Complete Methylation of Reducing Carbohydrates[2]

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Reducing carbohydrates can be completely methylated in a single step in good yield using silver oxide and methyl iodide in dimethylformamide. The ring structure and conformation of the principal glycosidic product obtained depends on the sugar used.

Preparation of completely methylated derivatives plays an important part in many carbohydrate structural studies. The Purdie silver oxide–methyl iodide[3] and the Haworth alkali–methyl sulfate[3] procedures have been frequently used, but in many cases, a simple method reported recently by Kuhn, Trischmann, and Löw[4] gives satisfactory results. In this newer procedure, silver oxide and methyl iodide are again the methylating agents, but the use of dimethylformamide (DMF) as solvent promotes complete methylation in a single step. Both the Purdie and Haworth techniques require several remethylations to obtain complete etherification.

Reducing carbohydrates are ordinarily converted to glycosides for protection during methylation. Conventionally, protective glycosidation is carried out in methanolic hydrochloric acid, but in the case of acid labile disaccharides and oligosaccharides, a large amount of unwanted hexoside is also produced. Alternatively, reducing carbohydrates can be methylated directly using the alkali–methyl sulfate procedure but undesirable side reactions cut down yields.[5]

For a study of certain sucrose derivatives,[6] it became necessary to prepare a number of methylated glycosides for gas chromatographic standards. We found that the silver oxide–methyl iodide–DMF method[4] could be used to prepare completely methylated glycosides directly from reducing carbohydrates in excellent yield, without prior protection of the reducing group. Reaction proceeds smoothly with a variety of aldoses, ketoses, and uronic acids. Either milligram or gram quantities of sugars can be handled easily. Good yields (80–90%) of completely methylated products are obtained after sixteen hours reaction time, although traces of incompletely methylated product can be shown even after forty hours if no new reagents are added. The composition of crude reaction product can be established simply and quickly by gas chromatography. For gas chromatographic analysis it is unnecessary to remove all traces of solvent and other accompanying low-boiling impurities.

It was somewhat unexpected that methylation of aldoses would proceed so smoothly in the presence of silver oxide. Glucose, shaken with silver oxide in DMF at room temperature, is partially decomposed in several hours.[7] When methyl iodide is present in the reaction mixture, however, no silver mirror (indicating oxidation-reduction) is formed; the silver is converted to silver iodide. Direct methylation of reducing carbohydrates is successful because protective glycosidation occurs more rapidly than competing degradative side reactions. The ability of DMF to accelerate certain alkylation reactions is well established.[8] In this reaction methylation of hydroxyl of the hemi-acetal group of glucose is essentially complete in one hour (as shown by loss of reducing function), but complete etherification of the remaining hydroxyls requires considerably longer. This difference in rate is expected from the difference in acidity (and ease of anion formation) of the hydrogens on the two different types of hydroxyl groups. Preliminary measurements of the rate of glycosidation of glucose have shown that the reaction is not simple, even in the presence of a large excess of silver oxide and methyl iodide. Because reaction conceivably occurs by both heterogeneous and homogeneous pathways, intensive kinetic studies have not been made. It appears, however, that the specific glycosidation reaction is rapid enough to allow the preferential preparation of methyl glycosides by suitable choice of reaction conditions. Work on this problem is in progress.

Reducing carbohydrates we have methylated completely in good yield include xylose, arabinose, glucose, mannose, galactose, galactuoronic acid,

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(3) F. J. Bates and associates, "Polarimetry, Saccharimetry and the Sugars," U. S. Government Printing Office, Washington, 1942, p. 506–507.

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

(5) F. J. Bates and associates, "Polarimetry, Saccharimetry and the Sugars," U. S. Government Printing Office, Washington, 1942, p. 519.

(6) M. Gee and H. G. Walker, Jr., *Chem. Ind.* (London), 829 (1961).

(7) At room temperature, reaction between silver oxide and DMF alone appears to be negligible. Kornblum and Blackwood [*J. Am. Chem. Soc.*, **78**, 4037 (1956)] have shown that methyl iodide and DMF react very slowly at room temperature; this reaction probably is accelerated by silver oxide. Despite this undesirable side reaction, carbohydrate methylation proceeds readily, but a considerable excess of methylating reagents should be used.

(8) H. E. Zaugg, B. W. Horrom, and S. Borgwardt, *ibid.*, **82**, 2895 (1960); H. E. Zaugg, *ibid.*, **82**, 2903 (1960).

fructose, maltose, cellobiose, melibiose, turanose, and inulin. Gas chromatographic analysis of the completely methylated material in every case shows a mixture of at least two, and sometimes four, completely methylated glycosides present, corresponding to the various possible anomeric pyranose and furanose forms. Some results for monosaccharides are given in Table I. Standard compounds necessary for anomeric analysis were not available in the disaccharide series so that results with these sugars are not shown. The table shows that production of a particular isomer is favored. Formation of this favored isomer is believed to result from stereochemical factors rather than from simple equilibrium.

