HAWORTH MEMORIAL LECTURE*

Structural Studies of Polysaccharides

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1 Introduction

Haworth and his co-workers laid the foundation for polysaccharide chemistry, a branch of carbohydrate chemistry that hardly existed before the Haworth era. Few subjects could therefore be more suitable for a Haworth memorial lecture than polysaccharide chemistry.

Polysaccharides are biopolymers composed of monosaccharide residues and, for several of them, non-carbohydrate substituents. They fulfil different functions in Nature, as construction materials, reserve nutrition, thickeners, and lubricants. Some microbial polysaccharides stimulate the production of antibiotic substances in plants, others show antitumour activity. Some polysaccharides are antigenic and are responsible for immunological reactions. Structural studies of polysaccharides are needed in order to correlate their structures with their biological and physical properties. Such studies should involve the determination of components, linkages, sequences, anomeric configurations, and conformation.

There is a vast number of immunologically active, bacterial polysaccharides, both cell-wall polysaccharides and extracellular polysaccharides. In the late 60s we took up studies of these polysaccharides. We felt, however, that in order to cover even a small part of this field, the methods in structural polysaccharide chemistry had to be improved so that they became more accurate and much faster. They also needed to be scaled down so that milligram quantities, rather than gram quantities, would suffice for a structural determination. This was obviously an opportune time for initiating such studies. The combination gas liquid chromatography–mass spectrometry (g.l.c.–m.s.) was better than anything we had before for qualitative and quantitative analysis of complex mixtures, and commercial instruments for g.l.c.–m.s. had become available. Somewhat later, nuclear magnetic resonance (n.m.r.) spectrometers operating in the Fourier transform mode became available and ^{1}H and ^{13}C n.m.r. rapidly became valuable tools in structural studies of polysaccharides.

In the following paper I shall discuss some aspects of structural polysaccharide chemistry. The examples will be taken from our studies of bacterial polysaccharides. These polysaccharides have regular structures and are composed of

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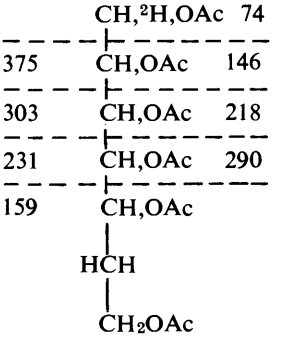
oligosaccharide repeating units, and it should therefore be possible to arrive at conclusive structures. Some other polysaccharides have less regular structures, and for these only average structures may be proposed.

2 Component Analysis

Some 100 different monosaccharide components and 20 different non-sugar components have been found in polysaccharides, and these numbers are increasing. Most sugars and other components are obtained as monomers on acidic hydrolysis, and the qualitative and quantitative analysis is preferably performed by g.l.c.–m.s. of suitable dervatives. The sugars are generally transformed into the acetylated alditols or aldononitriles. Stereoisomers give similar mass spectra, but for a known sugar the relative retention time gives further information, which permits identification, except for the absolute configuration.

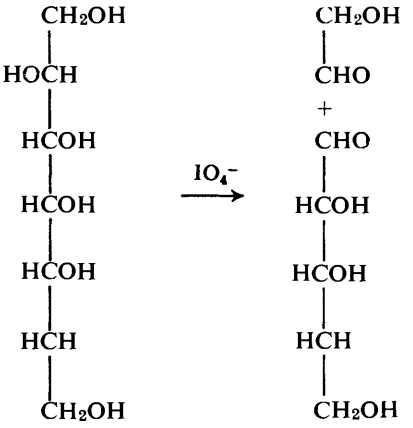
For a new sugar, the mass spectrum gives information on the class to which it belongs. Thus, two new sugars, from a *Yersinia pseudotuberculosis* lipopolysaccharide[1] and from a cell-wall antigen of *Eubacterium saburreum*,[2] were identified as 6-deoxyheptoses from the mass spectra of their [1-^{2}H]alditol acetates (1).[3]



(1)

The primary fragments on electron impact, formed by fission between two acetoxylated carbon atoms, are indicated in struture (1). Fission at the methylene group is insignificant. The sugar from the *Y. pseudotuberculosis* lipopolysaccharide was, according to immunochemical arguments, assumed to have the D-*manno*-configuration, and this was proved by synthesis. The alditol from the *E. saburreum* sugar (2) on oxidation with less than 1 mole of periodate yielded 4-deoxy-D-*erythro*-pentose (3). As oxidation of the glycol groupings with

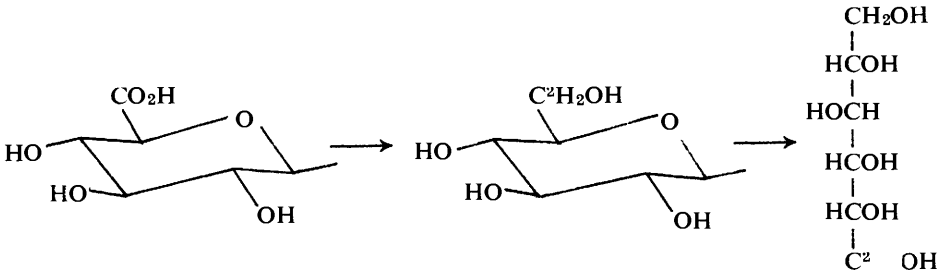
[1] C. G. Hellerquist, B. Lindberg, K. Samuelsson, and R. R. Brubaker, *Acta Chem. Scand.*, 1972, **26**, 1389.
[2] J. Hoffman, B. Lindberg, J. Lönngren, and T. Hofstad, *Carbohydr. Res.*, 1976, **47**, 261.
[3] N. K. Kochetkov and O. S. Chizhov, *Adv. Carbohydr. Chem.*, 1966, **21**, 39.



(2) (3)

trans-hydroxyls in alditols occurs most readily,[4] the sugar was assumed to have the *altro*-configuration. It was also identical with 6-deoxy-D-*altro*-heptose, prepared by an unambiguous route.

Uronic acid residues, *e.g.* (4), are preferably carboxy-reduced before the sugar analysis. The reduction is performed by treatment first with a water-soluble carbodi-imide and then with sodium borohydride.[5] When the carboxy-reduction is performed with borodeuteride, the dideuteriated alditol deriving from the uronic acid (5) is easily distinguished from the non-deuteriated analogue, obtained from the corresponding neutral sugar.

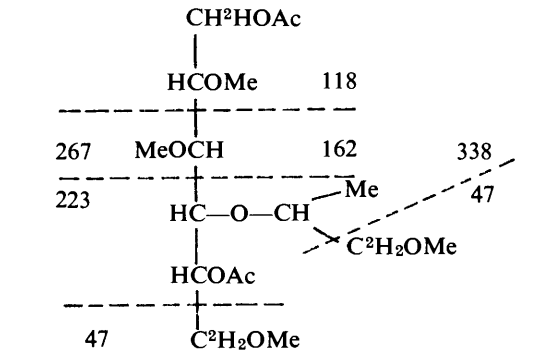


(4) (5)

An acidic sugar from the *Klebsiella* type 37 capsular polysaccharide was assumed to be a uronic acid. When analysed as outlined above, however, it gave

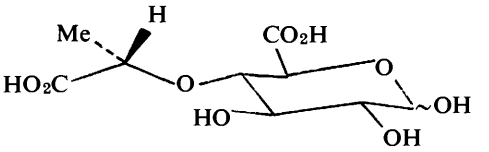
[4] J. C. P. Schwarz, *J. Chem. Soc.*, 1957, 276.
[5] R. L. Taylor, J. E. Shively, and H. E. Conrad. *Methods Carbohydr. Chem.*, 1976, **7**, 149.

a mass spectrum that was difficult to interpret. The mass spectrum of the alditol acetate (6) obtained on methylation analyses of the carboxy-reduced poly-



(6)

saccharide, however, gave valuable structural information. Analysis of this spectrum indicated that the sugar was a hexuronic acid, etherified at O-4 with lactic acid. Further studies demonstrated that the sugar was 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid (7).[6]

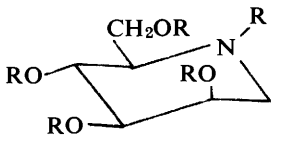


(7)

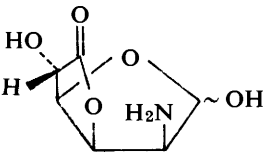
An artefact (8*a*) was observed in the sugar analysis of the capsular polysaccharide from *Streptococcus pneumoniae* type 12F.[7] The mass spectrum of the acetylated component (8*b*) showed, *inter alia*, that it was cyclic and contained one nitrogen atom and five acetyl groups. It was identified as the fully acetylated 1,5-dideoxy-1,5-imino-D-mannitol and is formed from D-mannosaminuronic acid *via* its lactone (9). On treatment of (9) with borohydride, reduction at C-1 probably occurs first. The aldehyde group formed on partial reduction at C-6 then reacts with the amino-group and the intermediate pyranoside, with nitrogen in the ring, is reduced to 8*a*.

