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Preliminary communication

Base-catalyzed degradations of methylated acidic polysaccharides: a modified procedure for the determination of sites of attachment of hexuronic acid residues*

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The base-catalyzed β-elimination of 4-*O*-substituted hexuronate derivatives results in the formation of hex-4-enopyranosiduronates[1]. The reaction has been applied to methylated polysaccharides[2], where chain cleavage results in the exposure of reducing groups which, unless protected[3], undergo further degradation. Although some cleavage of glycosyluronic acid linkages during alkaline treatment of methylated acidic polysaccharides has been reported[4], it has been assumed that the majority of chains are still terminated by hex-4-enopyranosyluronic acid residues, and that mild treatment of the degraded polysaccharide with acid is necessary to effect selective hydrolysis of the modified units in order to expose hydroxyl groups to which uronic acid residues were attached. We have now shown that treatment of methylated polysaccharides with M sodium methylsulfinylmethanide in methyl sulfoxide, under the conditions described by Lindberg *et al.*[2], results in complete loss of hexuronic acid residues, and that the subsequent acid hydrolysis is unnecessary. We therefore report a modified procedure for the determination of the site of attachment of uronic acid residues in polysaccharides. In *a single operation*, the methylated polysaccharide is treated with base[2] and then, without work-up, alkylated with trideuteriomethyl or ethyl iodide to label the site(s) to which uronic acid residues were attached. The procedure thus avoids intermediate isolation of degraded polysaccharide and possible loss of acid-labile glycosyl substituents which might occur during the acid treatment designed to achieve selective hydrolysis of unsaturated glycosyluronic acid units.

Methyl 2,3,4-tri-*O*-methyl-6-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-galactopyranosyluronate)-β-D-glucopyranoside[5] was kept overnight in 0.67M sodium methylsulfinylmethanide in methyl sulfoxide at room temperature. After neutralization of the reaction mixture, direct extraction with chloroform and chromatography of the extracted material on silica gel furnished crystalline methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside in 89% yield. In separate experiments, samples (5 mg each) of the methylated disaccharide and methyl 2,3,4,6-tetra-*O*-methyl-αβ-D-galactopyranoside (as internal standard) were treated

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.

TABLE I

METHYLATION ANALYSES OF NEUTRAL SUGAR COMPONENTS OF ORIGINAL AND MODIFIED *Klebsiella* TYPES 47 AND 81 CAPSULAR POLYSACCHARIDES

Methylated sugar[a]	T[b]	*Mole %*[c]			
		A	*B*	*C*	*D*
2,3,4-Rha[d]	0.42	24	3	–	26[f]
3,4-Rha	0.87	–	–	37	16
2,3-Rha	0.92	–	46[e]	–	–
2,4-Rha	0.94	–	–	42	31
2-Rha	1.37	37	4	–	–
2,4,6-Gal	2.03	39	47	21	28

[a] 2,3,4-Rha = 2,3,4-tri-*O*-methyl-L-rhamnose, *etc.* [b] Retention time of the derived alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an OV-225 column at 170°. [c] Polysaccharide: *A*, original type 47; *B*, type 47, degraded and trideuteriomethylated; *C*, original type 81; *D*, type 81, degraded and trideuteriomethylated. [d] Part of this volatile ether and derivative was probably lost during work-up. [e] Trideuteriomethylated at O-3. [f] Trideuteriomethylated at O-2.

with M sodium methylsulfinylmethanide in methyl sulfoxide (2 ml) for various periods of time. Methyl iodide (1 ml) was added dropwise with cooling and the resulting solutions were stirred for 0.5 h. Water (10 ml) was added, the solutions were extracted with chloroform, and the dried extracts were concentrated and examined by g.l.c. The results showed that loss of uronic acid residues was complete after 1 h, and that methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside was formed in 92% yield.

