärke*, 1974, **26**, 386.
[171] M. E. Carr, B. T. Hofreiter, and C. R. Russell, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 1441.
[172] G. Hollinger and R. H. Marchessault, *Biopolymers*, 1975, **14**, 265.
[173] E. Gruber, S. Alloush, K. John, and J. Schurz, *Stärke*, 1973, **25**, 325.
[174] H. D. Heath, B. T. Hofreiter, A. J. Ernst, W. M. Doane, G. E. Hamerstrand, and M. I. Schulte, *Stärke*, 1975, **27**, 76.

Xylans.—Depolymerization of xylan by chlorine in aqueous solution yielded D-xylonic acid and D-xylose end-groups.[175] Glyceric, threonic, and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acids were present in minor proportions. Some D-galacturonic acid residues glycosidically linked to potentially reducing D-xylose end-groups were cleaved during treatment of xylan with chlorine.

β-D-Xylan has been converted into the 2,3-di-*O*-toluene-*p*-sulphonate (43), which underwent an elimination with zinc dust and sodium iodide to give 2,3-dideoxy-β-D-*glycero*-pent-2-enopyranoglycan (44) (Scheme 7).[108] Treatment of (44) with aqueous acetic acid furnished 2-(hydroxymethyl)furan.



Reagents: i, TsCl–py; ii, NaI–Zn–DMF; iii, H_3O^+

Scheme 7

Modification of Glycoproteins and Uses of Modified Glycoproteins

Albumins.—Albumin has been converted into a cortisol-succinyl derivative, an immobilized form of which was used for affinity chromatography.[40] Bovine serum albumin has been polythiolated, by reaction with *N*-acetylhomocysteine thiolactone, and then covalently coupled to cellulose xanthate to form an immobilized derivative.[133]

Antibodies.—Antibodies to the milk oligosaccharide (40), *viz.* anti-(lacto-*N*-difucohexaose) antibody, have been complexed with succinylated haemocyanin as part of a study of the affinity chromatography of carbohydrate-specific immunoglobulins.[59]

Fetuins.—Desialylized fetuin has been used as a substrate for β-D-galactosidase.[176]

Mucins.—Porcine colonic mucin has been desialylized by mild hydrolysis with acid; the modified glycoprotein was used as a substrate for an *endo*-β-D-galactosidase.[177]

Phytohaemagglutinins.—Conditions have been established for the modification of concanavalin A with dextranase by a glutaraldehyde-mediated reaction.[178] The soluble, intermolecularly cross-linked conjugate possessed both concanavalin

[175] T. Ericsson and O. Samuelson, *Acta Chem. Scand.* (*B*), 1975, **29**, 309.
[176] A. G. W. Norden, L. L. Tennant, and J. S. O'Brien, *J. Biol. Chem.*, 1974, **249**, 7969.
[177] M. Fukuda and G. Matsumura, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 465.
[178] S. A. Barker, A. G. Giblin, C. J. Gray, and W. H. Bowen, *Carbohydrate Res.*, 1974, **36**, 23.

A and dextranase activities; hydrolysis of dextran by the conjugate afforded products different from those obtained with the free enzyme.

Orosomucoids.—The desialylized form of orosomucoid has been radioactively labelled and used to examine the role of glycosyltransferases in the hepatic binding of sialoglycoproteins.[179]

Ovalbumins.—Ovalbumin acted as an acceptor of D-[^{14}C]galactose in a method for the simultaneous determination of D-galactosyltransferase and 5′-nucleotidase activities in cell fractions.[180] Ovalbumin has also been modified by sulphitolysis.[181]

Immobile Glycoproteins.—The modification of glycoproteins to yield immunologically active, but immobilized, derivatives continues to receive attention.[13] The preparation of many immobilized, insolubilized, and coupled glycoproteins has been described; these derivatives, together with their applications, are summarized in Table 6.[182–184]

In addition to its use as an affinity-chromatography material, human serum albumin immobilized by reaction with agarose cyclic imidocarbonate has been used in the removal of contaminants during the purification of other glycoproteins.[41]

Immobilization of serum albumin by covalent coupling to 4-benzoquinone can be carried out over a broad pH-range; the amount of albumin coupled was comparatively high, and the product was very stable.[21]

Anti-(human serum albumin) antibody immobilized by reaction with agarose cyclic imidocarbonate has found general use as an immunoadsorbent.[32]

Anti-(human parotid α-amylase) and anti-(bovine-spleen deoxyribonuclease II) antibodies have been bound to various types of glass bead and challenged with human-parotid or pancreatic α-amylases or with deoxyribonuclease II, respectively.[183] Although the beads in a column bound these enzymes, the amounts of the enzymes recovered from the beads were not quantitative and were extremely variable. U.v.-active material was also eluted. The bound anti-(α-amylase) antibody was used to form an active insoluble derivative of the homologous antigen.

Human immunoglobulins IgA, [^{125}I]-IgG, and IgM have been used as markers in studies of the conversion of agarose into the cyclic imidocarbonate derivative and of coupling reactions thereof.[72] Immunofluorescence showed that the bound immunoglobulins are distributed uniformly throughout the agarose beads.

Immobilized forms of phytohaemagglutinins continue to be used in the separation and purification of glycoproteins by affinity chromatography. Concanavalin A immobilized on agarose cyclic imidocarbonate has been used to reveal the heterogeneity of carcinoembryonic antigen;[184] *Lens culinaris*,[65] wheat-germ,[65] and *Vicia cracca*[70] phytohaemagglutinins have also been used.

[179] R. L. Hudgin and G. Ashwell, *J. Biol. Chem.*, 1974, **249**, 7369.
[180] L. S. Freilich, M. E. Richmond, A. C. Reppucci, and J. E. Silbert, *Biochem. J.*, 1975, **146**, 741.
[181] A. L. Tarentino, T. H. Plummer, jun., and F. Maley, *J. Biol. Chem.*, 1974, **249**, 818.
[182] M. Nanjo and G. G. Guilbault, *Analyt. Chim. Acta*, 1974, **73**, 367.
[183] M. E. Hodes and J. T. Glier, *J. Chromatog.*, 1975, **104**, 379.
[184] G. T. Rogers, F. Searle, and K. D. Bagshawe, *Nature*, 1974, **251**, 519.

Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product* [a]	*Ref.*
Albumin	Glutaraldehyde cross-linked with L-amino-acid oxidase	Enzyme electrode for the measurement of L-amino-acids	182
	Glutaraldehyde cross-linked with D-glucose oxidase	Enzyme electrode for the measurement of D-glucose	182
Albumins (serum)	Reaction with agarose cyclic imidocarbonate	Removal of bilirubin and other albumin-bound substances from blood and plasma by affinity chromatography	41
		Selective binding of human and other interferons to immobilized albumnis	42
	Reaction with a 4-benzoquinone derivative of agarose (see p. 415)	Demonstration of a new, simple method for the immobilization of proteins	21
Albumin ([^{14}C]acetylated)	Reaction with a triazine derivative of cellulose	Purification of alkaline phosphatase by immunoadsorption	101
Albumin ([^{14}C]carboxymethylamido derivative)	Reaction with triazine derivatives of cellulose, carboxymethylcellulose, DEAE-cellulose		
Albumin (polythiolated)	Reaction with cellulose xanthate	Demonstration of cellulose xanthate as a matrix for immobilization with reversible coupling	133
Albumin (succinylated, cortisol derivative)	Reaction with agarose cyclic imidocarbonate	Isolation of a cortisol-binding immunoglobulin by affinity chromatography	40
Anti-(β-D-acetamidodeoxyhexosidases 'A' and 'B') antibody		Removal of β-D-acetamidodeoxyhexosidases 'A' and 'B' in the purification of human-brain β-D-acetamidodeoxyhexosidase 'C'	31
Anti-albumin (serum) antibody	Reaction with agarose cyclic imidocarbonate	Immunoaffinoelectrophoresis; a study of the interactions occurring during immunoadsorption in a simple system	32
Anti-(alkaline phosphatase) antibody		Purification of alkaline phosphatase by immunoadsorption	33

Anti-allotype serum	Reaction with agarose cyclic imidocarbonate provided with linkage-extension facilities [see (10), Scheme 3b]	Quantitative determination of allotypic markers by radioimmunoassay	34
Anti-(α-amylase) antibody Anti-(deoxyribonuclease II) antibody	Adsorption onto glass beads and onto zirconium glass beads	Immobilized antibodies for the production of an active immobilized derivative of the homologous enzyme antigen	183
Anti-idiotype serum	Reaction with agarose cyclic imidocarbonate provided with linkage-extension facilities [see (10), Scheme 3b]	Quantitative determination of idiotypic markers by radioimmunoassay	34
Anti-interferon antibody	Reaction with agarose cyclic imidocarbonate	Purification and concentration of interferon by immunoadsorption	37
Anti-prolactin antibody	Reaction with agarose cyclic imidocarbonate	Isolation of prolactin by immunoadsorption	38
Anti-(Tamm–Horsfall glycoprotein) antibody		Isolation of a pseudo-Tamm–Horsfall glycoprotein by affinity chromatography	36
Anti-(Tamm–Horsfall glycoprotein) serum		Isolation of a pseudo-Tamm–Horsfall glycoprotein by affinity chromatography	36
Blood-group A substance (partially deacetylated)		Immunoadsorption of anti-(blood-group A substance) antibody	39 [b]
Cell material		Purification and concentration of interferon by affinity chromatography	37
Concanavalin A	Glutaraldehyde cross-linked with dextranase	Active immobilized enzyme possessing haemagglutinating activity	178
	Reaction with agarose cyclic imidocarbonate	Fractionation of carcinoembryonic antigen by affinity chromatography	184
		Investigation of the interaction of interferon with immobilized concanavalin A; demonstration of carbohydrate recognition and of hydrophobic interactions	54
		Purification of β-D-acetamidodeoxyhexosidase 'A' by affinity chromatography	55
Goat immunoglobulin, anti-(bovine prothrombin) antibody	Reaction with agarose cyclic imidocarbonate	Purification of prothrombin by immunoadsorption	35

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product* [a]	*Ref.*
Human immunoglobulin IgA [^{125}I]-Human immunoglobulin IgG Human immunoglobulin IgM	Reaction with agarose cyclic imidocarbonate	Investigation of the coupling of macromolecules to agarose cyclic imidocarbonate	72
Lens culinaris phytohaemagglutinin	Reaction with agarose cyclic imidocarbonate	Fractionation of synaptic plasma-membrane glycoproteins by affinity chromatography	65
Luteinizing hormone	Reaction with divinylsulphonyl-agarose	Isolation of anti-(luteinizing hormone) antibodies by immunoadsorption	96
Rabbit anti-(human type-O red-blood cells) antibody	Reaction with dialdehydodextran	Polysaccharide-labelled antibody examined by electron microscopy	149
Rabbit immunoglobulin IgG anti-(α-amylase) antibody Rabbit immunoglobulin IgG anti-(deoxyribonuclease) antibody	Adsorption onto glass beads and onto zirconium glass beads	Immobilized antibody for the production of an active immobilized derivative of the homologous enzyme antigen	183
Rabbit immunoglobulin IgG anti-(human type-O) antibody	Reaction with dialdehydodextran	Polysaccharide-labelled antibody examined by electron microscopy	149 [c]
Rabbit immunoglobulin IgG anti-(Tamm–Horsfall glycoprotein) antibody	Reaction with agarose cyclic imidocarbonate	Isolation of a pseudo-Tamm–Horsfall glycoprotein by affinity chromatography	36
Vicia crassa phytohaemagglutinin		Affinity chromatography of blood-group A substance	70
Wheat-germ phytohaemagglutinin		Fractionation of synaptic plasma-membrane glycoproteins by affinity chromatography	65

[a] Unless otherwise indicated, the product is insoluble in water; [b] Agarose was cross-linked with 2,3-dibromopropanol prior to treatment with cyanogen bromide; [c] Water-soluble derivative.

The purification of glycoside hydrolases (*e.g.* β-D-acetamidodeoxyhexosidase) on immobilized concanavalin A demonstrated that these enzymes are glycoproteins rather than proteins.[55]

The complex formed by interaction of interferon with concanavalin A immobilized on agarose cyclic imidocarbonate could not be dissociated with methyl α-D-mannopyranoside, indicating that carbohydrate recognition and hydrophobic interactions both contribute to the binding.[54]

Macromolecules at the surface of erythrocytes have been derivatized, *via* the thiol groups, with modified forms of α-amylase to give active immobilized enzymes; the underivatized erythrocytes did not take up the unmodified enzyme.[185]

Modification of Enzymes and Uses of Modified Enzymes

β-D-Galactosidases.—A β-D-galactosidase from *Propionibacterium shermanii* has been derivatized with thiol-blocking groups; these modifications inhibited the enzymic activity, which was partly restored by the action of 1,4-dithiothreitol.[186]

β-D-Glucosidases.—The active site of the β-D-glucosidase from *Aspergillus wentii* has been labelled by reaction with the epoxide of [^{3}H]conduritol B.[187] The derivative was used in sequence studies of the enzyme. A reduced and alkylated derivative of the β-D-glucosidase from crude *Trichoderma viride* cellulase has been prepared.[188]

α-Amylases.—α-Amylase has been labelled by reaction with [^{14}C]formaldehyde without loss of enzymic activity.[183]

α-Amylase derivatized with 5,5′-dithiobis(2-nitrobenzoic acid) and 3-maleimidobenzoic acid can be immobilized by reaction with the thiol groups of a suitable matrix.[185] Both thiol groups of porcine pancreatic α-amylase have been derivatized by treatment of the enzyme with 5,5′-dithio-bis(2-nitrobenzoic acid), or iodoacetamidonaphthol, or Hg^{2+} ions.[189] The reaction with the first two reagents occurred only after tightly bound calcium ions had been removed, whereas this was unnecessary in the preparation of the mercury derivative. α-Amylase reacted with one mol of Hg^{2+} ion to form a —S—Hg—S— bridge, demonstrating that the thiol groups are very close together. Since all three derivatives of the enzyme were active, the thiol groups cannot be located at the active site. Both thiol groups of this α-amylase have also been derivatized with 5,5′-dithiobis(2-nitrobenzoic acid) in the presence of H_4edta and/or urea.[190] A part of the purified α-amylase molecule was found to contain disulphide bridges, again indicating the close proximity of the thiol groups. The reaction was first order with respect to the enzyme's concentration in the presence of H_4edta, and the two thiol groups possessed identical reactivities; complete blocking of the groups resulted in a total loss of enzymic activity. Deblocking of the thiol groups proceeded at the same rate.

[185] L. F. Chen and T. Richardson, *Pharmacol. Res. Comm.*, 1974, **6**, 581.
[186] J. C. Hartley and E. R. Vedamuthu, *Appl. Microbiol.*, 1975, **29**, 74.
[187] E. Bause and G. Legler, *Z. physiol. Chem.*, 1974, **355**, 438.
[188] L. E. F. Berghem and L. G. Pettersson, *European J. Biochem.*, 1974, **46**, 295.
[189] M. L. Steer, N. Tal, and A. Levitzki, *Biochim. Biophys. Acta*, 1974, **334**, 389.
[190] G. Pommier, P. Cozonne, and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1974, **350**, 71.

β-Amylases.—The thiol groups of the β-amylase from wheat reacted instantly with 4-chloromercuribenzoate and *N*-ethylmaleimide in the absence of a substrate.[191] Derivatization almost completely abolished the enzymic activity, and the kinetics indicated that inhibition by the mercury compound is non-competitive. Inhibition by these reagents was considerably less in the presence of a substrate, since the enzyme undergoes an induced conformational change that renders the internal thiol groups inaccessible. The free thiol groups of the enzyme were divided into two types in these reactions: two groups on the molecular surface that play no part in the catalysis, and two in the interior that do.

Dextranases.—The L-lysyl ε-amino-group and other amino-groups of a dextranase from *Penicillium funiculosum* have been acetylated; the L-arginyl and L-cysteinyl residues reacted with cyclohexane-1,2-dione and 4-chloromercuribenzoic acid, respectively; the L-histidyl residues have been modified by photo-oxidation; and the carboxy-groups have been amidated with glycine ethyl ester.[192] These modifications, which were carried out on separate batches of the enzyme, indicated that an L-cysteine residue outwith the active site is involved in activation of the enzyme by cobalt ions.

D-Glucose Oxidase.—The carbohydrate moieties of D-glucose oxidase from *Aspergillus niger* have been modified by oxidation with periodate ion.[193] Although *ca.* 60% of the carbohydrate residues were oxidized at pH 5.6, none of the amino-acid residues was oxidized. The oxidized enzyme retained its full activity and was converted into an immobilized form.

Lysozymes.—The effects of γ-radiation on an aqueous solution of egg-white lysozyme have been studied with a view to assessing the resistance of egg-white to irradiation.[194] The inactivation curve is exponential, and the amount of an inactive fraction (mol. wt. 7.0×10^4) increased with increasing dosage. Ethanol protected the enzyme from degradation, and it was concluded that hydroxyl radicals are primarily responsible for aggregation and inactivation of lysozyme in aqueous solutions brought about by γ-rays.

The reaction of the primary amino-groups in hen egg-white lysozyme with 7-chloro-4-nitrobenz-2-oxa-1,3-diazole displayed an induction period, due to the obligatory formation of a kinetic precursor arising from reaction of the L-tyrosyl residues.[195] The precursor was fully active enzymically. C.d. and fluorescence emission and excitation spectra of this precursor indicated that limited structural changes had taken place in the helical region on derivatization. The enhanced reactivity of the amino-groups towards the reagent was discussed in the light of possible conformational changes. The precursor underwent a conformational transition in alkali: the associated thermodynamic parameters ($pK_a = 9.89$, $\Delta H = 4.74$ kcal mol^{-1}, $\Delta S = -29.9$ cal deg^{-1} mol^{-1} at 300 K) suggested that the transition depends on the ionization of an L-tyrosyl residue.[196]

Treatment of egg-white lysozyme with cyclohexanedione resulted in derivatization of all the L-arginyl residues and in partial (65%) inactivation.[197] Similar

[191] E. László, J. Holló, and A. Hoschke, *Stärke*, 1973, **25**, 420.
[192] M. Sugiura, A. Ito, T. Ogiso, and K. Kato, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2953.
[193] O. R. Zaborsky and J. Ogletree, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 210.
[194] T. Kume, Y. Sato, and Y. Umemoto, *J. Agric. Chem. Soc. (Japan)*, 1973, **47**, 549.
[195] A. A. Aboderin, E. Boedefeld, and P. L. Luisi, *Biochim. Biophys. Acta*, 1973, **328**, 20.
[196] A. A. Aboderin, E. Boedefeld, and P. L. Luisi, *Biochim. Biophys. Acta*, 1973, **328**, 31.
[197] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 565.

modification of bovine pancreatic ribonuclease I resulted in derivatization of two or three of the L-arginyl residues and in 90% inactivation.

Hen egg-white lysozyme has been esterified specifically at L-aspartic acid-52 with the affinity label 2,3-epoxypropyl β-di-*N*-acetylchitobioside.[7] The disulphide and L-aspartic acid ester bonds of the derivatized enzyme were then reduced with 1,4-dithiothreitol and sodium borohydride, respectively, resulting in the removal of the affinity label and the formation of homoserine residues. The enzyme obtained on re-oxidation with glutathione was inactive, whereas reduction (with 1,4-dithiothreitol) and re-oxidation (with glutathione) of the original enzyme did not abolish its activity. It was concluded that L-aspartic acid-52 is essential for enzymic activity.

Lysozyme reacted with the α-D-galactopyranosylamine-ammonia complex, lactosylamine, and 2-amino-2-deoxy-D-glucose *via* the free carboxy-groups of L-aspartic and L-glutamic acids.[6]

Cross-linking of lysozyme with glutaraldehyde afforded a series of semi-synthetic agglutinins that simulated the action of the phytohaemagglutinins.[8] Haemagglutination of erythrocytes by these agglutinins was associated with higher 'oligomers' of the enzyme.

Hen egg-white lysozyme has been reduced and alkylated to aid in studies of long-range interactions between the *N*- and *C*-termini of the molecule.[198]

Stable complexes were formed when [^{14}C]cello-oligosaccharides were incubated with lysozyme in the pH range 3—9 (see also p. 432).[113]

Immobilized Derivatives of Enzymes.—The modification of enzymes to yield active water-insoluble or immobilized derivatives continues to receive attention. Reviews covering the principles of the technology of immobilized enzymes [16] and the preparation and uses of immobilized enzymes [13–15, 199] have appeared. A compendium of data on the techniques used for the immobilization of enzymes,[17] and books on the uses of immobilized enzymes in industrial reactors [200] and in food and microbial processes [12] have been published.

The progress made in the use of immobilized enzymes as industrial catalysts has been discussed in detail.[201] A number of natural and synthetic polymers have been selected for the immobilization of enzymes from a knowledge of the physical and chemical properties of the catalytically active protein.[202]

Immobilized enzymes have been considered from a number of theoretical viewpoints. The influence of the solvent on the motion of molecules 'immobilized' on polystyrene matrices and on glass surfaces has been studied,[203] and the effects of internal diffusion on Lineweaver–Burk plots for immobilized enzymes have been investigated.[204] Diffusion has been shown to influence the apparent rate of denaturation of gel-entrapped enzymes.[205] Many of the theoreti-

[198] G. E. Matthyssens and L. Kanarek, *European J. Biochem.*, 1974, **43**, 363.
[199] 'Insolubilized Enzymes', ed. M. Salmona, C. Saronio, and S. Garattini, Raven Press, New York, 1974.
[200] 'Immobilized Enzymes for Industrial Reactors', ed. R. A. Messing, Academic Press, New York, 1975.
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[205] R. A. Korus and K. F. O'Driscoll, *Biotechnol. and Bioeng.*, 1975, **17**, 441.