Previously, sugar and non-sugar components that were degraded during the hydrolysis of a polysaccharide might have been overlooked. Today, when

[6] B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 1976, **49**, 411.
[7] K. Leontein, B. Lindberg, and J. Lönngren, *Can. J. Chem.*, 1981, **59**, 2081.



(8*a*, R = H; *b*, R = Ac)



(9)

n.m.r. is used as a matter of routine, this should not happen. A good example is the *Vibrio cholerae* O-antigen, which on acid hydrolysis yielded only small amounts of sugars.[8] The ^{13}C n.m.r. spectrum (Figure 1) showed, however, that

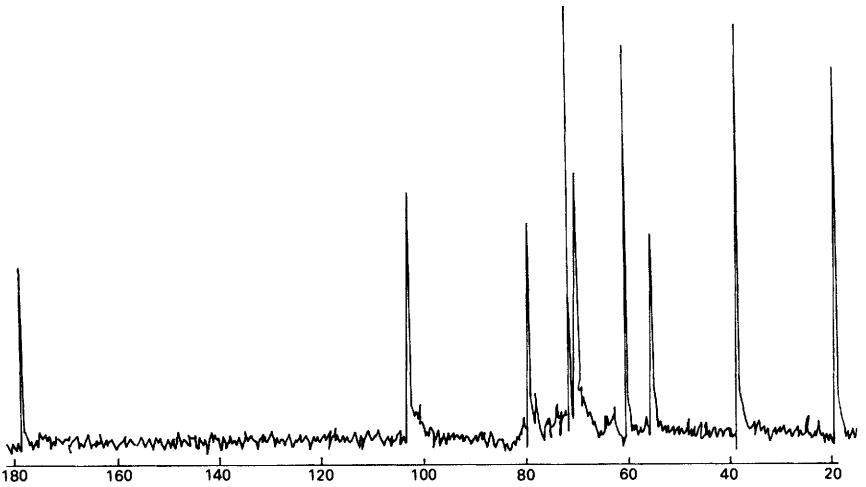
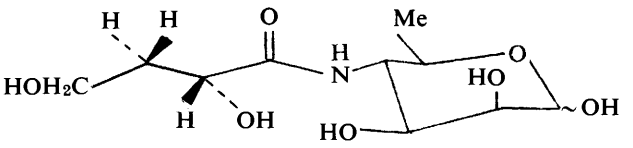


Figure 1 *The* ^{13}C *n.m.r. spectrum of the* Vibrio cholerae *O-antigen*

it had a simple structure and was composed of C_{10} repeating units. Further studies, using n.m.r. and different degradations, showed that the sugar component in the O-antigen is 4-amino-4-deoxy-D-mannose, *N*-acylated with *S*-2,4-dihydroxybutanoic acid (10). The free amino-sugar is decomposed during hydrolysis with acid. It could, however, be isolated as the *N*-acyl derivative by treatment with anhydrous hydrogen fluoride, followed by hydrolysis with acid under mild conditions. This treatment, during which glycosidic linkages are cleaved but amide linkages remain intact, has proved to be of general value in studies of polysaccharides containing *N*-acylamino-sugars.

Some sugars occur both as the D- and the L-form in Nature, and determina-

[8] L. Kenne, B. Lindberg, P. Unger, T. Holme, and J. Holmgren, *Carbohydr. Res.*, 1979,**68** C14.



(10)

tion of their absolute configurations is an essential part of the structural studies. We have devised a simple micro-method for doing this, which involves glycosidation with a chiral alcohol [*e.g.* (+)-octan-2-ol], acetylation, and g.l.c.[9] The products from a D- and an L-sugar are diastereomeric and readily separated on a good column. Thus, the galactan from the snail *Helix pomatia* contains both D- and L-galactose, which was confirmed by this method (Figure 2).

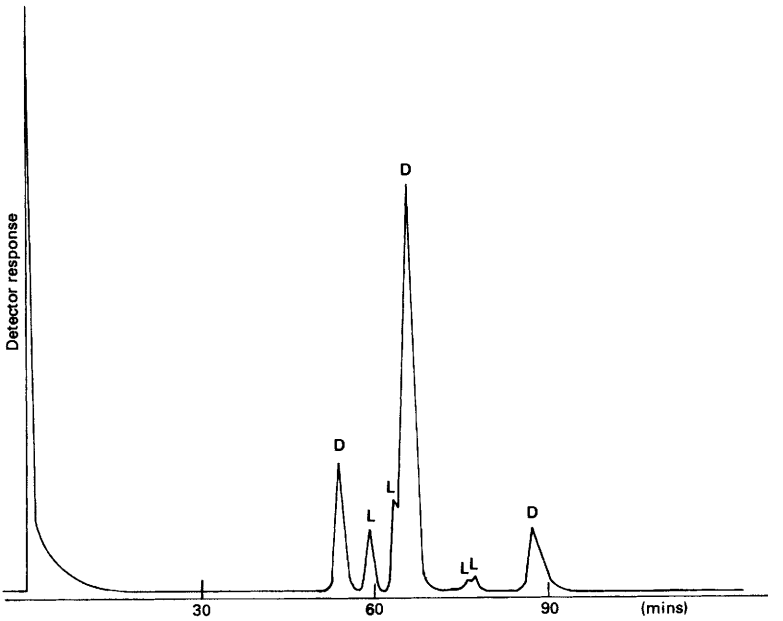
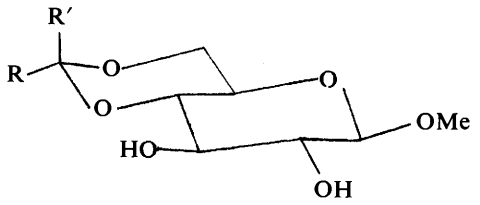


Figure 2 *G.l.c. separation of acetylated* (+)-2-*octyl galactosides obtained from the hydrolysate of the* Helix pomatia *galactan*
(Reproduced by permission from *Carbohydr. Res.*, 1978, **62**, 361.)

[9] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 1978, **62**, 359.

Several bacterial polysaccharides contain cyclic acetals of pyruvic acid. Acetals linked to O-4 and O-6 in hexopyranosides are the most common, but acetals linked to vicinal positions have also been observed. The absolute configuration at the acetalic carbon had only been determined for some polysaccharides, using degradation methods. We therefore synthesized model compounds, representative of the most common types, and showed that the isomeric acetals, *e.g.* (11*a*) and (11*b*), gave typical n.m.r. spectra. [10] Thus, in ^{13}C n.m.r. the equatorial methyl group in (11*a*) (the *S*-acetal) gives a signal at δ 25.5, while the corresponding signal for (11*b*) (the *R*-acetal) occurs at δ 17.7. This large



(11*a*, R = Me and R′ = CO_2H; *b*, R = CO_2H and R′ = Me)

difference allows assignment of the absolute configurations of pyruvic acid acetals with similar stereochemistry in polysaccharides.[11]

3 Methylation Analysis

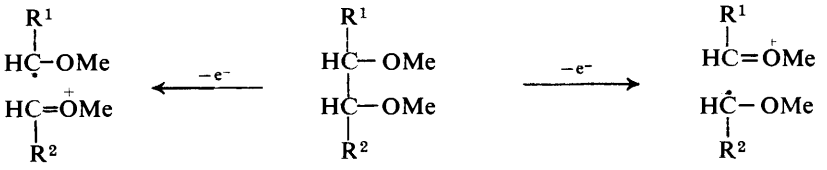
Methylation analysis, developed by Haworth and his co-workers, is still the most important method in structural carbohydrate chemistry. It involves methylation of all free hydroxy-groups in the polysaccharides and hydrolysis of the methylated polysaccharide to a mixture of partially methylated monosaccharides. The free hydroxy-groups in these mark the positions at which the corresponding sugar residues were substituted in the polysaccharide. Qualitative and quantitative analysis of this mixture therefore gives information on how the different sugar residues are linked. It does not, however, give information on sequences or on anomeric configurations.

