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DEGRADATION OF POLYSACCHARIDES CONTAINING URONIC ACID RESIDUES*

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ABSTRACT

A method for the selective degradation of polysaccharides containing uronic acid residues is described. It involves methylation of hydroxyl and carboxyl groups, base-catalysed elimination, and mild hydrolysis with acid. The degraded product is etherified with trideuteriomethyl or ethyl groups and hydrolysed, and the resulting mixture of etherified sugars is analysed, as the alditol acetates, by g.l.c.–m.s. Comparison of this analysis with the methylation analysis of the original polysaccharide gives information on the nature of the sugar residues on either side of the uronic acid residue.

INTRODUCTION

Alkaline degradation of pyranosiduronate residues substituted at position 4, in compounds of low molecular weight and in polysaccharides, has been investigated by Kiss[1], by Aspinall and Barron[2], and by others cited in Ref. 2. This type of degradation has obvious possibilities in structural polysaccharide chemistry and has also been applied in structural studies of a bacterial polysaccharide, colanic acid or M-antigen[3]. We now report a modification of this alkaline degradation, which may give valuable information on sequences of sugar residues in acidic polysaccharides.

RESULTS AND DISCUSSION

When polysaccharides containing hexopyranosyluronic acid residues are methylated by the Hakomori method[4] with methylsulphinyl sodium and methyl iodide in methyl sulphoxide, complete methylation and esterification is obtained in one step. Degradation by β-elimination or reaction of the ester group with the methylsulphinyl anion are insignificant, probably because of the faster reaction between the anion and the methyl iodide which is used in excess. A prerequisite for successful methylation is that the polysaccharide be soluble in methyl sulphoxide and that the

*Dedicated to Dr. Louis Long, Jr., in honor of his 70th birthday.

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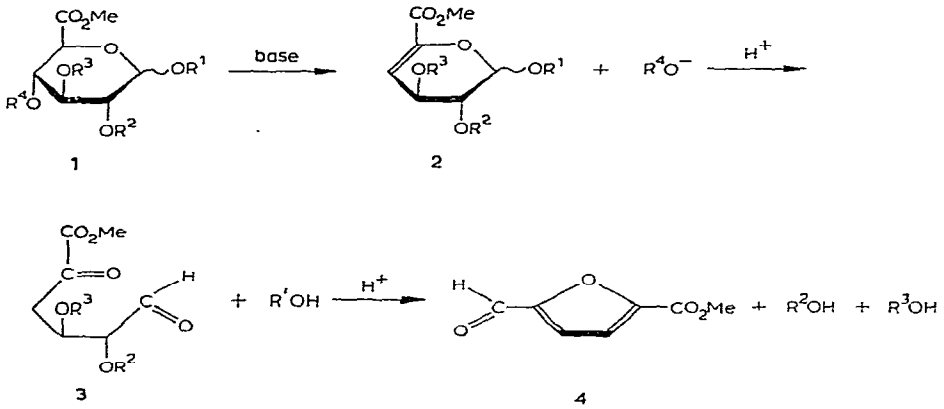
uronic acid residues in the starting material are non-esterified. The methyl uronate residues (*e.g.* **1**) in the methylated polymer carry a good leaving group at position 4, either a derivative of a sugar residue also present in the original polysaccharide or a methoxyl group. On treatment with base, this group should be eliminated and an unsaturated uronate residue (**2**) should be formed. The enol ether grouping in this moiety is acid labile, and mild hydrolysis with acid, using conditions under which glycosidic linkages are stable, should give a substituted 4-deoxyhex-5-ulosuronate (**3**)