Table 7 *Modification of enzymes by coupling to insoluble matrices and other macromolecules*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
Acetyl-cholinesterase	—	Entrapment in polyacrylamide gel	Active immobilized enzyme; assessment of the reaction kinetics of the immobilized enzyme	210
		Glutaraldehyde cross-linked onto partially hydrolysed nylon tubing	Active immobilized enzyme; assessment of the flow kinetics of the immobilized enzyme	211
Alcohol dehydrogenase	1.1.1.1	Covalent reaction on silanized alumina	Active immobilized enzyme for use in a continuous-flow reactor incorporating continuous electrochemical regeneration of nicotinamide adenine dinucleotide	212
L-Amino-acid oxidase	1.4.3.2	Glutaraldehyde cross-linked with albumin	Enzyme electrode for the measurement of L-amino-acids	182
Aminoacylase	3.5.1.14	Copolymerization of acrylate and acrylamide monomers in the presence of the enzyme	Active immobilized enzyme	213
Aminopeptidase (microsomal)	3.4.11.2	Reaction with agarose cyclic imidocarbonate Reaction with an aminopropyl derivative of glass	Active immobilized enzyme; hydrolysis of proteins	23
α-Amylase	3.2.1.1	Copolymerization of acrylate and acrylamide monomers in the presence of the enzyme	Active immobilized enzyme	213
		Reaction with the cyclic imidocarbonate derivative of carboxymethylcellulose	Active immobilized enzyme for the degradation of starch	122
α-Amylase (^{14}[C]-labelled)		Reaction of the enzyme with either 5,5′-dithio-bis(2-nitrobenzoic acid) or 3-maleimidobenzoic acid and coupling of the products with the thiol groups of erythrocytes	Active immobilized enzyme; demonstration of the use of erythrocytes as a matrix for immobilization	185

		Reaction with anti-(α-amylase) antibody immobilized on glass and zirconium-glass beads	Active immobilized enzyme; use in insoluble-enzyme reactor	183
β-Amylase	3.2.1.2	Adsorption onto alkyl derivatives of epichlorohydrin-cross-linked agarose	Active immobilized enzyme produced by hydrophobic interactions for the formation of an enzyme reactor bed	98
		Carbodi-imide assisted reaction with derivative (9) of agarose cyclic imido-carbonate (see Scheme 3a) Carbodi-imide assisted reaction with derivative (8) of agarose cyclic imido-carbonate (see Scheme 3b) Reaction with agarose cyclic imido-carbonate	Active immobilized enzyme used to investigate the catalytic activity of the insolubilized enzyme	24
L-Asparaginase	3.5.1.1	Adsorption onto alumina, titania-treated stainless steel, or nickel–nickel oxide sinters	Active immobilized enzyme	214
Aspartate 4-decarboxylase	4.1.1.12	Reaction of the subunits with agarose cyclic imidocarbonate	Studies of the structural relationship between the subunits of aspartate 4-decarboxylase, and of the activity of the subunits in immobilized form	25
Carboxy-peptidase A	3.4.12.2	Reaction with Sephadex cyclic imido-carbonate	Active immobilized enzyme used as a probe for studying the thermally induced unfolding of ribonuclease	71
Catalase	1.11.1.6	Adsorption onto alumina, titania-treated stainless steel, or nickel–nickel oxide sinters	Active immobilized enzyme	214
		Glutaraldehyde cross-linked on silica–alumina with (or without) nickel, silicon carbide, or Kieselguhr	Active immobilized enzyme; screening of inorganic supports for use in immobilized-enzyme reactors in industry	215
		Copolymerization of acrylate and acrylamide monomers in the presence of the enzyme	Active immobilized enzyme	213
		Reaction with a copolymer of glutaraldehyde and diaminobenzene	Active immobilized enzyme; production of D-gluconic acid from D-glucose	216 [b]

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
		Reaction with a diazo derivative of porous glass	Active immobilized enzyme; direct analysis of D-glucose in whole blood	217
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; purification of a catalase inhibitor from maize by affinity chromatography	26
Chymosin	3.4.23.4	Reaction with an alkylamine derivative of porous glass	Active immobilized enzyme incorporated into a fluidized-bed, continuous coagulation scheme for milk	218
Chymotrypsin	3.4.21.1	Coupling to cellulose-acetate membranes in a pressure-driven reaction	Active immobilized enzyme used in the production of enzyme-coupled ultrafiltration membranes	120
		Glutaraldehyde cross-linked with chitin	Active immobilized enzyme; microenvironmental alteration of the pH optimum of the enzyme	142
		Reaction with a 4-benzoquinone derivative of agarose (see p. 415)	Demonstration of a new, simple method for the immobilization of enzymes with retention of activity	21
		Reaction with 4-diazobenzylcellulose	Active immobilized enzyme used to demonstrate the protection of immobilized enzymes from thermal inactivation by denaturing agents	126
		Reaction with an alkylamine derivative of porous glass	Active immobilized enzyme incorporated into a fluidized-bed, continuous coagulation scheme for milk	218
		Reaction with macroporous cellulose *trans*-2,3-cyclic carbonate	Active immobilized enzyme with high activity against macromolecular substrates	219
Deoxyribonuclease I	3.1.4.5	Reaction with agarose cyclic imidocarbonate	Affinity-chromatography matrix for the isolation of the inhibitor of deoxyribonuclease I; identification of the inhibitor as actin	56

Deoxyribonuclease II ([^{14}C]-labelled)	3.1.4.6	Reaction with anti-(deoxyribonuclease II) antibody immobilized on glass or zirconium glass beads	Active immobilized enzyme	183
Dextranase	3.2.1.11	Glutaraldehyde cross-linked with concanavalin A	Active immobilized enzyme possessing haemagglutinating activity	178 [c]
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; kinetic studies and the hydrolysis of dextrans	27
β-D-Fructofuranosidase		Adsorption onto DEAE-cellulose	Active immobilized enzyme for a continuous fluidized-bed reactor	100
		Entrapment in polyacrylamide gel; adsorption onto carboxymethylcellulose	Active immobilized enzyme; comparison of the kinetic properties of the two immobilized forms	220
		Entrapment in spherical pellets of agar	Active immobilized enzyme used in a continuous fluidized-bed reactor	100
β-D-Galactosidase	3.2.1.23	Adsorption onto alumina or titania-treated stainless steel	Active immobilized enzyme for the treatment of cheese whey	214
		Encapsulation in cellulose nitrate collodion microcapsules	Active immobilized enzyme that permits free approach by low-molecular-weight substrates	129
		Glutaraldehyde cross-linked with chitin	Active immobilized enzyme; micro-environmental alteration of the pH optimum of the enzyme	142
Glucoamylase	3.2.1.3	Adsorption onto alumina, titania-treated stainless steel, or nickel–nickel oxide sinters	Active immobilized enzyme	214
		Copolymerization of acrylate and acrylamide monomers in the presence of the enzyme	Active immobilized enzyme	213
		Reaction with a copolymer of glutaraldehyde and diaminobenzene	Active immobilized enzyme; production of D-glucose from starch	216
		Reaction with beads of derivatized, macroreticular polystyrene	Active immobilized enzyme	221
D-Glucose isomerase	5.3.1.18	Reaction with a copolymer of glutaraldehyde and diaminobenzene	Active immobilized enzyme; production of D-fructose from D-glucose	216
		Reaction with alumina, porous glass, or titania treated with cyanogen bromide	Active immobilized enzyme; demonstration of a new coupling process	222

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
D-Glucose oxidase	1.1.3.4	Entrapment in polyacrylamide gel	Active immobilized enzyme; model for amperometric enzyme electrode	223
		Entrapment in polyacrylamide gel on platinum or on glutaraldehyde cross-linked on platinum	Active immobilized enzyme; electrodes for electrochemical assessment of the enzyme	224
		Glutaraldehyde cross-linked on silica–alumina with (or without) nickel, silicon carbide, or Kieselguhr	Active immobilized enzyme; screening of inorganic supports for use in immobilized-enzyme reactors in industry	215
		Glutaraldehyde cross-linked with albumin	Enzyme electrode for measuring D-glucose	182
		Reaction of the periodate-oxidized enzyme with poly(4-aminostyrene)	Active immobilized enzyme; demonstration of complete retention of activity and of increased thermal stability on immobilization	193
		Reaction with a copolymer of glutaraldehyde and diaminobenzene	Active immobilized enzyme; production of D-gluconic acid from D-glucose	216 [d]
		Reaction with a diazo derivative of porous glass	Active immobilized enzyme; direct analysis of D-glucose in whole blood	217
		Reaction with derivatives of nylon tubes	Active immobilized enzyme; automated analysis of D-glucose	225
D-Glucose-6-phosphate dehydrogenase	1.1.1.49	Entrapment in polyacrylamide gel	Active immobilized enzyme for the detection of hexokinase, D-glucose phosphate isomerase, and phospho-D-glucomutase after polyacrylamide-gel electrophoresis	226
		Reaction with *sym*-triazine derivative of cellulose	Active immobilized enzyme	132
Glutamate dehydrogenase [NAD(P)$^+$]	1.4.1.3	Reaction with succinamidopropyl derivative of glass beads mediated by a water-soluble carbodi-imide	Active immobilized enzyme; reversible allosteric modulation of the immobilized enzyme	227
Hexokinase	2.7.1.1	Reaction with *sym*-triazine derivative of cellulose	Active immobilized enzyme	132

α-Lactalbumin	see 2.4.1.22	Reaction with agarose cyclic imidocarbonate	Quantitative study of acceptor–ligand interactions in the lactose synthetase system; affinity chromatography of lactose synthetase A protein	64
Lactoperoxidase	—	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for the radioiodination of proteinaceous macromolecules	28
Microbial metalloenzyme	3.4.24.4	Reaction with agarose cyclic imidocarbonate Reaction with an aminopropyl derivative of glass	Active immobilized enzyme; hydrolysis of proteins	23
Pepsin A	3.4.23.1	Reaction with an alkylamine derivative of porous glass	Active immobilized enzyme incorporated into a fluidized-bed coagulator for milk	218
Phosphatase, acid	3.1.3.2	Glutaraldehyde cross-linked with chitin	Active immobilized enzyme; microenvironmental alteration of the pH optimum of the enzyme	142
Phosphorylase	2.4.1.1	Reaction with *sym*-triazine derivative of cellulose	Active immobilized enzyme	132
Proline dipeptidase	3.4.13.9	Reaction with agarose cyclic imidocarbonate Reaction with an aminopropyl derivative of glass	Active immobilized enzyme; hydrolysis of proteins	23
Pronase	(3.4.24.4)	Reaction with a derivative of zirconium oxide-coated porous glass	Active immobilized enzyme; hydrolysis of proteins	228
		Reaction with agarose cyclic imidocarbonate Reaction with an aminopropyl derivative of glass	Active immobilized enzyme; hydrolysis of proteins	23
		Reaction with *sym*-triazine derivative of cellulose	Active immobilized enzyme	132
Protease (from *Aspergillus* sp.)	—	Reaction with agarose cyclic imidocarbonate Reaction with an aminopropyl derivative of glass	Active immobilized enzyme; hydrolysis of proteins	23

Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product* [a]	*Ref.*
Protease (from *Mucor miehei*)	—	Reaction with an alkylamine derivative of porous glass	Active immobilized enzyme for incorporation into a fluidized-bed, continuous coagulation scheme for milk	218
Proteases	—	Copolymerization of acrylate and acrylamide monomers in the presence of the enzyme	Active immobilized enzyme	213
Ribonuclease	3.1.4.22/23	Reaction with a 4-benzoquinone derivative of agarose (see p. 415)	Demonstration of a new, simple method for the immobilization of enzymes with retention of activity	21
Trypsin	3.1.21.4	Adsorption onto alumina, titania-treated stainless steel, or nickel–nickel oxide sinters	Active immobilized enzyme	214
		Adsorption onto carbon	Experimental geometry in studies of the properties of the active immobilized enzyme	229
		Reaction with alumina, porous glass, or titania treated with cyanogen bromide	Active immobilized enzyme; demonstration of a new coupling process	222
		Reaction with 4-diazobenzylcellulose	Active immobilized enzyme used to demonstrate the protection of immobilized enzymes from thermal inactivation by denaturing agents	126
Trypsin (polythiolated)	(3.4.21.4)	Reaction with cellulose xanthate	Active immobilized enzyme formed with reversible coupling	133
Tryptophanase	4.1.99.1	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; kinetic studies on coenzyme binding and dissociation in immobilized tryptophanase	29
Tryptophanase (apo)	(4.1.99.1)	Reaction with pyridoxal 5′-phosphate derivatives of agarose cyclic imidocarbonate [see Table 4 and (28)—(30)]	Production of active immobilized enzyme with immobilized pyridoxal 5′-phosphate	30
Urease	3.5.1.5	Reaction with Enzacryl AA Reaction with Enzygel	Active immobilized enzyme; enzyme electrode specific for urea	230

[a] Unless otherwise indicated the product is insoluble in water; [b] Immobilized together with D-glucose oxidase; [c] Water-soluble derivative; [d] Immobilized together with catalase.

cal problems associated with immobilized enzymes are claimed to be solved by collocation methods.[206] A graphical procedure, which is sufficiently accurate for most practical purposes, provided a rapid estimate of the effectiveness factor.

When diffusion of a substrate to an immobilized enzyme is relatively slow, the concentration of substrate is lower in the microenvironment than in the macroenvironment: the resulting reduction in enzymic activity is referred to as diffusional inhibition, a factor attenuated by an inhibitor of the enzyme.[207] Consequently, inhibition by products is more pronounced in the presence, than in the absence, of diffusional resistances at a given concentration of product in the macroenvironment. The anti-energistic interaction between chemical and diffusional inhibitions, however, makes the activity of a bound enzyme less sensitive to changes in product concentration in the macroenvironment. The interplay between the various types of product and diffusional inhibitions was illustrated graphically. Another consequence of the anti-energistic interaction is that the activity of the bound enzyme at steady state is affected less by an inhibitor in the presence, than in the absence, of diffusion-limited transfer of the substrate.[208] These effects also apply to membrane-bound enzymes in cellular milieu.

The kinetic behaviour of a bound enzyme inhibited by the substrate shows multiple steady states within a particular range of concentrations of the substrate in the macroenvironment, provided diffusion to the enzyme is slow.[209] When the substrate has to cross a membrane to reach the enzyme, regulatory schemes showed that the enzyme's activity can be changed dramatically by small variations in substrate concentration, membrane permeability, and the kinetic constants of the enzyme. It was concluded that the regulatory properties of an enzyme in a cell are more effective than suggested by experiments with the enzyme in solution.

Many preparations of immobilized enzymes and of enzymes derivatized by coupling to other macromolecules to give water-soluble and -insoluble forms have been reported during the past year. These derivatives and their applications are summarized in Table 7;[210–230] immobilized enzymes are referred to in this

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[223] L. D. Mell and J. T. Maloy, *Analyt. Chem.*, 1975, **47**, 299.
[224] E. J. Lahoda, C. C. Liu, and L. B. Wingard, jun., *Biotechnol. and Bioeng.*, 1975, **17**, 413.

Table whether or not they are enzymically active. The following details of immobilized glycoside and polysaccharide hydrolases and carbohydrate oxidases are worthy of note.

Whole-cell β-D-fructofuranosidase from *Saccharomyces pastorianus* entrapped in spherical agar pellets can be used for the inversion of sucrose in a complete-mixing reactor, demonstrating that it is not necessary to isolate or to purify an enzyme before immobilization.[100] Up to 25% of the enzymic activity was retained when tomato β-D-fructofuranosidase was immobilized either by embedding it in polyacrylamide gel or by adsorbing it onto carboxymethylcellulose.[220] Substrates did not release the enzyme from either of the carriers; neither was the adsorbed enzyme released at pH values <5. No changes were observed in the pH optimum on embedding the enzyme in polyacrylamide gel, and the K_m values for the embedded and adsorbed enzymes towards sucrose were 1.4×10^{-3} and 6.9×10^{-3} mol l^{-1}, respectively.

Acid-tolerant β-D-galactosidases bound to chitin with glutaraldehyde exhibited maximum activities at one pH unit less than the free enzymes.[142] Approximately 60% of the enzymic activity was retained on immobilization, and a column of the immobilized enzyme in continuous use had a half-life of 27 days. Encapsulation of β-D-galactosidase in collodion microspheres resulted in little deactivation of the enzyme, which could be approached by 2-nitrophenyl β-D-galactopyranoside.[129]

The β-D-galactosidase from *Aspergillus niger* immobilized by adsorption onto either alumina or titania-treated stainless steel retained essentially all of its activity when stored under water at 23 °C for long periods; little activity was lost when cheese whey was treated with the immobilized enzyme at 55 °C over several weeks.[214] The catalytic activity of the immobilized enzyme (maximum activity at pH 3 and 60 °C) was unaffected by frequent sanitizations. Nickel–nickel oxide sinters were also used to immobilize this β-D-galactosidase. Glucoamylase was also successfully immobilized on these matrices.

Human parotid and pancreatic α-amylases have been immobilized on glass-bead matrices by immunoadsorption; this novel technique emerged from studies of the reproducibility of columns of antibodies on glass beads, when α-amylase was shown to retain its enzymic activity on binding to the immobilized homologous antibody.[183]

The coupling of α-amylase treated with either 5,5′-dithio-bis(2-nitrobenzoic acid) or 3-maleimidobenzoic acid with the surface thiol groups of human erythrocytes could be blocked with 1,4-dithiothreitol.[185] α-Amylase and glucoamylase immobilized by incorporation into polyacrylates generated *in situ* retained their enzymic activity, even when polymerization was induced by irradiation of the monomer.[213]

The merits of immobilizing β-amylase on various agarose derivatives have been compared.[24] β-Amylase has been used in a new type of reactor bed which

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[229] G. E. Stoner, E. Gileadi, J. C. Ludlow, and D. J. Kirwan, *Biotechnol. and Bioeng.*, 1975, **17**, 455.
[230] G. G. Guilbault and M. Tarp, *Analyt. Chim. Acta*, 1974, **73**, 355.

depends on hydrophobic interactions between the enzyme and alkylated agaroses.[98]

The specific activity of dextranase from *Penicillium funiculosum* immobilized on agarose cyclic imidocarbonate was as high as 30% of that of the free enzyme.[27] The pH and temperature optima of the enzyme were unchanged by immobilization, whereas the pH and temperature stabilities were enhanced. The immobilized dextranase was more active towards low-molecular-weight dextrans, and the Michaelis constants were two to five times greater than those for the free enzyme acting on substrates of the same chain length; the action patterns of the free and immobilized enzymes are virtually identical. Columns of the immobilized enzyme were suitable for repeated use.

Macroreticular polystyrene beads have been shown to be as good as porous glass beads for the covalent binding of such enzymes as glucoamylase.[221] The discovery that enzymes can be immobilized on copolymers of a diaminobenzene and glutaraldehyde appears to provide more economical use of enzymes in industries based on starch and sugar.[216] The immobilization of glucoamylase, D-glucose isomerase, and D-glucose oxidase plus catalase for the production of D-glucose, D-fructose, and D-gluconic acid, respectively, was investigated.

Mixed-bed reactors have been obtained by mixing D-glucose oxidase and catalase, each immobilized on diazoaryl derivatives of porous glass.[217] Coupling of D-glucose oxidase to nylon tubing has led to a new, 'reagentless', automated analysis for D-glucose.[223] A platinum screen has been used to support a polyacrylamide matrix in the construction of a D-glucose oxidase electrode; the effect of applying a DC voltage to the screen during the immobilization process was examined.[225]

A model for the amperometric enzyme electrode, obtained by digital simulation, has been applied to D-glucose oxidase immobilized within polyacrylamide gel.[224] D-Glucose oxidase was selected for screening a number of inorganic supports as matrices for immobilization.[215] The activity of the immobilized enzyme on matrices of silica–alumina (with or without nickel), or silicon carbide, or Kieselguhr depended on the size and nature of the supporting particles.

To avoid interference with the active site of the enzyme, D-glucose oxidase from *Aspergillus niger* has been immobilized by way of its carbohydrate moieties.[193] Thus, treatment of the periodate-oxidized enzyme with a styrene derivative afforded a water-insoluble material retaining full enzymic activity and exhibiting enhanced thermal stability at 60 °C.

Modification of Gangliosides and Glycolipids, and Uses of Modified Gangliosides and Glycolipids

Gangliosides.—Standard methodology has been adopted in the preparation of Tay-Sachs ganglioside G_{M_3} (45) specifically labelled in either the *N*-acetylneuraminic acid or 2-acetamido-2-deoxy-D-galactopyranosyl residue.[231]

Hydrolysis of ganglioside G_{M_1} (46) with acid removed the *N*-acetylneuraminic acid residue, yielding a material that was used in studies of antisera produced in response to the modified and normal gangliosides.[232]

[231] J. F. Tallman, E. H. Kolodny, and R. O. Braby, *Methods in Enzymol.*, 1975, **35**, 541.
[232] M. Naiki, D. M. Marcus, and R. Ledeen, *J. Immunology*, 1974, **113**, 84.

X-β-D-Gal*N*Ac*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
3
↑
2
α-Neu*N*Ac

(45)

(46) X = β-D-Gal*p*-(1 → 3)

Glycolipids.—2-*O*-Acyl-1-*O*-alkyl-3-*O*-(β-D-galactopyranosyl 3-sulphate)-glycerol has been labelled with [^{35}S]sulphate; the labelled glycolipid was used as the substrate in studies of the specificities of human arylsulphatases A and B.[233]

Oxidative ozonolysis of the alkenic bond of the sphingosine moiety of either 'globoside' (47) or the methyl ester of 'haematoside' (48) allowed the product to be

β-D-Gal*N*Ac*p*-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(47)

α-Neu*N*G-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(48)

coupled to an aminoethyl derivative of agarose (8) (see Scheme 3b) or to an amino derivative of glass.[82] The immobilized forms of the glycolipids were used in the purification of anti-glycosphingolipid antibodies.

[233] A. L. Fluharty, R. L. Stevens, R. T. Miller, and H. Kihara, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 348.

Author Index

Aach, H. G., 234
Aalto, M., 353
Aaronson, S. A., 272
Abbas, S. A., 28, 41
Abduazimov, K. A., 228
Abdel-Akher, M., 170
Abd El-Rahman, M. M. A., 84
Abdolrahimzadeh, H., 409
Abe, H., 300
Abe, Y., 138
Abigorov, B. D., 144
Aboderin, A. A., 379, 450
Aboul-Hosn, W. R., 314
Abou-State, M. A., 222
Abousteit, O., 226
Abraham, C. V., 318
Abraham, D. J., 188
Abrahamsson, K., 223, 229
Abramova, L. N., 143
Abramson, C., 373
Aburaki, S., 204
Achenbach, H., 131
Achmatowicz, B., 176
Achmatowicz, O., 17, 88
Achtardjieff, C., 230
Acton, E. M., 60
Adachi, R., 64
Adair, N. K., 416
Adam, A., 261
Adam, M., 298
Adam, S., 209
Adamany, A. M., 323, 324
Adams, C. A., 342
Adarich, E. K., 141
Adensámová, J., 242
Aerts, G., 176
Afanas'ev, V. A., 69, 144
Afanas'eva, T. I., 364
Agnihotri, J. P., 382
Ahmad, A., 279, 323
Ahmed, A. I., 321
Ahmed, A. U., 221
Ahrgren, L., 256
Aizawa, M., 459
Ajisaka, K., 167, 178
Akahori, T., 221
Akasak, M., 305
Akasaka, K., 184
Akatsu, M., 60
Akgerman, A., 14
Akhrem, A. A., 141, 159
Akim, G. L., 9
Akimoto, Y., 193, 293
Akiyama, F., 390
Aladin, A. A., 245
Albarracin, I., 403
Albert, S. O., 296
Albrecht, H. P., 25
Aldadeff, J. A., 299, 337
Alekseev, Yu. E., 103, 109
Alekseeva, V. G., 75, 199
Alenin, V. V., 146
Alexander, B. F., 459
Alföldi, J., 6, 117, 177
Allalouf, D., 373
Allams, A. K., 134
Allen, A., 351
Allen, A. K., 212
Allen, B. R., 344, 459
Allen, W. S., 291
Allison, M. J., 361
Alloush, S., 443
Allsobrook, A. J. R., 138
Almin, K. E., 362
Alonso, G., 151
Alpers, D. H., 345
Alt, K. O., 27
Alter, G. M., 277
Altona, C., 39
Alves, M. J. M., 275
Amakawa, A., 284
Amakawa, T., 305
Amemura, A., 346, 359
Aminoff, D., 330, 416
Amit, B., 161
Anand, S. R., 402
Anastassiades, T. P., 309
Anderle, D., 6, 102, 193, 213, 216
Anderson, A., 213
Anderson, A. J., 237
Anderson, C. B., 175
Anderson, D. M. W., 224
Anderson, J. C., 290
Anderson, L., 127
Anderson, M. A., 253, 327, 442
Anderson, R. C., 104, 200, 210
Anderson, W. A., 342
Anderson-Prouty, A. J., 227
Andersson, L-O., 415
Andre, A., 191
Andre, F., 319
Andreoli, M., 310
Andrews, P., 392, 416
Andrianova, I. P., 160
Andrievskaya, E. A., 87
Anfinsen, C. B., 416
Angelides, K. J., 19, 343
Angyal, S. J., 10, 39, 129, 271
Anjaneyalu, Y. V., 226
Ankel, H., 274
Anno, K., 321, 390
Anselstetter, V., 322
Anstee, D. J., 281
Anteunis, M., 174, 176, 180
Anthonsen, T., 122
Antikainen, P. J., 190
Antkowiak, W. Z., 8
Antonakis K., 115, 156, 158, 159
Antosz, F. J., 134
Antus, S., 18
Aoyagi, T., 352
Arai, K., 219, 222, 223, 249
Arao, T., 336, 459
Arbilla, S., 440
Arbogast, B., 299, 331
Arcamone, F., 138
Archibald, A. R., 237, 239, 240
Arisawa, M., 42
Arita, H., 404
Armstrong, G. T., 212
Arnarp, J., 28, 215
Arneberg, P., 319
Arnett, A. T., 258
Arnheim, N., 375, 376
Arnold, W., 14, 165, 177
Arnon, R., 380, 414
Arnott, S., 233, 291
Aro, A., 301
Aronoff, S., 8
Aronson, J. M., 266, 304
Arsenis, C., 389
Arthur, jun., J. C., 189
Aruna, R. M., 399
Arvor-Egron, M.-J., 158
Asano, T., 152
Ashwell, G., 305, 311, 325, 445
Ashworth, J. M., 335
Askenasi, R., 320
Aslam, J., 221
Aspinall, G. O., 18, 115, 118
Atalla, R. H., 199, 221
Atanasiu, P., 272
Atkins, E. D. T., 291
Atkinson, P. H., 304, 308, 323
Atsukawa, Y., 368, 384
Atsumi, K., 438
Audhya, T. K., 296
Audichya, T. D., 113, 146
Aunis, D., 300
Aurebekk, B., 416
Avenel, D., 187
Avigad, G., 210, 213
Avila, J. L., 298
Awasthi, Y. C., 332
Axelrod, B., 19, 347
Axelsson, I., 290
Axén, R., 361, 430
Axerio, P., 27
Aylsworth, A., 350
Azerbaez, I. N., 144
Aziz, K., 222
Azuma, I., 245, 246
Azzar, G. J.-C., 285

