

Partial Methylation of Dextran

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Dextran has been partially methylated with dimethyl sulphate in alkaline solution and the distribution of methoxyl groups has been determined by hydrolysis and resolution of the hydrolysate by paper and gas chromatography. The ratio of the relative rate constants for methylation of the hydroxyl groups at C(2), C(3) and C(4) was found to be 8:1:3.5. The changes of the relative rate constant at the C(3)-hydroxyl group during the course of the reaction have also been determined.

Studies on the distribution of substituents in partially alkylated polysaccharides have so far only been carried out on cellulose,¹⁻⁵ amylose,⁶ and xylan.⁷ In general, the C(2)-hydroxyl group appears to possess a higher reactivity than the C(3)-hydroxyl group. The present paper is concerned with the reactivity of dextran towards methylation.

The dextran used in this investigation is synthesized by *Leuconostoc mesenteroides* strain B-512 and is partially hydrolyzed to a molecular weight (\bar{M}_w) of 40 000. The methylations were performed with dimethyl sulphate in 19 % sodium hydroxide solution. Complete hydrolysis of the partially methylated dextran gave a mixture of glucose and glucose methyl ethers. The glucose, mono-, di-, and tri-*O*-methylated glucoses were separated by chromatography on thick paper. The mono- and di-*O*-methyl fractions were analysed by GLC as their trimethylsilyl ethers (Figs. 1 and 2). The identity of the peaks was established by simultaneous injection of reference substances. Table 1, column 2, presents the results of the analysis of an *O*-methyl dextran containing 1.35 methoxyl groups per anhydro glucose unit.

Spurlin⁸ has shown how the relative rate constants for etherification, in homogeneous reaction, can be calculated from the substitution pattern. The reactivity at C(3) may depend upon whether the hydroxyl at C(2) is free or etherified, and *vice versa*, and this has to be accounted for. Spurlin's approach, with suitable modifications, may be applied to related problems.

In dextran with three adjacent hydroxyl groups in each glucose unit the calculations are more complex than for cellulose. Substitution at C(3) may not only change the reactivity at C(2) but also that at C(4); conversely, substitution at C(4) and C(2) individually and combined may effect the reactivity at

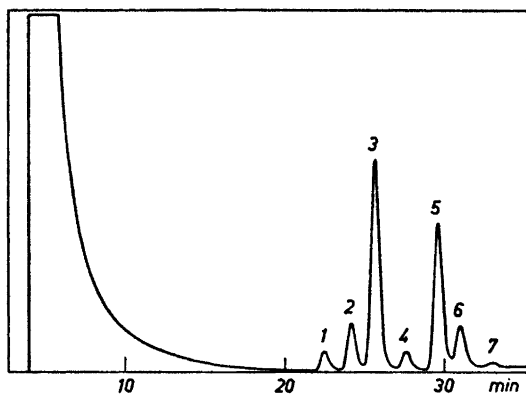


Fig. 1. Gas chromatogram of the trimethylsilyl ethers of a mono-*O*-methyl glucose fraction obtained from a partially methylated dextran.

1. 3-*O*-methyl- α -D-glucopyranose.
2. 4-*O*-methyl- α -D-glucopyranose.
3. 2-*O*-methyl- α -D-glucopyranose.
4. 3-*O*-methyl- β -D-glucopyranose.
5. 2-*O*-methyl- β -D-glucopyranose.
6. 4-*O*-methyl- β -D-glucopyranose + 6-*O*-methyl- α -D-glucopyranose.
7. 6-*O*-methyl- β -D-glucopyranose.

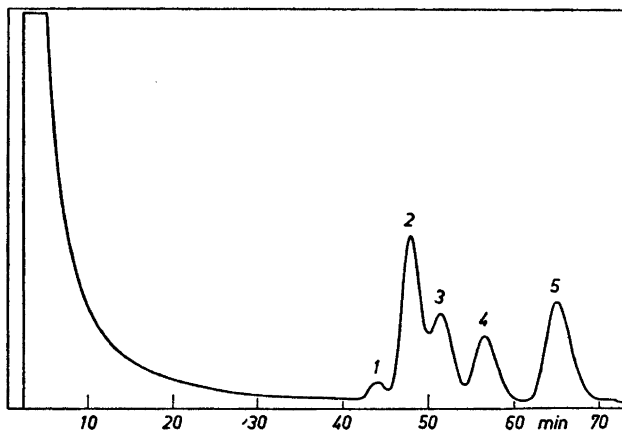
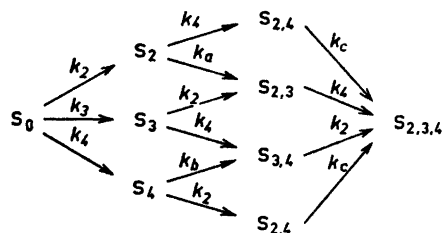


Fig. 2. Gas chromatogram of the trimethylsilyl ethers of a di-*O*-methyl glucose fraction obtained from a partially methylated dextran.

