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MODIFICATION OF POLYSACCHARIDES CONTAINING URONIC ACID RESIDUES*†

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ABSTRACT

Side chains, consisting of single or substituted uronic acid residues, on a number of polysaccharides have been eliminated by acetylation, treatment with acetic anhydride–triethylamine, and deacetylation.

INTRODUCTION

Degradation of methylated polysaccharides containing uronic acid residues by β-elimination under basic conditions, followed by mild acid hydrolysis, has proved valuable in structural studies[2,3]. If an analogous reaction could be performed with removable protecting groups, the reaction could be used for the modification of polysaccharides. This would be of interest both for structural and immunochemical studies. For instance, in structural studies, the modified product may be degraded by enzymes more readily than the original polysaccharide.

Previously, polysaccharides have been protected as mixed acetals by treating the hydroxyl and carboxyl groups with methyl vinyl ether and an acidic catalyst[1]. The method was not, however, entirely satisfactory as two consecutive degradations had to be performed in order to achieve elimination of the majority of the uronic acid residues. Kitagawa *et al.*[4] observed that glucopyranosiduronic acid linkages in some saponins are selectively cleaved on treatment with acetic anhydride in pyridine. We now report similar studies with polysaccharides containing uronic acid residues in the side chains.

RESULTS AND DISCUSSION

Preliminary studies were performed with the *Klebsiella* type 28 capsular polysaccharide (K 28), having the repeating unit[5] **1**. The polymeric material obtained by uronic acid degradation of K 28 should be linear and have structure **2**.

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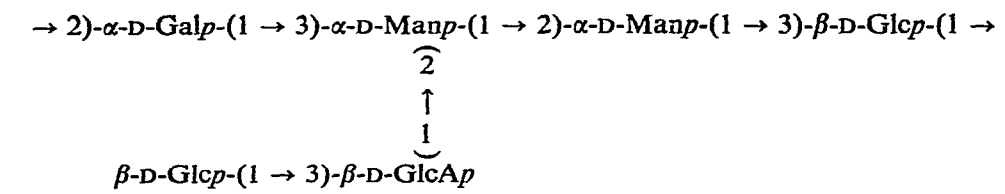
*Dedicated to Professor Dexter French on the occasion of his 60th birthday.
†Part II of a series. For Part I, see ref. 1.



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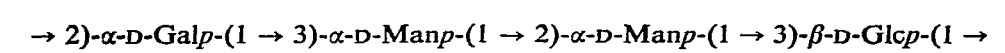

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The polysaccharide, in formamide, was first acetylated by treatment with acetic anhydride and pyridine. The acetylated polysaccharide was then treated with acetic anhydride and a base at elevated temperature, after which the product was deacetylated, and purified by gel filtration. The result of the reaction was determined by methylation analysis. Little elimination was observed when pyridine, sodium acetate, or 1,8-bis(dimethylamino)naphthalene was used as the base. When, however, the reaction was performed in 9:5 acetic anhydride–triethylamine for 2.5 h at 100°, followed by treatment with 90% acetic acid for 2 h at 100°, essentially all of the side chains were eliminated, as revealed by methylation analyses of original and modified material. (Table I). In agreement with this, signals from four anomeric protons were observed in the ^{1}H-n.m.r. spectrum of the modified polysaccharide.

When the *Klebsiella* capsular polysaccharides types[6] 9 (K 9, **3**), 47 (K 47, **4**) (ref. 7), and 59 (K 59, **5**) (ref. 8) were subjected to the foregoing treatment, only partial elimination of the side chains was achieved. The results of the methylation analyses are given in Tables II, III, and IV, and the yields and optical rotations of the

TABLE I

METHYLATION ANALYSIS OF ORIGINAL (A) AND DEGRADED (B) *Klebsiella* TYPE 28 POLYSACCHARIDE

Methylated sugar[a]	*Mol %*	
	A	B
2,3,4,6-Glc	25	
3,4,6-Man	19	27
2,4,6-Glc	18	27
2,4,6-Man		23
3,4,6-Gal	19	23
4,6-Man	19	

[a]2,3,4,6-Glc = 2,3,4,6-tetra-*O*-methyl-D-glucose, and so on.

TABLE II

METHYLATION ANALYSES OF ORIGINAL (A) AND DEGRADED (B) *Klebsiella* TYPE 9 POLYSACCHARIDE

Methylated sugar[a]	*Mol %*	
	A	*B*
3,4-Rha	23	17
2,4-Rha	23	35
2-Rha	29	7
2,4,6-Gal	25	41

[a]See footnote *a*, Table I.

TABLE III

METHYLATION ANALYSES OF ORIGINAL (A) AND DEGRADED (B) *Klebsiella* TYPE 47 POLYSACCHARIDE

Methylated sugar[a]	*Mol %*	
	A	*B*
2,3,4-Rha[b]	25	5
2,3-Rha		26
2-Rha	38	17
2,4,6-Gal	37	52

[a]See footnote *a*, Table I. [b]This component and its derivatives are volatile and are partially lost during concentration.