Babiarz, P. S., 56
Bach, G., 391
Bachhawat, B. K., 279, 323, 398
Bachner, L., 424
Bachur, N. R., 278
Backinowsky, L. V., 251

Bacon, J. S. D., 229
Baddiley, J., 238, 239, 240, 241, 409
Bade, M. L., 363
Badurski, J., 398
Bächi, T., 317
Bäckström, G., 294
Baehler, B., 110
Baenziger, J., 315, 325
Baer, H. H., 12, 73, 124
Bagshawe, K. D., 445
Bahl, O. P., 310, 335
Bailey, R. W., 227, 228
Bailon, P., 415
Baimbridge, C. L., 21
Bakari, T. I., 256
Bakeev, N. F., 433
Baker, A. P., 325
Baker, D. A., 11, 246
Baker, D. S., 172
Baker, R. S., 253
Bakerman, S., 318
Bako, P., 33
Bal, A. K., 362
Baldo, B. A., 255, 280
Balduini, C., 296
Ball, D. B., 23
Ball, D. H., 81
Ball, W. D., 357
Ballou, C. E., 264, 265, 274
Banaszek, A., 88
Banerjee, D. K., 320, 333, 416
Banerjee, S. K., 376
Banjo, C., 303
Bánky, B., 361, 430
Bánky-Elöd, E., 218
Bannister, J. V., 335
Banoub, J., 16
Barascut, J. L., 141
Barbalat-Rey, F., 97, 173
Barclay, M., 404
Bardsley, J., 7
Barengo, R., 258, 285
Bar-Guilloux, E., 17
Barka, T., 305
Barker, S. A., 329, 367, 414, 435, 444
Barlow, J. J., 15, 20
Barnard, A. J. jun., 123
Barnett, J. E. G., 9, 127
Barnoud, F., 231
Barondes, S. H., 275, 278, 306
Barrales-Rienda, J. M., 256
Barran, L. R., 269
Barrantes, F. J., 440
Barratt, M. E. J., 296
Barrett, T. W., 298
Barry, C. D., 183
Barry, D. N., 297
Barsanti, L., 349
Bartell, P. F., 410
Bartholomew, D. G., 164
Bartling, G. J., 381
Bartnicki-Garcia, S., 263, 266, 275
Barton, D. H. R., 42, 86, 114, 170
Barton, L. L., 359
Barton, N. W., 400
Bartsch, W., 120, 121
Barwald, G., 229
Basch, A., 219
Bass, H., 318
Bastard, J., 90
Basu, D., 333, 399, 416
Basu, M., 401
Basu, S., 407
Baszynski, T., 409
Batchelor, J. R., 299
Batey, J. F., 41, 107, 234
Batrakov, S. G., 186, 398
Battarbee, H. D., 285
Batte, E. A., 334
Batyuk, V. S., 18
Bauer, H., 273
Bauer, Š, 88, 264, 265, 324
Baugh, P. J., 7
Baum, G., 342, 372, 459
Baumler, A., 303
Bause, E., 346, 449
Baxter, E., 293
Bayard, B., 320, 322
Bayramova, N. E., 17
Beachey, E. H., 241, 283
Beck, E., 102
Becker, J. W., 277
Beckey, H. D., 186
Bedetti, C., 356
Beerens, H., 263
Beg, M. M., 221
Begbie, R., 313
Begg, G. S., 375
Behre, H., 44
Behrenwald, E., 7
Belaya, Y. A., 252
Bel-Ayche, J., 219
Belcourt, A., 319
Bellisario, R., 310
Belogortseva, N. I., 186
Belokon, Z. S., 433
Belue, G. P., 227
Belyavin, G., 352
Bemporad, M., 27
Bendelow, V. M., 229
Bender, V. J., 271
Bendetskii, K. M., 358
Benitez, T., 266
Bennett, G. N., 163
Bennett, V., 306
Benraad, Th. J., 297
Benson, B. J., 312
Benson, P. F., 299
Benson, R. L., 195, 212
Bensusan, H. B., 283
Benyesh-Melnick, M., 273
Benz, H. U., 391
Benzing-Nguyen, L., 64
Ber, A., 373
Beran, K., 266
Beránek, J., 71, 156, 160
Beratis, N. G., 337
Berenson, G. S., 292, 298, 299
Berezin, I. V., 435
Bergamini, E., 285
Bergel'son, L. D., 18, 398
Bergen, B., 188
Bergenholtz, A., 296
Berger, G., 219
Berger, R. L., 212
Bergère, J. L., 258
Bergeron, R., 256
Berghem, L. E. F., 347, 449
Bergstein, P. E., 259
Beringer, V., 79
Berk, P. D., 416
Berliner, L. J., 328
Berman, H. M., 178
Bernacki, R. J., 325
Bernstein, D., 242
Berra, B., 302
Berrang, B., 19
Berthillier, G., 285
Berthoud, D., 316
Bessler, W., 280, 372
Bessodes, M., 158
Bettelheim, F. A., 297
Beulter, E., 331, 332
Bevan, M. J., 306
Beveridge, R. J., 228
Beving, H. F. G., 109
Bewick, M., 299
Beyer, C. F., 306
Bhaskar, K. R., 281
Bhatt, R. S., 64
Bhattacharjee, A. K., 117, 261
Bhattacharjee, S. S., 170, 435
Bhattacharya, G., 344
Bhavanandan, V. P., 290, 301
Bielat, K., 303
Biely, P., 264, 265, 324
Bierther, M., 439
Bihari, M., 54
Bilik, V., 5, 6
Biol, M. C., 22
Birch, G. G., 28, 29
Bird, G. W. G., 317
Birdsell, D. C., 237
Birnbaum, G. I., 188
Bischof, E., 68
Bischofberger, K., 36, 56, 113
Bishara, R. H., 194
Bishop, C. T., 14
Bissett, D. J., 210
Bissett, F. H., 81
Bister, K., 405
Bittinger, H., 229
Black, P. H., 296
Blackwell, J., 218, 222
Blandin, M., 178
Blank, G. E., 189
Blaskó, G., 297
Blau, K., 208
Bleaney, R. C., 164
Bleha, T., 173
Bless, P., 437
Bliss, C. A., 194
Bloch, A., 22, 136, 146
Bloch, K., 256
Blostein, R., 316
Blount, J. F., 136
Bloxman, D. L., 211
Blumbergs, P., 62
Bobbio, F. O., 71
Bobbio, P. A., 71
Bobek, M., 22, 142, 146
Bochkov, A. F., 5, 15, 43, 44, 45, 173
Bociek, S. M., 185, 208
Bock, K., 32, 54, 55, 181, 183
Bock-Hennig, S. B., 380
Bockish, M., 119
Bodkin, C. L., 10, 129
Boedefeld, E., 379, 450
Boeder, C., 172
Boegman, R. J., 424
Böhm, M., 288
Böniger, R., 372, 459
Boersma, A., 319
Bössler, H. M., 219
Bogdanov, I. G., 244

Bogdanova, G. V., 117
Bøg-Hansen, T. G., 358
Bognar, R., 54, 60, 75, 135
Boigegrain, R., 47, 77
Boman, H. G., 247
Bonner, T. G., 197
Bontemps, F., 287
Boone, C. W., 216
Boothby, D., 244
Borchardt, R. T., 146
Bordet, C., 245
Borén, H. B., 15
Borisova, N. P., 8
Bornstein, P., 283
Borovsky, D., 220
Borst, P., 339
Boruch, M., 190
Bose, J. L., 41
Bose, S., 28, 416
Bosmann, H. B., 307
Bostick, D. T., 211
Bouillant, M. L., 22
Boullanger, P., 91
Bouquelet, S., 311
Bourgeois, J.-M., 101
Bourne, E. J., 197, 236
Bourrillon, R., 280
Boutry, M. C., 263
Bøvre, K., 209
Bowanko, A., 377 432
Bowen, W. H., 367, 444
Bowers, B., 266
Bowers, W. E., 306
Bowness, J. M., 297, 300
Boyd, R. N., 212
Boyse, E. A., 308
Bozzini, A., 356
Bracha, R., 245
Brady, R. O., 326, 330, 331, 397, 399, 406, 461
Braidman, L., 331, 416
Brailovsky, C., 405
Brandolin, C., 424
Brandt, A. E., 295
Brandt, H., 287, 288
Brandt, I. K., 299
Brandt, J., 415
Brandt, K. D., 296
Brandt, R. B., 274
Brandtzaeg, P., 300
Brannon, J. H., 234
Brass, L. F., 283
Braun, E., 234
Braun, V., 275
Braund, K. G., 212
Brazhnikova, M. G., 135
Breckenridge, W. C., 324
Breer, H., 407
Breimer, M. E., 403
Breitmaier, E., 135
Brennan, P. J., 409, 410
Brenner, M., 335
Breslow, R., 437
Brett, M. J., 295
Bretthauer, R. K., 324
Brewer, C. F., 291
Brežný, R., 28
Briles, E. B., 240
Brimacombe, J. S., 28, 67, 100, 106, 119
Brink, A. J., 56, 68, 105
Brocas, H., 416
Brock, J. H., 238
Brodelius, P., 416
Brodersen, R., 424
Brody, S., 270
Broido, A., 33, 222
Brokeš, J., 156, 160
Brombacher, P. J., 416
Bron, C. I., 316
Broom, A. D., 164
Broquet, P., 324
Brosseau, J. D., 424
Brossmer, R., 18, 181, 191
Brown, B. D., 114
Brown, H. D., 381
Brown, H. E., 226
Brown, J. C., 306
Brown, M. C., 309
Brown, R. C., 318
Brown, R. G., 258, 366, 439
Brown, W., 325
Brownell, A. G., 282
Bruel-Groleas, A., 290
Brunfeldt, K., 158
Brunner, W. C., 191
Brunngraber, E. G., 301, 302, 404
Bryce, T. A., 233
Bryn, K., 209
Bubbar, G. L., 142
Buc, H. C., 287
Buc-Caron, M.-H., 287
Buchanan, J. G., 25, 27, 172
Buchardt, O., 39
Buchowicz, I., 316
Buchs, A., 186, 187
Buddecke, E., 282, 296, 297, 334
Budějsínský, M., 61, 66
Büyük, G., 65
Buffe, D., 313
Bugg, C. E., 188
Bujtas, G., 60
Bukowski, P., 17
Bull, A. T., 371
Bullis, C. M., 306
Bullock, C., 41, 107
Buma, T. J., 190
Bundle, D. R., 11
Buonassisi, V., 296
Buravleva, V. I., 7
Burden, I. J., 38
Burdykova, R. S., 7
Burge, B. W., 272
Burger, M. M., 277, 303, 304
Burgers, P. M. J., 161
Burgess, A. W., 422
Burnaru, R., 436
Burns, H. D., 65
Burns, R. O., 263
Burschtein, K. Ya., 43
Burton, K. A., 271
Burton, R. M., 314
Burtseva, T. I., 213
Buscher, H.-P., 212, 322
Bush, C. A., 398
Bushek, J., 56
Bussey, H., 256, 273
Butler, L. G., 19, 347
Butnaru, R., 436
Butt, N. M., 221
Butters, T. D., 308
Button, L. R., 299
Byram, S. K., 25
Byrne, H., 362
Byszewska, E., 314
Byval'tsev, Yu. A., 20
Cabib, E., 266
Cacan, M., 279
Cacan, R., 279
Cadenas, R. A., 42
Cadet, J., 142, 178
Cadmus, M. C., 271
Cael, J. J., 218
Caen, J. P., 437
Cagliani, I., 276
Calatroni, A., 302
Caldes, G., 196
Caldwell, K. D., 361, 430
Callahan, J. W., 341
Campbell, G. L., 241
Campbell, J., 385, 460
Cano, F. R., 275
Cantara, J. L., 175
Cantor, J. O., 298
Cantz, M., 299, 333
Capdevila, A., 138
Čapek, K., 32, 105
Cappuccinelli, P., 276
Capulong, Z. L., 287
Caputto, R., 403
Carbonell, R. G., 344, 435
Carceller, M., 220
Cardini, C. E., 185, 220
Carlier, A. R., 384
Carlson, D. M., 322
Carlson, S., 307
Carlstedt, I., 440
Caron, M., 416
Carr, M. E., 443
Carraway, K. L., 309, 316, 318
Carroll, F. I., 19
Carroll, M., 331, 416
Carruthers, C., 303
Carter, J. P., 398
Carter, W. A., 416
Carter, W. G., 278, 279
Carty, T. J., 287
Carubelli, R., 301
Casals-Stenzel, J., 212, 322
Casazza, A. M., 138
Casey, M., 45
Casinovi, C. G., 180
Casparie, A. F., 297
Cass, K. H., 416
Cassinelli, G., 45
Castagne, A. E., 195, 213
Castellino, F. J., 313
Castillo, F., 304
Castro, B., 47
Casu, B., 184, 208, 217, 289
Caterson, B., 294
Catlin, J. C., 178
Cavallo, G., 276
Ceccarini, C., 304, 309
Čech, D., 164
Čeh, M., 442
Celada, F., 343
Cerda, J. J., 339
Cerezo, A. S., 27
Černý, F. J., 374
Černý, M., 20, 61, 66
Chacón-Fuertes, M., 15, 40
Chairez, R., 272
Chakraborty, A. K., 231
Chalkley, R. A., 9
Chaloupka, J., 242
Chambers, J., 324
Chan, B., 344, 437
Chan, P. H., 424
Chan, S., 23

Chang, K.-J., 306
Chang, L. B., 297
Chang, M. M. Y., 221
Chanrasekharam, V., 289
Chanzy, H., 263
Chao-Min Liu, 136
Chaplin, M. F., 194, 211
Chapman, V. M., 349
Charbonnière, R., 219
Charles, M., 344, 459
Charlton, K. M., 248
Charm, S. E., 416
Charollais, E. J., 187
Chatterjee, A. N., 237
Chatterjee, G. C., 344
Chattopadhyay, S. K., 381
Chaudhari, A. S., 118
Chaudhuri, K. L., 362
Cheeseman, G. W. H., 142
Chekunchikov, V. N., 13
Chen, A. B., 314
Chen, A. L., 289
Chen, C.-M., 87
Chen, F. C., 255
Chen, G. C., 80
Chen, L. F., 360, 449
Chen, M., 84
Chen, T. C., 8
Chen, W. W., 326
Chênevert, R., 41
Cheng, T. C., 380
Chenon, M.-T., 181
Cherkasov, I. A., 364
Chernetskii, V. P., 144
Chernetsky, V. N., 15, 45
Cherniak, R., 253
Chernyak, A. Ya., 17, 38, 46
Cheryan, M., 459
Chesnut, R. W., 309
Chessen, J. H., 234
Chester, I. R., 249
Chet, I., 362
Cheveldayoff, M., 8
Chiang, T. M., 283
Chiba, T., 41, 64
Chibata, I., 416
Chien, J. L., 401
Child, J. J., 264
Chin, C. C. Q., 416
Chipman, D. M., 290, 373, 440
Chirgwin, J. M., 111
Chiron, A., 38, 46
Chitharanjan, D., 62, 95
Chittenden, G. J. F., 28
Chittenden, R. A., 49
Chiu, T. H., 240
Chizhov, O. S., 19, 38, 46, 186
Chludzinski, A. M., 386
Chmielewski, M., 81, 84
Choi, H. U., 320
Chojnacki, T., 252
Chopin, M. J., 22
Chorpenning, F. W., 238
Choy, Y. M., 254
Chrastil, J., 214
Chrispeels, M. J., 276
Christensen, A. T., 25
Christensen, J. E., 60
Christensen, L. F., 165
Christensen, T. B., 279, 300, 321
Christou, N. V., 362
Chu, B., 255
Chu, C. K., 148
Chu, C. Y. T., 351
Chu, G. H., 283
Chu, S.-H., 146
Chuba, J. V., 282
Chung, D., 428
Chursina, N. A., 69
Cifonelli, J. A., 297
Cintron, C., 20
Ciorbaru, R., 261
Cisar, J., 316
Claeyssens, M., 19, 20
Clamp, J. R., 193, 300
Clardy, J., 99
Clark, A. H., 233
Clark, C. C., 282, 283, 339
Clark, D. G., 416
Clarke, A. E., 210
Clarke, W. C., 428
Clay, M. G., 300
Clayton, C. J., 82
Clayton, S. D., 31, 148
Clegg, W., 82, 189
Cleophax, J., 62, 67
Cleve, H., 317, 318
Cleveland, L., 385
Cline, B. L., 143
Closset, J., 310
Coan, M. H., 312
Coccioli, F., 20
Cocker, D., 19
Codington, J. F., 308, 309
Cöster, L., 293
Coffman, R. E., 385
Cogliati, R., 209
Cohen, G. H., 303
Cohen, L. A., 143
Cohen, P., 287, 288
Cohn, Z. A., 334
Colbert, J. C., 4
Coleman, P. L., 406
Coles, E., 403
Coligan, J. E., 262, 302, 303
Collart, M., 323
Colli, W., 275
Collins, P. M., 35, 36, 62, 69, 93, 95
Colman, R. W., 312
Colonna, W. J., 275
Colson, P., 182
Colvin, J. R., 259, 260
Comoglio, P. M., 309
Conant, N.-T., 263
Conde, A., 188
Conigliaro, P. J., 160
Conlee, R. K., 284
Conn, P. M., 376
Connolly, D. T., 304
Conrad, M. E., 318
Constantopoulos, G., 299
Conuit, J., 298
Cook, G. M. W., 303
Cook, P. D., 143, 144
Coombs, J. P., 318
Coon, P., 385
Cooper, A., 377
Cooper, A. G., 309
Cooper, D. J., 61, 260
Cooper, H. R., 238
Cooreman, W., 355, 442
Coppa, G. V., 333, 440
Corbett, C., 283
Corley, L., 416
Cornblath, M., 399
Cornillot, P., 416
Cornoiu, I., 361, 416
Cosgrove, D. J., 46
Costantino-Ceccarini, E., 406
Costello, A. J. R., 217, 271
Cottett, C., 97
Couchman, R., 210
Coughlin, R. W., 344, 459
Couperwhite, I., 253
Courtenay, B. M., 257
Courtney, R. J., 273
Courtois, B., 261
Courtois, J. E., 231
Courtois, Y., 306
Coutsogeorgopoulos, C., 136
Couturier, D., 175
Covi, A., 349
Cowen, D. M., 334
Cowles, C. R., 173
Cowley, D. E., 139
Cox, A. J., 288
Coxon, A. C., 34
Coxon, R. V., 288
Coyette, J., 244
Coykendall, A. L., 258
Cozonne, P., 357, 449
Crain, P. F., 139, 158
Cramer, F., 165
Crane, R. K., 339
Crea, R., 161, 296
Creeth, J. M., 281
Cresswell, P., 304
Crisel, R. M., 253
Crites, S. K., 283
Croneiss, I., 178
Crook, C., 377, 432
Cross, B. P., 179
Cross, J., 283
Crouch, M. S., 299
Croxen, R. L., 375
Csuros, Z., 33
Cuatrecasas, P., 306
Culling, C. F. A., 300
Cuminge, D., 305
Cunningham, B. A., 277
Cunningham, R. L., 234
Curtis, W. D., 198
Curtius, H. C., 354
Curvall, M., 215, 254
Custardoy, V., 101
Cutler, B., 322
Cyr, N., 181, 184, 217
Cyr, T., 175
Czech, M. P., 304, 439
Czernecki, S., 88
Czichocki, G., 111
Czlonkowska, A., 399

Da'Aboul, I., 67
Dabbagh, R., 263
Dadenkova, M. N., 7
Dahl, L. F., 139
Dahlhoff, W. V., 53
Dahlqvist, A., 284
Dahms, A. S., 116
Dahr, W., 317
Da Lage, C., 289
Dalev, P. G., 244
Dalferes, E. R., 299
Dalion, J., 294
Dance, N., 331, 416
Daneo-Moore, L., 244, 246
Daniel, T. M., 261
Daniels, J. R., 283
Daniels, P. J. L., 131, 134, 137

