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MODIFICATION OF POLYSACCHARIDES CONTAINING URONIC ACID RESIDUES

MARGARETA CURVALL, BENGT LINDBERG AND JORGEN LONNGREN

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-104 05 Stockholm (Sweden)

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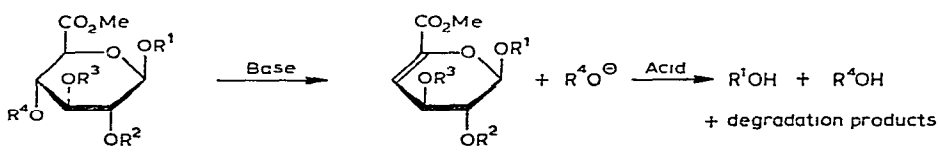
ABSTRACT

Klebsiella Type 47 capsular polysaccharide has side chains attached to the main chain *via* D-glucuronic acid residues The side chains have been removed to yield an essentially linear polysaccharide by the following sequence of reactions (*1*) substitution of hydroxyl and carboxyl groups with methyl vinyl ether, (*2*) β-elimination by treatment with base, (*3*) removal of modified uronic acid residues and protecting groups by mild acid hydrolysis The possibility of modifying other uronic acid-containing polysaccharides by this method is discussed

INTRODUCTION

We recently demonstrated that polysaccharides containing uronic acid residues may be specifically degraded by methylation of hydroxyl and carboxyl groups, base-catalysed elimination, and mild hydrolysis with acid[1]



1 2

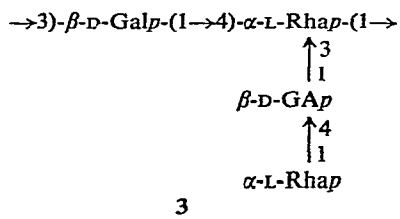
$R^1 - R^4$ = Me or sugar residue

Treatment with base eliminates the substituent at C-4 in the uronic acid residue (**1**) On subsequent mild hydrolysis with acid, the substituent at C-1 is released from the unsaturated uronic acid residue (**2**) Presumably, R^2OH and R^3OH are also released during this treatment Thus, the procedure should cleave not only the uronidic linkage but any other glycosidic linkage in any position of the uronic acid residue This degradation has been applied to structural studies of the *Klebsiella* Type 52 capsular polysaccharide[2] and of other acidic polysaccharides[3] Methyl ethers

are not easily cleaved, but if the methoxyl groups could be replaced by other, more easily removed groups, this method offers possibilities for structural modification of polysaccharides without modifying constituent sugars Experiments aimed at such modifications are reported in the present communication

RESULTS AND DISCUSSION

Klebsiella Type 47 capsular polysaccharide has a tetrasaccharide repeating-unit[4,5] (3)



The hydroxyl groups were protected as mixed acetals by reaction with methyl vinyl ether and a catalytic amount of toluene-*p*-sulphonic acid in methyl sulphoxide, as devised by De Belder and Norrman[6] Similar reactions with dihydropyran and carboxylic acids predict that carboxyl groups should react with the vinyl ether, yielding esters[7] The modified polysaccharide was treated with methylsulphinyl anion in methyl sulphoxide and then with aqueous acetic acid The latter treatment hydrolysed the modified uronic acid residues and the protecting acetal groups Gel filtration yielded the final products, portions of which were methylated according to

TABLE I

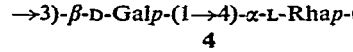
METHYLATION ANALYSES OF ORIGINAL AND MODIFIED *Klebsiella* TYPE 47 CAPSULAR POLYSACCHARIDES

Sugar[a]	T[b]	*Mole %*[c]							
		A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
2,3,4-Rha[d]	0 49	25	26	8	5	3	<1	12	21
2,3-Rha	0 94	—	—	29	36	42	35	28	5
2-Rha	1 24	38	26	18	12	5	10	18	33
2,4,6-Gal[e]	2 01	37	26	45	41	50	55	42	41
2,3,6-Glc[e]		—	22	—	6	—	<1	—	—

[a]2,3,4-Rha = 2,3,4-tri-*O*-methyl-L-rhamnose, etc [b]Retention time of the corresponding alditol acetate, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an SP-1000 glass capillary column at 220° [c]Material: *A*, original polysaccharide, *B*, original polysaccharide, reduced before methylation, *C*, polysaccharide degraded once, *D*, as *C* but reduced before methylation, *E*, polysaccharide degraded twice, *F*, as *E* but reduced before methylation, *G*, polysaccharide acetalated twice and degraded, *H*, polysaccharide degraded with methoxide [d]Part of this volatile ether and derivatives was lost during concentrations [e]These components were separated on an OV-225 S C O T column at 190°

Hakomori[8–10] Hydrolysis yielded methylated sugars which were analysed, as their alditol acetates, by g l c–m s as previously described[9,10] (Table I) In some experiments, carboxyl groups in the polysaccharides were subjected to carboxyl-reduction, as described by Taylor and Conrad[11], before methylation

A linear polysaccharide, composed of disaccharide repeating-units (**4**) was predicted



