Short Communication

Methylation Analysis of Polysaccharides with Butyllithium in Dimethyl Sulfoxide*

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The most common method for methylation of polysaccharides in recent years has involved the use of methylsulfinyl carbanion, introduced by Hakomori,[1] as alkoxide-forming reagent. This reagent may be obtained by treatment of dry dimethyl sulfoxide (DMSO) with sodium[1] or potassium[2] hydride. Recently, the lithium salt of methylsulfinyl carbanion, obtained by treatment of DMSO with butyllithium, has been shown to have superior performance compared with the sodium and potassium salts.[3,4]

A direct method for sugar alkoxide formation using solid base (NaOH and KOH) in DMSO solution has been reported by Ciucanu *et al.*[5] This communication reports a convenient method for direct sugar alkoxide formation using butyllithium (15 % in hexane) in DMSO. After addition of the reagent, methyl iodide is added to the same reaction vial without evaporation of the butane and hexane introduced with the butyllithium solution.

Ten different glucans of known structure were chosen in order to test the viability of the method. Complete and very clean methylation was achieved for all ten polysaccharides.

Hydrolysis of the methylated polysaccharides was performed with a combination of formic acid/trifluoroacetic acid. Hydrolysis with trifluoroacetic acid alone, as described by Harris *et al.*,[6] was found to be insufficient for the complete degradation of 1,6-linked polysaccharides.

The methylated monosaccharides were all analysed quantitatively by GLC as alditol acetates, using the effective carbon response (e.c.r.) factors introduced by Ackman[7]. Dextran, pustulan and pullulan all gave two extra peaks with relative retention times close to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. As analysed by GLC-MS, they were found to be α- and β-1,6-di-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucopyranose. Together they accounted for 14 % of the 2,3,4-tri-*O*-methylated glucose not being reduced. More than 96 % reduction was obtained for all other methylated glucoses with the method used. Increasing the sodium borohydride concentration or the reduction time gave no further reduction.

When using base-catalysed (1-methylimidazole) acetylation in the analysis of dextran, 10 % of the 2,3,4-tri-*O*-methyl-D-glucose was found as 1,6-anhydro-2,3,4-tri-*O*-methyl-β-D-glucopyranose, giving the same MS data as described by Heyns *et al.*[8] In acidic solution, 2,3,4-tri-*O*-methyl-D-glucose therefore exists as an equilibrium mixture of 10–14 % 1,6-anhydro and 90–86 % hemiacetal forms. 1,6-anhydro sugars have been suggested by others to be artifacts formed during methylation analysis,[9] but without any real evidence.

The results summarized in Table 1 are in good agreement with results obtained by others for the same glucans. Rather large amounts of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitols were found in some cases. This may be due to some degradation of polysaccharides during solubilization, as suggested by Kaufmann *et al.*[10] for dex-

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Table 1. Molar percentages of partially methylated glucitol acetates (Glc) obtained using the e.c.r. response factors. Positions of methyl groups indicated. In quantifying total 2,3,4-Glc, 1,6-di-*O*-acetyl-2,3,4-tri-*O*-methyl-α- and -β-D-glucopyranose are included. Retention times (Rel.r.t.) are relative to myo-inositol hexaacetate.

Glucan	2,3,4,6-Glc	2,4,6-Glc	2,3,4-Glc	2,3,6-Glc	2,4-Glc	2,3-Glc	3,4-Glc
Pullulan P-200	1.3		30.4	67.4		0.9	
Pullulan P-800	0.5[a]		31.5	67.4		0.6	
Nigeran	1.0	49.4	0.4[a]	48.1	0.7[a]	0.5[a]	
Pustulan	4.1	1.1	93.4		0.5	0.4[b]	0.5[b]
Dextran	4.4	0.3[a]	91.7		3.1	0.5[a]	
Lichenan	1.1	33.9	1.6[a]	63.1	0.2[a]	0.2[a]	
A.L. Glycogen	9.3	0.9	0.5[a]	82.6		6.7	
H.L. Glycogen	17.0		2.4[a]	67.8		12.8	
Amylopectin	3.9			92.7		3.4	
Cellulose	0.6[a]			99.0		0.4[a]	
Rel.r.t.	0.407	0.536	0.590	0.615	0.739	0.762	0.762

[a]Identified by GLC only. [b]Quantified on basis of MS-data.

tran. With pustulan, small amounts of dimethylated glucitols were found. Since none of the other polysaccharides display extensive undermethylation using this procedure, pustulan may be slightly branched, as indicated earlier.[11]