Danielson, T. J., 194
Danileiko, V. M., 7
Danilov, B., 91
Danyluk, S. S., 178
Daoust, V., 193, 216
Daruwalla, E. H., 432
Darzynkiewicz, E., 179, 188
Das, B. C., 42, 243
Das, K. G., 186
Dasdia, T., 138
Dashunin, V. M., 44, 45, 173
Dastidar, P. G., 344
Dasu, B., 320
Datunashvili, E. I., 227
Daussant, J., 358
Dausset, J., 401
Daves, G. D., jun., 79
Davey, M. W., 416
David, S., 17, 18, 123
Davidson, E. A., 290
Davie, E. W., 312
Davies, A. M. C., 210
Davies, D. B., 178, 179
Davies, D. H., 61, 134
Davis, L., 385
Dawes, J., 209, 260
Dawson, G., 302, 320, 345
Dax, K., 111
De, K. K., 292, 430
Dea, P., 143
Deak, G., 33
Dean, A. C. R., 346
Dean, M. F., 298, 299
Dean, R. T., 334, 349
Deangelis, N. J., 199
DeBelder, A. N., 256
de Bie, M. J. A., 176, 216
Debray, H., 279
De Bruyn, A., 19, 174, 176, 180
De Bruyne, C. K., 19, 20, 278, 351
DeBusk, A. G., 273
de Carlin, M. C. L., 440
Decker, J. M., 343
Decker, R. F. H., 231
Decker, R. V., 316
Deckers, S. H. C. M., 416
Declercq, J. P., 188
de Clerq, E., 424
Dedonder, R., 336
Defaye, G., 424
Defaye, J., 17, 42, 147, 169, 172
Deferrari, J. O., 74, 174
Degand, P., 302, 319
DeGregorio, R. R., 388
Dejter-Jusynski, M., 15
Dekaban, A. S., 299
Dekker, R. F. H., 361, 372
De Las Heras, F. G., 148, 151
Delautre, M., 311
Delbaere, L. T. J., 187
De Lederkremer, R. M., 112
de Leeuw, H. P. M., 164
Deleyn, F., 20
Delmotte, F. M., 15, 379
Delpy, S., 27
De Luca, G. C., 296
De Lucas, L., 188
de Macario, E. C., 343
Dempsey, A. M., 66
Den, H., 326, 405
Denamur, R., 311
Denborough, M. A., 210
Depew, C., 140
Depmeier, W., 189
Depper, R., 213
De Prijcker, J., 351
Dergel'son, L. D., 186
Derieux, J. C., 261
de Roberts, E., 440
De Rooy, J. F. M., 164
De Rousset-Hall, A., 266
Deryabin, V. V., 186
Desai, P. R., 281
Descos, F., 319
Descotes, G., 91, 97, 435
Deslauriers, R., 182, 216
Deslongchamps, P., 41, 170
Desnick, R. J., 348
Desnuelle, P., 345
Dessein, A., 383
Destree, A., 316
Detar, C. C., 385, 459, 460
Deudon, E., 290
Deulofeu, V., 69
De Vault, R. L., 134
de Vellis, J., 424
Deverson, E. V., 316
de Villiers, O. G., 93, 117
De Vries, O. M. H., 266
Dewar, M. D., 366
De Wit, G., 7
Dey, P. M., 342
de Zeeuw, R. A., 194, 210
Di Cesare, J. L., 398
Dick, W. E., jun., 36, 37
Dickerson, A. G., 15
Dickerson, J. P., 62
Dickinson, D. B., 230
Dickinson, H. R., 398
Dickson, I. R., 314
Dieterle, W., 27
Dietrich, C. P., 291, 292, 293
Dietzler, D. N., 259
Dietzsch, B., 143
Di Ferrante, N., 298, 333, 440
Dillard, C. J., 331
Dillon, J., 190
Di Marco, A., 138
Dimick, B. E., 221
Dimitrijević, R., 236
Dimond, R. L., 335
Dimov, K., 433
Dingle, J. T., 296
Dinh, N. D., 185
Dintzis, F. R., 219
Dinya, Z., 19
Dion, H. W., 139
Di Palma, S., 302
Distler, J. J., 290, 295
Dixit, S. N., 283
Dixon, G. H., 287
Dixon, M., 342
Dixon, S. N., 208
Dizdaroglu, M., 8
Djuričić, B. M., 288
Dmitriev, B. A., 17, 38, 46, 251, 252
Dmytraczenko, A., 102
Dnistrian, A. M., 404
Doane, W. M., 79, 80, 443
Dobson, C. M., 183
Dodd, G. H., 31
Dodge, C. S., 208
Doi, A., 370
Doi, K., 370
Dolan, P., 298
Dolgopolova, N. A., 109
Dominick, H.-C., 356
Domkin, V. D., 146
Don, M., 424
Donohue, T. M., 213
Dooley, K., 103
Dorfman, A., 294, 295, 296, 299, 331
Dorman, D. E., 253
Dorner, M. M., 316
Doron'kina, O. V., 117
Doss, S. H., 177
Douglas, A. P., 300
Douglas, S. D., 281, 282
Downton, W. J. S., 220
Doyle, R. J., 237
Drach, J. C., 194
Dreesman, G. R., 272
Drewes, L. R., 270
Drewry, D. T., 250
Dreyfus, H., 407
Dreyfus, J. C., 331, 332
Driguez, H., 11, 17
Dritschilo, W., 211, 385, 459
Droege, W., 305
Dropkin, D. J., 370
Drysdale, J. W., 322
Drzeniek, Z., 317
D'Souza, R., 19, 88
Duarte, J. H., 321
Dubois, R., 305
Duckworth, M., 240, 241
Duckworth, T., 296
Ducolomb, R., 178
Ducruix, A., 188, 189
Duffy, M. J., 55
Duk, M., 281
Dull, G. G., 194, 210
Dumont, J. E., 416
Duncan, H. J., 408
Dunn, A., 376, 413
Dunn, A. D., 25
Dunn, I. J., 329
Dunn, J. T., 310
Dunn, W. L., 300
Dunstan, D. R., 416
Duong, K. N. V., 156
Duprat, F., 219
Durán, A., 266
Durante, M., 349
Dure, L. S., 138
Durham, L. J., 139
Durham, N. N., 309
Durocher, J. R., 318
Duschinsky, R., 165
Dutta, S. K., 111
Dutton, G. G. S., 192, 215
Dworak, A., 38, 179
Dybkaer, R., 327
Dymova, S. F., 51
Dyong, I., 30, 90
Dzhanakhmetova, Zh. K., 144
Dziegielewski, J., 184, 416
Dzierzkowa-Borodej, W., 317
Dziewiatowski, D. D., 283
Dzizenko, A. K., 186, 192, 216

Eagon, R. G., 249
Earl, R. A., 142
Eberlein, K., 208
Ebermann, R., 219
Eberstein, K., 31, 108
Ebert, W., 317

Ebisu, S., 262
Ebner, K. E., 311
Eby, R., 13, 412
Eckhart, W., 307
Eckstein, F., 137
Edelev, M. G., 51
Edel-Harth, S., 407
Edelman, G. M., 276, 277
Eder, H., 27
Edgar, A. R., 25, 27, 172
Edmur, L., 240
Edson, C. M., 270
Edward, M., 370
Edwards, J. R., 14
Egami, F., 390
Egan, R. S., 134
Egan, W., 180
Egli, H., 401
Ehrenberg, L., 162
Ehrlich, K. C., 292
Einstein, F. W. B., 188
Eisenberg, F., 47, 213
Eisenstein, R., 375, 437
Ejima, A., 137
Ekborg, G., 15
Eklind, K., 90
Ektova, L. V., 146
El Ashry, E. S. H., 75, 113
Elbein, A. D., 20, 274, 324, 383, 409
Elekes, I., 32
El-Kafrawy, A., 175
El Khadem, H. S., 113, 146
El'kin, Yu. N., 192, 216
Elliot, T. S. J., 239
Ellis, R. B., 330
El Saadany, F. M., 219
El Saadany, R. M., 219
El-Saied, H. M., 359
Elwell, M., 380
Elyakov, G. B., 17, 45
Emert, J., 437
Emes, J. H., 294
Emmelot, P., 308
Emori, M., 236, 271
Emoto, S., 50, 65, 136, 170, 200
Endersen, C., 238
Endo, M., 290, 299, 300
Endo, T., 30
Endres, G. F., 312
Enevoldsen, B. S., 220
Enfield, D. L., 312
Engasser, J. M., 330, 459
Engels, J., 163
Engen, R. L., 213
Englert, G., 111, 165
Engvall, E., 302
Ensley, B., 359
Entlicher, G., 278, 304
Erbing, B., 169
Erbing, C., 248
Ericsson, L. H., 312
Ericsson, T., 444
Eriksson, K.-E., 362, 370
Ermol'eva, Z. V., 364
Ermolinskii, V.-G., 9
Ernst, A. J., 443
Ernst, L., 163
Esaki, S., 15
Eschenfelder, V., 18, 181, 191
Eshdat, Y., 376, 413
Espinoza, J., 357
Essner, E. S., 404
Esterbauer, H., 92
Etchison, J. R., 212
Eto, M., 161
Eto, Y., 390
Etzler, M. E., 278
Eustace, E. J., 79
Evans, F. E., 179
Evans, J. E., 33, 399
Evans, M. E., 39
Evans, R. H., jun., 136
Evans, R. T., 242
Eventoff, W., 424
Everly, J. L., 326
Every, D., 335
Evett, M., 33, 222
Evstigneeva, R. P., 128, 129
Excoffier, G., 15, 42
Ezhov, V. N., 227

Faigle, J. W., 27
Fairclough, P. H., 50
Fan, D. P., 243
Fang, P., 227
Farkas, I., 18, 19, 54
Farmer, V. C., 229
Faulstich, H., 273
Faure, A., 97, 416, 435
Favre, S., 272
Fayolle, J., 321
Feather, M. S., 8, 66
Fedin, E. I., 256
Fedorcsak, I., 162
Fedoreeva, L. I., 19
Fedoroňko, M., 7
Feeney, R. E., 321
Feijó, M. A. L., 321
Feit, H., 301
Fell, H. B., 296
Fellous, M., 401
Felsted, R. L., 278
Felton, J., 341
Fengel, D., 228
Fenichel, L., 33
Feniksova, R. V., 342
Feres, A., 357
Fernandez-Bolanos, J., 74
Ferris, C., 277
Fetizon, M., 90
Fialkiewiczow, Z., 27
Fialová, D., 280
Fiddler, M. B., 348
Fiedler, F., 71, 241
Fietzek, P. P., 283
Filippova, T. M., 51
Finch, P., 76
Fincher, G. B., 227
Fink, A. L., 19, 343
Fischer, E. H., 287
Fischer, G., 143
Fischer, W., 410
Fishman, P. H., 397, 399, 406
Fissekis, J. D., 23
Fitch, F. W., 294
Fitzgerald, O., 356
Flawiá, M., 258
Fleet, G. H., 368
Flengsrud, R., 312
Fletcher, H. G., jun., 112, 334
Fletcher, T. F., 338
Flippen, J. L., 187
Flohr, K., 438
Florencio-Sabate, F., 188
Florent, J. C., 216
Flowers, H. M., 15, 30, 317 342, 416
Fluharty, A. L., 388, 389, 462
Foces-Foces, C., 188
Foda, Y. H., 219
Fomina, V. N., 75, 199
Fondy, T. P., 56
Fong, D. H. T., 129
Fong, J. W., 398, 400
Fontaine, M., 314
Foote, J. L., 403
Forbes, G. P., 170
Formal, S. B., 252
Forman, M., 234
Formelli, F., 138
Fornstedt, N., 280
Forrest, T. P., 175
Forsee, W. T., 409
Forsén, S., 180
Forstner, G. G., 319
Forstner, J. F., 319
Foster, A. B., 177
Foster, J. F., 424
Fouque, B., 142
Fox, C. F., 353
Fox, J. J., 54, 55, 56, 58, 136, 139, 148
Franchi, G., 138
Francis, G., 283
Frangione, B., 314
Franjić-Mihalić, I., 91
Frank, N., 254, 272
Frank, R. N., 322
Franke, W. W., 229, 403, 406
Franklin, E. C., 314
Franklin, L. E., 373
Fransson, L.-Å., 293, 295, 440
Fraser, D., 300
Fraser-Reid, B., 3, 13, 91, 92, 93, 99, 104, 170, 200
Frazier, W. A., 278, 306
Fréchet, J. M. J., 12, 30, 38
Freedman, M. L., 258
Freedman, O., 303
Freestone, A. J., 142, 151, 164
Fréhel, D., 41
Freilich, L. S., 295, 445
French, A. D., 218
Fressmann, L., 333
Freund-Mölbert, E., 372
Freysz, L., 405
Frezal, J., 401
Frgala, J., 20
Friebolin, H., 181
Fried, W., 424
Friedman, Y., 389
Friesen, H., 416
Fromme, H. G., 296
Fromme, I., 249
Frostell, G., 373
Frush, H. L., 168
Fuchs, E.-F., 112, 114
Fuertes, M., 143, 144, 151
Fukagawa, K., 271
Fukimbara, T., 360
Fuks, A., 303
Fukuda, A., 71, 408
Fukuda, H., 437
Fukuda, M., 367, 444
Fukuda, T., 169
Fukui, K., 337, 387
Fukui, S., 416
Fukui, T., 370
Fukui, Y., 337, 387

Fukunaga, Y., 296
Fukuoka, F., 236, 271, 276
Fukuyama, J., 368, 370
Fujikawa, T., 208, 234
Fujimori, S., 343
Fujimoto, M., 270
Fujimura, K., 194
Fujino, Y., 408
Fujisawa, K., 138, 312
Fujita, S., 317, 318
Fujita, Y., 280
Fuller, N. A., 247
Funabashi, M., 98, 100, 118
Funaki, Y., 127
Funakoshi, I., 303
Funasaka, W., 194
Funatsu, G., 276
Funatsu, M., 276
Funcke, W., 181
Fundiler, I. N., 43
Fung, D., 388
Furda, I., 18, 115
Furia, M., 355
Furie, B., 416
Furie, B. C., 416
Furness, G., 304
Furthmayr, H., 316
Furukawa, M., 136
Fusinato, L., 409
Futami, T., 299

Gabel, D., 422
Gadelle, A., 169, 172
Gagnaire, D., 15, 259
Gahmberg, C. G., 308, 405
Gahrton, G., 284
Gaillard, B. D. E., 228
Gal, A. E., 331
Gal, S., 220
Galanos, C., 246
Galbis, Perez, J., 74
Galbraith, L., 249, 432
Gale, D. J., 174
Gall, R. E., 114
Gallagher, J. T., 326
Galle, K., 22
Galligani, L., 296
Galmarini, O. L., 69
Galun, E., 265, 279
Gan, J. C., 310
Gander, J. E., 270
Ganfield, M. C. W., 241, 410
Ganguly, A. K., 131
Garcia, M. M., 248
Garcia, R., 408
Garcia Acha, I., 266
Garcia-Blanco, S., 188
Garcia Gonzalez, F., 74
Garcia-Munoz, G., 151
Gardiner, M., 276
Gardner, K. H., 222
Garegg, P. J., 7, 15, 90, 100
Garg, H. G., 72
Garibaldi, A., 346
Garnett, J. L., 129
Garrido, E. F., 17
Garrido, J., 307
Garves, K., 222
Gascón, S., 336
Gassmann, N., 65
Gatti, G., 184, 217
Gaudemer, A., 156
Gaudin-Harding, F., 289
Gautheron, D. C., 424
Gautier, A., 209
Gauwerky, C. H., 282
Gawronski, T. H., 365, 439
Gear, A. R. L., 288
Geier, S. S., 304
Geis, M. P., 175
Gelas, J., 36
Gelb, N. A., 296
Gelman, R. A., 319
Gelpi, M. E., 42
Gemski, P., 252
Gemzell, C., 310
Gennaro, U., 208, 289
Gensler, W. J., 23
Gent, P. A., 15, 18, 29
Gentile, B., 97, 105
Geoffroy, M., 75
Georges, M., 67
Georgoulis, C., 88
Gerbal, A., 401
Gerbaut, L., 213
Gerber, D. A., 298
Gercken, G.,
Gerecs, A., 69
Gerlach, U., 356
Gerlt, J. A., 47
Germaine, G. R., 188, 258, 386
Gero, S. D., 62, 67, 127, 180, 183
Gerrie, J., 341
Gert, E. V., 433
Gerwig, G. J., 193
Gessner, W. P., 8
Ghali, J., 170
Ghidoni, R., 398
Ghora, B. K., 362
Ghosh, P., 212, 296
Gianfreda, L., 355
Gibbons, L. N., 346
Gibbons, R. A., 208
Gibbons, R. J., 319
Giblin, A. G., 367, 444
Gibson, K. D., 296
Giebens, G., 376
Gigg, R., 15, 18, 29
Gijzen, A., 416
Gilardi, R. D., 187
Gileadi, E., 460
Gilham, P. T., 163
Gill, M. C. L., 224
Gillard, G. C., 294
Gilleland, H. E., 249
Gilliam, E. B., 309
Gillier-Pandraud, H., 187
Ginsburg, V., 72, 416, 441
Giovanoli, R., 220
Girotra, R. N., 20
Givol, D., 314
Glacet, C., 175
Glangetas, A., 186
Glaser, J. H., 347
Glaser, L., 241, 242, 387
Glaudemans, C. P. J., 117, 316
Glauser, S., 242
Glebko, L. I., 213
Glebova, Z. I., 34
Glick, H., 102
Glier, J. T., 360, 445
Gligorijević, M., 44, 103
Glimcher, M. J., 298
Glinsmann, W. H., 288
Glišin, D., 44, 103
Glöckner, W. M., 321
Glonek, T., 217, 271
Glossmann, H., 322
Gmetner, J., 249
Gnidt-Szulzycka, J., 389
Go, N., 7
Gockerman, J. P., 401
Godinot, C., 424
Godwin, R. A., 142
Gold, D., 303
Gold, P., 303
Goldberg, A. R., 277
Goldberg, M., 437
Goldberg, R. N., 212
Goldhaber, P., 298
Golding, B., T., 31
Goldmacher, V. S., 435
Goldstein, I. J., 279, 280, 302, 317
Goldstein, J., 297
Goldstone, A., 349
Golecki, J. R., 251
Golova, O. P., 87
Gomez, Z., 301
Gondo, S., 330, 451
Gonzalez, A., 101
González, D. I., 278
Gonzalez, F., 92
Gooday, G. W., 266
Goodman, L., 60
Goodrich, J. E., 194
Goodwin, J. C., 34
Gordon, A. H., 229
Gordon, J. K., 416
Gordon, S., 334
Gorin, P. A. J., 217, 398, 774
Gorshkova, R. S., 252
Gospodinov, N., 116
Got, R., 285
Goto, Y., 212
Gotthammar, B., 15, 90
Gottlieb, A. B., 416
Gottlieb, C., 325
Gould, R. O., 188
Gould, S. E. B., 188
Goulding, R. W., 193, 209
Gouyette, C., 27, 148
Gowland, G., 245
Graf, R., 160
Graffi, A., 18
Graham, I., 313
Grand, R. J. A., 369
Grandolini, G., 180
Grant, A. M. S., 416
Grant, D. M., 181
Grasdalen, H., 122
Graslund, A., 189
Graves, D. J., 287
Gray, C. J., 367, 444
Gray, G. W., 250
Gray, M. W., 139
Green, B. S., 200
Green, E. A., 188
Green, M. R., 323
Green, S., 416
Greenberg, E., 259
Greene, G. L., 30
Greenfield, P. F., 386, 459
Greenwald, R. A., 298, 375
Greeves, D., 131, 134
Gregor, H. P., 435
Gregor, I., 165
Greiling, H., 289
Gressel, J., 226
Griffin, M., 363
Griggs, L. J., 84

Grimaldi, J. J., 174, 277
Grimmonprez, L., 311
Grinberg, V. Y., 256
Grindley, T. B., 39
Grisebach, H., 102
Grønnerød, O., 284
Groome, N. P., 352
Gros, E. G., 69, 174
Gross, B., 47, 77
Gross, S. K., 33, 399
Grossman, H., 322
Grov, A., 238
Grozinger, K., 23
Gruber, E., 227, 443
Grynkiewicz, G., 71
Gsell, R., 351
Guenther, H. E., 375, 437
Guenther, H. L., 375, 437
Guerrera, J., 357, 431
Guilbault, G. G., 212, 386, 445, 460
Guilbot, A., 219
Guilliard, W., 146
Guinto, E., 331
Gunson, H. H., 317
Gupta, K. C., 8
Gupta, S. K., 430
Gupta, V. S., 142
Gurari-Rotman, D., 416
Gurd, J. W., 416
Gurevich, A. I., 244
Guschlbauer, W., 178, 191
Guseva, A. S., 51
Guss, J. M., 291
Gustafsson, B., 298
Gustavsson, R., 223
Guterman, G. E., 103
Gutz, C. G., 277
Guzman, G. M., 222
Györgydeák, Z., 75

Hackert, M. L., 424
Hackett, J. A., 409
Hackler, R. E., 122
Hackman, R. H., 437
Hadzi, D., 167
Hadžija, O., 212
Haga, M., 18, 41, 64, 79
Hageman, T. C., 312
Haglid, K. G., 301
Hahn, H., 439
Haines, A. H., 28, 41, 122, 174
Hakomori, S.-I., 281, 308, 397, 401, 405, 424
Halford, M. H., 81
Hall, C. R., 49
Hall, J., 107
Hall, L. D., 50, 56, 173, 183, 184
Hall, R. H., 36, 68, 93, 113
Hallén, A., 294
Hallgren, P., 319
Halliwell, G., 363
Halmann, M., 49
Halmos, T., 115, 156, 159
Halpern, B. C., 290
Halpern, J. A., 290
Halpern, R. M., 290
Hamada, Y., 10
Hamano, K., 249
Hamberg, U., 311
Hamers, M. N., 339
Hamerstrand, G. E., 443
Hammarström, S., 248, 279, 302
Hampton, A., 159
Hanada, K., 366
Hanahan, D. J., 312
Hanai, T., 194, 210
Hancock, I. C., 238
Hanessian, S., 16, 30, 45
Hanfland, P., 401
Hanke, D. E., 226
Hankiewicz, J., 374
Hanoune, J., 428
Hansen, J., 218
Hansen, S. A., 194, 209
Hanson, T. E., 210
Hansske, F., 165
Hanuza, J., 185
Haque, K. E., 30
Hara, A., 336
Harada, F., 139
Harada, K., 356
Harada, T., 346, 359
Harai, H., 416
Haralambie, G., 374
Harata, K., 255
Harcke, E., 245
Hardegger, E., 65
Hardie, D. G., 383
Harding, G. B., 314
Hardingham, T. E., 365
Harfouche-Obeika, C., 195
Harlander, S. K., 258
Harmon, R. E., 58, 165, 292, 430
Harpaz, N., 317, 342, 416
Harris, D. W., 8
Harris, J. A., 189
Harris, R. K., 174
Harrison, J., 324
Harrison, J. M., 49
Harrison, R., 311
Harrison, R. A. P., 460
Harrold, D., 319
Hart, D. A., 410
Hartenstein, J., 18
Hartigan, A., 14
Hartley, J. C., 344, 449
Hartmann, R., 380
Harwood, J. L., 408
Haryu, K., 270
Harzer, K., 391
Hascall, V. C., 283, 292
Hasegawa, A., 38, 60
Hasegawa, I., 274
Hasegawa, N., 38
Hash, J. H., 380
Hashimoto, Y., 123
Hasilik, A., 266
Haskins, R. H., 264
Haškovec, C., 304
Hasselberger F. X., 344, 459
Hassid, W. Z., 424
Hassner, A., 42
Hata, T., 78, 161, 165, 188, 346, 393
Hata, Y., 137
Hatae, Y., 289
Hatanaka, C., 382
Hatanaka, H., 390
Hatano, H., 184
Hatfield, G., 256
Hatton, M. W. C., 297, 313, 351, 416
Hattori, K., 157
Hattori, Y., 246
Haug, A., 234
Haug, R. H., 349
Hausman, R. E., 307
Haustveit, G., 6
Haverkamp, J., 176, 216
Havlicek, J., 209
Hawes, E. M., 194
Hawker, J. S., 220
Hay, A. J., 307
Hay, G. W., 84
Hayakawa, I., 136
Hayashi, J., 221, 260
Hayashi, O., 216
Hayes, A. W., 270
Hayes, C. E., 317
Heath, E. C., 247, 372
Heath, H. D., 443
Heckels, J. E., 238, 239
Heckendorf, A. H., 236
Hederstedt, B., 363
Hedges, A., 362
Hedrick, J. L., 321
Heerma, W., 216
Heffernan, C., 300
Hehre, E. J., 259, 412
Heidelberger, C., 142
Heikkilä, H., 223
Heinegård, D., 290, 292, 293
Heindel, N. D., 65
Heinrikson, R., 278
Hejna, C. J., 416
Helali, S. E., 65
Helgeland, K., 262
Heller, D., 143
Hellman, W., 294
Helman, J. R., 237
Hemerick, G. A., 234
Hemming, F. W., 324, 325
Hems, D. A., 284, 286
Henderson, G. B., 416
Hendricks, K. B., 11
Henis, Y., 362
Henkel, W., 101
Henkels, W.-D., 181
Henneberg, D., 8
Hennen, G., 310
Hennessee, G. L., 19
Henry, D. W., 60
Hepp, K. D., 428
Herbold, D. R., 242, 387
Hercules, D. M., 211
Herd, J. K., 373
Heremans, J. F., 314
Herman, G., 284
Heřmánková, V., 88
Hermann, T., 136
Hermelin, B., 290, 296
Hernádi, S., 227
Herries, D. G., 334
Herrmann, K. M., 116
Hers, H.-G., 287
Herscovici, J., 115
Herscovics, A., 323, 324
Hershgold, E. J., 289
Herschkowitz, N. N., 390
Herth, W., 229
Herzog, K.-H., 322
Hess, G. P., 377
Hessle, H., 303
Hetzler, C. E., 370
Hewett, M. J., 241
Hewson, K., 160
Heymer, B., 242