Even if the principle is the same, the technique has been changed. Previously, several methylation steps were necessary in order to achieve complete methylation. On treatment of the polysaccharide, in dimethyl sulphoxide, with sodium methylsulphinylmethanide and subsequently with methyl iodide, as devised by Hakomori,[12] complete methylation is now obtained in one step. This method has therefore more or less replaced all other methylation methods. Methylation with methyl trifluoromethane sulphonate in trimethyl phosphate, and with 2,6-di-(t-butyl)-pyridine as proton scavanger, is an alternative when the polysaccharide contains alkali-labile substituents which should be preserved.[13]

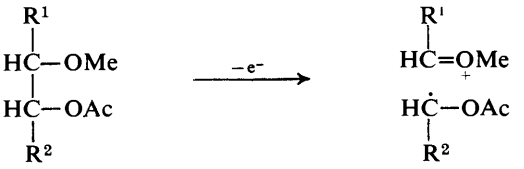
[10] P. J. Garegg, B. Lindberg, and I. Kvarnström, *Carbohydr. Res.*, 1979, **77**, 71.
[11] P. J. Garegg, P.-E. Jansson, B. Lindberg, F. Lindh, J. Lönngren, I. Kvarnström, and W. Nimmich, *Carbohydr. Res.*, 1980, **78**, 127.
[12] S. Hakomori, *J. Biochem. (Tokyo)*, 1964, **55**, 205.
[13] P. Prehm, *Carbohydr. Res.*, 1980, **78**, 372.

Haworth and his co-workers separated the methylated sugars, as their methyl glycosides, by distillation and identified them as crystalline derivatives. Considerable effort and skill was devoted to the synthesis and characterization of all possible methyl ethers of the common monosaccharides. Later, the fractionation was performed by partition chromatography on paper or cellulose columns, carbon column chromatography, or thin-layer chromatography, but the necessity of preparing crystalline derivatives remained. We therefore developed a technique by which the sugars, as their alditol acetates, were analysed by g.l.c.–m.s.[14] The methylation pattern of a component is evident from its mass spectrum. Stereoisomers give almost identical mass spectra but it has always been possible to find columns on which they are separable. All components in a mixture may therefore be identified by g.l.c.–m.s., provided that the sugar composition of the polysaccharide is known. Mass spectra and relative retention times of partially methylated alditol acetates have been compiled.[15]

The interpretation of the mass spectra is simple. Primary fragments are formed by fission of the alditol chain. When a component contains vicinal methoxylated carbons (12), fission between these is preferred and either fragment may carry the positive charge. When a methoxylated carbon has acetoxylated neighbours (13), fission between the methoxylated and an acetoxylated carbon



(12)



(13)

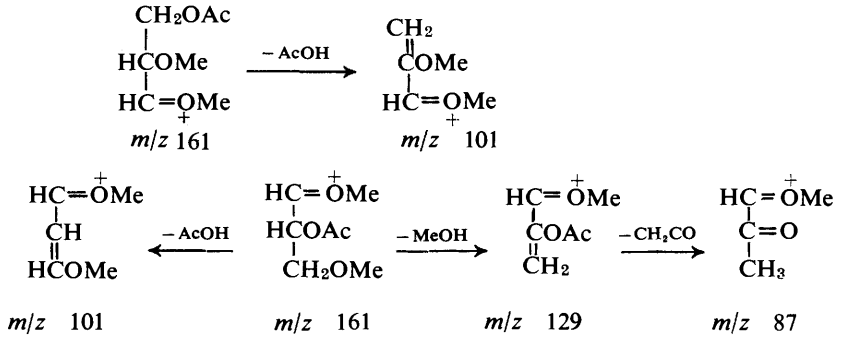
becomes significant and the fragment with the methoxylated carbon carries the positive charge. When a component contains methoxy-groups, fragmentation between acetoxylated carbon atoms becomes insignificant.

The primary fragments give secondary fragments, and the formation of these has been studied in detail, using specifically deuteriated derivatives. The most important reactions giving secondary fragments are single or consecutive

[14] H. Björndal, C. G. Hellerquist, B. Lindberg, and S. Svensson, *Angew. Chem., Internat. Ed. Engl.*, 1970, **9**, 610.

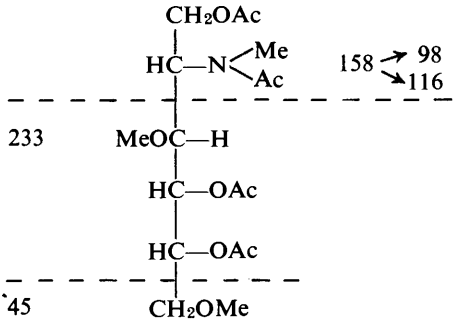
[15] P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun. (Stockholm Univ.)*, 1976, **8**.

eliminations of methanol, acetic acid, and ketene. Eliminations of methanol, ketene, and acetic acid from the two primary fragments of *m/z* 161 are illustrated in Scheme 1.



Scheme 1

Acetamido-sugars become, to a considerable extent, *N*-methylated during the Hakomori methylation. The fission of the derived alditol acetates is governed by the *N*-methylacetamido-group. Thus, for alditol acetates derived from 2-acetamido-2-deoxysugar residues [*e.g.* (14)] the primary fragment *m/z* 158 and the two secondary fragments *m/z* 116 and *m/z* 98 predominate. Other fragments are, however, strong enough to admit the identification of the methylation pattern.



(14)

As discussed above, deuterium may be introduced at C-1 in order to get unsymmetrical derivatives. Other modifications in the procedure, such as dideuteriation of C-6, trideuteriomethylation or ethylation give derivatives, the fragmentations of which are quite analogous to those discussed above. As the methylation analysis has become so simple, it is also used in connection with different specific degradations of polysaccharides, performed in order to determine sequences of the sugar residues (see below).

Table 1 *Methylation analysis of varianose*

Methylated Sugar	*Mole %*	
	A[a]	*B*[b]
2,3,4,6-Glc[c]	14	19
2,3,4,6-Man	—	2
2,3,5,6-Gal	—	5
3,4,6-Man	—	6
3,5,6-Gal	—	20
2,3,6-Gal	Major	28
2,3,5-Gal	—	4
2,4-Man	—	2
3,5-Gal	—	14
Tri-OMe-Hex,n.i.[d]	14	—

[a] Haworth *et al.*, 1935. [b] Jansson *et al.*, 1980. [c] 2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, *etc.* [d] not identified.

In 1935, Haworth, Raistrick, and Stacey reported on the methylation analysis of the fungal polysaccharide varianose.[16] They methylated 50 g of polysaccharide and identified two main components as crystalline derivatives (Table 1). One of these components was 2,3,4,6-tetra-*O*-methyl-D-glucose, and this was the first accurate estimation of end groups in a polysaccharide by methylation analysis. In 1980 we repeated this analysis, starting from 1 mg of polysaccharide.[17] The analysis was completed in two days and four major and five minor components were identified. Another difference is that while the former analysis required considerable experimental skill, the latter mainly required access to some rather advanced equipment.

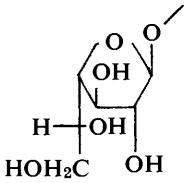
4 Sequence Analysis

Different degradation techniques are used for sequence analysis of polysaccharides. There is no standard method but each polysaccharide presents its own problems. The oldest method is partial acid-hydrolysis and analysis of the oligosaccharides formed. The most commonly used method is the Smith degradation, devised by Fred Smith, a former co-worker of Haworth. There are further methods based upon β-elimination and upon deamination of amino-sugar residues. The different methods have been summarized.[18]

A. Partial Hydrolysis with Acid.—Some polysaccharides contain linkages that are especially sensitive to acidic hydrolysis, the most common being the furanosidic linkages, *e.g.* that of the β-D-galactofuranosyl group (15). Others contain linkages that are only slowly hydrolysed. Thus 2-amino-2-deoxy-glycosides are almost completely resistant, due to the inductive effect of the protonated amino-group [as in (16)]. Uronidic linkages in polysaccharides

[16] W. N. Haworth, H. Raistrick, and M. Stacey, *Biochem. J.*, 1935, **29**, 2668.
[17] P.-E. Jansson and B. Lindberg, *Carbohydr. Res.*, 1980, **82**, 97.
[18] B. Lindberg, J. Lönngren, and S. Svensson, *Adv. Carbohydr. Chem Biochem.*, 1975, **31**, 185.