TABLE I
ISOMERIC COMPOSITION OF COMPLETELY METHYLATED PRODUCTS FROM VARIOUS MONOSACCHARIDES

Sugar	Per Cent[a] Pyranose	Furanose
L-Arabinose	69α, 17β	14
D-Xylose	34α, 66β	Trace
D-Mannose	76α, 24β	Trace
D-Glucose	16α, 84β	Trace
D-Galactose	10	80α, 10β
D-Fructose	Trace	19α, 81β
D-Galacturonic acid	Trace	93α, 7β

[a] Estimated from gas chromatographic analysis.

The mechanism of this methylation reaction has not been investigated extensively; however, certain experimental facts give some clues about the course of the reaction. For example, the solubility of silver oxide in DMF is low (about 0.02%). Rates of glycosidation and subsequent etherification appear to depend on the rate of stirring or shaking. Polymeric materials insoluble in DMF are not methylated successfully, and crude rate runs on soluble materials do not follow simple pseudo-first-order kinetics (see for example, Table II). These facts all indicate that reaction occurs predominately by a heterogeneous pathway. Kornblum[9] has already established that reactions involving silver can proceed readily on crystal surfaces.

From his work it also seems evident that the transition state leading to methylation involves a cyclic intermediate wherein silver oxide, methyl iodide, and carbohydrate hydroxyl become oriented to permit reaction to proceed by a "pull-push" mechanism.[8] Silver ion contributes a significant pull to break the carbon-iodine bond of methyl iodide and hydroxyl (or its anion) contributes a push as it simultaneously attacks the electron deficient carbon of methyl iodide. The anion of the hydroxyl group appears to be implicated because reaction is fastest at the most acidic hydroxyl in the carbohydrate (hemi-acetal hydroxyl at carbon 1).

(9) N. Kornblum, R. A. Smiley, R. K. Blackwood, and D. C. Iffland, *J. Am. Chem. Soc.*, **77**, 6269 (1955).

The predominant formation of a particular anomeric glycoside during reaction is interesting. We know that initial reaction leading to glycoside formation is apparently irreversible because methyl glycosides of known structure can be completely methylated without isomerization. It has been shown that mutarotation of reducing sugars in dry DMF is slow, but does occur in the absence of strong acids or bases.[10] The optical rotation of a solution containing α-D-glucose and silver oxide in DMF decreases even more rapidly than α-D-glucose alone in DMF, although this may not be due entirely to mutarotation. Thus at carbon 1, in aldoses, where isomerization is possible through mutarotation, the carbohydrate ring has a chance at the time of reaction to assume the stablest conformation available, no matter what the ring form was in the original solution. Table I shows that, in every case where pyranose ring formation predominates, the major anomeric product has the ring conformation containing the fewest "instability factors."[11] Hence, derivatives of β-D-glucose, β-D-xylose, and α-D-arabinose are formed because the oxygen atom at carbon 1 is equatorial. An α-D-mannose derivative is formed, even though it has an axial group at carbon 1, because the β derivative with an equatorial group at carbon 1 has an even more unfavorable conformation due to the "Δ 2 condition."[11]

Why fructose, galactose, and galacturonic acid give predominantly furanose derivatives when completely methylated by this procedure is not understood at present. The tendency of fructose and galactose to form furanoside structures on direct methylation with alkali and methyl sulfate has been noted previously.[12] In the furanoside series also, steric requirements in the reacting complex seem to govern production of a favored anomer (see Table 1).

The structure and properties of the methyl tetra-*O*-methyl-D-galactofuranosides and methyl (methyl tri-*O*-methyl-D-galactofuranoside) uronates will be described in a separate publication.

Experimental

Starting Materials.—Reducing sugars and reagents were commercial products, used without special purification. Freshly prepared silver oxide gave no better results than commercial "purified grade" material. Known standards were prepared from commercial methyl glycosides or from samples available in this laboratory.

General Procedure.—The method of Kuhn was followed without extensive modification.[3] The reducing sugar was dissolved in 10 to 25 times its weight of DMF in a flask protected from moisture. Heat was used to effect solution if necessary. At room temperature, methyl iodide (3 equivalents per —OH) and silver oxide (2 equivalents per —OH) were added and the mixture stirred or shaken in a water bath at room temperature. After at least 16 hr.,

(10) R. Kuhn and F. Haber, *Ber.*, **86**, 722 (1953).

(11) R. E. Reeves, *J. Am. Chem. Soc.*, **72**, 1499 (1950); R. B. Kelly, *Can. J. Chem.*, **35**, 149 (1957).