TABLE IV

METHYLATION ANALYSES OF ORIGINAL (A) AND DEGRADED (B) *Klebsiella* TYPE 59 POLYSACCHARIDE

Methylated sugar[a]	*Mol %*	
	A	*B*
3,4,6-Man	27	24
2,4,6-Glc	25	26
2,4,6-Man	26	25
2,4,6-Gal		17
2,6-Gal	22	8

[a]See footnote *a*, Table I.

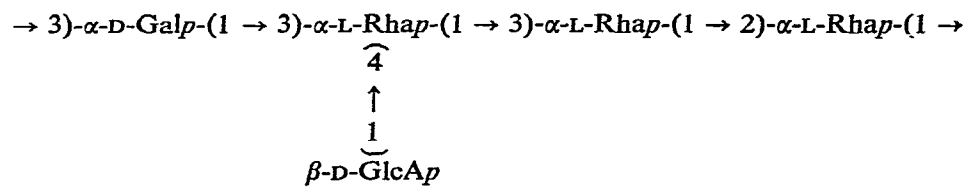
TABLE V

RECOVERY OF DEGRADED POLYSACCHARIDE (A), DEGREE OF ELIMINATION (B), AND SPECIFIC ROTATION OF MODIFIED MATERIAL (C)

Klebsiella *polysaccharide*	*A%*	*B%*	*C*[a]
Type 9	67	67	−13°
Type 28	77	100	+106°
Type 47	90	60	ND[b]
Type 59	100	68	+41°

[a]$[\alpha]_{578}$ (*c* 1, water). [b]ND, not determined.

modified polysaccharides are given in Table V. The degree of elimination, which is not very accurate, is calculated from the percentages of methylated sugars derived from branching points and former branching points in the methylation analysis, for example 2-*O*-methyl- and 2,3-di-*O*-methyl-L-rhamnose for K 47.



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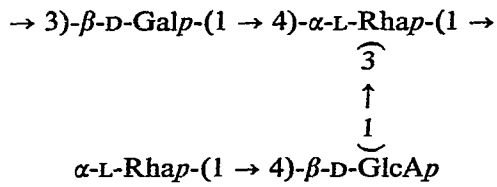

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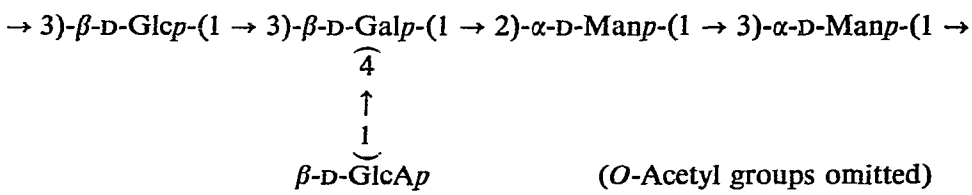

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In order to achieve complete elimination of the side chains, the reaction time and/or temperature were increased. The nonspecific degradation, resulting in discoloration and lower recovery, increased under these conditions but, unexpectedly, the degree of elimination was essentially unchanged. The most unsatisfactory results (only 60% elimination) were obtained with K 47. When modified K 47 was carboxyl-reduced[9] and subjected to methylation analysis, 2,3,6-tri-*O*-methyl-D-glucose, derived from residual uronic acid residues, was detected. The percentage of this component indicated that the remaining uronic acid residues in degraded K 47 were present as such, and had not been modified during the reaction. The glycosyloxy

group at C-4 of the uronic acid residue in K 47 is presumably a poorer leaving-group than the acetate anion.

The mechanism of the reaction has not been investigated. It seems reasonable to assume, however, that the first step is an elimination from the mixed anhydride formed between the uronic acid residue and acetic acid, for example **6**, of the substituent at O-4. The intermediate **7** should be acid labile. It probably reacts further

6 **7**

during the treatment with base, however, as only a slight decrease in the degree of elimination was observed when the step of mild acid hydrolysis was omitted. Similar results were obtained by Aspinall and Rosell[10] when fully methylated polysaccharides containing uronic acid residues were treated with sodium methylsulfinylmethide.

The formation of intramolecular, uronic ester linkages may explain why the degree of elimination is not improved when the reaction time or temperature is increased. The uronic ester groups are less electron-withdrawing than the mixed anhydride groups and therefore β-elimination may not occur on reaction with the relatively weak base.

As the degradation is performed under acetylating conditions, reducing-sugar residues that are released should be protected from further degradation. A similar reaction of methylated polysaccharides has been investigated by Aspinall and Chaudhari[11]. It should, therefore, be possible to use this reaction for degrading polysaccharides, having uronic acid residues in the main chain, into oligosaccharides.