1. 3,4-Di-*O*-methyl-D-glucopyranose "A".
2. 2,4-Di-*O*-methyl-D-glucopyranose "A".
3. 2,3-Di-*O*-methyl-D-glucopyranose "A".
4. 2,3-Di-*O*-methyl-D-glucopyranose "B".
5. 2,4-Di-*O*-methyl-D-glucopyranose "B" + 3,4-Di-*O*-methyl-D-glucopyranose "B".

C(3). Thus, five additional rate constants would have to be inserted besides those prevailing at C(2), C(3) and C(4) in unsubstituted dextran. It was decided that the total effort demanded for the systematic derivation of all rate constants did not justify the relatively small effects involved and so the following assumptions were made: substitution at C(3) does not change the reactivity at C(4) and C(2). These assumptions are consistent with results obtained by Croon^{1,8,7} and other investigators.^{9,10} According to their investigations, substitution at C(3) does not affect the reactivity at C(2) significantly.

In the analysis of the results the following reaction scheme was employed:



k_a = the rate constant at position 3 when position 2 is methylated

k_b = the rate constant at position 3 when position 4 is methylated

k_c = the rate constant at position 3 when positions 2 and 4 are methylated

Since this is a series of first order reactions of the type

A $\xrightarrow{k_1}$ B $\xrightarrow{k_2}$ C,¹¹ the following expressions may be derived:

$$d[S_2]/dt = k_2[S_0] - (k_a + k_4)[S_2] \quad (1)$$

$$d[S_3]/dt = k_3[S_0] - (k_4 + k_2)[S_3] \quad (2)$$

$$d[S_4]/dt = k_4[S_0] - (k_b + k_2)[S_4] \quad (3)$$

$$d[S_{2,3}]/dt = k_a[S_2] + k_2[S_3] - k_4[S_{2,3}] \quad (4)$$

$$d[S_{2,4}]/dt = k_2[S_4] + k_4[S_2] - k_c[S_{2,4}] \quad (5)$$

$$d[S_{3,4}]/dt = k_b[S_4] + k_4[S_3] - k_2[S_{3,4}] \quad (6)$$

$$d[S_0]/dt = -(k_2 + k_3 + k_4)[S_0] \quad (7)$$

$$S_{2,3,4} = 1 - S_2 - S_3 - S_4 - S_{2,3} - S_{2,4} - S_{3,4} - S_0 \quad (8)$$

The initial rate constants k_2 , k_3 , and k_4 were obtained by investigating the proportions of 2-*O*-, 3-*O*- and 4-*O*-methyl glucopyranose in the hydrolysate from an *O*-methyl dextran with a low degree of substitution (D.S. 0.14) in which the di- and trimethyl fractions were negligible. The ratio of the rate constants $k_2:k_3:k_4$ was found to be 8:1:3.5.

Eqns. (1)–(7) were integrated, programmed and computed. As can be seen in Table 1, column 3, the best agreement between the experimental and calculated values was obtained when k_a , k_b , and k_c were assigned values of 3, 4, and 5.2, respectively. In view of the limitations of this method a better agreement could hardly be expected. The values given in Table 1, column 4, have been calculated on the basis of an unchanged ratio of the relative initial rate constants during the course of the reaction.

Table 1. Composition of the hydrolysate of a partially methylated dextran.

1	2	3	4
Substance ^a	D.S. 1.35 ^d		
	Exptl.	Calc. ^b	Calc. ^c
S ₀	17.6	17.4	17.4
S ₂	30.1	30.3	35.9
S ₆	2.8	2.5	2.5
S ₄	8.7	8.7	11.0
S _{2,3}	10.6	10.9	5.4
S _{3,3}	16.3	16.3	22.7
S _{3,4}	2.2	4.0	1.6
S _{2,3,4}	11.7	9.9	3.5
D.S.	1.35	1.34	1.19

^a S₀ = glucose, S₂ = 2-O-methyl glucose, etc.