Heyneman, R. A., 340
Heyns, K., 107, 108, 114
Hickman, R. J., 129
Hicks, D. R., 93, 104
Hicks, K. B., 66
Hidaka, H., 360
Hierowski, M., 424
Higginbotham, J. D., 311
Higgins, M. L., 242
Higgs, S. J., 7
Highsmith, S., 290, 373, 440
Hillegass, L. M., 325
Hindenburg, A., 375, 376
Hingerty, B., 255
Hino, S., 272
Hinojosa, O., 189
Hirano, S., 74, 169, 193, 293, 322, 408
Hirayama, H., 210
Hirose, A., 239, 366
Hirsch, J., 29, 118, 177, 193, 216
Hirschberg, C. B., 410
Hirschhorn, K., 337
Hisamatsu, M., 196, 213, 217, 218
Hiura, M., 264
Hiyama, K., 365
Hizukuri, S., 220
Hjerpe, A., 297
Hjertén, S., 329, 415
Hjertquist, S. O., 291
Ho, M. W., 344
Ho, T.-L., 62
Hobbs, G., 245
Hodes, M. E., 360, 445
Hodge, J. E., 34, 36, 37, 193, 210
Hodges, C. C., 33, 222
Höltje, J. V., 238, 240
Hoenig, H., 66
Höök, M., 294, 295
Høstmark, A. T., 284, 285
Hof, H., 301
Hof, L., 69
Hoffman, J., 345
Hoffman, K. R., 250
Hoffmann-Ostenhof, O., 424
Hofman, I. L., 251
Hofreiter, B. T., 431, 443
Hofstad, T., 246, 248, 261
Hogsett, W. E., 234
Hohnjec, M., 141
Holan, Z., 266
Holcenberg, J. S., 322
Holland, J. J., 212
Hollenberg, D. H., 56, 58, 139
Holler, E., 377
Hollingdale, M. R., 248
Hollinger, G., 443
Holló, J., 361, 430, 450
Holloway, M. R., 328
Holly, S., 33
Holm, M., 403
Holman, G. D., 9
Holme, T., 373
Holmgren, J., 400
Holmquist, J., 191, 323, 353, 416
Holt, C., 221
Holý, A., 140, 164
Hon, N. S., 432
Honda, S., 195, 210, 213, 214
Hongo, M., 243
Honig, H., 41
Honjo, M., 163
Honma, T., 12
Hooghwinkel, G. J. M., 344
Hope, G. C., 346
Hopfinger, A. F., 180
Hopfinger, A. J., 294
Hopwood, J. J., 294, 296
Hořejšě, V., 413
Hori, T., 407
Horikoshi, K., 255, 368, 384
Horiuchi, S., 126
Horn, R. S., 284, 285
Hornby, W. E., 209, 385, 460
Hornez, J. P., 261
Horning, E. C., 209
Horning, M. G., 209
Horowitz, M. I., 365, 402
Horsefield, P., 296
Horstmann, H.-J., 322
Horta, A., 256
Horton, D., 36, 62, 77, 147, 172, 174, 183, 431
Horton, H. R., 460
Horvath, C., 330, 459
Horvath, G. Y., 153
Horwitz, J. P., 174
Hoschke, A., 450
Hoshi, M., 408
Hoske, H., 416
Hough, L., 41, 50, 57, 64, 66, 151, 164, 193, 214
Hough, T. L., 142
Houngbadjii, L., 294
Housset, E., 295
Houston, C. W., 351
Hovingh, P., 292
Howard, J. G., 257
Howell, D. S., 375
Hranisavljević-Jakovljevic, M., 236
Hřebabecký, M., 71
Hsu, R., 278
Huang, C. C., 416
Huang, F. L., 288
Huang, G.-F., 165
Huang, J. W., 416
Huber, J. F. K., 194
Hudgin, R. L., 325, 445
Hudson, B. G., 311, 318
Hue, L., 287
Huggins, J. W., 309
Hughes, H. N., 404
Hughes, N. A., 82
Hughes, R. C., 303, 306, 308
Huijing, F., 284
Hukins, D. W. L., 291
Hulsebos, T. J. M., 408
Hultberg, B., 336
Humbel, R., 323
Humphreys, R. E., 318
Hunsmann, G., 272
Hunt, R. C., 306
Hunt, S. M., 306
Hurford, J. R., 93, 95
Hurst, R. E., 290
Huseby, R. M., 314
Huynh-Dinh, T., 27, 148, 188
Hvoslef, J., 188
Hwang, P., 416
Hyakutake, H., 194, 210

Iacono, V. J., 262
Ichihara, A., 128
Ide, G. J., 379
Igarashi, K., 12
Ignarro, L. J., 348
Ignatov, V. V., 238
Igolen, J., 27, 188
Iinuma, F., 438
Iinuma, K., 131
Iio, M., 161
Iitaka, Y., 136, 188
Ikeda, D., 138
Ikeda, H., 308
Ikeda, K., 30, 156, 438
Ikeda, S. I., 416
Ikeda, T., 64
Ikeda, Y., 251
Ikehara, M., 153, 155
Ikekawa, T., 251, 271
Ikenaka, T., 291
Ikonne, J. U., 330
Iley, D. E., 13, 104
Il'Ina, E. F., 186, 398
Imada, K., 75
Imahori, K., 280
Imamura, T., 382
Imbach, J. L., 141, 142, 167
Imoto, T., 184
Imre, Z., 408
Inch, T. D., 49, 82
Ingerbretsen, W. R., jun., 373
Inman, D. J., 209
Inokawa, S., 85
Inomata, M., 276
Inouye, S., 21, 160, 289
Inouye, Y., 408, 440
Ioannou, P. V., 31
Ionescu-Stoian, F., 211
Ionina, S. N., 8
Irani, R. K., 41
Irimura, T., 308
Irisawa, J., 12
Iritani, N., 20
Irwin, D., 309
Irwin, L. N., 301, 397
Isaacs, R., 309
Isayama, S., 330, 451
Isbell, H. S., 168
Ise, N., 219, 222
Isemura, M., 291
Ishay, J., 373
Ishido, Y., 51, 160
Ishigami, M., 193, 293
Ishiguro, M., 276
Ishitoya, Y., 212
Ishizaki, T., 71
Ishizuka, I., 398, 407
Isono, K., 158
Itasaka, O., 407
Ito, A., 366, 416, 450
Ito, E., 266
Ito, I., 143
Ito, S., 314, 336, 408, 459
Ito, S. I., 243
Ito, T., 71, 408
Ito, Y., 274
Itoh, N., 301
Ivanov, K. K., 252
Ivanov, M. A., 8
Ivanova, E. A., 193
Ivanović, J. P., 358
Ivanovics, G., 143
Iwai, M., 380
Iwakawa, M., 75
Izumi, K., 183

Jachymczyk, W., 116
Jackson, R. C., 318
Jackson, R. L., 298
Jacquinet, J.-C., 15
Jacobs, J. W., 416
Jacobs, M., 231
Jacobson, G., 310
Jacobus, J., 180
Jaffé, W. G., 278
Jain, J. P., 382
Jain, Y. C., 402
Jakob, K. M., 226
Jakovljevic, I. M., 194
Jakubowski, H., 416
James, K., 11
James, V. J., 187
Jamieson, J. C., 314
Jancik, J., 318
Janeček, F., 102, 231
Janković-Hladni, M. I., 358
Jankowski, W., 252
Jann, B., 247
Jann, K., 246
Janota-Bassalik, L., 362
Janson, J. C., 429
Janssens, H. H. P., 416
Jansson, L., 292, 294
Jantzen, E., 209
Japelj, M., 141
Jaquet, H., 315
Jarchow, O. H., 189
Jaret, R. S., 131
Jarman, M., 84
Jarrell, H. C., 102, 140, 182
Jarvis, M. C., 408
Jarý, J., 29, 32, 88, 90, 105
Jatzkewitz, H., 389, 390
Javaid, J., 301
Jaworek, D., 329, 451
Jeanes, A., 271
Jeanloz, R. W., 17, 47, 280, 302, 308, 309, 323, 324
Jedlinski, Z., 38
Jefferson, W., 241
Jeffrey, A. M., 72, 416
Jeffrey, G. A., 187
Jegou-Aumont, E., 62, 67
Jelsma, J., 264
Jenkins, I. D., 139
Jenner, M. R., 57
Jenness, R., 392
Jennings, A. L., 377, 432
Jennings, G. C., 290
Jennings, H. J., 117, 182, 216, 261
Jennings, W. G., 209
Jensen, L. H., 187
Jensen, R. B., 39
Jermyn, M. A., 211
Ježo, I., 64
Jezowska-Trzebiatowska, B., 185
Ji, T. H., 401
Jirgensons, B., 279
Jochims, J. C., 80
Jodál, I., 36
Johansson, B. G., 302
Johar, G. S., 214
John, K., 443
Johnsen, G. S., 238
Johnson, G., 65
Johnson, K. G., 353
Johnson, M. D., 156
Johnson, N. P., 179
Jolly, R. D., 350
Jones, A S , 164
Jones, C. S., 335
Jones, C. W., 301
Jones, D. W., 188
Jones, G. H., 25, 198
Jones, I. L., 291
Jones, J. K. N., 102, 140
Jones, L. B., 80
Jones, R. G., 194, 212, 213
Jones, R. L., 227
Joniak, D., 228
Jordaan, A., 56, 68, 93, 105, 113, 117
Jordan, J., 212
Jorgensen, S. E., 39
Joseph, K. C., 401
Joseph, R., 238, 241
Jost, R., 424
Jourdian, G. W., 290, 295
Jovanović, S., 223
Jowitt, R., 7
Juláková, O., 66
Juliano, B. O., 219
Juliano, R. L., 305
Julliard, J. H., 424
Jungkind, D., 246
Junqua, S., 321
Jurczak, J., 71
Jurnak, F. A., 187
Just, G., 23, 148
Justice, P., 299

Kabat, E. A., 279, 281, 316
Kaboré, I., 216
Kadentsev, V. I., 186
Kärkkäinen, J., 212
Kaifu, R., 17, 280
Kainosho, M., 167
Kainuma, K., 220
Kaiser, E. T., 438
Kaji, E., 62
Kakehi, K., 195, 210, 213
Kakie, T., 336
Kakimoto, K., 210
Kakinuma, K., 134
Kakudo, M., 188
Kalimov, B. K., 144
Kalinevich, V. M., 15, 45
Kalinovskii, A. I., 186
Kaliss, N., 358, 431
Kalra, A. J., 18
Kalvoda, L., 136
Kam, B. L., 142, 167
Kamanabroo, D., 356
Kamenar, B., 187
Kamerling, J. P., 186, 193, 216
Kamisaku, M., 178
Kamiya, S., 15
Kamiyama, Y., 209
Kamprath-Scholz, U., 68
Kanada, K., 289
Kanada, Y., 438
Kanarek, L., 376, 451
Kanbe, M., 276
Kandler, O., 220
Kane, J. A., 262
Kaneko, M., 188
Kanfer, J. N., 334, 345, 398
Kang, A. H., 283
Kania, J., 343
Kannan, Y., 335
Kanno, C., 311
Kano, I., 339
Kano, S., 358, 372
Kappler, F., 159
Karácsonyi, Š., 119, 226
Karakawa, W. W., 262
Karasaki, S., 309
Kardos-Balogh, Z., 18
Kardošová, A., 224, 367
Kareem, H. A., 280
Karg, J., 146
Karl, W., 131
Karlsson, K. A., 400, 403, 407
Kasai, H., 139
Kasai, N., 253
Kashimura, N., 74, 108, 195, 215
Kason, C. M., 377
Katan, J., 362
Kato, H., 312
Kato, K., 191, 222, 262, 359, 366, 450
Kato, M., 273
Kato, T., 143
Katterman, R., 211
Katz, D. J., 142
Kaufman, B., 326, 407
Kaul, K., 408
Kaverzneva, E. D., 315, 387
Kawaguchi, H., 138
Kawai, K., 274
Kawai, M., 359, 381
Kawai, Y., 321
Kawakishi, S., 8
Kawanami, J., 404
Kawana, M., 50, 255
Kawasaki, T., 301, 305
Kawase, M., 276
Kawashima, K., 360, 459
Kawazoe, Y., 165, 166
Kay, I. M., 194, 211, 329, 385, 414
Keenan, R. W., 409
Keenan, T. W., 304, 397, 403, 406
Kefalides, N. A., 282
Kefurt, K., 29, 90
Kefurtová, Z., 29, 88, 90
Keilich, G., 191, 210, 229, 322
Keiser, H., 291
Keller, J. M., 292
Keller, P. J., 356, 443
Keller, R., 127
Kellerman, R., 256
Kelly, J. J., 345
Kemp, R. B., 303
Kempf, W., 226
Kemsley, K., 19
Kenne, L., 28, 215, 230, 253
Kennedy, J. F., 194, 211, 214, 298, 329, 385, 414, 435, 436, 459
Kenny, C. P., 117, 261
Kent, P. W., 326
Kent, S. P., 438
Kernohan, J. C., 288
Keys, A. J., 114
Khakhanova, T. S., 358
Khalkhali, Z., 325
Khan, D. P., 122
Khan, M. K. A., 29
Khan, M. U., 408, 409
Khan, Q. H., 221
Khan, R., 28, 38, 57, 115
Kharyanov, E. N., 226
Khodyreva, N. V., 8

Khomenko, N. A., 251
Khorlin, A. Ya., 60
Khuong-Huu, Q., 216
Kia'ng, S. W., 212
Kiburis, J., 177
Kidby, D. K., 336
Kidroni, G., 273
Kieboom, A. P. G., 7, 199
Kiely, D. E., 64, 124
Kieras, F. J., 290, 293
Kihara, H., 388, 389, 462
Kijima, H., 7, 345
Kikuchi, H., 349
Kikuchi, T., 269
Kikuchi, Y., 437, 439
Kikugawa, K., 143
Killilea, S. D., 288
Kim, C.-B., 146
Kim, J. C., 146
Kim, U., 303
Kim, Y.-B., 343
Kim, Y. S., 309
Kimble, B. K., 257
Kimura, J., 123
Kimura, M., 65
Kindinger, J. I., 230
Kindt, T. J., 262, 416
King, M., 319
King, R. R., 14
Kingsbury, C. A., 173
Kinoshita, M., 203, 204
Kinoshita, T., 289, 438
Kirino, Y., 119
Kirk, K. L., 143
Kirkacharian, B. S., 195
Kirsch, A., 129
Kirschbaum, J., 282
Kirwan, D. J., 460
Kishore, G. S., 301
Kisiel, W., 312
Kislov, M. B., 412
Kiso, M., 60
Kiss, J., 14, 19, 64, 88, 118, 177
Kiss, L., 88
Kisseleva, V. V., 387
Kitahara, K., 156, 165
Kitahara, N., 249
Kitahara, T., 118
Kitahata, S., 358, 386, 413
Kitamura, M., 356
Kitano, S., 178
Kitchen, B. J., 392, 416
Kito, Y., 8
Kittrell, J. R., 386, 459
Kivrikko, K. I., 282
Kiyohara, T., 352, 416
Kjosbakken, J., 259
Kleefeldt, L., 30
Klein, R. S., 54, 56, 148
Kleine, R., 315
Kleinschmidt, A. K., 294
Klemer, A., 30, 93, 181
Klibanov, A. M., 435
Klibansky, C. H., 345
Klier, K., 256
Klimov, E. M., 15, 17, 33, 412
Kloppenborg, P. W. C., 297
Knapp, A., 405
Knirel, Y. A., 251, 252
Knoll, W. M. J., 134
Knollmann, R., 90
Knopf, P. M., 316
Knox, K. W., 240, 241
Knufermann, H., 372
Knutson, C. A., 208
Ko, A. M. Y., 193, 214
Koaze, C. Y., 360
Kobata, A., 210, 281, 314, 336, 397
Kobayashi, A., 336
Kobayashi, R., 368, 369, 371
Kobayashi, T., 20, 231
Kobayashi, Y., 161
Kobe, J., 141, 167
Koch, A. L., 342
Koch, H. J., 181
Koch, K. F., 131
Kochetkov, N. K., 13, 15, 17, 19, 33, 38, 44, 45, 46, 47, 173, 186, 251, 252, 412
Kocourek, H., 413
Kocourek, J., 238, 278, 280, 304
Koebernick, H., 27, 177
Koekemoer, J. M., 200
Köll, P., 27, 107, 108
Koeltzow, D. E., 252
Koenig, H., 301, 349
Koenig, J. L., 218
Koenig, W. A., 135
Königstein, J., 7
Köster, R., 53
Koetz, R., 8, 215
Koga, T., 273
Koga, Y., 193
Kogure, T., 281
Kohama, T., 270
Kohno, H., 62
Kohoutova, M., 238
Koide, N., 303
Koizumi, J., 289
Koizumi, O., 345
Kojima, H., 407
Kokrady, S. S., 147
Kolb, A., 27, 148
Kolb, H. J., 428
Koleva, M., 230
Kolka, S., 71
Kolodkina, I. I., 193
Kolodny, E. H., 461
Kolomyitseva, E. N., 165
Kolosov, M. M., 244
Komaki, T., 219, 370, 442
Komura, H., 51
Kondo, L. E., 209
Kondo, S., 131, 177
Kondo, Y., 408
Konecny, J., 327, 451
Konigsberg, W. H., 314
Konigstein, J., 102
Konishi, F., 15
Konishi, N., 204
Konovalov, S. A., 370
Korableva, N. P., 346
Korbukh, I. A., 141, 142, 143
Korchagina, G. T., 358
Korman, R. Z., 245, 262
Kornblatt, M. J., 404, 405
Kornfeld, R., 277
Kornfeld, S., 315, 325
Kornilov, A. N., 86, 93
Kornilov, V. I., 90
Korol'kov, I. I., 9
Koroteev, M. P., 47
Korsch, B., 181
Korus, R. A., 330, 451
Koshland, M. E., 316
Košík, M., 117, 228
Košiková, B., 228
Koskela, P., 289
Kosman, D. J., 335
Kosov, Yu. Yu., 13
Kostelian, L. I., 87
Koszalka, T. R., 282
Kotani, S., 262, 273
Kotelko, K., 249
Kothbauer, H., 280
Koto, S., 10
Kotowycz, G., 167
Koubek, I., 442
Kováč, P., 28, 29, 117, 118, 177, 186, 193, 213, 216
Kováčik, V., 29, 118, 186, 216
Koval, S. F., 250
Kovář, J., 73, 124
Kowollik, G., 156
Kozlov, N. A., 433
Kozlov, P. V., 433
Krantz, M. J., 213
Krasnobajew, V., 372, 459
Krasnokutskaya, D. M., 142
Krátký, Z., 264, 265, 324
Krause, E.-G., 288
Krause, R. M., 242
Kravchenzo, N. A., 364
Křečková, P., 242
Kreger, D. R., 264
Kremer, B. P., 194, 214
Kresse, H., 296, 297, 332, 333
Krestianov, V. Y., 238
Kriemler, H.-P., 27
Krishnamurthy, T. N., 18, 115, 118
Krishnamurti, M., 18
Krisman, C. R., 258, 285
Kristiansen, T., 414, 416
Kritzyn, A. M., 140
Krivit, W., 399
Krolikiewicz, K., 141, 143
Kruczek, M., 409
Krueger, W. C., 134
Krupa, Z., 409
Krupenskii, V. I., 9
Kuan, J. W., 212
Kubačková, M., 226, 231
Kubala, J., 224, 367
Kubota, H., 436
Kubota, Y., 136
Kučár, Š., 88
Kudryashov, L. I., 19, 66
Kuduk-Jaworska, J., 185
Kühbauch, W., 229
Kühlwein, H., 245
Künstler, K., 181
Küster, J. M., 30
Kuettner, K. E., 375, 437
Kuge, T., 431
Kuhl, W., 331
Kuhne, H., 120
Kuhns, W. J., 282
Kujawski, M., 371
Kuksi, A., 408
Kula, M.-R., 163
Kulikova, A. K., 342
Kulinkovich, L. N., 141
Kulonen, E., 353
Kulp, K., 329
Kulpa, C. F., 247
Kulshreshtha, A. K., 436
Kulyaeva, V. V., 134
Kumar, A., 7

Kume, T., 379, 450
Kundu, S. K., 398, 402
Kunimoto, S., 352
Kuninaka, A., 270
Kunst, F., 336
Kuntz, G. P. P., 167
Kuo, C.-H., 20
Kuppel, A., 229
Kuramitsu, H. K., 258, 387
Kurono, G., 42, 236
Kurtz, M. J., 295
Kusakabe, I., 20, 231
Kushida, H., 296
Kusumoto, S., 203
Kusunoki, K., 330, 451
Kuszmann, J., 153
Kutnevich, A. M., 9
Kuusela, P., 305
Kuznetsova, Z. P., 129
Kuzovkov, A. D., 135
Kuzuhara, H., 65, 136, 170
Kwak, K. S., 281
Kwan, T., 119
Kwiatkowski, B., 392

Låås, T., 429
Labbé, J. P., 274
Lacombe, M.-L., 428
Lacorbiere, M., 307
Lagrue, G., 341
Lahoda, E. J., 386, 459
Lai, D., 301
Lai, H.-Y. L., 19, 347
Lai, Y.-Z., 108, 111
Laidlaw, J., 283
Laidler, D. A., 198
Laidler, K. J., 459
Laine, R. A., 397, 398, 401, 409, 424
Lais, C. J., 259
Laishley, E. J., 258
Lalley, P. A., 333, 349
Lallier, R., 405
Lambert, B. E., 373
Lambert, P. A., 238
Lamblin, G., 302, 319
Lamed, R., 424
Lamm, M. E., 315
Lampen, J. O., 275
Landgraf, H. R., 410
Landmann, L., 289
Lando, J. B., 437
Landolfo, S., 309
Lange, S. M., 139
Langen, P., 156
Langenbach, R., 405
Langworthy, T. A., 248
Lapanje, S., 376
Lapuk, V. A., 315
Larm, O., 27, 253
Larner, J., 286
Larraga, V., 274
Larsen, B., 122
Larsen, L. H., 212
Larsson, K., 34
Larsson, S. E., 291, 295
Laseter, A. G., 160
Lassaga, F. E., 403
László, E., 361, 430, 450
Latimer, S. B., 351
Lau, P.-Y., 99
Laumonier, R., 294
Laurent, M., 306
Laurent, T. C., 298
Lauterbach, J. H., 174
Lauwers, A., 355, 442
Lavallee, P., 30
Lavintman, N., 185, 220
Law, A. R., 194, 213
Lawhead, C. L., 194
Lawrence, J. C., jun., 304, 439
Lazarides, E., 416
Lazarova, P., 116
Lazdunski, M., 328
Lea, E. E., 14, 40
Lea, E. J. A., 282
Leaback, D. H., 334
Leaver, C. J., 114
Leavitt, R. D., 278
Leblond-Larouche, L., 309
Leboul, J., 62, 67
Leckie, M. P., 259
Ledeen, R. W., 398, 400, 402, 461
Leder, I. G., 391
Lederer, E., 261
Le Dizet, P., 231
Lee, A. S. K., 163
Lee, E. E., 14, 40
Lee, E.-S., 146
Lee, E. Y. C., 287, 288
Lee, C.-H., 179
Lee, C.-K., 169
Lee, H. Y., 266
Lee, R. I., 65
Lee, Y. C., 18, 20, 65, 213
Leech, R. M., 409
Lee-Own, V., 298
Lee, W.-L., 270
Lee, W. W., 60
Lefebvre-Soubeyran, O., 188
Legaz, M. E., 312
Leger, G., 449
Legler, G., 346
Legrand, Y., 437
Lehle, L., 325
Lehman, E. D., 311
Lehmann, J., 80, 112, 114, 343
Le-Hong, N., 27
Lehrfeld, J., 40
Leibowitz, S., 399
Leive, L., 247
Lelievre, J., 218
Lemieux, R. U., 11, 38
Lemos, E. M., 306
Lemperg, R., 291, 295, 296
Lenarčič, S., 376
Lenhoff, H. M., 436
Lennarz, W. J., 242, 248, 323, 324, 326
Leonard, G. J., 256
Leonenko, V. A., 364
Lepesant, J. A., 336
Leppard, G. G., 260
Lequin, R. M., 310
Lerch, T. J., 287
Lerner, L. M., 6, 88, 140, 150, 151
Lesage, S., 92
Lessin, S., 335
Lester, R. L., 408
Letham, D. S., 139
Letsinger, R. L., 30
Leung, S., 408, 409
Levai, A., 190
Levenberg, M. I., 134
Levi, Sh., 116
Levine, M., 404, 405
Levine, M. J., 262
Levitan, G. E., 109
Levitzki, A., 356, 449
Levy, A., 278
Levy, P., 296
Lewin, M., 219
Lewis, A. W., 41
Lewis, D., 197
Lewis, G. J., 49
Lewis, P. W., 298
Lewis, R. G., 295
Li, C. H., 428
Li, L. H., 134
Li, S. C., 400, 403
Li, Y.-T., 302, 400
Liao, J., 316
Liau, Y. H., 365
Liberti, J. P., 404
Lichtenthaler, F. W., 131, 136, 156, 165
Liefländer, 322
Liehr, J. G., 139
Lienhard, G. E., 377
Liga, A., 233
Lilley, G., 371
Lim, T. W., 298, 391
Lin, C., 347
Lin, L. J., 424
Lin, T., 165
Lin, T.-S., 58, 160
Lind, J., 363
Lindahl, U., 292, 294, 295
Lindberg, A. A., 250
Lindberg, B., 7, 28, 169, 215, 253, 254, 258, 366
Lindberg, K. B., 188
Lindberg, U., 416
Linden, J. C., 194, 210, 220
Linder, L., 373
Lindley, M. G., 28, 29
Linker, A., 292
Linkies, A., 114
Linko, M., 359, 435
Linko, Y.-Y., 359, 435
Linsley, K. B., 308
Linzer, R., 262
Lipták, A., 17, 28, 36, 88, 190
Lis, H., 279
Lisker, N., 362, 381
Lisman, J. J. W., 344
Lisowsita, E., 317
Lisowska, E., 281
Litter, M. I., 112
Little, C., 416
Litwiller, R. D., 194
Liu, C. C., 386, 459
Liu, H. H., 20
Liu, I. Y., 323
Liu, T. Y., 255
Llewellyn, J. W., 32, 66
Lloyd, C. W., 303
Lloyd, K. O., 270, 271
Lodi, W. R., 306
Lönngren, J., 15, 28, 215, 254
Lönnroth, I., 400
Løutrup, S., 294
Löwendahl, L., 115, 223
Lofroth, G., 189
Logardt, I. M., 244
Logue, M. W., 156
Loh, T.
Lohmander, S., 293, 294, 297
Lokshin, G. B., 135
Lomakina, N. N., 135

