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METHYLATION OF CARBOHYDRATES[1]

A CONVENIENT METHOD FOR THE METHYLATION OF POLYSACCHARIDES[2]

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One of the important methods of structural determination of polysaccharides is the methylation analysis. In the Haworth method[3] of methylation, which is used most widely, the polysaccharide is dissolved in 30-40% NaOH followed by dropwise addition of dimethyl sulfate with vigorous stirring.[4] Inert atmosphere and low temperatures are used initially to avoid the degradation of the polymer. The method, although quite successful, suffers from the following disadvantages: (i) the working up of the reaction mixture is laborious and the process as a whole is time consuming (ii) small amounts of polysaccharides are difficult to handle and (iii) since there is a strong alkaline environment, alkali sensitive linkages are likely to be ruptured.

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1 Part II. For Part I, see H.C. Srivastava, S.N. Harshe and Prem Pal Singh, Indian J. Chem. 1, 305 (1963).

2 The results of this investigation form part of a provisional Indian patent application No.85356.

3 W.N. Haworth, J. Chem. Soc. 107, 8 (1915).

4 F. Smith and R. Montgomery, "Methods of Biochemical Analysis," Vol. III p. 153. Interscience Publishers, Inc., New York (1956).

Another important method of methylation of polysaccharides is due to Freudenberg.[5] In this method, the polysaccharide in liquid ammonia is treated with metallic sodium to give the sodio salt which is then methylated with methyl iodide. The method has given good results in a number of cases[6,7] and can be used on small amounts of carbohydrate polymers.

We now report a method of methylation of polysaccharides which is based upon the Kuhn methylation procedure[8,9,10] for lower molecular weight carbohydrates and on our modification[1] of the Kuhn method. The general method of methylation is as follows:

The dry polysaccharide (1 g.) is dissolved in the minimum amount (ca 20 ml.) of dry dimethyl sulfoxide (DMSO),[11] the solution is cooled to 20°C and barium oxide (5 g.) and methyl iodide (15-20 ml.) are added. The mixture is stirred at room temperature (30°C) for 48 hrs.[12] after which time it is centrifuged and the residue washed

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5 K. Freudenberg and H. Boppel, Ber. 71, 2505 (1938).

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7 S.A. Barker, M. Heidelberger, M. Stacey and D.J. Tipper, J. Chem. Soc. 3468 (1958).

8 R. Kuhn, H. Trischmann and I. Löw, Angew. Chem. 67, 32 (1955).

9 R. Kuhn, Angew. Chem. (Int. edn.) 1, 19 (1961).

10 H.G. Walker Jr., Mildred Gee and R.M. McCready, J. Org. Chem. 27, 2100 (1962)

11 DMSO was dried by azeotropic distillation with benzene and the fraction distilling at 189-190°C was used. The dissolution of the polysaccharides was facilitated by warming the DMSO-polysaccharide mixture.

12 The 48 hrs. time of reaction was chosen arbitrarily in the initial stages of the development of this method. Later studies (see below) have shown that 24 hrs. is a long enough time for the reaction.

with chloroform. The supernatant and the chloroform extracts are mixed and more chloroform is added till the precipitation of the inorganic salts is complete. The salts are removed by centrifugation and the solution, which has a red-brown colour, is washed with a 15% aqueous solution of sodium thiosulfate. The resulting colourless chloroform solution is washed with a small quantity of water, dried over Na_2SO_4 and evaporated to dryness *in vacuo*.

Employing the above method (hereinafter referred to as the DMSO method), maize starch, beechwood xylan,[13] tamarack arabogalactan[14] and oyster glycogen have been methylated. These are only representative examples and many other polysaccharides, which are soluble in DMSO, can be methylated likewise. The analytical data on the methylated products are given in Table 1.

TABLE 1

Methylation of Polysaccharides by the DMSO Method

Polysaccharide	Reaction Time in Hours	Methylated Polysaccharide			$[\alpha]_D$ in $CHCl_3$
		% Yield	% OCH_3		
			Calc'd	Found	
Maize Starch	24	100	45.6	28.0	+ 125°
Maize Starch	48	100	45.6	28.5	+ 120°
Maize Starch	72	95	45.6	30.5	+ 122°
Maize Starch*	24	100	45.6	29.1	+ 123°
Beechwood Xylan	48	100	38.7	31.5	- 42.4°
Tamarack Arabogalactan	44	80	43.0	30.5	- 15.7°
Oyster Glycogen	45	100	45.6	25.5	+ 132°

* Barium oxide was added in small portions over a period of 4 hrs.

13 G.A. Adams, Can. J. Chem. 35, 556 (1957).

14 G.A. Adams, Can. J. Chem. 38, 280 (1960).

In the case of maize starch, it was observed that no significant improvement in the degree of methylation took place if the time of reaction was varied from 24 to 72 hrs. or if the addition of BaO was done portionwise. In all cases of polysaccharides so far investigated, after one methylation by the DMSO method, the partially methylated polysaccharides were completely soluble in methyl iodide. Table 2 gives analysis of the products obtained by remethylation of partially methylated starch (by the DMSO method) by different methylation

TABLE 2

Remethylation of Starch Once Methylated by the DMSO Method

Partially Methylated Starch	% Yield	% OCH_3	$[\alpha]_D$ in $CHCl_3$
One Purdie Methylation	100	35.2	+ 125°
Two Purdie Methylations	100	39.1	+ 125°
One Methylation by the DMSO Method	100	33.3	+ 124°
One Kuhn Methylation (DMF* + Ag_2O + MeI)	96	34.7	+ 124°

* N,N-Dimethyl Formamide

techniques. Since remethylation showed increased degree of methylation, complete methylation could be achieved by repeating the process adequate number of times. From our experience, the most convenient procedure for the methylation of polysaccharides would be to give one methylation by the DMSO method followed by Purdie methylations until the theoretical value of $-OCH_3$ is obtained. The infrared spectrum of the methylated starch prepared by the DMSO-Purdie methods was identical with that obtained by the conventional Haworth-Purdie procedures.

The DMSO method of methylation is time saving, the reaction is carried out under mild conditions and the recovery of the product is

easy. Furthermore, the procedure is applicable to milligram quantities of the polysaccharides.

In our previous modification[1] of the Kuhn method, the use of drierite was recommended to maintain anhydrous reaction conditions. In the present DMSO method for the methylation of polysaccharides, the use of drierite has been purposely avoided so as to produce barium hydroxide in situ. The latter has been shown[9] to be a necessary adjunct of BaO for efficient methylation. In the DMSO method of methylation of polysaccharides (ROH), the following reactions are believed to take place:

$$ROH + CH_3I \longrightarrow ROCH_3 + HI$$

$$(CH_3)_2SO + 2HI \longrightarrow (CH_3)_2S + H_2O + I_2$$

$$BaO + H_2O \longrightarrow Ba(OH)_2$$

It is possible that the DMSO in this method and DMF in the Kuhn's procedures[8,9] besides being solvents for the carbohydrate materials, also assist the reaction by solvating the cations.[15]

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