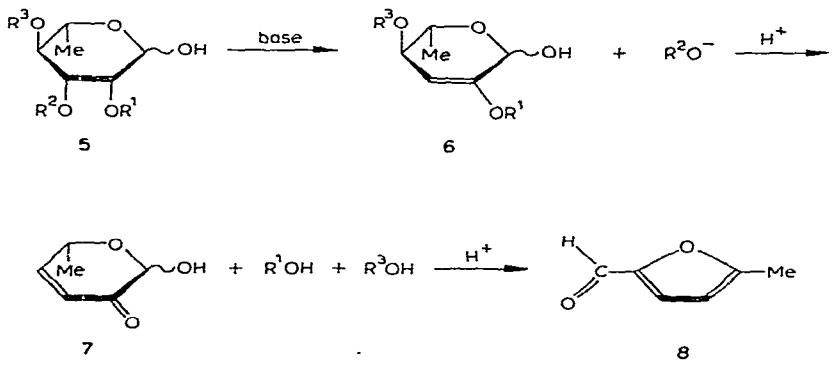
1 2 3 4

R^1 = sugar residue

R^2, R^3, R^4 = Me or sugar residue

and the free aglycon. The ester **3** would probably react further, ultimately yielding a furan derivative (**4**). The closely related 3-deoxyulosonic acids react in this manner[5]. By this sequence of reactions, a selective cleavage of the uronidic linkage, the linkage at C-4, and possibly also the linkages at C-2 and C-3 should be achieved.

When a sugar, *e.g.* L-rhamnose, is linked to C-4 in the uronate residue and after methylation is eliminated by reaction with base, the resulting reducing-sugar



5 6 7 8

R^1, R^2, R^3 = Me or sugar residue

residue (**5**) has a good leaving group at position 3 and will undergo a further β-elimination. The enol ether **6** thus formed is sensitive to acids and will, by analogy with similar etherified monomers[6], give a furan (**8**) *via* the *cis*-glyc-3-enosulose derivative **7**. In the latter reaction, the substituents linked to positions 2 and 4 of **5** will be released.

When the polysaccharide contains terminal uronic acid residues, the only result of the degradation will be elimination of these residues. This reaction has been investigated with two polysaccharides of known structures. One of these is the *Klebsiella* type 9 capsular polysaccharide[7], composed of pentasaccharide repeating units with the structure **9**. The methylated polysaccharide was treated with base, either sodium methoxide in methanol or methylsulphinyl sodium in methyl sulphoxide, and then with aqueous acetic acid. The product was then re-methylated, using trideuteriomethyl iodide, and hydrolysed, and the methylated sugars in the hydrolysate were analysed, as their alditol acetates, by g.l.c.–m.s.[8]. In these and other experiments, the degradation products of uronic acid residues and later also from sugar residues, such as furans, were not accounted for. The results, together with methylation analyses of the original polysaccharide, are given Table I. The decrease in 2-*O*-methyl-L-rhamnose and the concomitant appearance of 2,4-di-*O*-methyl-L-rhamnose trideuteriomethylated at position 4 is in agreement with the previous result that the terminal D-glucuronic acid residue is linked to position 4 of the branching L-rhamnose residue. Most of the uronic acid residues (80–90%) were eliminated, the reaction with sodium methoxide giving the somewhat better result.

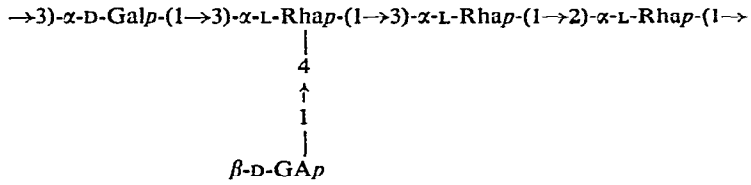


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TABLE I

METHYLATION ANALYSIS OF NATIVE AND DEGRADED *Klebsiella* TYPE 9 CAPSULAR POLYSACCHARIDE

Polysaccharide material	*3,4-Rha*[b] (T *0.92*[c]) (*mole %*)	*2,4-Rha* (T *0.98*)	*2-Rha* (T *1.52*)	*2,4,6-Gal* (T *2.28*)	*2,3,4-G* (T *2.50*)
Native	23	23	29	25	—
Native, reduced[a]	22	18	24	19	18
Degraded, MeO^-, 2 h	24	49[d]	3	24	—
Degraded, $CH_3SOCH_2^-$	29	45[d]	5	22	—

[a]Carboxyl reduced after methylation; see Ref. 7. [b]3,4-Rha = 2,3-di-*O*-methyl-L-rhamnose, *etc.* [c]Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an ECNSS-M column at 170°. [d]~45% of deuterium labelling in OMe-4.