Lombardo, A., 351
Lombart, C. 213
Lonchampt, M.-O., 306
Londesborough, J. C., 311
Long, L., jun., 81
Long, M. A., 129
Longchambon, F., 187
Loomis, W. F., 335
Loontiens, F. G., 278
Lorans, G., 295
Lotan, R., 265, 279
Lott, J. A., 211
Louisot, P., 324
Lourens, G. J., 117, 200
Louvard, D., 345
Lowther, D. A., 294, 295
Lubineau, A., 18
Lucas, J. J., 324
Lucas, T. J., 13
Ludlow, J. C., 460
Luedemann, H. D., 178, 184
Lüderitz, O., 47, 246, 252, 305
Luessow, K. F., 7
Luetzow, A. F., 109
Luger, P., 188
Luisi, P. L., 379, 450
Lukacs, G., 180
Lukevits, O. K., 174
Lukin, O. V., 63
Lundblad, A., 319
Lundblad, G., 363
Lundt, I., 54
Lunney, J., 305
Lusebrink, T. R., 179
Lust, W. D., 283
Luttenberger, F., 280
Luyten, W. C. M. M., 161
Luzzati, D., 306
L'vov, V. L., 251
Lyle, L. R., 304
Lynn, W. S., 304, 439
Lyutik, A. I., 128

Ma, G. Y., 284
Macario, A. J. L., 343
McBride, W. H., 209, 260
McBride-Warren, P. A., 387
McCabe, M., 298
McCabe, M. M., 258
McCallum, M. F., 253
McCleary, B. V., 230
McCloskey, J. A., 139, 156, 158, 187
McCloskey, J. T., 223
McCluer, R. H., 33, 399
McComb, E. A., 6
McCombie, S. W., 42
McCune, J. M., 318
McDaniel, M. L., 237
McDonald, I. J., 353
McDonnell, M. ,239
McGeeney, K. F., 355, 356, 357, 416, 442
McGinnis, G. D., 227
McGinnis, J. F., 424
McGlothlin, C. D., 212
McGuire, E. J., 326
Machida, Y., 256, 435
Machigaki, E., 380
Machon, Z., 147
Machovich, R., 297
McHugh, J. J., 359
McKay, K. A., 248
Mackay, M. F., 188
McKelvey, H., 339
Mackender, L. R. O., 409
Mackenzie, G., 146
McKibbin, J. M., 400, 403
MacLachlan, G. A., 224, 362
Maclaren, N. K., 399
MacLaughlin, D. T., 314
McLendon, J. H., 226, 381
MacLeod, J. K., 139
McMorris, F. A., 406
McNab, C. G. A., 233
McNiel, M., 227
Macri, A., 355
McWilliams, M., 315
Maddox, M. L., 25
Madelian, V., 424
Madhosingh, C., 269
Madsen, G. B., 358
Madumelu, C. B., 73
Maeda, M., 165, 166
Maestre, M. R., 191
Maeta, T., 300
Magar, N. G., 219
Maghuin-Rogister, G., 310
Magnani, J. L., 259
Magnuson, J. A., 277
Mahajan, K. P., 56
Maher, G. G., 443
Mahler, H. R., 416
Mahley, R. W., 325
Mahmood, S., 100
Mahuzier, G., 195
Maijs, L., 174
Main, J. S., 249
Majoor, C. L. H., 297
Majumder, A. L., 47
Majumder, G. C., 334, 335
Majumder, P. K., 344
Makinoda, M., 336
Makita, A., 289
Malaisse, W. J., 214
Maley, F., 315, 326, 336, 445
Malinen, R., 19, 187, 215
Mal'kova, V. P., 244
Mallams, A. K., 61
Malmendier, C. L., 314
Malmström, A., 293, 295
Maloy, J. T., 386, 459
Maluszynska, G. M., 362
Malysheva, N. N., 17, 33
Mandel, P., 300, 405, 407
Mankowski, T., 252
Manley, R. J., 260
Manners, D. J., 368, 383
Mannik, M., 423
Manoucheri, M., 223, 229
Manscher, O., 158
Månsson, J. E., 400, 403
Mansy, S., 166
Marc-André, L., 214
Marchalonis, J. J., 306
Marchesi, V. T., 316
Marchessault, R. H., 263, 443
Marchis-Mouren, G., 357, 449
Marcus, D. M., 400, 401, 461
Margolis, R. K., 297, 301
Margolis, R. U., 297, 301
Mariat, F., 267
Maritz, V. M., 210
Mariyama, S., 203
Markey, P. E., 386, 459
Markham, J. L., 241
Markwalder, H., 230
Maroteaux, P., 299
Maroux, S., 345
Márquez, R., 188
Marsden, N. V. B., 256, 438
Marsh, J. J., jun., 60
Marsh, R. E., 188
Marshall, A. G., 416
Marshall, J. J., 215, 329, 361, 369
Marshall, R. D., 325, 416
Martel, A., 23
Martin, A., 261
Martin, J.-C., 91, 97, 435
Martin, M. A., 436
Martin, O., 27
Martinel, K., 435
Martinez, E., 101
Martín-Lomas, M., 15, 40
Martynov, V. S., 142
Marumoto, R., 163
Maruo, B., 359
Maruyama, T., 153
Marx, F., 119
Marx, G., 297
Marx-Figini, M., 260
Maslinska-Solich, J., 38
Mason, R. D., 460
Masson, F., 245
Masson, P. K., 330
Masters, C. J., 424
Masubuchi, M., 301
Masuda, M., 300
Mašura, V., 222
Matalon, R., 299, 320, 331, 388
Mateescu, M. A., 361, 416
Matern, U., 102
Matheson, N. K., 230
Mathews, C. H., 229
Mathewson, N. S., 213
Matoth, Y., 345
Matsuda, K., 151, 270
Matsuda, T., 321
Matsuhashi, M., 239
Matsui, M., 60, 118, 141
Matsumura, G., 291, 367, 444
Matsuzaki, K., 436
Matsuzawa, T., 336, 459
Matta, K. L., 15, 20
Matthews, B. W., 380
Matthews, T. R., 144
Matthieu, J.-M., 326
Matthyssens, G. E., 376, 451
Mattox, V. R., 194
Matuo, Y., 416
Matz, L. L., 411
Matzuzawa, M., 255
Max, S. R., 399
Mayberry, W. R., 248
Mayer, H., 60
Mayer, R. M., 258
Mazliak, P., 408
Mazurek, M., 180
Mazza, G., 276
Mazza, J. C., 14
Meadow, P. M., 249, 250
Medcalf, D. G., 234
Medved, Z. N., 433
Megarry, M. L., 236
Mehrishi, J. N., 359
Mehrotra, R. N., 7, 8
Meier, A., 65
Meisler, M., 341
Melcher, U., 315, 424
Melchers, F., 316

Meldrum, J. B., 142
Mell, L. D., 386, 459
Melnick, J. L., 272
Mel'nik, S. Ya., 146, 162
Melvaer, K. L., 262
Menard, D., 339
Mendiara, S., 220
Menyhart, M. M., 54
Menzel, H. M., 131
Mercer, J. M., 344, 437
Merchant, Z., 76
Mercier, C., 219, 220, 258
Mercier, D., 67, 127
Merész, M., 153
Merlis, N. M., 87
Mersmann, G., 334
Mescher, M. F., 273
Mesentsev, A. S., 134
Meshreki, M. H., 431
Mester, L., 65
Mester, M., 65
Mestres, R., 101
Metlitskii, L. V., 346
Metzger, R. P., 334
Meyborg, H., 44
Meyer, C., 424
Meyer, F. A., 319
Meyer, K., 320
Meyer, R. B., 143
Meyer, R. B., jun., 163, 166
Meyer zu Reckendorf, W., 68
Mian, A. M., 143
Mian, N., 334
Michalska, M., 47
Michalski, J., 47
Michel, G., 245
Michel, J. P., 219
Michelacci, Y. M., 291
Michell, A. J., 222
Michishita, K., 42
Mickey, C. D., 21
Mićović, V., 236
Midtvedt, T., 349
Mifuchi, I., 246
Mihaly, E., 75
Mikami, K., 98
Mikhailopulo, I. A., 141, 159
Mikheyskaya, L. V., 236
Milanović, M. P., 358
Milas, M., 226
Milecki, J., 8
Milijković-Stojanović, J., 236
Miljković, M., 44, 103
Miller, A., 343
Miller, A. L., 299, 337
Miller, D. C., 50
Miller, F., 303
Miller, J. A., 106
Miller, J. B., 278
Miller, J. N., 277
Miller, J. P., 163, 166
Miller, R. T., 389, 462
Miller, R. W., 269
Millett, F., 375
Mills, J. A., 39
Milstein, C. P., 316
Milyukin, M. V., 7
Mimaki, K., 339
Minakova, A. L., 345
Minamoto, K., 152, 157
Miniker, T. D., 142
Minnikin, D. E., 409
Minor, R. R., 282
Minshall, J., 100
Miras-Portugal, M.-T., 300
Mirelman, D., 245, 265, 270
Mirnyi, V. N., 199
Misaki, A., 262
Mitchell, A. J., 185
Mitchell, E. D., 351
Mitchell, J., 218
Mitsunobu, O., 123
Mitura, W., 118
Mitzner, R., 7
Miura, S., 301
Miyahara, T., 283
Miyake, A., 275
Miyake, M., 152
Miyatake, T., 338, 341, 404
Miyawaki, M., 289
Miyawaki, T., 299
Miyazaki, T., 216, 267, 269
Mizuno, Y., 30, 156, 178
Mo, F., 187
Mochalin, V. B., 86, 93
Moczar, E., 413
Moennig, V., 272
Moffatt, J. G., 25, 59, 96
Mohammed, J., 227
Moisy, M., 341
Mokrushin, V. S., 142
Molasky, D., 314
Molenaar, J. L., 339
Mollard, A., 231
Møller, I., 218
Molodtsov, N. V., 19, 350
Mompon, B., 42
Monaco, F., 310
Monaco, G., 310
Monbaliu-Bawens, D., 340
Monner, D. A., 247
Monneret, C., 216
Mononen, I., 212
Monro, J. A., 227
Monsey, J. B., 320
Monsigny, M. L. P., 15, 379
Montgomery, J. A., 31, 141, 148, 160
Montreuil, J., 311, 320
Mookerjea, S., 325
Moonim, S. M., 432
Moor, J., 15, 187, 216
Moore, J. S., 19
Moore, P. M., 266
Moorhouse, R., 291
Moo-Young, M., 328
Mora, P. T., 326, 406
Moragues, M. J., 17
Morais, R., 309
Moreau, C., 41
Morel, E., 274
Morelis, R., 324
Morell, P., 406
Moreno, E., 188
Moreno, F., 336
Morgan, E. H., 305
Morgan, F. J., 375
Morgan, H. R., 307
Morgenlie, S., 36, 37, 192, 209
Morgenstern, M., 237
Mori, M., 18
Morikawa, H., 226
Morikawa, K., 188
Morino, T., 131, 136
Morishima, I., 408
Morita, H., 345
Morita, N., 42
Moriya, N., 299
Moriyama, T., 337, 387
Morozova, E. V., 346
Morr, M., 163
Morré, D. J., 304, 397
Morrell, A. G., 311
Morris, D. L., 385, 460
Morris, E. J., 229
Morris, E. R., 191
Morris, H. P., 288
Morris, J. G., 259
Morrison, D. C., 247
Morrison, I. M., 192, 217, 228
Morrison, K. E., 314
Morrod, P. J., 416
Mort, M., 50
Morton, J., 131, 134
Mory, H., 27
Mosbach, K., 416, 460
Moscona, A. A., 307
Mosher, C. W., 60
Mossesson, M. W., 314
Motycka, L., 373
Moulik, S. P., 122
Mowery, D. F., jun., 10
Mraz, W., 390
Mršulja, B. B., 285, 288
Mudd, J. B., 408
Mühlradt, P. F., 251
Mueller, A., 178
Müller, H. E., 352
Mueller, S. L., 134
Müller-Hill, B., 343
Mufti, K. S., 38, 57
Muhlrad, A., 424
Muir, H., 293, 298, 299, 365
Mukai, S., 208
Mukasa, H., 262
Mukherjee, A. K., 224
Mumford, R. A., 345
Munasinghe, V. R. N., 35
Munavu, R. M., 35
Munday, K. A., 9
Munns, T. W., 194
Muñoz, E., 274
Munro, M. H. G., 138
Murai, A., 283
Murakami, K., 433
Murakami, M., 52
Murakami, T., 87
Muramatsu, T., 303, 304, 314, 336
Murao, S., 380
Murase, K., 52
Murat, J. C., 208
Murata, K., 290
Murgia, E., 212
Murphy, V. G., 218
Murray, A. W., 119
Murray, J. B., 416
Murray, R. K., 326, 405, 406
Mushika, Y., 162
Mushran, S. P., 8, 118
Musolan, C., 230
Muzzarelli, R. A. A., 437
Myers, T. D., 284
Myher, J. J., 408
Myklestad, S., 234
Myllylä, R., 282
Mynott, A. R., 7

Naccarato, W. F., 20
Nachbar, M. S., 282
Nader, H. B., 293
Nadler, H. L., 388

Nadzhimutdinov, S. H., 435
Nagabhushan, T. L., 131, 134, 137
Nagai, T., 435
Nagai, Y., 408
Naganawa, H., 131
Naganuma, T., 118
Nagasaki, S., 368, 369, 370, 371
Nagasawa, K., 281, 440
Nagata, Y., 277, 359
Nagayama, H., 362
Nagel, J., 164
Nagel, S. C., 221
Naiki, M., 400, 401, 461
Naito, T., 138
Nakabayashi, T., 260
Nakada, H., 303
Nakae, T., 245
Nakagawa, A., 180
Nakagawa, H., 336, 459
Nakagawa, I., 78
Nakagawa, S., 138
Nakahara, M., 249
Nakahara, T., 408
Nakai, C., 286
Nakai, S., 377
Nakajima, T., 264, 265, 274
Nakamura, N., 255
Nakamura, T., 9, 20, 87
Nakanishi, K., 190
Nakashima, K., 234
Nakashima, Y., 298
Nakatsuka, S., 226
Nakayama, T., 356
Nakazaki, N., 160
Nakazawa, K., 293, 335, 367
Namiki, M., 8
Nanasi, P., 17, 28, 36, 88
Nanjo, M., 386, 445
Nanno, S., 276
Naoi, Y., 267
Narasimhan, S., 325
Narayanan, P., 178
Naroska, L., 211
Nassr, M. A. M., 75
Natelson, S., 211
Naumova, I. B., 265
Naves, R. G., 168
Navia, E., 357
Navon, R., 330
Navratil, J. D., 212
Necco, A., 138
Nedeltscheva, M., 218
Nedoborova, L. I., 19
Needleman, S. B., 301
Nelboeck, M., 451
Nelboeck, N., 329
Nelson, T. E., 288, 369
Neri, A., 304
Neri, G., 279, 309
Nesawibatko, W. N., 191
Nesbitt, L. R., 264
Neskovic, N. M., 407
Nesmeyanov, V. A., 15
Nesnow, S., 142
Neszmélyi, A., 180
Neuberger, A., 416
Neufeld, E., 298
Neufeld, E. F., 391
Neujahr, H. Y., 244
Neukom, H., 8, 215, 230
Neuman, A., 187
Neurath, H., 312
Neville, D. M., 322
Nevins, D. J., 229
Newman, A. D., 277
Newman, A. R., 144
Newman, R., 311
Newman, R. A., 321
Newton, D. J., 289
Nexø, E., 424
Nghiêm, H. O., 251
Ngo, T. T., 459
Niall, H. D., 416
Nichol, L. W., 298
Nickel, J. B., 357, 431
Nicolson, G. L., 307
Niebes, P., 289
Niedballa, U., 140, 141, 143
Niedieck, B., 399
Nieduszynski, I. A., 291
Nielsen, J. U. R., 39
Niemann, H., 254
Niemczura, W. P., 62
Nifant'ev, E. E., 47, 160
Nigam, V. N., 309, 405
Nigrelli, R. F., 282
Niida, T., 360
Nikaido, H., 245
Nikitin, V. M., 9
Nimmich, W., 254
Ninomiya, E., 366
Nirkko, P., 220
Nishikawa, A. H., 415
Nishikawa, Y., 42, 236
Nishikaze, O., 348
Nishimura, S., 139
Nishimura, T., 163
Nishimura, Y., 137
Nishiyama, S., 64
Nisizawa, K., 230
Nitta, K., 376
Niwa, T., 360
Noack, K., 19
Nógrádi, M., 18
Noguchi, K., 135
Noguchi, T., 274
Nokin, P., 411, 416
Nolan, R. A., 362
Noltmann, E. A., 111
Norberg, T., 100
Norden, A. G. W., 336, 444
Norman, B. E., 358
Norris, F. A., 163
Norris, K. E., 158
Northcote, D. H., 226
Nosova, V. V., 161
Notkins, A. L., 357
Novellie, L., 342
Novikova, L. E., 45
Novotny, A. M., 234
Nowak, T. P., 280
Nozawa, Y., 274
Nunez, J., 285
Nunn, J. R., 234
Nusbacher, J., 282
Nwokedi, G. I. C., 277
Nyberg, L., 310

Obata, Y., 308
O'Brien, E., 40, 41
O'Brien, J. S., 299, 336, 337, 444
Obruchnikov, I. V., 15
Ochoa, A. G., 336
O'Colla, P. S., 14
Oda, K., 128
Oda, N., 143
O'Donnell, M. D., 355, 356, 357, 416, 442
O'Driscoll, K. F., 330, 451
Öckerman, P. A., 336
Oeding, P., 238
Oegema, T. R., 283
Ögren, S., 292
Oemrawsingh, I., 319
Oeltmann, T. N., 372
Ötvös, L., 32
Ofek, I., 241
Ofitserov, V. I., 142
Ogamo, A., 281
Ogata, S., 243
Ogata-Arakawa, M., 303
Ogawa, S., 127
Ogawa, T., 60, 118, 141
Ogawa, Y., 273, 390
Ogiso, T., 366, 450
Ogiso, Y., 153, 155
Ogiwara, Y., 219, 223, 436
Ogletree, J., 385, 450
Ogura, H., 149
Ogura, M., 270
Ogura, N., 336, 459
Ohannessian, J., 187
Ohashi, Y., 49, 188
Ohashi, Z., 139
Ohba, R., 358
Ohe, Y., 193, 322
Ohki, E., 49, 138
Ohki, K., 236
Ohnishi, A., 191, 222
Ohrui, H., 25, 54, 200
Ohta, G., 136
Ohtsuru, M., 346, 393
Oida, S., 49
Oishi, K., 280
Okada, G., 259
Okada, H., 359
Okada, S., 339, 358, 365, 386, 413
Okado, G., 412
Okamoto, T., 165
Okamura, Y., 98
Okawa, K., 209
Okayama, M., 295
Okazaki, H., 249
Okazaki, K., 293
Okuda, N., 299
Okuda, S., 246
Okuda, T., 6
Olden, K., 243
Oldmixon, E. H., 242
Oleinik, D. M., 162
Oleinikova, A. Ya., 7
Olesker, A., 127
Oliphant, G., 321
Oliver, G. J. A., 324
Ollapally, A., 158
Ollis, J., 187
Olsen, C., 307
Olsen, K. W., 424
Olsnes, S., 279
Olson, N. F., 459
Olsson, I., 284
O'Malley, B. W., 376
Omura, H., 161
Omura, S., 180
Onodera, K., 74, 108, 195, 215, 307
Oparaeche, N. N., 35, 36
Oplatka, A., 424

Oppenheim, J. D., 282
Oppenheimer, N. J., 139
Oppenheimer, S. B., 304, 308
Oprea, S., 436
Ordin, L., 230
Orenstein, N. S., 99
Orhanovic, Z., 168
Orlich, I., 47
Orme, T. W., 216
Orrego, H., 357
Ortiz, J. M., 335
Orzaez, M., 101
Osawa, T., 17, 280, 308, 352, 416
Osborne, W. R. A., 439
Oshitok, G. I., 17
Ostman, B., 323
Osuga, D. T., 321
Ota, K., 143
Otagiri, M., 438
Otnaess, A. B., 416
Ototani, N., 292, 300
Ouchi, S., 161
Ouyang, C., 321
Overend, W. G., 62, 69, 93, 95
Ovodov, Yu. S., 186, 213, 236, 252
Ovodova, R. G., 236
Owen, P., 261
Owen, W. G., 312