(12) W. N. Haworth, D. A. Ruell, and G. C. Westgarth, *J. Chem. Soc.*, 2468 (1924).

the mixture was vacuum filtered and the insoluble salts washed with DMF. The combined filtrates were evaporated to small volume *in vacuo* at 100°. Chloroform was added and the mixture refiltered or centrifuged to remove as much solid material as possible. The chloroform filtrate was then washed several times with water. From measurements of the apparent distribution ratios of completely methylated glucose and maltose between chloroform and water (15:1 and 30:1, respectively), it was concluded that washing losses were not large. For convenience, however, an aqueous cyanide extraction to remove traces of silver salts was omitted. After drying the chloroform solution over sodium sulfate and filtering, it was evaporated carefully on a rotary vacuum evaporator using an aspirator and a water bath at a final temperature of 60–70°. Traces of residual solvent did not interfere with gas chromatographic analysis of crude product.

Yields.—No attempt was made to investigate all reaction variables influencing yield. Kuhn reported an 85% yield of completely methylated sucrose isolated by vacuum distillation.[3] From one experiment we obtained 7.90 g. of crude completely methylated glucose from 5.00 g. of glucose. Aliquots of this material were hydrolyzed overnight in 1 *N* hydrochloric acid in sealed tubes on the steam bath. Hypoiodite analysis[13] indicated that the crude product contained 78% of a mixture of methyl tetra-*O*-methyl-D-glucosides; that is, a yield of 88% for the overall methylation reaction. Admittedly, this analysis would include also any incompletely methylated products, but significant amounts of these materials were shown to be absent by gas chromatographic analysis. The hypoiodite method was more satisfactory than colorimetric or copper methods for analysis of tetra-*o*-methylglucose; results of an analysis of a standard sample were in excellent agreement with polarimetric data. DMF did not interfere with the hypoiodite determination.

The infrared spectrum of a crude completely methylated glucose sample (78% purity by hypoiodite analysis) was measured. Absorption in the carbonyl region was so small that oxidation of glucose to gluconic acid could not have been a major factor in the reaction. The amount of absorption could be attributed to traces of side products and DMF which were already known to be present from other analyses.

Gas Chromatographic Analysis of Reaction Products.—The equipment, methods, and polar substrates used (polyesters and polyethers) have been described in a separate publication.[14] Generally, component identifications were based on chromatographic comparison of known and unknown samples, but in certain cases peaks were collected and confirmed by rotational measurements. The isomeric composition of crude reaction products was estimated from peak height measurements of chromatograms run at temperatures high enough to eliminate significant peak broadening.

Rate of Glycosidation.—The rate of glycosidation of D-glucose was followed by measuring the disappearance of reducing power (measured by hypoiodite) as a function of time. Samples of the reaction mixture were withdrawn periodically, filtered, and an aliquot titrated. Although the method was not altogether suitable for precise kinetic work, the results of a typical experiment are shown in Table II.

TABLE II

REACTION OF D-GLUCOSE (0.222 *M*), METHYL IODIDE (3.31 *M*) AND SILVER OXIDE (1.13 *M*) IN DIMETHYLFORMAMIDE AT 21°

Time, Min.	Reducing Function Remaining, %
0	100
6	94
10.5	92
15	88
20	82
25.5	69
31	65
35.5	49
43.5	28
52	20
63.5	8

A plot of these data shows an S-shaped curve that could be expected from a heterogeneous reaction requiring an equilibration before rapid reaction occurs. Results from both glucose and maltose show definitely that glycosidation occurs more rapidly than complete etherification. In an experiment using only glucose and silver oxide at the above concentration levels in DMF, hypoiodite analysis showed that 30 and 45% of the reducing groups were destroyed in 1 and 2 hr., respectively.

(13) E. F. Jansen and L. R. MacDonnell, *Arch. Biochem.*, **8**, 97 (1945).

(14) M. Gee and H. G. Walker, Jr., *Anal. Chem.*, in press.

The Synthesis and Acid Hydrolysis of Methyl α-D-Glucopyranosiduronic Acid[1]

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Methyl α-D-glucopyranosiduronic acid was synthesized by a catalytic air oxidation of methyl α-D-glucopyranoside followed by: (a) conversion of the oxidation product into crystalline methyl α-D-glucopyranosiduronic hydrazide and (b) hydrolysis of the hydrazide to yield barium (methyl α-D-glucopyranosid)uronate. Methyl α-D-glucopyranosiduronic acid and methyl α-D-glucopyranoside were hydrolyzed in *N* sulfuric acid at 70, 80, and 90°. The results of the hydrolysis suggested that the uronoside and neutral glycoside did not hydrolyze *via* identical mechanisms and that the uronoside was not stabilized by a significant inductive effect. Results from a study of the degradation of methyl α-D-glucopyranosiduronic acid support the reaction sequence: hydrolysis of methyl α-D-glucopyranosiduronic acid followed by degradation of D-glucuronic acid.

Methyl α-D-glucopyranoside (methyl α-glucoside) was oxidized by air in the presence of a platinum-carbon catalyst; the acidic products of the oxidation were isolated as their barium salts. Paper chromatographic analysis of the crude products indicated that, in addition to the expected methyl α-D-glucopyranosiduronic acid (methyl α-glucuronide), the oxidation produced several unidentified compounds. Thus, it appears that the catalytic

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