[as in (17)] are also more resistant to acid hydrolysis than most other glycosidic



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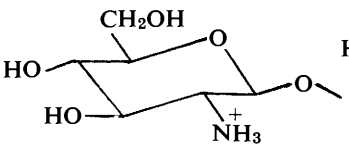

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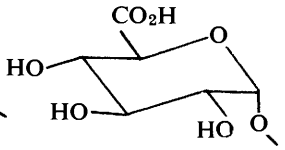
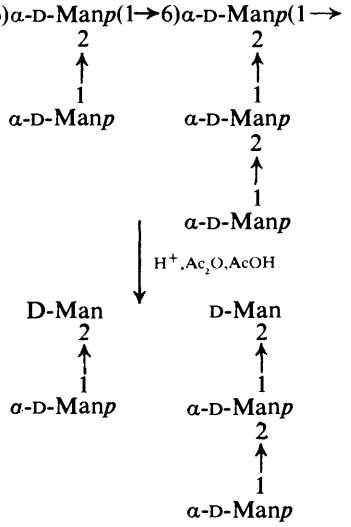


(17)

linkages. Polysaccharides containing linkages of either type and also 'normal' glycosidic linkages therefore give good yields of oligosaccharides on partial hydrolysis. For other polysaccharides the yields of individual oligosaccharides on partial hydrolysis are low, but isolation and indentification of oligosaccharides is nevertheless a common method in structural polysaccharide chemistry.

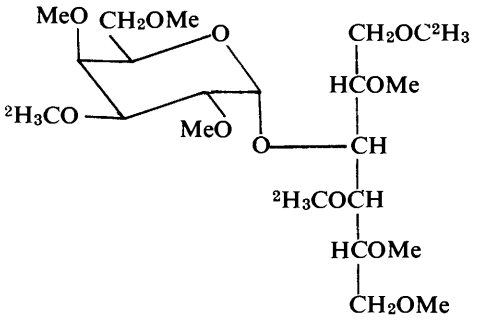
An alternative to hydrolysis is acetolysis, and the relative rates by which different glycosidic linkages are cleaved differ for the two methods. Pyranosidic (1 → 6)-linkages are thus cleaved more readily than other types of pyranosidic linkages on acetolysis. Acetolysis has been a valuable tool in structural studies of fungal α-D-mannans (Scheme 2, in which the mannosyl residues are assumed to be fully acetylated).



Scheme 2

The relative rates of cleavage of different types of glycosidic linkages also seems to differ for acidic hydrolysis and treatment with liquid hydrogen fluoride, but the practical consequences of this remain to be explored.[7]

If the polysaccharide is methylated before acidic hydrolysis and the oligosaccharides are reduced and realkylated, using trideuteriomethyl or ethyl iodide, more structural information is obtained. Thus, the isolation of the alkylated disaccharide alditol (18) demonstrates that the polysaccharide contains the disaccharide element (19), but also that the non-reducing galactosyl group in (19) was linked to the 3-position in the polysaccharide, that the reducing residue was furanosidic, and that both residues were chain residues and not



(18)

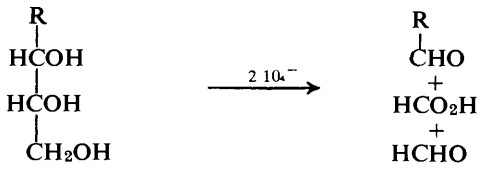
α-D-Gal*p*-(1→3)-D-Gal

(19)

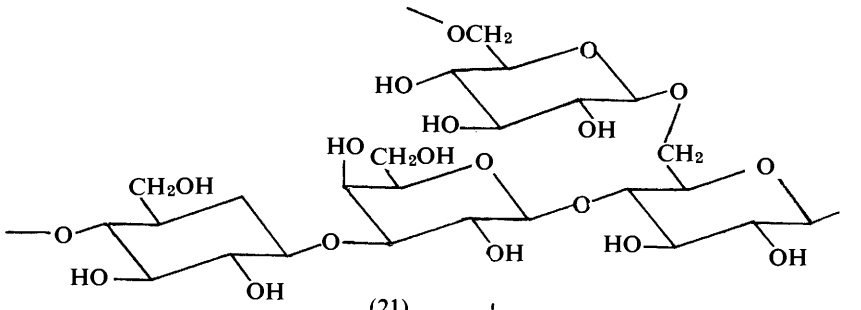
branching residues.[19] The technique has been further developed by Albersheim and his co-workers,[20] who separate the methylated, reduced, and re-ethylated oligosaccharides formed by h.p.l.c. and characterize them. In favourable cases, when the relative rates of hydrolysis for the different types of glycosidic linkages involved do not differ too much, the complete polysaccharide structure may be determined by this method.

B. Smith Degradation.—Substances containing vicinal hydroxy-groups, such as (20), are oxidized by periodate with the formation of carbonyl compounds, the simplest being formaldehyde and formic acid. Several of the methods that have been used in structural polysaccharide chemistry are based upon periodate oxidation, the most important being the specific degradation devised by F. Smith.[21] In this degradation, the periodate-oxidized polysaccharide is reduced with borohydride to a 'polyalcohol'. The acetal linkages in the modified, non-

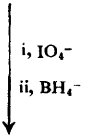
[19] B. Lindberg, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 1972, **23**, 47.
[20] B. S. Valent, A. G. Darvill, M. McNeil, B. K. Robertsen, and P. Albersheim, *Carbohydr. Res.*, 1980, **79**, 165.
[21] I. J. Goldstein, G. W. Hay, B. A. Lewis, and F. Smith, *Methods Carbohydr. Chem.*, 1965, **5**, 361.

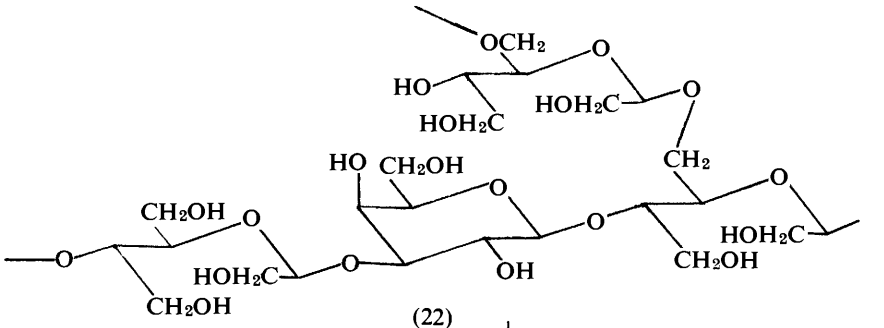


(20)

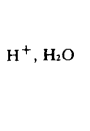


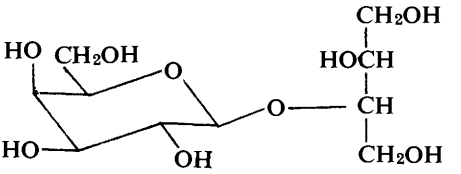
(21)





(22)

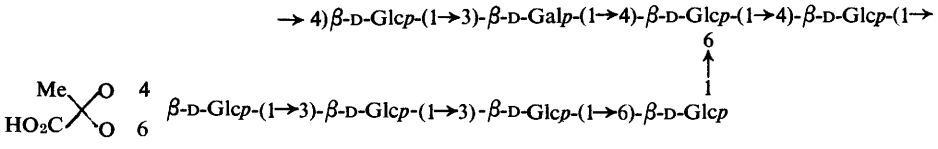




(23)

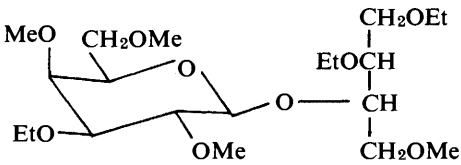
cyclic residues are much more sensitive to acid hydrolysis than the glycosidic linkages of the intact sugar residues, and can be selectively cleaved under mild conditions. Characterization of the product, which may be polymeric or consist of low-molecular weight glycosides, often gives considerable structural information. Smith degradation of a galactoglucan containing the structural element (21) thus gives 2-*O*-β-D-galactopyranosyl-D-erythritol (23) *via* the polyalcohol (22). The identification of this substance demonstrates that the polysaccharide contains β-D-galactopyranosyl residues resistant to periodate oxidation (probably because they are substituted in the 3-position) and linked to O-4 of D-glucopyranosyl residues.