EXPERIMENTAL

General methods. — Concentrations were carried out under diminished pressure at temperatures not exceeding 50°. For g.l.c., a Hewlett–Packard 5830 A instrument equipped with flame-ionisation detection was used. Separations were performed on an SP-1000 glass-capillary column (25 m × 0.25 mm) at 220°. For g.l.c.–m.s., a Varian MAT 311–SS100 m.s.–computer system was used. Separations were performed with a packed column of OV-225, and spectra were recorded at 70 eV. ^{1}H-N.m.r. spectra were recorded on a Varian XL-100 instrument operated in the pulsed, Fourier-transform mode.

Polysaccharide materials. — These were the same as used for structural studies[5–8].

Methylation analysis. — Methylations were performed with sodium methylsulfinylmethide–methyl iodide in dimethyl sulfoxide[12,13]. Methylated materials were recovered by dialysis against tap water, hydrolysed, and transformed into alditol acetates as described earlier[13]. Analysis of methylated sugars was performed by

g.l.c.–m.s. of their alditol acetates[13,14]. The identifications were unambiguous and will not be discussed.

Acetylation of polysaccharides. — The polysaccharide (100 mg) was dissolved in formamide (20 ml) and pyridine–acetic anhydride (1:1, 10 ml) was added. The solution was kept for 15 h at room temperature and then evaporated. The residue was dialysed against tap water overnight and then freeze-dried. Yields were essentially quantitative. In some instances, i.r. spectra (KBr) revealed incomplete reaction, and the acetylation was then repeated.

Degradation of acetylated polysaccharides. — The acetylated polysaccharide (40 mg) was dissolved in acetic anhydride (16 ml) by ultrasonic treatment for 30 min. Triethylamine (13.5 ml) was added and the solution was kept for 2.5 h on a boiling-water bath. The reagents were distilled off and the residue was treated with 90% aqueous acetic acid (10 ml) for 2 h at 100°. (This step was omitted in some experiments without significant changes in the yield of degradation.) The mixture was cooled, dialysed against tap water, and freeze-dried. The recovered material was dissolved in dimethyl sulfoxide (9 ml), and 0.4M sodium hydroxide (3 ml) was added. The solution was kept at room temperature overnight and then dialysed and freeze-dried as before. The i.r. spectrum (KBr) revealed complete deacetylation. The recovered polysaccharide was finally purified by gel filtration on a column (2.5 × 90 cm) of Sephadex G-25 eluted with water. The modified polysaccharide was eluted in the void volume. Results of methylation analyses are given in Tables I–IV and yields, extents of degradation, and optical rotations of the products in Table V. In the ^{1}H-n.m.r. spectrum (D_2O, 80°) of the modified K 28, anomeric protons resonated at δ 5.32, $J_{1,2} \leqslant 2$ Hz (2 H), δ 4.92, $J_{1,2} \leqslant 2$ Hz (1 H), and δ 4.51, $J_{1,2} \geqslant 7$ Hz (1 H).

Attempted degradations by using the bases sodium acetate, pyridine, and 1,8-bis(dimethylamino)naphthalene were performed essentially as in the foregoing.

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REFERENCES

1 M. CURVALL, B. LINDBERG, AND J. LÖNNGREN, *Carbohydr. Res.*, 41 (1975) 235–239.
2 B. LINDBERG, J. LÖNNGREN, AND J. L. THOMPSON, *Carbohydr. Res.*, 28 (1973) 351–357.
3 B. LINDBERG, J. LÖNNGREN, AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 31 (1975) 185–240.
4 I. KITAGAWA, M. YOSHIKAWA, Y. IKENISHI, K. S. IM, AND I. YOSIOKA, Report at the Eighth International Symposium on Carbohydrate Chemistry, Kyoto, Japan, August 1976.
5 M. CURVALL, B. LINDBERG, J. LÖNNGREN, AND W. NIMMICH, *Carbohydr. Res.*, 42 (1975) 95–105.
6 B. LINDBERG, J. LÖNNGREN, J. L. THOMPSON, AND W. NIMMICH, *Carbohydr. Res.*, 25 (1972) 49–57.
7 H. BJÖRNDAL, B. LINDBERG, J. LÖNNGREN, K.-G. ROSELL, AND W. NIMMICH, *Carbohydr. Res.*, 27 (1973) 373–378.

8 B. LINDBERG, J. LÖNNGREN, U. RUDÉN, AND W. NIMMICH, *Carbohydr. Res.*, 42 (1975) 83–93.
9 R. L. TAYLOR AND H. E. CONRAD, *Biochemistry*, 11 (1972) 1383–1388.
10 G. O. ASPINALL AND K.-G. ROSELL, *Carbohydr. Res.*, 57 (1977) C23–C26.
11 G. O. ASPINALL AND A. S. CHAUDHARI, *Can. J. Chem.*, 53 (1975) 2189–2193.
12 S. HAKOMORI, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
13 B. LINDBERG, *Methods Enzymol.*, 28B (1972) 178–195.
14 J. LÖNNGREN AND S. SVENSSON, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 41–106.