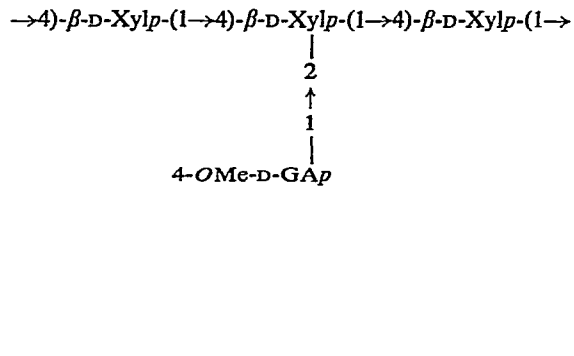
TABLE II

METHYLATION ANALYSES OF NATIVE AND DEGRADED BIRCH XYLAN

Polysaccharide material	*2,3,4-Xyl*[b] (T *0.68*[c]) (*mole %*)	*2Et-3Me-Xyl*[d] (T *1.38*)	*2,3-Xyl* (T *1.54*)	*3-Xyl* (T *2.92*)	*2,3,4-G* (T *2.50*)
Native	1.5	—	92.9	5.5	—
Native, reduced[a]	1.3	—	83.8	7.7	7.3
Degraded, MeO^-, 2 h	1.7	3.8	92.0	2.4	—
Degraded, $CH_3SOCH_2^-$	1.5	4.7	92.3	1.5	—

[a]Carboxyl reduced after methylation, see Ref. 14. [b,c]As in Table I. [d]2Et-3Me-Xyl = 2-*O*-ethyl-3-*O*-methyl-D-xylose.

Hardwood xylan contains chains of (1→4)-linked β-D-xylopyranose residues, some of which[9] carry a 4-*O*-methyl-α-D-glucopyranosyluronic acid residue at position 2 (**10**). A fully methylated birch xylan was subjected to treatments first with base and then with acid, as described above, and the product was ethylated, hydrolysed, and analysed (Table II). The disappearance of 3-*O*-methyl-D-xylose and the appearance of 2-*O*-ethyl-3-*O*-methyl-D-xylose demonstrated that ~60% of the terminal uronic acid residues had been eliminated, the better results now being obtained with methylsulphinyl sodium. Several attempts to increase the yield of the elimination were unsuccessful, and the reason for the relatively low yield is not understood.



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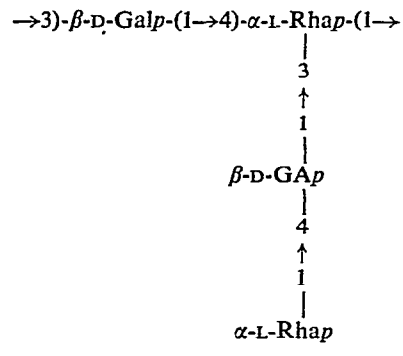

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In the *Klebsiella* type 47 capsular polysaccharide[10], the tetrasaccharide repeating unit has the structure **11**. After degradation of the fully methylated polysaccharide by treatment with methylsulphinyl sodium followed by mild hydrolysis with acid, a polymeric material was recovered by gel chromatography on Sephadex LH-20 (lipophilic). This product represented ~50% of the starting material and, in contradistinction to the latter, showed absorption in the hydroxyl region of the i.r. spectrum, but only faint absorption in the carbonyl region. These results indicate that the elimination of the side chains was essentially complete. The results of different degradations, followed by methylation analysis using trideuteriomethyl iodide, are given

in Table III. The disappearance of 2-*O*-methyl-L-rhamnose and the appearance of 2,3-di-*O*-methyl-L-rhamnose, with a trideuteriomethyl group at position 3, confirms the previous conclusion that the D-glucuronic acid residue is linked to position 3 of the branching L-rhamnose residue. The 2,3,4-tri-*O*-methyl-L-rhamnose from the terminal L-rhamnose residue should be released during the β-elimination and subjected to a further β-elimination, accounting for the decrease of this sugar. With sodium methoxide as base, the eliminations seem to be essentially complete after 15 min. The sugar sequence **12** could be deduced from these results and consequently also the structure of the tetrasaccharide repeating unit, except for the anomeric natures of the sugar residues.