Pacák, J., 61, 66, 280
Padeh, B., 330
Page, W. J., 253
Pahlman, S., 329, 415
Paigen, K., 341
Paine, C. M., 253
Paine, M. A., 344, 435
Pal, S., 294
Palamarczyk, G., 325
Palescandolo, R., 355
Palma, A., 223
Palmer, T. N., 383
Palmoski, M. J., 296
Pálos, L. A., 297
Palovčík, R., 29, 117, 177
Panchenko, S. I., 193
Panzica, R. P., 143, 181
Papin, J. P., 214
Pardoe, G. I., 315
Pargaonkar, P. S., 298
Parish, E. J., 227
Park, J. T., 262
Park, R. W. A., 246
Parker, C. W., 139, 304
Parks, E. W., 168
Parolis, H., 56, 234
Parrish, F. W., 10, 39
Parthasarthy, R., 146
Paruchuri, E. K., 344, 459
Pasanen, S., 289
Pascal, M., 336
Pascard-Billy, C., 188, 189
Pascher, I., 400, 403
Pass, G., 55
Passonneau, J. V., 283, 286
Pastan, I., 305
Pastewka, J. V., 323
Paszkiewicz, E., 72
Patchornik, A., 42, 161
Patil, D. L., 219
Paton, R. M., 438
Patthy, L., 376, 450
Paul, I. C., 134
Paul-Gardais, A., 296
Paulsen, H., 14, 27, 31, 43, 44, 120, 121, 177, 188
Pavlenko, A. F., 186
Payne, R. C., 318
Pazoles, C. J., 247
Pazur, J. H., 370
Peak, S., 388
Pearce, C. J., 127
Pearce, R. H., 294
Pearson, C. M., 273
Pearson, M. L., 406
Peberdy, J. F., 266
Peciar, C., 117, 177
Pedersen, C., 32, 54, 55, 181
Peer, H. G., 5
Peitersen, N., 363
Pellé, G., 38
Pellmont, B., 165
Pellon, G., 245
Pelyvas, I., 60
Pencheva, E., 116
Penco, S., 138
Penhoet, E., 307
Penny, D., 227
Penton, E., 332
Percival, E. E., 236
Perdomo, J. M., 309
Perdon, A. A., 219
Pereira, M., 279
Perelygin, B. G., 20
Perena, J. M., 222
Perkins, H. R., 243
Perlin, A. S., 92, 170, 181, 184, 186, 217, 293, 435
Perraud, R., 175
Perret, F., 27
Perrin, P., 272
Perry, M. B., 193, 216
Pertoft, H., 298
Petersen, J. B., 158
Petersson, G., 115, 215
Petit, J. F., 261
Petitou, M., 14, 17
Petrukhin, V. G., 252
Pettersson, B., 362, 370
Pettersson, L. G., 347, 449
Pezzanite, J. O., 99
Pfaffenberger, C. D., 209
Pfeilsticker, K., 119
Phadnis, S. P., 50, 57
Phaff, H. J., 214, 368
Philip, G., 288
Phillips, B. A., 238
Phillips, G. O., 7, 19, 55
Phillips, M. E., 288
Phillips, N. C., 350, 416
Phillips-Quagliata, J. M., 315
Phizackerley, P. J. R., 335
Picard, J., 290, 296
Pichuzhkina, E. I., 193
Pickering, M. V., 79
Pickmere, S. E., 228
Piekarska, B., 10
Pieringer, R. A., 241, 410
Pignaud, G., 437
Pihl, A., 279
Pike, L. M., 29
Pillai, C. G. P., 264
Pillai, P. M., 62
Pinter, I., 190
Pion, B. G., 260
Piriou, F., 180
Pischel, H., 164
Pita, J. C., 375
Pitarra, G. C., 416
Pitcher, R. G., 103
Pittner, F., 424
Pitts, M. J., 84
Pitzner, L. J., 199
Pivetta, O. H., 358, 431
Planche, G., 22
Plantner, J. J., 322
Plas, C., 285
Plattner, R. D., 192, 216
Playtis, A. J., 23
Plemyannikova, L. A., 244
Pless, D. D., 242, 248, 324
Plessy, B., 297
Pletcher, J., 189
Plotz, P. H., 416
Plummer, T. H., jun., 315, 336, 445
Pocock, D. H., 208
Podder, S. K., 398
Podolsky, D. K., 304
Podrazký, V., 298
Poduslo, J. F., 326
Poenaru, L., 331, 332
Pokorný, J., 242
Pokorny, M., 112, 334
Pokorny, V., 266, 267
Polacheck, Y., 267
Poland, J. L., 284
Poling, M. D., 116
Polyakov, A. I., 53
Polyanskii, K. K., 7, 20
Pommier, G., 357, 449
Ponce de Leon, M., 303
Poncet, J., 66
Poole, A. R., 314
Pooley, H. M., 237
Poon, J. P., 296
Popa, V. I., 233, 234
Popova, L. V., 346
Popova, S., 218
Poppleton, B. J., 187
Porath, J., 280, 361, 414, 428, 429, 430
Porter, W. H., 212
Pospišilová, J., 304
Posshchast'eva, E. B., 141
Postel-Vinay, M.-C., 428
Potenzone, R., 294
Potenzone, R., jun., 180
Poverennyi, A. M., 165
Powell, D. A., 241
Power, M. J., 27, 172
Pownall, H. J., 298
Prager, E. M., 375
Prat, M., 309
Pravdić, N., 91, 112, 334
Praznik, W., 219
Prehm, P., 247
Preiser, H., 339
Preiss, J., 259
Preobrazhenskaya, M. E., 345
Preobrazhenskaya, M. N., 141, 142, 143, 146, 162
Prescott, B., 196
Presser, J., 210
Preston, B. N., 298
Preston, C. M., 173, 184
Preti, A., 351
Preti, C., 297, 301
Price, H. C., 398, 402
Pridham, J. B., 342
Prieels, J.-P., 392, 428

Priest, F. G., 359
Prigent, M. J., 280
Prijcker, J. D., 19
Prinzbach, H., 126, 127
Pritchard, D. G., 290
Privat, J.-P. D. J., 15, 379
Prohaska, R., 317
Prokazova, N. V., 18
Prosen, E. J., 212
Protsenko, Z. I., 226
Prout, K., 187
Provelenghiou, C., 88
Prusiner, P., 179
Prusoff, W. H., 160
Prydz, H., 312, 416
Prystaš, M., 136, 143
Przylenk, M., 228
Pugmire, R. J., 181
Pulkownik, A., 367
Puskas, M. M., 135
Putnam, S. L., 342
Pvorotilo, S., 370

Quarfoth, G. J., 392
Quarles, R. H., 326
Quatrano, R. S., 234
Que, L., jun., 56
Quillivic, R., 156
Quirk, J. M., 331

Rab, A., 355
Rabczenko, A., 179
Rabinovich, D., 187
Rabinsohn, Y., 200
Racz, V. M., 62, 69
Radatus, B., 91
Radhakrishnamurthy, B., 292, 298, 299
Radin, N. S., 345
Raftery, M. A., 375
Raghaven, S. S., 345
Rahemtulla, F., 294
Raine, D. N., 298
Raizada, M. K., 274
Rakhit, S., 67
Ralph, B. J., 271
Ramachandran, P. A., 330, 459
Ramamurthi, R., 289
Ramey, C. W., 300
Ramjeesingh, M., 23, 148
Ranganathan, R., 146
Rao, C. V. N., 224
Rao, G. V. K., 56, 377, 432
Rao, V. S. R., 173
Raouf, M. S., 170
Rapin, A. M. C., 305
Rapport, M. M., 398
Rasmussen, R., 54, 55
Ratcliffe, M., 104
Rathbone, E. B., 102, 217
Rattazzi, M. C., 333
Rauf, P. W., 435
Ray, P. M., 231
Ray, R. E., 20
Ray, S. C., 310
Raymond, Y., 224
Rebel, G., 208, 405
Rebeyrotte, P., 274, 341
Redaelli, S., 138
Reddy, B. S., 188, 349
Redmond, J. W., 252
Reed, M. J., 242
Reeke, G. N., jun., 277
Reepmeyer, J. C., 143
Rees, D. A., 175, 180, 188, 191, 233, 291
Reese, C. B., 161, 164
Reeve, J. N., 239
Refsnes, K., 279
Regen, S. L., 329, 451
Regoeczi, E., 297, 313, 351, 416
Reichmann, U., 55
Reichstein, E., 316
Reid, D. S., 233
Reid, P. E., 300
Reinhardt, R., 188
Reinhold, V. N., 33, 75, 399
Reistad, R., 244
Reitherman, R. W., 278, 306
Rejholcová, M., 238
Rejtö, M., 200
Remin, M., 179
Remington, S. J., 380
Rendig, V. V., 6
Rendleman, J. A., jun., 193, 210
Rengevich, E. E., 144
Renkonen, O., 398
Rennecke, R.-W., 108
Renner, R., 428
Renwrantz, L., 282
Repke, D. B., 25
Reppucci, A. C., jun., 295, 445
Retig, N., 381
Reusser, F., 134
Rey, E., 213
Reynolds, L. W., 350
Reznick, A. Z., 303
Riande, E., 222
Ribadeau-Dumas, B., 311
Rich, G. T., 282
Richards, A. G., 322
Richards, F. F., 314
Richards, G. N., 228, 231, 256, 361, 372
Richardson, A. C., 50, 64, 151, 164
Richardson, T., 360, 449, 459
Riche, C., 189
Richmond, M. E., 445
Richter, W. J., 27
Rick, P. D., 270
Rickert, W. S., 387
Ridley, D. D., 114
Rieche, H., 7
Rietra, P. J. G. M., 339
Rietschel, E. T., 252
Righetti, P. G., 322
Rigouard, M., 219
Rimbaut, C., 313
Rimerman, R. A., 256
Rinaudo, M., 226
Rinehart, K. L., 134, 138
Rios, A., 353
Rist, C. E., 443
Risteli, L., 282
Ritchie, R. G. S., 181
Ritzmann, G., 56
Rivest, R., 188
Robbins, J., 310
Robbins, P. W., 410
Robert, B., 437
Robert, L., 321, 437
Roberts, E. J., 221
Roberts, G. P., 318
Roberts, J., 208, 322, 405
Roberts, S. H., 300
Robertson, M., 304
Robic, D., 17
Robin, J. P., 219
Robins, M. J., 163
Robins, R. K., 143, 144, 163, 166
Robineaux, R., 295
Robinson, D., 331, 338, 350, 397, 416
Robinson, D. S., 210, 320
Robinson, H. C., 295
Robinson, H. K., 334
Robinson, I. C. A. F., 416
Robinson, J. M., 209
Robinson, J. P., 380
Robinson, R. D., 380
Roblin, R., 296
Robos, V., 51
Robson, R. L., 259
Robson, R. M., 259
Robyt, J. F., 193, 210, 257
Rocchetti, R., 437
Rodbard, D., 322
Rodemeyer, G., 93
Rodén, L., 295
Rodionov, A. V., 5, 45
Rodrick, G. E., 380
Roe, J., 410
Röd, T. O., 349
Roeder, O., 178, 184
Roelcke, D., 317
Rölla, G., 262
Rönnbäck, L., 301
Rönnemaa, T., 353
Rogers, G. T., 445
Rogers, H. J., 237, 239
Rogovin, Z. A., 432
Rolandson, S., 221
Rolf, L., 68
Roller, P. P., 216
Rollins, A. J., 100
Romagnoli, E., 208, 289
Romond, C., 263
Rood, J. I., 352, 416
Rooney, S. A., 325
Roos, E., 307
Roos, P., 310
Root, M., 296
Roppel, J., 60
Rose, J. E., 275
Rosell, K. G., 230
Roseman, S., 326, 397, 405, 407
Rosen, S., 238
Rosen, S. D., 275, 278, 306
Rosenberg, A., 379, 400
Rosenberg, L., 294
Rosenberger, R. F., 267
Rosenblit, P. D., 334
Rosenbusch, J. P., 243
Rosenfeld, E. L., 345
Rosenfeld, L., 264
Rosengren, J., 329, 415
Rosenstein, R. D., 188
Rosenstein, R. W., 314
Rosenthal, A., 103, 104, 246
Rosevear, A., 459
Rosić, N., 285
Rosik, J., 224, 367
Ross, M. E., 357
Rossi, C., 180
Rossignol, B., 284
Rottenberg, D. A., 286

Rottman, F., 29
Roukema, P. A., 319
Roushdi, M., 170
Rousseau, M., 258
Rousseau, R. J., 143
Roussel, P., 302
Roux, D., 322
Rouze, L. L., 213
Rowland, S. P., 221
Roy, A., 224
Roy, A. B., 389
Roy, N., 117
Rozynov, B. V., 186, 398
Rubasheva, L. M., 134
Rubinstein, M., 42, 161
Ruddle, F. H., 406
Rudén, U., 254
Rudick, M. J., 274
Rudolph, C., 372
Rugg, P. W., 188
Ruggeri, A., 296
Ruiz-Herrera, J., 266
Ruoslahti, E., 305, 314
Rupley, J. A., 376, 377
Rusan, V., 233
Russell, C. R., 79, 80, 443
Rutkowski, J., 227
Ryan, M. D., 411
Ryter, A., 243
Ryuzaki, M., 407

Saarinen, P., 359, 435
Sabraw, A., 259
Sachs, L., 405
Sachtleben, P., 351
Sadoff, H. L., 253
Sadovnikova, M. S., 128, 129
Saeki, H., 49, 138
Saenger, W., 255
Saint-Lèbe, L., 219
Sairam, M. S., 428
Saito, H., 300
Saito, N., 299, 358
Saito, S., 6
Saitoh, Y., 284
Sakai, Y., 209
Sakakibara, E., 348
Sakakibara, T., 105
Sakamoto, A., 336
Sakamoto, M., 298
Sakamoto, S., 298
Sakamoto, W., 348
Sakamura, S., 128
Sakata, K., 131
Sakata, M., 77
Sakurai, A., 131
Sakurai, T., 38
Salahuddin, A., 320
Salfner, J., 282
Salkie, M. L., 373
Salmon, C., 401
Salsas, E., 286
Salton, M. R. J., 261
Salvatore, G., 310
Salz, E., 256
Saman, E., 20, 176
Samee, A., 55
Samek, Z., 105
Samoshina, N. F., 17, 45
Samshina, E. N., 63
Samuelson, O., 115, 209, 223, 229, 444
Samuelsson, B. E., 400, 403, 407
Sanders, E. B., 92
Sanderson, G. R., 191
Sandford, P. A., 271
Sanehi, R., 8
Sanemitsu, Y., 136
Sanin, P. I., 162
Sano, H., 65
Sano, K., 346
Sano, R., 416
Sanwal, B. D., 406
Sanyal, M., 218
Saraswathi, S., 312
Sarkar, M., 224
Sarko, A., 218
Sarma, R. H., 179
Sarmadjiewa, W., 433
Sarre, O. Z., 131
Sarymsakov, A. A., 435
Sasada, Y., 188
Sasai, Y., 299
Sasaki, A., 410
Sasaki, M., 211
Sasaki, T., 152, 157
Sasaki, Y., 433
Sasso, E., 316
Sastry, P. S., 397
Sato, K., 170, 288
Sato, M., 169, 246
Sato, S., 188
Sato, T., 288, 416
Sato, Y., 379, 450
Satoh, N , 226, 229
Satoh, T., 103
Satyanarayana, K., 289
Sattler, M., 339
Satzinger, G., 18
Satzke, L. O. G., 188
Saul, M., 79
Saunders, J., 104
Saunders, J. R., 142
Sayare, M., 244
Sax, M., 189
Scandalois, J. G., 416
Scarborough, G. A., 9
Scardi, V., 355
Schachtele, C. F., 258, 365, 386, 439
Schachter, H., 211, 325, 405
Schafer, W., 272
Scharpé, S., 355, 442
Scharschmidt, B. F., 416
Schauer, R., 186, 212, 318, 322
Schell, H. D., 361, 416
Schellman, J., 380
Schenkel-Brunner, H., 280, 317
Scheraga, H. A., 312, 380, 414, 422
Scherz, H., 219
Schifflers, M. H., 289
Schilling, G., 181
Schlecht, S., 250
Schleich, T., 179
Schleifer, K. H., 242, 261
Schmer, G., 322
Schmickel, R. D., 290
Schmid, E., 406
Schmid, H., 7
Schmidt, C. L., 142
Schmidt, D., 255
Schmidt, F., 220
Schmidt, R. R., 146
Schmit, A. S., 242, 248
Schmit, J. C., 270
Schnebli, H. P., 317
Schneider, E. F., 269
Schneider, H.-W., 127
Schneider, I., 272
Schneider, J. E., 18, 20
Schneider, W., 288
Schnute, W. C., 262
Schomburg, G., 8
Schott, H., 435
Schrader, W. P., 243
Schröter, E., 343
Schroll, G., 39
Schubert, J., 92
Schuerch, C., 13, 412
Schulte, M. I., 443
Schulte-Frohlinde, D., 129
Schulten, H.-R., 186
Schultz, A. M., 326, 406
Schultz, J. C., 324, 409
Schultz, R. C., 219
Schulz, G. V., 223
Schurz, J., 227, 443
Schutzbach, J. S., 274
Schwabe, K., 18
Schwarcz, J. A., 170, 186
Schwartz, A. L., 140
Schwartz, C. E., 298, 375
Schwartz, J. P., 286
Schwartz, M., 383
Schwartz, N. B., 295, 296
Schwartz, P. M., 194
Schwarz, R., 219
Schwarz, U., 380
Schwarzmaier, U., 196
Schwesinger, R., 126, 127
Schwick, H. G., 398
Scocca, J. J., 248
Scott, J. E., 289
Scott, J. R., 234
Scott, W. E., 188, 233
Searle, F., 445
Secemski, I. I., 377
Secrist, J. A., tert., 152
Sefton, B. M., 272
Segal, H. L., 286
Seglen, P. O., 285
Segrest, J. P., 282
Seichertová, O., 266
Seide, R. K., 416
Seiter, C. W., 289
Sekiguchi, J., 359
Sekine, M., 161, 165
Sekiya, M., 160
Sekizawa, Y., 131
Sela, B.-A., 276, 405
Seldes, A. M., 74, 174
Sellen, D. B., 221
Sellmair, J., 102
Selvarajan, V. R., 289
Selvendran, R. R., 227
Semin, Yu. A., 165
Semjenowa, M. N., 191
Senatorova, T. P., 358
Senchenkova, T. M., 66
Senda, M., 226
Senehi, R., 118
Sener, A., 214
Seno, N., 390
Sensakovic, J. W., 410
Sensenbrenner, M., 405
Seo, K., 85
Sepulchre, A.-M., 180, 183
Séquin, U., 165
Serafini-Cessi, F., 195

Serafty, A., 208
Seshadri, T. R., 18
Seto, S., 151
Seyfried, H., 317
Seymour, F. R., 192, 216, 217, 271
Shaban, M. A. E., 72, 75
Shabarova, Z. A., 161
Shafizadeh, F., 108, 111
Shah, J., 234
Shah, R. H., 335
Shaltiel, S., 416
Shapira, E., 388
Shapiro, L. J., 298
Shapiro, R. H., 192, 215, 216
Shapiro, S. S., 296
Sharma, R. A., 22, 142
Sharon, N., 245, 265, 279, 317, 342, 376, 413, 416
Sharpe, M. E., 238
Shashkov, A. S., 45, 173
Shaw, G., 146, 188
Shcherbakov, A. A., 238
Shcherbakov, V. A., 174
Shcherbakova, L. L., 174
She, C. Y., 185
Sheehan, J. K., 291
Sheinin, R., 326, 406
Shelanski, M. L., 301
Shepeleva, E. S., 162
Sherman, W. R., 124, 186
Sherry, A. D., 277
Shestov, A. G., 7
Shiba, T., 203
Shibaev, V. N., 13
Shibaeva, V. I., 236
Shibata, S., 184, 276
Shibazaki, K., 276
Shida, M., 270
Shih, T. Y., 436
Shimada, E., 291
Shimada, Y., 138
Shimahara, H., 230, 233
Shimazaki, K., 279
Shimazu, T., 284
Shimizu, B., 188
Shimizu, M., 42, 311
Shimizu, Y., 236
Shimokawa, S., 122
Shimonaka, H., 274
Shimono, T., 273
Shimura, M., 131
Shinjyo, S., 300
Shinohara, T., 231
Shiobara, Y., 6
Shioiri-Nakano, K., 352, 416
Shiomi, M., 208
Shiono, R., 188
Shiozawa, S., 283
Shipp, D. W., 300
Shirai, S., 407
Shiraishi, H., 151
Shirataki, Y., 42
Shiue, C.-Y., 146
Shmakova, F. V., 315
Shmyrev, I. K., 51
Shobe, C. R., 424
Shockman, G. D., 238, 241, 244, 246
Shoda, M., 337, 430
Shomura, T., 360
Shore, G., 224
Shows, T. B., 333, 349
Shugar, D., 163, 179, 188
Shul'ga, N. I., 192, 216
Shuster, J., 303
Shvets, V. I., 128, 129
Siddiqui, I. R., 192, 195, 209, 213
Sidebotham, R. L., 256
Sidorczyk, Z., 249
Sidwell, R. W., 143
Sieben, A., 214
Siefring, G. E., 313
Sietsma, J. H., 264
Sigler, K., 430
Silano, V., 355, 356
Silbert, J. E., 295, 445
Silva, M. E., 292
Silverman, J. B., 56
Silvieri, L. A., 199
Siminovitch, L., 325
Simionescu, C. I., 233, 234, 436
Simmonds, R. L., 353
Simon, L. N., 143, 163, 166
Simon, M., 47
Simoncsits, A., 162
Simpson, D. L., 275
Sims, H. F., 194
Sims, W., 352
Sinaÿ, P., 14, 15, 17
Sinclair, A. C., 134
Sindric, R., 113
Sing, V. O., 266, 275
Singer, R., 220
Singh, A. K., 8
Singh, H. S., 8
Singh, J., 333, 440
Singh, M. P., 8
Singh, P. P., 305
Singh, R. K., 8
Singh, V. P., 8
Singy, A., 187
Sinha, A. M., 344
Sinkey, J. D., 223
Sinnema, A., 199
Sinnott, M. L., 19, 328
Sisman, J., 272
Sjöberg, L. A., 229
Sjöberg, Z., 293
Sjöblad, S., 336
Sjöström, E., 19, 187, 215, 223
Skehel, J. J., 272
Skelton, P. P. C., 324
Skipper, N., 273
Skipski, V. P., 404
Slade, H. D., 262
Slayter, H. S., 302
Slodki, M. E., 192, 216, 217, 271
Slomiany, A., 398, 402
Slomiany, B. L., 398, 402
Slott, S., 358
Sly, W. S., 347
Smestad, B., 234
Smets, L. A., 308
Smiatacz, Z., 72
Smidsrod, O., 122
Smirnova, L. S., 228
Smit, P., 74
Smith, C. H., 213
Smith, C. W., 100
Smith, D. F., 309
Smith, D. J., 262
Smith, E. E., 220, 258
Smith, E. L., 376, 400, 403, 450
Smith, G. A., 135
Smith, G. D., 298
Smith, I. C. P., 182, 216, 261
Smith, K. A., 135
Smith, N. L., 436
Smith, P. F., 248
Smith, P. J. C., 175, 180, 291
Smith, R., 416
Smith, R. A., 290
Smith, T. W. D., 296
Smith, W. L., 264, 275
Smith-Verdier, P., 188
Snaith, S. M., 351
Snatzke, G., 190
Snell, E. E., 416
Snyder, S. L., 213
Sobiczewski, W., 137
Sobocinski, P. Z., 213
Sobue, M., 296
Soderling, T. R., 286
Sømme, R., 261
Sohar, P., 153
Sohma, J., 122
Sokolova, N. I., 161
Solanki, J. S., 382
Soldat, W.-D., 107
Solov'eva, N. P., 63
Solymosy, F., 162
Soman, G., 288
Somers, K., 87
Somers, P. J., 194, 212, 213
Somogyi, L., 75
Sondack, D. L., 214
Sonenberg, M., 428
Soni, P. L., 28
Sonnenschein, W., 214
Soranzo, C., 138
Sorenson, J. C., 416
Sorgente, N., 375, 437
Šorm, F., 136
Soulier, S., 311
Southgate, D. A. T., 227
Southworth, D., 230
Sowa, T., 152, 161
Sowden, J. M., 188
Sowden, L. C., 260
Špan, J., 376
Spataro, A. C., 307
Spengler, G. A., 315
Speziale, P., 296
Spielvogel, C. H., 334
Spiro, R. G., 323, 324
Spitznagel, J., 376
Spoormaker, T., 199
Spranger, J., 299
Springer, G. F., 281, 282
Srinivasan, S. R., 298
Srivastava, H. C., 436
Srivastava, P. C., 144
Srivastava, S. K., 332
Srivastava, V. K., 436
Sroczynski, A., 9
Staat, R. H., 258, 365, 439
Stacey, B. E., 50
Stachnyk, O., 408
Stage, D. E., 423
Stalmans, W., 287
Stanaszek, R. S., 134
Staněk, J., 20, 29, 32
Stanescu, V., 299
Stang, J., 304
Stanley, P., 305, 325
Stanley, W. L., 344, 437
Staples, B. R., 212