The glycoside (23) was actually isolated after Smith degradation of the capsular polysaccharide, composed of octasaccharide repeating units (24), from



(24)

Rhizobium meliloti.[22] Four of the sugar residues in this unit are parts of the linear backbone. From the isolation of (23), however, it could not be decided if the β-D-galactopyranosyl residue in this backbone was linked to O-4 of the branching β-D-glucopyranosyl residue or to O-4 of one of the chain β-D-glucopyranosyl residues. In order to decide between those possibilities, the 'polyalcohol' was methylated before the mild hydrolysis and the product ethylated. In the glucoside (25), the ethyl groups mark the positions that were substituted in the original



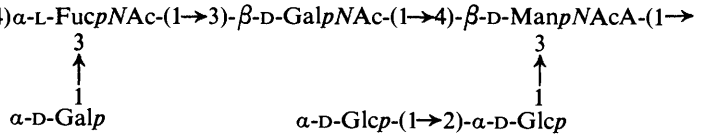
(25)

polysaccharide, namely the 3-position in the β-D-galactopyranosyl residue and the 5- and 6-positions of the β-D-glucopyranosyl residue (which become the 3- and 4-positions of the D-erythritol residue). This modified Smith degradation

[22] P.-E. Jansson, L. Kenne, B. Lindberg, H. Ljunggren, J. Lönngren, U. Rudén, and S. Svensson, *J. Am. Chem. Soc.*, 1977, **99**, 3812.

therefore gives further information and demonstrates that the β-D-galactopyranosyl residue is linked to the branching β-D-glucopyranosyl residue, as in (24). In the conventional Smith degradation, acetal migration with the formation of cyclic acetals often becomes a side reaction,[21] but this complication is avoided in the modified procedure discussed above.

The Smith degradation is especially suitable for polysaccharides containing 2-acetamido-2-deoxyhexopyranosyl residues, as such residues, when substituted in either the 3- or the 4-position, are not oxidized by periodate. In studies of the capsular polysaccharide from *Streptococcus pneumoniae* type 12F,[7] which is composed of hexasaccharide repeating units (26), the side chains were eliminated

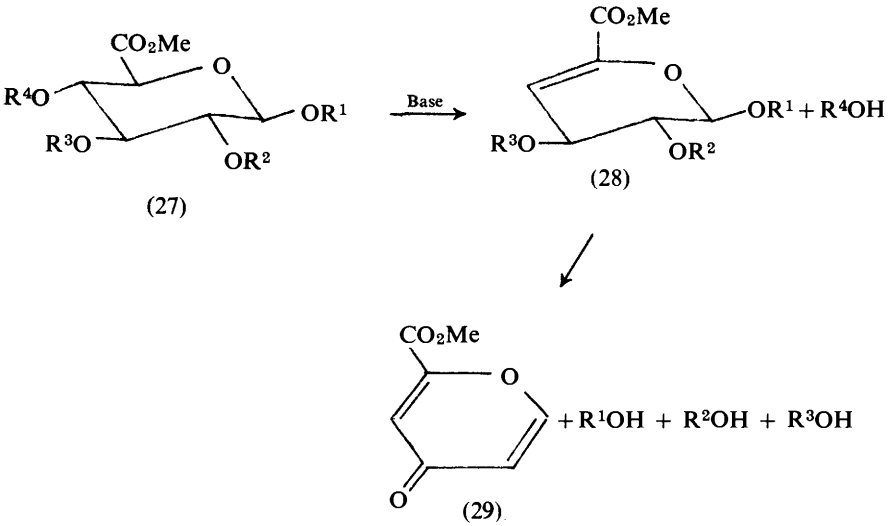


(26)

by Smith degradation, and a linear polymer was obtained. Analysis of this product demonstrated that the 3-positions of the 2-acetamido-2-deoxy-α-L-fucopyranosyl and 2-acetamido-2-deoxy-β-D-mannopyranosyluronic acid residues had become exposed. The side chains were consequently linked to these positions. The hydroxyls at C-3 and C-4 in the α-D-galactopyranosyl group in (26) are the only *cis*-oriented vicinal hydroxyls in this polysaccharide. The α-D-galactosyl group could therefore be selectively oxidized by periodate, and on Smith degradation of this material, the 3-position of the 2-acetamido-2-deoxy-α-D-fucopyranosyl residue became exposed, demonstrating that the α-D-galactopyranosyl group was linked to this position. The possibility of performing similar periodate oxidations increases the usefulness of the Smith degradation.

C. β-Elimination Reactions.—(*i*) *Uronic Acid Degradation.* Several polysaccharides contain uronic acid residues. During the Hakomori methylation of such polysaccharides, the carboxy-groups are simultaneously esterified. On treatment of the methylated product [*e.g.* (27)] with base, β-elimination occurs, and we have used this reaction in structural studies of several acidic polysaccharides.[18] It was first believed that the primary product (28), which contains a vinyl ether linkage, should be sensitive to acid and that the aglycon should be released during hydrolysis with acid under mild conditions. Upon further reaction of the derived 4-deoxy-5-ulosuronide, with formation of a furan derivative, the substituents at C-2 and C-3 should also be released. The unsaturated uronide (28) is, however, remarkably stable to acid.[23] The substituents may nevertheless be split off, but during the alkaline treatment, with the formation of a 4-pyrone derivative.[24] This is in agreement with the results of Aspinall and Rosell,[25]

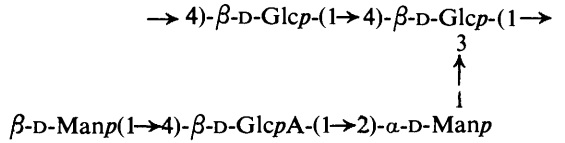
[23] K. Shimizu, *Carbohydr. Res.*, 1981, **92**, 219.
[24] P. Kovác, J. Hirsch, and V. Kovácik, *Carbohydr. Res.*, 1977, **58**, 327.
[25] G. O. Aspinall and K. G. Rosell, *Carbohydr. Res.*, 1977, **57**, C23.



(27) (28) (29)

who demonstrated that the glycosidic linkage may be cleaved during the treatment with base.

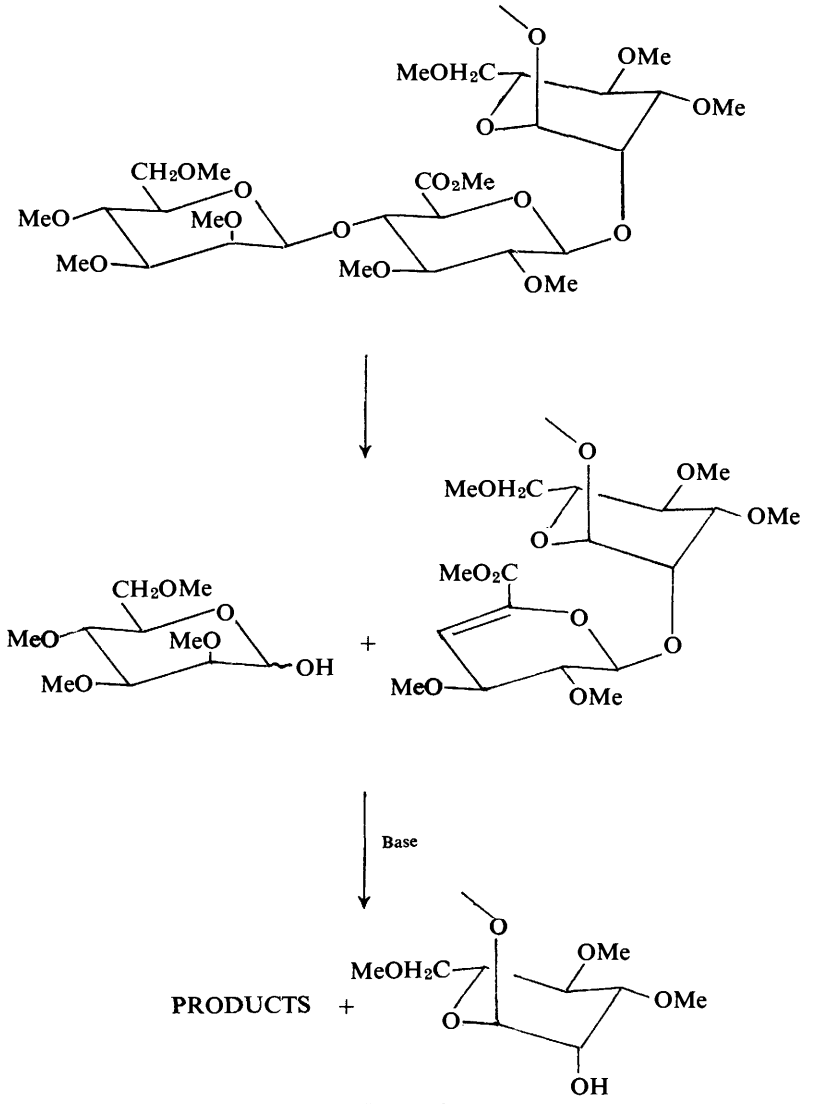
Solutions of xanthan gum, a polysaccharide elaborated by *Xanthomonas campestris*, have unique physical properties and the polysaccharide is produced industrially.[26] It is composed of pentasaccharide repeating units (30). In about