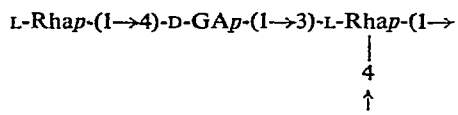

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TABLE III

METHYLATION ANALYSES OF NATIVE AND DEGRADED *Klebsiella* TYPE 47 CAPSULAR POLYSACCHARIDE

Polysaccharide material	*2,3,4-Rha*[b] (T *0.46*[c]) (*mole* %)	*2,3*-Rha*[d] (T *0.98*)	*2-Rha* (T *1.52*)	*2,4,6-Gal* (T *2.28*)	*2,3-G* (T *5.39*)
Native	21	—	37	42	—
Native, reduced[a]	19	—	32	27	22
Degraded, MeO^-, 15 min	8	43	5	44	—
Degraded, MeO^-, 45 min	6	43	2	50	—
Degraded, MeO^-, 2 h	3	43	2	52	—
Degraded, $CH_3SOCH_2^-$	9	41	3	47	—
Degraded, $CH_3SOCH_2^-$[e]	3	51	2	44	—

[a]Carboxyl reduced after methylation; see Ref. 10. [b,c]As in Table I. [d]3* indicates deuterium labelling in OMe-3. [e]Material purified by gel chromatography.

The examples above illustrate the value of the degradation in structural studies of polysaccharides containing uronic acid residues. It is easy to perform, does not require much material, and gives considerable structural information. It has been applied in structural studies of the capsular polysaccharide from *Klebsiella* type 52, composed of hexasaccharide repeating units, and has given information on the sequence of four of the six sugar residues[11].

It should be possible to use this degradation for other purposes. The hydroxyl group released on mild hydrolysis of the unsaturated uronate (**2–3**) with acid could be used as a starting point for further degradation. Replacement of the methyl ether groups by other groups which are more readily removed, *e.g.* acetal groups obtained by reaction of a polysaccharide with methyl vinyl ether[12], would also open interesting possibilities.

EXPERIMENTAL

General methods. — G.l.c., g.l.c.–m.s., and other methods were performed as previously described[7,10].

Methylation of polysaccharides. — The isolation of the capsular *Klebsiella* polysaccharides (types 9 and 47), performed by Dr. W. Nimmich, has been reported[13]. The birch xylan was isolated as described by Timell[9]. The polysaccharide (100 mg) in methyl sulphoxide (100 ml) and 2M methylsulphinyl sodium in methyl sulphoxide (30 ml) under nitrogen, was agitated in an ultrasonic bath for 30 min and kept at room temperature overnight. Methyl iodide (30 ml) was then added dropwise with external cooling. After the turbid solution had been agitated ultrasonically for 30 min, a clear solution was obtained. Excess methyl iodide was then distilled off and the solution was dialysed against running tap water for 24 h and then against distilled water overnight. The dialysed solution was freeze-dried; the yield of methylated polysaccharide was quantitative. I.r. of the methylated products, in chloroform, showed strong absorption in the carbonyl region (1740 cm^{-1}), but no absorption in the hydroxyl region.

Methylation analysis. — Natural and modified polysaccharides, with or without carboxyl reduction of the former, were subjected to methylation analysis as previously described[7,10,14]. The identifications of the different methylated sugars, as the alditol acetates, and the location of trideuteriomethyl or ethyl groups by g.l.c.–m.s.[8] were unambiguous and will not be discussed.