Methylated derivatives of the capsular polysaccharides from *Klebsiella* types 47[2,6] and 81[7], and of birch xylan[2,8], were degraded with M sodium methylsulfinylmethanide in methyl sulfoxide at room temperature for 16 h, and then directly re-alkylated. The modified polysaccharides were hydrolyzed, and the resulting methylated sugars were analyzed by g.l.c.–m.s. as the alditol acetates[9]. The results were in good agreement with those previously reported. Table I shows that, for the *Klebsiella* type 47 polysaccharide **1**,

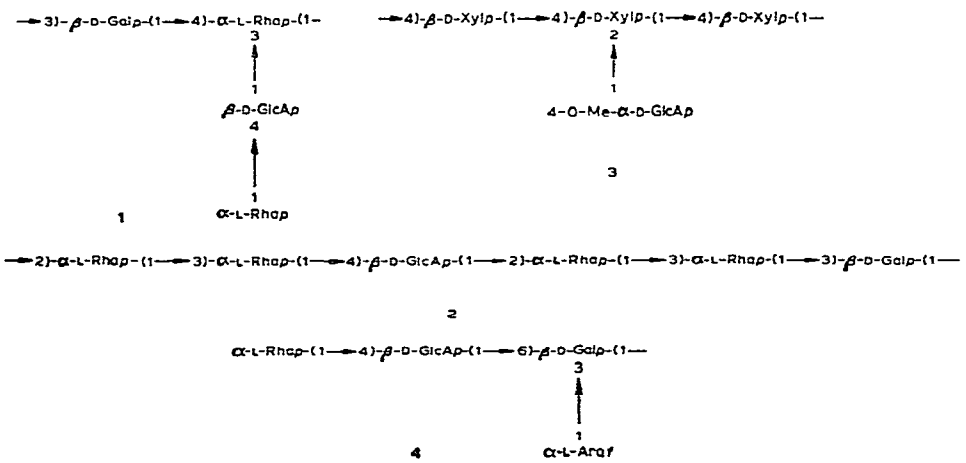

TABLE II

METHYLATION ANALYSES OF NEUTRAL SUGAR COMPONENTS OF NATIVE AND MODIFIED BIRCH-XYLAN

Polysaccharide	*Mole %*				
	2,3,4-Xyl[a] *(T = 0.68*[c]*)*	*2-Et-3-Me-Xyl*[b] *(T = 1.38)*	*2,3-Xyl (T = 1.54)*	*2,3,4-Glc (T = 2.50)*	*3-Xyl (T = 2.92)*
Native	1		93		6
Native, reduced[d]	1		82	8[e]	9
Base treatment, 0.5 h, ethylated	1	7	90		2
Base treatment, 1 h, ethylated	1	8	90		1
Base treatment, 4 h, ethylated	1	8	90		1

[a]2,3,4-Xyl = 2,3,4-tri-*O*-methyl-D-xylose, *etc.* [b]2-Et-3-Me-Xyl = 2-*O*-ethyl-3-*O*-methyl-D-xylose. [c]Retention time of the derived 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]Carboxyl-reduced ($LiAlD_4$) after methylation. [e]Deuterium labelling at C-6.

virtually complete loss of glucuronic acid residues and attached rhamnose end-groups occurred, and that label was incorporated at O-3 of L-rhamnose to which the uronic acid was formerly attached. With the type-81 polysaccharide **2**, loss of D-glucuronic acid residues involved cleavage of the main chain and was accompanied by some further degradation of exposed reducing-groups. However, in the absence of the acid hydrolysis step, the recovery of virtually all the galactose residues indicated that these residues were probably protected from further degradation by carrying glycosidically bound hex-2-enopyranose units. For the birch xylan **3** (Table II), the recovery of 2-*O*-ethyl-3-*O*-methylxylose after *O*-ethylation, in proportions corresponding to those of the original uronic acid, indicated that complete loss of acid units took place during the base treatment. Further experiments on this methylated polysaccharide showed that loss of uronic acid residues was complete after treatment with base for only 1 h.

Gum arabic contains terminal L-rhamnopyranose residues, and the isolation of 4-*O*-α-L-rhamnopyranosyl-D-glucopyranose on partial acetolysis of the carboxyl-reduced polysaccharide[10] showed that at least some of the L-rhamnose end-groups were linked to D-glucuronic acid residues. Treatment of methylated gum arabic with sodium methylsulfinylmethanide in methyl sulfoxide, followed by direct ethylation, furnished a modified polysaccharide from which all the 2,3,4-tri-*O*-methylrhamnose residues had been removed without change in the relative proportions of arabinofuranose and galactopyranose residues. Additionally, the location of ethyl groups at O-6 of galactose residues confirmed the site of attachment of glucuronic acid residues. These galactose residues were non-uniformly substituted, presumably as a consequence of the attachment of side-chains (probably of arabinofuranosyl units) to some of these residues in this portion (**4**) of the structure of the gum. In other experiments, it was shown that some loss of arabinofuranose residues occurred when methylated gum arabic was heated in aqueous 10% acetic acid at 100° for 1 h.

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