(30)

half of these units, pyruvic acid is acetalically linked to the 4- and 6-positions of the terminal β-D-mannopyranosyl group. In the structural studies of this polysaccharide,[27] uronic acid degradation (Scheme 3) was an essential tool. The terminal and next-to-terminal sugars, D-mannose and D-glucuronic acid, were eliminated when the fully methylated polysaccharide was treated with base. In the polymeric residue, the hydroxy-group at C-2 in the second D-mannosyl residue had become exposed, as demonstrated by trideuteriomethylation and analysis of a hydrolysate. This result, in conjunction with the methylation analysis of the original polysaccharide, gave the structure of the trisaccharide side-chain in (30).

[26] P. A. Sandford, *Adv. Carbohydr. Chem.*, 1979, **36**, 265.
[27] P.-E. Jansson, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 1975, **45**, 275.

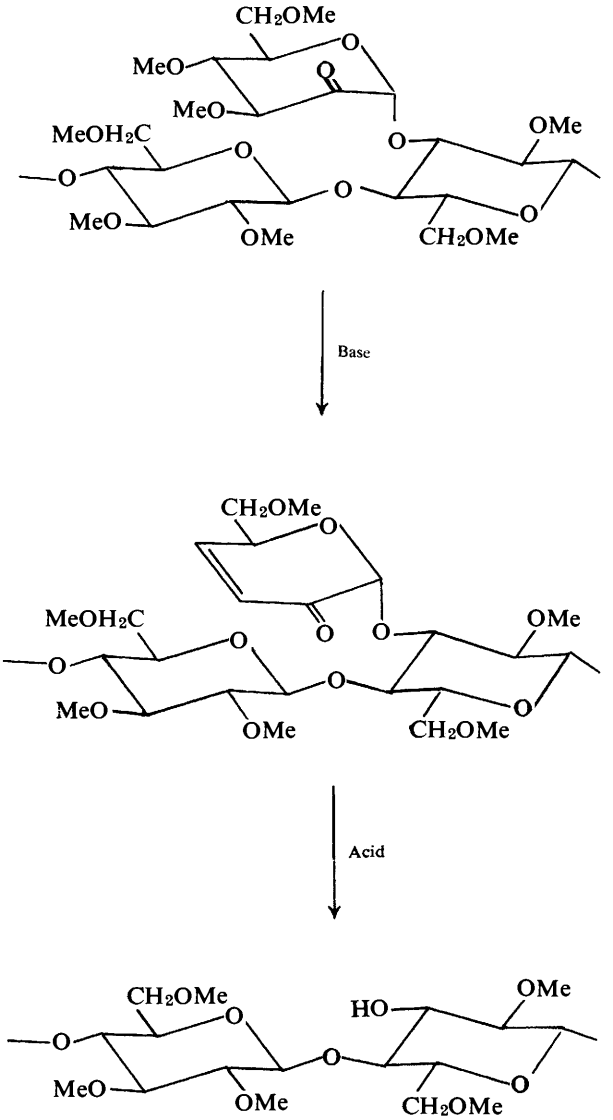


Scheme 3

The course of the uronic acid degradation becomes more complicated when the uronic acid residue is part of the main chain. For such examples, however, it may also give increased structural information.[18]

(*ii*) *Oxidation–β-Elimination.* There are different methods for preparing polysaccharides in which all hydroxy-groups, except a limited number in defined positions, are methylated. One such method is the uronic acid degradation

discussed above. These hydroxyls can be oxidized to carbonyl groups and the product subjected to β-elimination by treatment with base. The unsaturated sugars formed are sensitive to acid hydrolysis. This type of degradation was used in structural studies of xanthan gum (Scheme 4).[27] The partially methylated polysaccharide obtained on uronic acid degradation (Scheme 3) was oxidized,



Scheme 4

using chlorine–dimethyl sulphoxide–triethylamine,[28] treated with base and subsequently with acid under mild conditions. Analysis of the polymeric product showed that it consisted of methylated cellulose, in which every second glucosyl residue contained a free hydroxy-group in the 3-position. The trisaccharide side-chain in xanthan gum is consequently linked to this position.

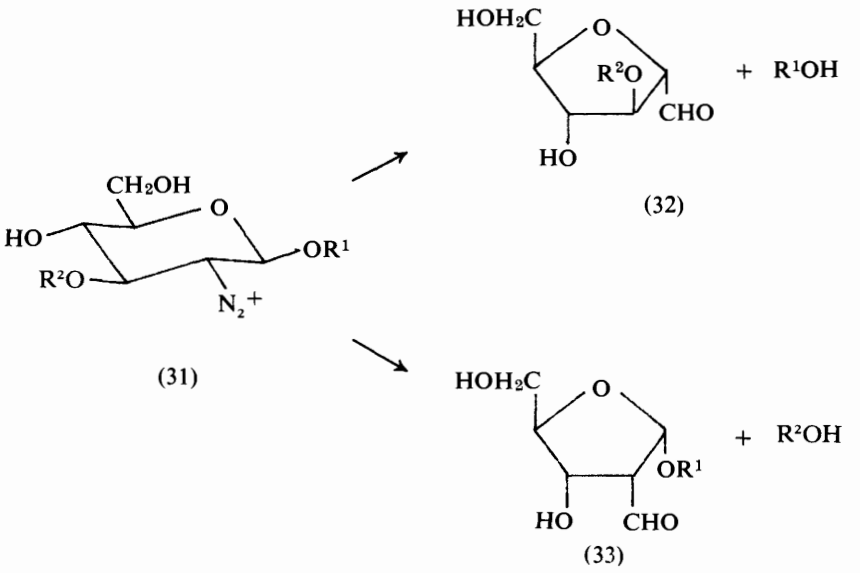
In structural studies of the capsular polysaccharide from *Rhizobium meliloti*, the sugar residues in the tetrasaccharide side-chains (24) were eliminated one after the other by this method, thereby establishing the sequence.[22]

D. Deamination.—Several polysaccharides contain amino-sugars, which offer starting points for specific degradations. The amino-groups are most often acetylated, and have first to be deacetylated. This is generally done by treatment with base, *e.g.* aqueous barium hydroxide or hydrazine. We showed that sodium hydroxide in aqueous dimethylsulphoxide, containing thiophenol, is an efficient *N*-deacetylating agent.[29] A different method is treatment with trifluoroacetic acid and trifluoroacetic anhydride, and hydrolysis of the resulting trifluoroacetamides, which can be done under mild conditions.[30] Because of the inductive effect of the introduced *O*-trifluoroacetyl groups, the glycosidic linkages are stable during the reaction conditions. Some polysaccharides are, however, degraded during attempted *N*-deacetylation by either method, and there is a need for milder, but still efficient methods for *N*-deacetylation.

The deamination of amino-sugars and their glycosides is accompanied by rearrangements.[31] Thus, deamination of a 2-amino-2-deoxy-D-glucopyranoside, *via* the diazonium ion (31), was known to give 2,5-anhydro-D-mannose (32, $R^2 = H$), with release of the aglycon. We showed that part of the reaction ($\sim$ 20 %) takes another course, with formation of a 2-deoxy-2-*C*-formyl-D-*ribo*-hexoside (33).[32] A substituent at C-3 is eliminated during this reaction, which explained some unexpected results in the literature.