Degradation of methylated polysaccharides, using sodium methoxide as base. — The methylated polysaccharides did not dissolve in methanol–2,2-dimethoxypropane, which was used by Aspinall and Barron[2] to ensure anhydrous conditions, and therefore dichloromethane was added to the system. Carefully dried, methylated polysaccharide (15 mg) and toluene-*p*-sulphonic acid (trace) were dissolved in a mixture (20 ml) of methanol, 2,2-dimethoxypropane, and dichloromethane (18:1:2), and the solution was refluxed for 30 min. A piece of freshly cut sodium (250 mg) was then added to the cooled solution, and the resulting, turbid solution was refluxed for different times (compare Tables I, II, and III). After cooling, the pH was adjusted to 6 by the addition of 50% aqueous acetic acid, water (50 ml) was added, and the mixture was extracted with chloroform (3 × 25 ml). The combined organic phases were washed with water (25 ml) and concentrated to dryness. The product was suspended in 10% aqueous acetic acid (10 ml), kept at 100° for 1 h, and cooled; the modified polysaccharide was recovered by freeze-drying. This material was re-methylated, using trideuteriomethyl iodide (*Klebsiella* types 9 and 47), or ethylated (birch xylan), following the methylation procedure devised by Hakomori[4] and described above. The re-etherified products were recovered by partition between chloroform and water, hydrolysed, transformed into alditol acetates, and analysed by g.l.c.–m.s.[8].

Degradation of methylated polysaccharides, using methylsulphinyl sodium. — A solution of carefully dried, methylated polysaccharide (10 mg) and toluene-*p*-sulphonic acid (trace) in a mixture (2 ml) of methyl sulphoxide and 2,2-dimethoxypropane

(19:1) was prepared in a serum vial which was sealed with a rubber cap. The vial was flushed with nitrogen and agitated in the ultrasonic bath for 30 min, and 2M methylsulphinyl sodium in methyl sulphoxide (1 ml) was added with the aid of a syringe. The solution was agitated for 30 min and kept at room temperature overnight, after which an excess of 50% aqueous acetic acid was added with external cooling. The mixture was poured into water (50 ml) and extracted with chloroform (3 × 25 ml). The combined organic phases were washed with water (4 × 25 ml) and concentrated to dryness. The product was hydrolysed with aqueous acetic acid, re-etherified, and analysed as described above.

In a separate experiment, a larger amount (75 mg) of methylated *Klebsiella* type 47 polysaccharide was treated with methylsulphinyl sodium and subsequently with aqueous acetic acid as described above. The lyophilised product was fractionated on a column (23 × 4.5 cm) of Sephadex LH-20, using chloroform–acetone (2:1) as irrigant. The eluate was monitored polarimetrically. All the optically active material (36 mg) was eluted with the void volume and consequently should be polymeric. This material, in chloroform, showed absorption in the hydroxyl region (3600–3300 cm^{-1}) of the i.r. spectrum, but only faint absorption in the carbonyl region. Part of this material (3.6 mg) was methylated with trideuteriomethyl iodide and analysed as described above.

ACKNOWLEDGMENTS

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REFERENCES

1 J. Kiss, *Carbohyd. Res.*, 10 (1969) 328.
2 G. O. Aspinall and P. E. Barron, *Can. J. Chem.*, 50 (1972) 2203.
3 C. J. Lawson, C. W. McCleary, H. I. Nakada, D. A. Rees, I. W. Sutherland, and J. F. Wilkinson, *Biochem. J.*, 115 (1969) 947.
4 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205.
5 B. A. Dmitriev, L. V. Backinovski, and N. K. Kochetkov, *Dokl. Akad. Nauk SSSR*, 193 (1970) 1304.
6 E. F. L. J. Anet, *Chem. Ind. (London)*, (1963) 1035.
7 B. Lindberg, J. Lönngren, W. Nimmich, and J. L. Thompson, *Carbohyd. Res.*, 25 (1972) 49.
8 H. Björndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610.
9 T. E. Timell, *Advan. Carbohyd. Chem.*, 19 (1964) 247.
10 H. Björndal, B. Lindberg, J. Lönngren, K.-G. Rosell, and W. Nimmich, *Carbohyd. Res.*, 27 (1973) 373.
11 H. Björndal, B. Lindberg, J. Lönngren, M. Mészáros, W. Nimmich, and J. L. Thompson, unpublished results.
12 A. N. de Belder and B. Norrman, *Carbohyd. Res.*, 8 (1968) 1.
13 W. Nimmich, *Z. Med. Microbiol. Immunol.*, 154 (1968) 117.
14 M. Han and B. Swan, *Sv. Papperstidn.*, 71 (1968) 552.