The percentages of 2,3,4-tri-*O*-methyl-L-rhamnose given in Table I are not very accurate due to the volatility of this ether and its derivatives The completeness of the elimination is best estimated from the percentages of 2-*O*-methyl-L-rhamnose, in the native polysaccharide, and 2,3-di-*O*-methyl-L-rhamnose in the degraded polysaccharide Only ~70% of the side chains were eliminated by one treatment (Table I, columns *C* and *D*). The reason only ~70% of the side chains were removed could not be incomplete esterification, as repetition of the treatment with methyl vinyl ether was without effect (Table I, column *G*) Incomplete elimination is most probably caused by small quantities of water in the reaction mixture when saponification of the ester competes with elimination However, after two consecutive eliminations, most of the side chains were removed, yielding an essentially linear polysaccharide (Table I, columns *E* and *F*) The p m r spectrum of this material contained two signals in the anomeric region in the ratio 1 1 When the elimination was performed using sodium methoxide at room temperature in place of methylsulphinyl anion, the reaction was less complete (Table I, column *H*). However, when the strong, uncharged base 1,8-bis(dimethylamino)naphthalene[12] was tried, no elimination occurred

Thus, by the procedure described, uronic acid-containing side chains in polysaccharides may be shortened or removed, depending on the position of the uronic acid residue Polysaccharides containing uronic acid residues in the main chain may be specifically degraded into oligosaccharides for further study Isolation of a single oligosaccharide, for example, will furnish evidence for the existence of a repeating unit

EXPERIMENTAL

The general methods[4,10], and methods for methylation analysis[8–10] and carboxyl-reduction[2,11] have been described previously G l c of partially methylated alditol acetates was performed on SP-1000 glass capillary columns (25 m × 0 25 mm) at 220° or OV-225 S.C O T. columns[13] (15 m × 0 5 mm) at 190° For g l c–m s , an OV-225 S C O T column was used P m r was performed on a D_2O-solution (80°), using a Varian HA-100 instrument operated in the PFT-mode at 100 MHz with internal sodium 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionate as reference.

Degradation of polysaccharide — Carefully dried polysaccharide (130 mg) was dissolved in Me_2SO (20 ml), methyl vinyl ether (condensed at −35°, 10 ml) and

toluene-*p*-sulphonic acid (5 mg) were added, and the mixture was kept at 15° for 4 h in a sealed serum-vial The reaction was quenched by addition of pyridine (0 5 ml), and excess methyl vinyl ether was flushed away with a gentle stream of nitrogen The reaction mixture was chromatographed using a column (40 × 4 cm) of Sephadex LH-20 developed with anhydrous acetone The eluate was monitored polarimetrically, and the acetalated polysaccharide recovered as a light-yellow syrup (194 mg, 73%) The product, after drying at reduced pressure over phosphorus pentaoxide, was dissolved in Me_2SO (10 ml) contained in a sealed serum-vial flushed with nitrogen Methylsulphinyl anion in Me_2SO (2M, 10 ml) was added, and the reaction mixture was placed in an ultrasonic bath for 30 min and then kept overnight at room temperature Aqueous acetic acid (50%, 5 ml) was added, and the reaction mixture was placed in an ultrasonic bath for 15 min, then dialysed overnight, and freeze-dried The resulting product was suspended in aqueous acetic acid (25%, 10 ml) and kept at 100° for 1 h The solution was evaporated to dryness, and the residue was dissolved in water (2 ml) and chromatographed on a column (90 × 2 5 cm) of Sephadex G-25 developed with water The eluate was continuously monitored by differential refractometry, and the modified polysaccharide, contained in the void volume, was collected and freeze-dried Yield 58 mg, 71% The analyses of the modified polysaccharide, $[\alpha]_{578}^{21}$ −36° (*c* 0 10, water), are shown in Table I, columns *C* and *D*

Sequential degradation — The polysaccharide (46 mg) which had been degraded as described above was treated again with the same reagents, in appropriate quantities The yield of the polysaccharide (25 mg) receiving two treatments was 43% (calculated on the amount of original polysaccharide) The product showed $[\alpha]_{578}^{21}$ −27° (*c* 0 15, water) The p m r spectrum (~10 mg/ml of D_2O at 80°) of this material contained, *inter alia*, signals at δ 5 08 ($J_{1,2}$ ~1 5 Hz, 1 H), 4 72 ($J_{1,2}$ ~7 0 Hz, 1 H), and 1 24 p p m ($J_{5,6}$ ~6 0 Hz, 3 H) The signals were attributed to the anomeric protons of the L-rhamnose and D-galactose residues and to the methyl group of the L-rhamnose residue, respectively The methylated product contained the sugars reported in Table I, columns *E* and *F*

Degradation of the polysaccharide sequentially treated with methyl vinyl ether — Carefully dried polysaccharide (25 mg) was acetalated as described above, using appropriate amounts of reagents The acetalated material was chromatographed using a column of Sephadex LH-20 and then immediately subjected to a second acetalation procedure The product was recovered, and degraded by β-elimination and hydrolysis The degraded material was recovered and a portion was subjected to methylation analysis (Table I, column *G*)

Degradation using sodium methoxide as base — A sample of acetalated polysaccharide (from 25 mg of original material) was dissolved in Me_2SO (2 ml), methanolic sodium methoxide (1 5M, 2 ml) was added, and the reaction mixture was kept overnight An excess of aqueous acetic acid (50%) was added and the mixture dialysed The product was recovered by freeze-drying and further treated as described for the other degradations, and part of the product was subjected to methylation analysis (Table I, column *H*)

Treatment with 1,8-bis(dimethylamino)naphthalene as base — A sample of acetalated polysaccharide (from 25 mg of original material) was dissolved in dichloromethane (5 ml), and 1,8-bis(dimethylamino)naphthalene (50 mg) was added The reaction mixture was placed in an ultrasonic bath for 30 min and then kept at room temperature overnight After neutralisation, the reaction mixture was dialysed overnight and the freeze-dried product further treated as described previously Part of the product was used for methylation analysis

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