^b Assuming $k_2:k_3:k_4 = 8:1:3.5$ and $k_2:k_3 = 3$; $k_1:k_3 = 4.0$; $k_1:k_2 = 5.2$.

^c Assuming $k_2:k_3:k_4 = 8:1:3.5$.

^d Determined by Zeisel's methoxyl analysis.

It is evident that the C(2)-hydroxyl group throughout the reaction is more reactive than the C(3)-hydroxyl group. This has also been observed in the methylation of different mono-^{6,9,10} and polysaccharides.^{1-3,6,7} The difference in reactivity between the C(2)- and C(3)-hydroxyl groups has previously been explained by the higher acidity of the C(2)-hydroxyl group^{1-3,6,7,9,10} due to the inductive effect from the acetal oxygens at C(1). The low reactivity of the C(3)-hydroxyl group appears to be a characteristic not only of polymeric glucans but also of glucose derivatives.^{3,6,9,10} The high initial reactivity of the C(4) hydroxyl group could depend on the inductive effect from the acetal oxygen in the glycopyranosyl ring which not only influences the electron density and consequently the reactivity of the C(2)-hydroxyl group but also that of the C(4)-hydroxyl group. The fact that the reactivity of the C(3)-hydroxyl group is enhanced when the C(2)- and C(4)-hydroxyl groups are methylated and that the reactivities of the C(2)- and C(4)-hydroxyl groups are virtually unaffected by substitution at C(3) is not easily interpreted. The explanation of these experimental results demands a more profound study of this problem.

EXPERIMENTAL

Evaporations were performed at reduced pressure at a bath temperature below 40°.

The paper chromatographic separations were carried out on Whatman No. 3 paper (butanol:ethanol:water 40:11:19).

The gas chromatographic separations were carried out on an F & M 810 gas chromatograph using a steel column (8 feet) packed with 5 % butandiol succinate polyester (BDS) on Chromosorb W.

Partial methylation of dextran. 1 g of dextran (dextran B-512, \bar{M}_w 40 000) was dissolved in 50 ml of 19 % sodium hydroxide solution. 1 and 15 ml, respectively, of dimethyl sulphate was added over 60 min with stirring under an atmosphere of nitrogen. The stirring was continued for 4 h. The reaction mixture was then neutralised with 5 N sulphuric acid, desalted on a Sephadex G-25 column and evaporated to dryness (0.9 and 1 g).

Hydrolysis of partially methylated dextran. 0.9 g of *O*-methyl dextran was dissolved in 50 ml of 2 N sulphuric acid and kept at 100° for 5 h. The solution was neutralised (IR-4B, free base), filtered and evaporated to dryness (0.8 g).

Analytical procedure. The hydrolysis products were fractionated by chromatography on thick paper. The glucose, mono-, di-, and tri-*O*-methyl glucose fractions were localized, eluted quantitatively with water, evaporated to dryness and weighed. The separation of the trimethylsilyl (TMS) derivatives¹² of the mono-*O*-methyl glucoses was carried out by gas chromatography (90–170°, 2°/min). The TMS derivatives of the di-*O*-methyl glucoses were also separated by gas chromatography (100°).

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REFERENCES

1. Croon, I. *Svensk Papperstid.* **63** (1960) 247.
2. Lenz, W. R. *J. Am. Chem. Soc.* **82** (1960) 182.
3. Sugihara, J. M. *Advan. Carbohydrate Chem.* **8** (1953) 16.
4. Roberts, E. J. and Rowland, S. P. *Can. J. Chem.* **45** (1967) 261.
5. Roberts, E. J. and Rowland, S. P. *Carbohydrate Res.* **5** (1967) 1.
6. Croon, I. *Acta Chem. Scand.* **13** (1959) 1235.
7. Croon, I. and Timell, T. *J. Am. Chem. Soc.* **82** (1960) 82.
8. Spurlin, H. M. In Ott, E., Spurlin, H. M. and Grafflin, M. W. *Cellulose and Cellulose Derivatives, II, High Polymers, Vol. V*, Interscience, New York 1954, IX A.
9. de Belder, A. N., Lindberg, B. and Theander, O. *Acta Chem. Scand.* **16** (1962) 2005.
10. Garegg, P. J. *Acta Chem. Scand.* **17** (1963) 1343.
11. Frost, A. A. and Pearson, R. G. *Kinetics and Mechanism*, Wiley, New York 1953, p.153.
12. Sweely, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Am. Chem. Soc.* **85** (1963) 2497.

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