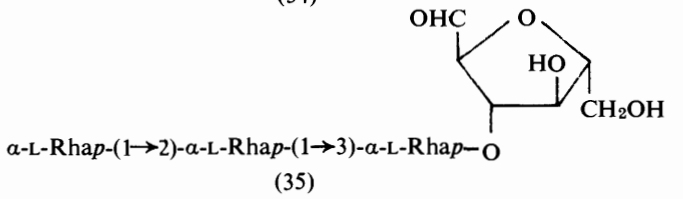
The O-specific polysaccharide from *Shigella flexneri*, variant Y, is composed of tetrasaccharide repeating units (34) and deamination was used in the structural studies of this polysaccharide.[33] The *N*-deacetylated polysaccharide was deaminated by treatment with nitrous acid, yielding tetrasaccharide (35) as the main product. Methylation analysis of the reduced tetrasaccharide showed that one of the 2-substituted α-L-rhamnopyranosyl residues in the original polysaccharide had become the terminal α-L-rhamnopyranosyl group in the tetrasaccharide. The 2-acetamido-2-deoxy-D-glucose is consequently linked to O-2 of L-rhamnose. On treatment of tetrasaccharide (35) with base, the trisaccharide linked to O-3 of the 2,5-anhydromannose residue was eliminated. Methylation analysis of the trisaccharide alditol (36) gave the sequence of the three α-L-rhamnopyranosyl residues in (34).

[28] E. J. Corey and C. U. Kim, *Tetrahedron Lett.*, 1973, 919.
[29] C. Erbing, K. Granath, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 1976, **47**, C5.
[30] B. Nilsson and S. Svensson, *Carbohydr. Res.*, 1978, **62**, 377.
[31] J. M. Williams, *Adv. Carbohydr. Chem.*, 1975, **31**, 9.
[32] C. Erbing, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1973, **27**, 3699.
[33] L. Kenne, B. Lindberg, K. Petersson, and E. Romanowska, *Carbohydr. Res.*, 1977, **56**, 363.



→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-β-D-Glc*p*N*A*c-(1→

(34)

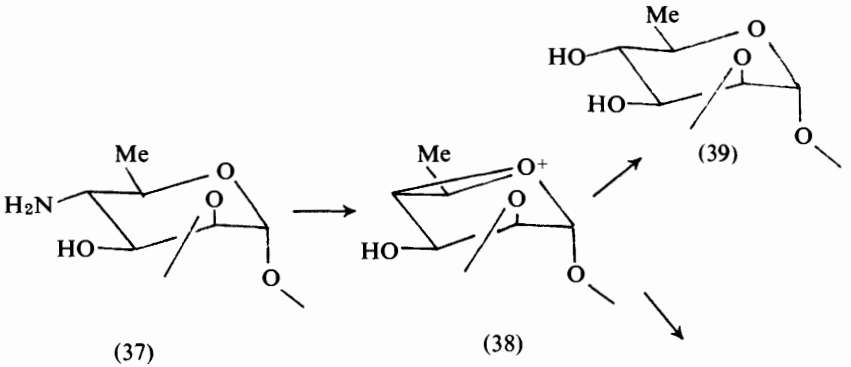


(35)

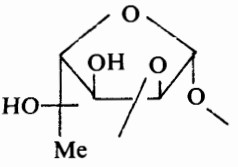
α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-L-rhamnitol

(36)

Deamination reactions have also been applied to polysaccharides containing other amino-sugars. *N*-Deacylation of a polysaccharide from *Vibrio cholerae* yielded a polymer composed of (1 → 2)-linked 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues (37).[8] The main products of deamination, formed *via* the epoxonium ion (38), are the 6-deoxy-α-D-mannopyranosyl (39) and 6-deoxy-β-L-allofuranosyl (40) residues, as confirmed by sugar and methylation analyses of the product. This established the nature of the amino-sugar and its mode of linkage.

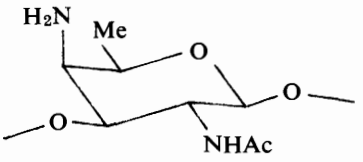


(37) (38) (39)



(40)

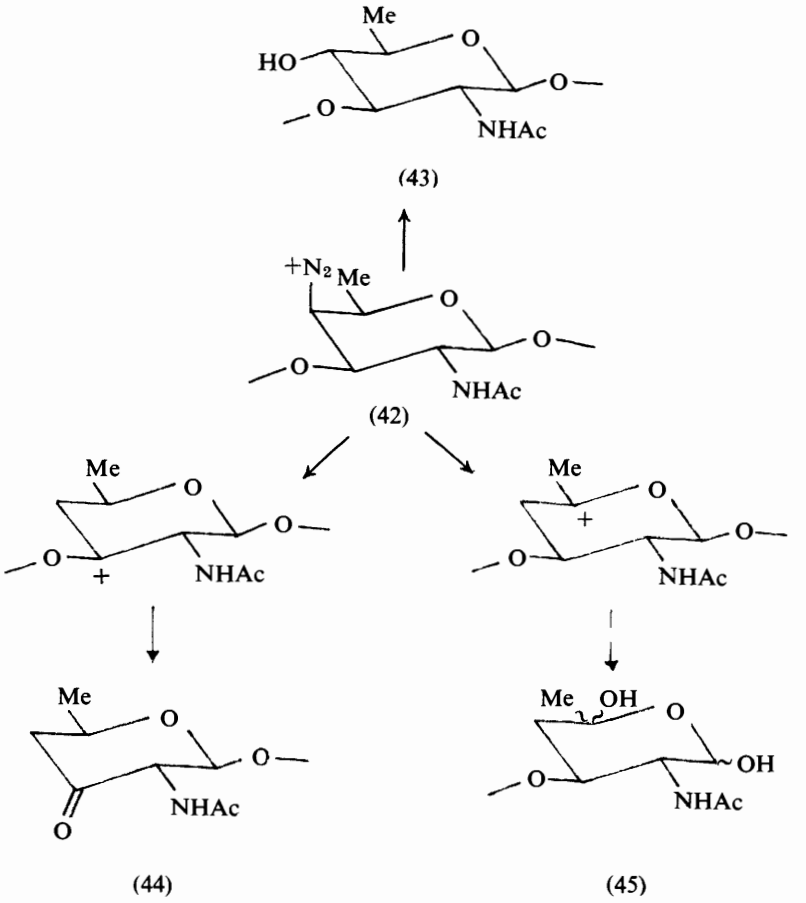
In two polysaccharides containing 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranosyl residues (41) this sugar was degraded during acid hydrolysis



(41)

and could therefore not be isolated, but was identified through its deamination products.[34,35] As the free amino-group is axial, the deamination follows a different course from those discussed above. A direct attack by water upon the diazonium ion (42) gives a 2-acetamido-2,6-dideoxy-D-glucopyranosyl residue (43). Alternatively, hydride shifts of H-3 or H-5 gives 2-acetamido-2,4,6-trideoxyhexos-3- or 5-ulose residues [(44) and (45)], with simultaneous cleavage of the linkage at O-3 or O-1, respectively. Sugar analysis of the deaminated and

[34] B. Lindberg, B. Lindqvist, J. Lönngren, and D. A. Powell, *Carbohydr. Res.*, 1980, **78**, 111.
[35] L. Kenne, B. Lindberg, K. Petersson, E. Katzenellenbogen, and E. Romanowska, *Carbohydr. Res.*, 1980, **78**, 119.



reduced product gave 2-acetamido-2,6-dideoxy-D-glucose and a mixture of 2-acetamido-2,4,6-trideoxyhexoses, which established the structure of the new amino-sugar.