Experimental

Polysaccharides. Dextran B 512 ($\overline{M}_w$ 24057), Pharmacia, pullulan P-200 ($\overline{M}_w$ 186000) and P-800 ($\overline{M}_w$ 853000), Showa Denko, nigeran, Koch-Light and methyl cellulose (ca. 1.8 methyl groups per glucosidic unit), Aldrich, were commercial samples. Glycogen from *Ascaris lumbricoides* (A.L. glycogen, $\overline{CL}$ = 12) and from human liver (H.L. glycogen, $\overline{CL}$ = 6) were gifts from professor D. J. Manners, while amylopectin, lichenan and pustulan were isolated, purified and analysed in this laboratory.

Methylation procedure. Polysaccharides (2 mg) were dried overnight at 60 °C in vacuum over phosphorous pentoxide in Reacti-vials (5 ml) containing magnetic stirrers. After addition of DMSO (400 µl), flushing with argon and sealing, the vials were stirred for 2 h at 50 °C. After cooling to room temperature, butyllithium (100 µl, 15 % in hexane) was added with a syringe and the vials stirred for 1 h at 40 °C. After cooling in an ice-bath (until frozen), methyl iodide (200 µl, ice-cold) was added with a syringe. The samples were allowed to thaw and then stirred for 1 h at room temperature. Extraction, hydrolysis, reduction and acetylation were carried out according to Harris *et al.*[6] except for the following details: If a copious white layer appeared at the interface during extraction, methanol was added in amounts up to 1.0 ml and the sample centrifuged. The organic layer remaining after the final washing was transferred to an 18 ml screw-cap tube and the sample evaporated to dryness in a stream of nitrogen at room temperature. No attempts were made to remove residual water. The samples were hydrolysed first with formic acid (600 µl, 90 %) at 100 °C for 1 h and then with trifluoroacetic acid (600 µl, 2 M), containing myoinositol, at 120 °C for 1 h. The hydrolysis was carried out under argon atmosphere. The acids were removed after hydrolysis by evaporation in a stream of nitrogen while warming in a water bath at 40 °C. The reduction was performed with sodium borohydride (1 ml, 1.0 M in 2 M NH_3) for 90 min at 60 °C.

Gas chromatography. The samples were separated on a SP 2340 fused silica capillary column (30 m × 0.24 mm i.d., Supelco Inc., Pennsylvania) using a Varian 3400 gas chromatograph with a flame-ionization detector. Myoinositol hexaacetate was used as internal standard. Helium was used as carrier gas at a flow-rate of approx. 210 cm min^{-1}, determined with dichloromethane. Splitless injection for 45 s was employed. The temperature program was: 80 °C

(hold time 30 s), increasing by 20 °C min^{-1} up to 160 °C (hold time 1 min) and then increasing by 2 °C min^{-1} up to 240 °C. The injection port and detector were held at 250 °C. The identity of peaks was confirmed using a Carlo Erba model 4200 gas chromatograph equipped with the same type of column in conjunction with a V.G. Micromass 7070F mass spectrometer in the electron-impact mode and a V.G. data system.

References

1. Hakomori, S. *J. Biochem. (Tokyo) 55* (1964) 205.
2. Phillips, L. R. and Fraser, B. A. *Carbohydr. Res. 90* (1981) 149.
3. Blakeney, A. B. and Stone, B. A. *Carbohydr. Res. 140* (1985) 319.
4. Pas Parente, J., Cardon, P., Leroy, Y., Montreuil, J. and Fournet, B. *Carbohydr. Res. 141* (1985) 41.
5. Ciucanu, I. and Kerek, F. *Carbohydr. Res. 131* (1984) 209.
6. Harris, P. J., Henry, R. J., Blakeney, A. B. and Stone, B. A. *Carbohydr. Res. 127* (1984) 59.
7. Ackmann, R. G. *J. Gas Chromatogr. 2* (1964) 173.
8. Heyns, K. and Scharmann, H. *Carbohydr. Res. 1* (1966) 371.
9. Jansson, P. E., Kenne, L., Liedgren, H., Lindberg, B. and Lønngren, J. *Chem. Commun. Univ. Stockholm* (1976) No. 8.
10. Kaufmann, W. and Bauer, K. *Ger. Pat.* 1,007, 951 (1957); *Chem. Abstr. 54* (1960) 16757.
11. Kjølberg, O. and Kvernheim, A. L. *Acta Chem. Scand., Ser. B 38* (1984) 735.

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