Starkey, B. J., 351
Stauub, A. M., 251
Stavrou, D., 301
Stead, A., 249
Steber, J., 261
Steby, M., 363
Steen, G. O., 407
Steer, M. L., 356, 449
Steigerwald, J. C., 407
Stein, E. R., 226
Steiner, M. R., 87
Steiner, S., 87, 272
Steinhausen, G., 280
Stella, E., 355
Stellner, K., 397
Stellwagen, E., 416
Stenzel, W., 14, 27
Stephen, A. M., 217
Stepanenko, B. N., 143
Stephens, R. W., 296
Stephenson, J. R., 272
Sternberg, M., 341
Sternglanz, H., 188
Stevens, C. L., 62, 95
Stevens, J. D., 37, 39, 173, 187
Stevens, P., 250
Stevens, R. L., 388, 389, 462
Stevenson, R. E., 350
Stewart, A. B., 219
Stewart, J. C. M., 164
Stewart-Tull, D. E. S., 273
Stick, R. V., 11, 38, 86
Sticzay, T., 117
Stidworthy, G. H., 295
Stiefel, D. J., 356, 443
Stinnett, J. D., 249
Stinshoff, K., 389
Stirm, S., 247, 254, 372
Stock, C. C., 404
Stockert, E., 308
Stoddart, J. F., 34, 38, 198
Stoeckl, P., 66
Stoffel, W., 405
Stoffyn, A., 404
Stoffyn, P., 404
Stoilkov, G., 218
Stone, B. A., 253, 327, 442
Stone, T. E., 79
Stoner, G. E., 460
Stoolmiller, A. C., 295, 345
Stoos, F., 65
Stork, G. A., 74
Stout, E. I., 79, 80
Stout, M. G., 143
Strand, G., 42
Strecker, G., 320
Streeter, D. G., 143
Streicher, H.-J., 318
Streips, U. N., 237
Strel'tsova, I. F., 69
Streshinskaya, G. M., 265
Strider, W., 294
Strobel, K., 156
Strombeck, D. R., 319
Strominger, J. L., 273, 305, 318, 323, 328
Stropnik, C., 442
Strosberg, A. D., 428
Stuart, W. D., 273
Stud, M., 151
Sturgeon, P., 281
Sturgeon, R. J., 370
Sturtevant, J. M., 47
Suami, T., 64, 126, 127
Suciu, N., 151
Sudo, K., 195, 210, 213, 214
Sudo, T., 307, 362
Sudoh, R., 105
Suehiro, H., 143
Suga, K., 328
Sugai, S., 376
Sugawara, S., 138
Sughrue, M. J., 259
Sugimoto, T., 356, 359
Sugimura, K., 245
Sugimura, T., 348
Sugita, M., 407
Sugita, T., 339
Sugiura, M., 366, 416, 450
Sugiura, T., 152, 157
Sugiyama, H., 151, 270
Sugiyama, N., 230, 233
Sugiyama, T., 248
Sukhanov, V. I., 128
Sulkowski, E., 416
Sumi, Y., 416
Sumida, S., 409
Sumimoto, T., 431
Summer, G. K., 287
Summons, R. E., 139
Sund, M. L., 373
Sundaralingam, M., 178, 179, 187
Sundblad, G., 279, 302
Surolia, A., 279, 323, 398
Susz, J. P., 301
Suvorov, N. N., 146
Suzuki, H., 230
Suzuki, K., 280, 338, 339, 341, 404
Suzuki, N., 296, 335
Suzuki, S., 267, 293, 296, 335, 367
Suzuki, Y., 338, 339, 340, 343, 346, 349
Svennerholm, L., 400
Svensson, S., 15, 169, 230, 248, 250, 253, 302, 319
Swahn, C.-G., 7
Swaisgood, H. E., 460
Swami, K. S., 289
Swan, B., 223
Swann, R. W., 301
Swanston, J. S., 361
Sweeley, C. C., 399
Sweet, D. P., 192, 215, 216
Sweet, F., 416
Swierczek, E., 374
Swislocki, N. I., 428
Sygusch, J., 175
Sykes, B. D., 174, 277
Symes, K. C., 122, 250
Szabo, L., 116
Szabo, P., 38, 46
Szafranek, J., 209
Szarek, W. A., 39, 102, 140, 176, 182
Szczeklik, H., 392
Szechner, B., 88
Szejtli, J., 218
Szilágyi, L., 75
Szlamka, I., 341
Szmant, H. H., 35
Sztaricskai, F., 60, 135

Tabakoff, B., 424
Tabata, S., 220
Tadano, K., 126
Tager, J. M., 339
Taguchi, Y., 161, 162
Tahara, Y., 243
Tai, T., 314
Taieb, C., 178
Taiz, L., 227
Takada, N., 359
Takagi, E., 191, 222
Takahara, K., 211
Takahara, Y., 380
Takahashi, H., 246
Takahashi, K., 236, 276
Takahashi, Y., 129, 136
Takai, H., 90
Takai, M., 260
Takasaki, S., 210
Takayama, K., 324
Takeda, N., 267
Takeda, T., 160, 188, 276
Takehana, H., 336, 459
Takeo, K., 431
Takeuchi, T., 352
Taki, M., 196, 213, 217, 218
Takiura, K., 90, 195, 210, 213, 214
Tal, I., 226
Tal, M., 449
Tal, N., 356
Talbot, P., 373
Tallman, J. F., 330, 331, 397, 399, 461
Tam, S. Y.-K., 91, 92, 104, 148
Tamai, Y., 407
Tamari, K., 362
Tamas, J., 54
Tamboskar, P. K., 432
Tamby, C., 141
Tamm, C., 165
Tamura, C., 188
Tamura, S., 131
Tanaka, K., 177
Tanaka, N., 87
Tanaka, Y., 9, 20, 87
Tandecarz, J., 220, 285
Tani, N., 346, 393
Taniguchi, M., 138
Taniguchi, N., 299
Taniyama, T., 245, 246
Tanner, M. J. A., 281
Tanner, W., 220, 325
Tapiero, C., 142
Tappel, A. L., 331
Tarakanov, O. G., 433
Tarasoff, L., 114
Taravel, F. R., 181, 259
Tarelli, E., 50, 57
Tarentino, A. L., 315, 326, 336, 445
Tarnai, A. K., 54
Tarp, M., 460
Taschner, P., 88, 118
Tashian, R. E., 439
Taubman, M. A., 262
Taylor, A., 243, 392
Taylor, C., 237, 239, 288
Taylor, H. A., 350
Taylor, K. G., 62, 263
Taylor, P. N., 246
Taylor, T. K. F., 212, 296
Techukhrova, A. I., 387
Teichner, E., 380, 414
Tejima, S., 18, 41, 64, 79
Tekolf, W., 297
Teller, D. C., 322

Temmink, J. H. M., 307
Templeton, D. H., 187
Tennant, L. L., 336, 444
Teoule, R., 142
Terao, T., 352, 416
Terui, T., 216, 269
Terzis, A., 188
Tesse, J., 175
Tessier, C., 401
Tettamanti, G., 351, 398
Texter, J. A., 256
Tezuka, T., 87, 155
Thanbichler, A., 102
Thang, M. N., 424
Tharanathan, R. N., 226
Thayumanavan, B., 186
Theander, O., 109
Thieffry, A., 123
Thiel, I. M. E., 74, 174
Thom, D., 191
Thoma, J. A., 360, 377, 432
Thomas, G. H., 350
Thomas, H. J., 141, 148
Thomas, J. A., 283, 286
Thompson, C. R., 80
Thompson, N. S., 223
Thorpe, R., 338
Thorpe, S. R., 348
Thung, S., 416
Thurman, P. F., 237, 239
Thurow, H., 254, 372
Thurston, C. F., 365
Thyberg, J., 293
Ticha, M., 238, 280
Tierney, B., 50
Tikhomirova, A. S., 342
Timoshchuk, V. A., 141, 159
Timpe, W., 111
Tipton, C. M., 284
Titani, K., 312
Tiwari, S. C., 8
Tkach, R. W., 134
Tobias, R. S., 166
Tobin, R., 219
Tocanne, J.-F,, 42
Toda, K., 337, 430
Todaro, G. J., 406
Todd, C. W., 303
Todd, J., 334
Todriya, G. K., 18
Tokunaga, M., 299
Tolstoguzov, V. B., 256
Toman, R., 226, 231
Tomasz, A., 238, 239, 240
Tomasz, J., 162
Tomichek, E. A., 282
Tominaga, Y., 380
Tomisek, A. J., 211
Tomita, M., 316
Tomoda, M., 226, 229
Tomshich, S. V., 252
Torgov, V. I., 17, 33
Tori, K., 178
Toriello, C., 267
Torii, K., 188
Tortolani, G., 208, 289
Tosa, T., 416
Toubiana, M.-J., 42
Toubiana, R., 42
Townsend, L. B., 142, 143, 181
Toyoshima, S., 280
Toyota, T., 212
Tralaggan, P. J., 295
Tran Dinh Son, 178
Travassos, L. R., 271
Treffry, T. E., 267
Trémolières, A., 408
Trevisan, L. M. V., 71
Triantaphylides, C., 49
Trigalo, F., 116
Trimnell, D., 79
Trisnadi, J. A., 219
Trnka, T., 61
Tronchet, J., 105, 160, 173
Tronchet, J. M. J., 27, 66, 75, 97, 105, 110, 160, 173, 186
Trowbridge, I. S., 306
Trummlitz, G., 25
Trus, B. L., 188
Tsang, W.-C., 300
Tsay, G. C., 302, 320
Tscharnuter, W., 255
Tschida, J., 373
Tseng, T. C., 362
Tsiang, H., 272
Tsitsishvili, V. G., 256
Tsuboi, M., 188
Tsuchiya, T., 65, 129, 136, 137, 138
Tsuji, A., 289, 438
Tsuji, S., 203
Tsujimoto, T., 271
Tsujisaka, Y., 380
Tsunoda, K., 152
Tsuta, Y., 260
Tsuyama, N., 386
Tsuzuki, Y., 177
Tu, A. T., 185
Tu, J.-I., 287
Tucker, L. C. N., 28, 67
Tulsiani, D. R. P., 301
Tunstall, A. M., 245
Tuppy, H., 317
Turchen, B., 314
Turchin, K. F., 63, 162
Turik, S. V., 90
Turk, V., 416
Turkington, R. W., 334, 335
Turner, B. M., 337
Turner, K., 211
Turner, M. D., 351
Turner, W. N., 131
Turner, V. S., 337
Turpin, M., 274
Turvey, J. R., 234
Tuyau, J. E., 352
Tvaroška, I., 173
Twine, C. E., 19
Twinter, W., 291
Tynecka, Z., 243
Tze-Fei Wong, J., 328

Uchida, K., 135, 343
Uchino, H., 127
Uchiyama, H., 246
Uchiyama, M., 349
Udiman, M., 214
Ueda, M., 409
Ueda, S., 358, 372
Uedaira, H., 255
Uekama, K., 438
Uemori, U., 439
Ueyama, M., 178
Uhlenbruck, G., 255, 280, 282, 317, 321
Uhr, J. W., 315
Ui, M., 284
Ulsperger, E., 111
Umarova, M. K., 433
Umeda, K., 360, 459
Umemoto, Y., 379, 450
Umezawa, H., 137, 138, 352
Umezawa, S., 65, 129, 131, 136
Underhill, C. B., 292
Unger, P. D., 270
Uno, H., 163
Uotani, D., 271
Urban, P. F., 407
Urbanek, H., 382
Uretsky, S. C., 281
Usatyi, A. F., 191
Usenko, L. S., 144
Usmanov, K. H. V., 435
Usov, A. I., 186
Usui, T., 136
Uthne, K., 208
Utille, J.-P., 15, 42
Utkina, E. A., 146
Utsumi, H., 119
Uvarova, N. I., 17, 45
Uzlova, L. A., 34

Vafina, M. G., 19, 350
Vaheri, A., 305, 314
Vaillant, R., 288
Vairel, E. G., 294
Vakorina, T. I., 192, 216
Valcavi, U., 141
Valfré, F., 355
Valz, G., 143
van Assche, J. A., 384
Van Beek, W. P., 308
van Beeumen, J., 174
van Bekkum, H., 7, 199
van Boom, J. H., 161, 164
Vance, D. E., 399
Vandalem, J.-L., 310
van Dedem, G., 328
Vandenhoff, G. E., 376
van der Hart, M., 317
van der Plaat, J. B., 384
van der Plas, H. C., 74
van der Toorn, J. M., 199
van der Veen, H. K. C., 190
van der Wal, S., 194
Van der Woude, W. J., 266
Van Gessel, G., 79
van Heijenoort, J., 243
Van Hummel, H. C., 408
Van Husen, H., 356
Vannier, C., 345
Van Schaik, F. W., 409
van Soligen, P., 384
Van Wauwe, J. P., 278
van Wyk, P. J., 459
Varga, J. M., 314
Varma, R., 291
Vassart, G., 416
Vasyanovich, G. P., 53
Vatèle, J. M., 18
Vauquelin, G., 428
Vaver, V. A., 18
Vedamuthu, E. R., 344, 449
Veerkamp, J. H., 409
Vegarud, G., 321
Veis, A., 282, 314
Vel'moga, I. S., 161
Veno, K., 407
Vercauteren, R. E., 340
Verenikina, S. G., 51
Vergella, J., 416
Verhegge, G., 174, 176, 180

Verheyden, J. P. H., 59, 96
Verma, D. P. S., 362
Versluis, C., 186
Vervoort, A., 351
Vessey, D. A., 325
Veyrieres, A., 17
Vibert, M., 331
Vicari, G., 257
Vicedomini, M., 20
Vichanska, J., 408
Vigdorchik, M. M., 63
Vigevani, A., 138
Vignais, P. M., 424
Vignais, P. V., 424
Vignon, M., 15
Vikha, G. V., 315
Vilarem, M.-J., 295
Villa, T. G., 266
Villalba, M., 331
Villanueva, J. R., 336
Villiger, W., 289
Vilter, V., 289
Vink, A. B. J., 161
Vink, J., 216
Viswamitra, M. A., 188
Viswanathan, A., 221
Vitetta, E. S., 315
Vittozzi, L., 356
Vliegenthart, J. F. G., 176, 186, 193, 216
Volk, W. A., 252
von Döhren, H. H., 137
von Figura, K., 296, 297, 332, 334
von Goldammer, E., 178, 184
von Minden, D. L., 139
von Muenchhausen, W., 416
von Nicolai, H., 352
von Sonntag, C., 8, 129
von Wartburg, J. P., 424
Vorbrüggen, H., 140, 141, 143
Vořišek, J., 267
Voronkov, M. G., 53
Vorontsova, L. G., 173
Vottero, P. J. A., 181
Voytenko, I. L., 432
Voznyi, Ya. V., 45
Vyas, D. M., 84, 176, 182

Waaland, J. R., 234
Wachter, M., 18
Wada, M., 208
Waechter, C. J., 324, 402
Wagle, S. R., 373
Wagner, G., 143, 164
Wagner, H., 22
Waheed, A., 320
Wahl, H. P., 102
Waight, E. S., 187, 216
Wakamatsu, E., 299
Waks, S., 240
Walborg, E. F., 209, 279, 309
Walbot, V., 138
Waldman, A. A., 297
Walker, D. L., 99, 104
Walker, G. J., 366, 367
Walker, H., 165
Walker, J. M., 416
Walker, R. T., 164
Walker-Nasir, E., 302
Walle, J., 336
Wallin, R., 312, 416
Walseth, T. F., 257
Walsh, K. A., 312
Walton, H. F., 212
Wander, J. D., 183
Wang, A. H.-J., 134
Wang, J. L., 276, 277
Wang, M. C., 263
Ward, J. B., 239, 243
Ward, M. E., 249
Wardi, A. H., 291
Warecka, K., 301
Warren, C. D., 47, 323, 324
Warren, W. A., 424
Wassiliadou-Micheli, N., 68
Wasteson, A., 208, 294
Watanabe, H., 281
Watanabe, I., 137
Watanabe, K., 360, 401
Watanabe, K. A., 55, 58, 136, 139, 148
Watanabe, S., 221, 260
Watanabe, T., 321
Watanabe, Y., 156
Watarai, A., 135
Waterfield, M. D., 272
Watson, D. C., 287
Watson, G. R., 408, 409
Watson, P. R., 271
Watson, R. R., 99
Watson, S. W., 273
Watt, P. J., 249
Watters, G. G., 344, 437
Watts, D. J., 267
Watts, P., 49
Waxdal, M. J., 277
Weber, A., 331
Weber, P., 69
Weckauf-Bloching, M., 273
Weckerle, W., 62, 80
Weckesser, J., 60
Wedgwood, J. F., 323
Weeks, C., 306
Weeks, G., 276, 306
Weetall, H., 329, 385, 459, 460
Weete, J. D., 264
Wegner, L. J., 318
Weibel, M. K., 211, 385, 459
Weicker, H., 317
Weidmann, H., 41, 66, 111
Weill, C. E., 357, 431
Weinbaum, G., 273
Weinges, K., 181
Weinhouse, S., 288
Weinstein, J., 137
Weinstein, L. I., 422
Weisburger, J. H., 349
Weiser, M. M., 304
Weisleder, D., 36, 37
Weislow, O. S., 431
Weiss, L., 428
Weissman, I. L., 306
Weisz, J., 192, 209
Welbourn, A. P., 250
Welinder, K. G., 276
Wells, A. G., 28, 122
Wells, W. W., 20
Welti, D., 183, 208
Wenger, D. A., 299, 337, 339
Wenn, R. V., 322
Wentworth, D. F., 343
Werries, E., 333
Wessels, J. G. H., 266
Wessman, G. E., 262
Westheimer, F. H., 47
Westhof, E., 178
Westley, J. W., 136
Westmark, B., 208
Westphal, M., 239
Westphal, O., 246
Westphal, U., 314
Westwood, J. H., 177
Wetmore, S., 325
Wheelock, E. F., 431
Whelan, W. J., 220, 383
Whistler, R. L., 81, 84, 146, 414
White, D. A., 402
White, R. C., 288
Whiteman, P., 289
Whitescarver, J., 304
Whitton, P. D., 284, 286
Wick, A. N., 334
Wicken, A. J., 240, 241
Wickett, R. R., 379
Widdowson, D. A., 114
Wide, L., 310
Wiecko, J., 186
Wiederschain, G. J., 338
Wiegandt, H., 403, 406, 414
Wieland, O. H., 428
Wiesman, U., 390
Wietzerbin, J., 261
Wilkie, K. C. B., 224
Wilkinson, H. W., 263
Wilkinson, R. G., 49, 247, 352, 416
Wilkinson, S. G., 249, 250
Will, H., 288
Williams, D. H., 135
Williams, G. C., 27, 172
Williams, G. J. B., 187
Williams, J., 313
Williams, J. M., 32, 41, 65, 66, 107
Williams, J. P., 408, 409
Williams, R. C., 319
Williams, R. J. P., 183
Wilson, A. C., 375
Wilson, D. E., 289
Wilson, D. V., 438
Wilson, G., 353, 368
Wilson, H. R., 188
Wilson, J. T., 214
Wilson, M. M., 139
Wilson, M. S., 187
Wilson, T. H., 243
Winand, R., 306
Winchester, B., 350, 416
Winchester, R. V., 219
Wingard, L. B., jun., 386, 459
Wingate, J. M., 318
Winkler, T., 27
Winter, W. T., 218
Winterfeldt, W., 136
Wintermans, J. F. G. M., 408
Winzler, R. J., 303
Winzor, D. J., 392, 416
Wise, D. S., 142
Witassek, F., 346
Withers, S. G., 328
Witkowski, J. T., 143, 144
Witorsch, R. J., 284
Wöber, G., 383
Woelter, W., 123
Wojnarowski, W., 165, 315
Wold, F., 416
Wolf, N., 111
Wolf, R. O., 357
Wolfe, L. S., 324
Wolfe, R. S., 362

Wolfenden, R., 343
Wolfenstein-Todel, C., 314
Wolfrom, M. L., 21, 160
Wollenberger, A., 288
Wong, C. M., 62
Wong, K.-L., 313
Woo, P. W. K., 138, 139
Wood, C. J., 82
Wood, G., 99
Wood, P. J., 192, 209, 269
Wood, S., 338
Wood, T., 185
Wright, J., 238
Wright, J. A., 405
Wright, K., 226
Wu, H. Y., 60
Wu, M.-C., 127, 247
Wu, S., 324
Wu, T. C. M., 262
Wu, Y. S., 146
Wudl, L., 349
Wusteman, P., 193, 214
Wyke, A. W., 239, 243
Wynder, E. L., 349
Wyss, P. C., 14, 64

Xavier, A. V., 183

Yabuuchi, H., 339
Yachnin, S., 278, 313
Yadomae, T., 216, 269
Yagisawa, M., 137
Yaguchi, M., 193, 216
Yakhontov, L. N., 142
Yakunina, N. G., 141
Yamada, H., 216, 269
Yamada, K. M., 305
Yamada, M., 239
Yamada, S. S., 305
Yamada, T., 196, 213, 217, 218
Yamagata, T., 293
Yamagishi, T., 321
Yamaguchi, H., 263, 271
Yamaguchi, K., 359
Yamaguchi, T., 366
Yamakawa, T., 339, 407
Yamamoto, I., 165
Yamamoto, K., 79, 358, 416
Yamamoto, R., 299
Yamamoto, S., 231, 368, 369, 370
Yamamura, Y., 245, 246, 273
Yamanaka, K., 211, 385
Yamane, K., 359
Yamaoka, N., 151
Yamasaki, K., 321
Yamasaki, R., 74
Yamasaki, T., 136
Yamasaki, Y., 346
Yamashina, I., 301, 303, 305
Yamashita, K., 314
Yamauchi, F., 276
Yamauchi, K., 311
Yamazaki, S., 100
Yamazaki, T., 151
Yanagawa, R., 253
Yanagihara, Y., 246
Yang, F. Y., 321
Yaron, A., 424
Yarovenko, V. L., 358
Yartseva, I. V., 146
Yashiro, H., 376
Yasuda, S., 246
Yasui, T., 20, 231
Yasukawa, T., 433
Yathindra, N., 178, 179
Yeaman, S. J., 287
Yeh, Y., 321
Yehaskel, A. S., 61
Yip, M. C. M., 404
Yocum, R. R., 318
Yogeeswaran, G., 326, 405, 406, 424
Yogesh, C. A., 332
Yokono, T., 122
Yonezawa, D., 271
Yoshida, A., 332
Yoshida, K., 74, 195, 215, 360
Yoshida, R., 409
Yoshikawa, M., 188
Yoshimoto, K., 42
Yoshimura, J., 75, 98, 100
Yoshino, H., 270
Yoshino, T., 51, 160
Yoshioka, Y., 271
Yosizawa, Z., 290, 292, 299, 300, 301
Young, D. S., 212
Young, D. W., 188
Young, F. E., 237
Young, J. C., 116
Young, L. S., 250
Yudina, O. N., 143
Yüceer, L., 197
Yuki, H., 90, 213
Yung, J. W. M., 325
Yunker, M. B., 104, 170
Yurchenco, P. D., 323
Yurewicz, E. C., 321
Yurkevich, A. M., 51, 193
Yutaka, T., 339

Zaborsky, O. R., 385, 450
Zagalak, B., 354
Zahur-ul-Haque, 119
Zaizov, R., 345
Zajac, A., 371
Zakim, D., 325
Zakir, U., 106
Zakrzewski, K., 314, 316
Zalewska-Sobczak, J., 382
Zambotti, V., 351, 398
Zamir, A., 435, 436
Zámocký, J., 88
Zamojski, A., 91
Zanati, W., 239
Zaslow, B., 218
Zatta, P., 325
Závadova, M., 242
Zeeck, A., 10
Žemlička, J., 27, 174
Zen, S., 10, 62
Zenkova, V. A., 135
Zglinski, W., 9
Zhane, Z. K., 47
Zhdanov, Yu. A., 34, 75, 90, 103, 109, 117, 199
Zhdanovich, Yu. V., 135
Zhmyrya, L. P., 7
Ziboh, V. A., 404
Ziderman, I., 219
Zief, M., 123
Ziegler, D., 209, 322
Ziegler, W., 414
Zilliken, F., 352
Zingaro, R. A., 21, 80
Zissis, E., 112, 334
Ziukova, L. A., 370
Živković, R. V., 288
Zobáčová, A., 88
Zolotarev, B. M., 186
Zonneveld, B. J. M., 263, 358
Zoon, K. C., 248
Zopf, D. A., 72, 416, 441
Zubchenko, A. V., 7
Zuckerman, S. H., 281, 282
Zumwald, J.-B., 27
Zupan, J., 167
Zurabyan, S. E., 15, 60
Zwierzchowska-Nowakowska, Z., 91
Zylber, J., 156

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