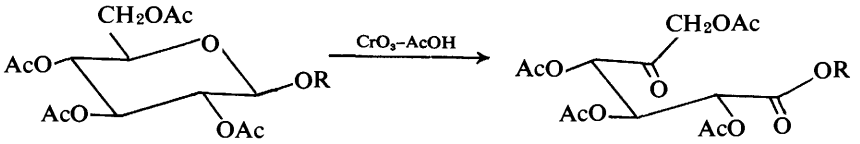
5 Anomeric Configuration

Investigation of oligosaccharides obtained by graded acid hydrolysis, using either enzymic methods or determination of their optical rotations, was until recently the most important method for determining the anomeric configurations of the sugar residues in a polysaccharide. Now ^{1}H and ^{13}C n.m.r. of the polysaccharide and its degradation products has become the method of choice.[36] It is often sufficient to determine chemical shifts and $J_{H\text{-}1,H\text{-}2}$. For pyranosides

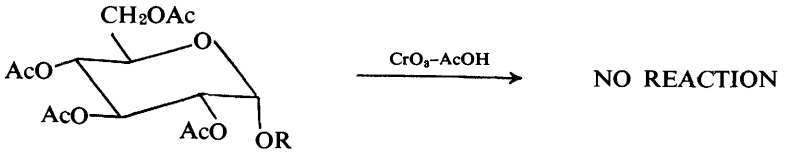
[36] D. R. Bundle and R. U. Lemieux, *Methods Carbohydr. Chem.*, 1976, **7**, 79.

$J_{C\text{-}1,H\text{-}1}$ also has considerable diagnostic value, being ~160 Hz when H-1 is axial and ~170 Hz when it is equatorial.[37]

Angyal and James[38] showed that a fully acetylated glycopyranoside with an equatorial aglycon, *e.g.* (46), is oxidized by chromium trioxide in acetic acid, yielding the ester of a glyc-5-ulosonic acid (47). The corresponding glycopyranoside with an axial aglycon (48) is not reactive. We have adopted this method



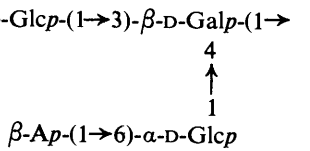
(46) (47)



(48)

for oligo- and polysaccharides.[39] Oxidation of the fully acetylated product, using *myo*-inositol hexa-acetate as an internal standard, and sugar analysis of the product, reveals the anomeric configurations of the different sugar residues. It is essential that the acetylation is exhaustive and also that there is considerable difference in energy between the two alternative chair forms of the sugar residues involved. The method cannot be used for furanosides.

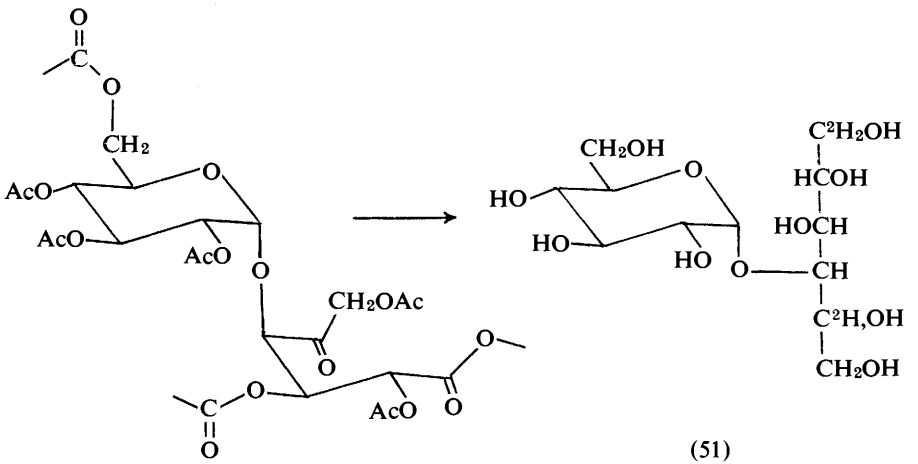
Chromium trioxide oxidation may also, in favourable cases, be used for sequence analysis. The capsular polysaccharide from *Klebsiella* type 37 is composed of tetrasaccharide repeating units (49),[40] in which A is the lactic acid



(49)

[37] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, 1974, 293.
[38] S. J. Angyal and K. James, *Carbohydr. Res.*, 1970, **12**, 147.
[39] J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 661.
[40] B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 1977, **58**, 443.

ether of D-glucuronic acid (7) previously discussed. At the final stage of the structural studies, the only unknown feature was which of the two D-glucopyranosyl residues was α-linked. In order to determine this, the acetylated polysaccharide was allowed to react with chromium trioxide in acetic acid. On treatment of the product (50) with sodium borodeuteride the carbonyl and the aldonate ester groups were reduced. The deacetylated product contained a mixture of two disaccharide alditols (51), of which the D-galactitol derivative predominated.



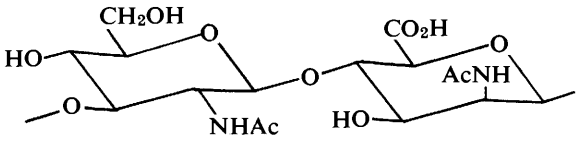
(50) (51)

The structure, determined by g.l.c.–m.s. of the permethylated material and confirmed by methylation analysis, demonstrated that the α-D-glucopyranosyl residue is actually linked to O-4 of the β-D-galactopyranosyl residue, as in (49).

6 Conformation Analysis

In studies aiming at a better understanding of the biological and physical properties of a polysaccharide, determination of its structure is only the first step. The next step should be the determination of its conformation in solution. This has been rather difficult and has only been done for a limited number of polysaccharides; the pioneers in this field being E. D. T. Atkins and D. A. Rees. Recently, however, Lemieux *et al.*[41] have used simple, hard-sphere calculations, with correction for the exoanomeric effect, for the determination of oligosaccharide conformations. They have also demonstrated, using high-field ^{1}H n.m.r. studies, that there is a good agreement between the calculated structures and those actually present in solution. Using their program, we have determined

[41] R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto, and V. S. Rao, *Can. J. Chem.*, 1980, **58**, 631.



(52)

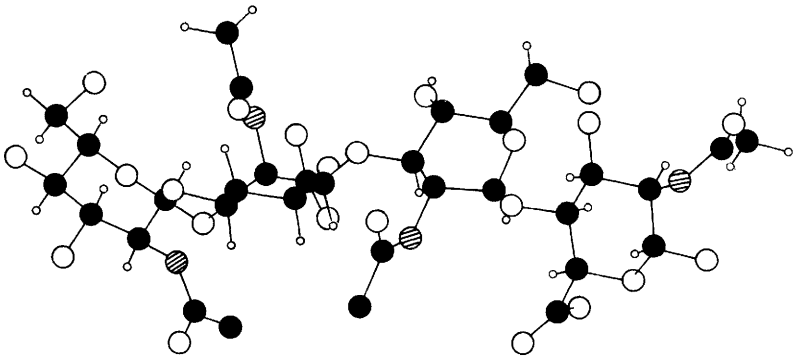


Figure 3 *Conformation of the capsular polysaccharide from* Haemophilus influenzae *type e*

the conformation of some bacterial polysaccharides. One of these is the capsular polysaccharide from *Haemophilus influenzae* type e, composed of disaccharide repeating units (52),[42] which has the conformation depicted in Figure 3.[43]

It is my firm belief that, in the near future, determination of conformation will be included in all studies of biologically important polysaccharides and oligosaccharides.

7 Conclusion

There has been considerable development in structural polysaccharide chemistry and even rather complicated structures can now be determined with moderate effort. Before 1970 only about 10 complete and wellfounded structures of oligosaccharide repeating units in bacterial polysaccharides had been published. As a result of the improved methods, this number has now increased to about 200. There are also good methods for the determination of polysaccharide conformations. As less time has to be devoted to the structural studies one may predict that polysaccharide chemists will pass on to other problems. The most fascinating of these is probably the specific interaction between polysaccharides

[42] P. Branefors-Helander, L. Kenne, B. Lindberg, K. Petersson, and P. Unger, *Carbohydr. Res.*, 1981, **88,** 77.
[43] P.-E. Jansson and L. Kenne, *Personal communication.*

(or oligosaccharide chains in glycoconjugates) and proteins, such as antibodies, lectines, and enzymes.

It is a great honour to present the Haworth Memorial Lecture and I am both glad and grateful for this privilege. I am, however, also well aware that an organic chemist does not stand alone but depends heavily upon his co-workers. I have been especially fortunate and have had a number of skilled and enthusiastic co-workers. Several of these, from Great Britain, Canada, and the U.S.A. are, scientifically, grandchildren or great grandchildren of Sir Norman Haworth and have brought with them something of the spirit of the Haworth carbohydrate school to our laboratory.