55. *The Acetolysis of Methylated Starch.*

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An expeditious method for the determination of the end group in methylated starch is described. It is shown that the acetolysis of methylated starch by means of acetyl bromide in cold chloroform solution proceeds with great rapidity. A selective removal of the end group is observed, the whole of the tetramethyl glucose being separated within five minutes of the beginning of reaction. Treatment of the bromine-containing product with methyl alcohol yields a mixture of methylglucosides, from which the tetramethyl methylglycoside is separated by distillation in the usual manner. A careful search failed to reveal the presence of any trimethyl sugar other than 2 : 3 : 6-trimethyl glucose.

THE use of acetyl bromide for the degradation of methylated starch enabled Haworth and Percival (J., 1931, 1342) to demonstrate the pre-existence of maltose residues in starch. The mixture of acetolysis products was oxidised with bromine water and after further methylation it was possible to isolate octamethyl methylmaltobionate.

The method offers a convenient and simple way of effecting the hydrolysis of methylated starch in that it is conducted in chloroform solution and at room temperature. We have therefore made use of this method of cold acetolysis of methylated starch and have submitted the products to a careful examination with a view to determining (i) the degree of hydrolysis that can be effected, (ii) the fate of the end group (tetramethyl glucose), and (iii) the presence of any trimethyl sugar other than 2 : 3 : 6-trimethyl glucose. The methylated starch, prepared by the exhaustive methylation of potato starch, contained 45% OMe. In an exploratory experiment, this material was treated with acetyl bromide in chloroform solution and polarimetric observation showed that the reaction was completed in about ten hours.

Subsequently, samples of the acetolysis product were prepared by arresting the reaction after 5, 10, 20, and 260 minutes. After the interval specified, the reaction was stopped by pouring the acetolysis mixture on ice. The mixture of bromohydrins so produced was converted into the corresponding methylglycoside mixture by treatment with methyl alcohol. By distillation, the glycoside mixture was separated into fractions, the boiling points and other properties of which showed them to consist of methylated monosaccharides, disaccharides, trisaccharides and dextrins. The proportions in which these fractions occurred in the different cases are shown in Table I.

TABLE I.

Expt. no.	Weight of methylated starch (g).	Acetolysis period (mins.).	% Composition of glycoside mixture produced. Monosacch.	Disacch.	Trisacch.	Residue.
III	12·30	5	17	25	22·5	35·5
IV	5·23	10	11	23	18	48
I	10·46	20	23	34	18	18
II	26·16	20	46	34	9	11
V	2·61	260	53	—	—	—

This table shows that no simple relation exists between the proportion of monosaccharides produced and the time of reaction. Obvious factors which influence this relationship are the scale of working (cf. expt. I and II) and the initial heating effect on mixing the reagents. The latter effect will be predominant when the time of reaction is short (cf. expt. III and IV).

A detailed examination was now made of the fractions obtained in expt. II. Redistillation of the monosaccharide fraction showed it to be a mixture of tetramethyl methylglucoside and 2 : 3 : 6-trimethyl methylglucoside with a small amount of dimethyl methylglucoside. The disaccharide fraction was submitted to hydrolysis with methyl-alcoholic hydrogen chloride, and the hydrolysate shown to consist of 2 : 3 : 6-trimethyl methylglucoside and a small amount of dimethyl methylglucoside; no tetramethyl methylglucoside was present. In a similar manner the trisaccharide fraction and higher fractions yielded, on hydrolysis, mixtures of trimethyl and dimethyl methylglucosides in which the former substance preponderated. Two facts of importance emerge: (1) The acetolysis of methylated starch for 20 minutes in the cold suffices to remove the whole of the end group as tetramethyl methylglucoside which is found exclusively in the monosaccharide fraction. (2) The only trimethyl methylglucoside found in any of the fractions was the 2 : 3 : 6-derivative.

The preferential removal of the end group when methylated starch is treated with acetyl bromide as demonstrated by the above observations is further confirmed by an examination of the products obtained in expt. III. The methylated starch in this case was in contact with the acetolysing agent for only a very short period (5 minutes). Nevertheless the whole of the end group was found, as tetramethyl methylglucoside, in the monosaccharide fraction and in an amount which corresponded to a chain length of 27 units. This figure is in excellent agreement with that calculated from expt. II and also with the chain length as determined by the standard method in which methylated starch is hydrolysed by means of boiling methyl-alcoholic hydrogen chloride. A method for the determination of the chain length of starch, which is more convenient and expeditious than the earlier method of complete hydrolysis, is thus made available.

Experimental.

Methylation of Potato Starch.—The methylation was carried out by the usual procedure adopted in this laboratory. The starch, in 30 g. lots, was treated at 50° in 5% sodium hydroxide solution with methyl sulphate (360 c.c.) and 30% sodium hydroxide solution. After the solution had been boiled at the end of the methylation, the whole of the partially methylated starch separated as a gelatinous mass, which was collected on a cloth filter. The products from the methylation of two lots (30 g.) of starch were combined and submitted to remethylation by the process described, acetone being added as an additional solvent. In the third and subsequent methylations the partially methylated starch was dissolved initially in acetone.

The methylation process was repeated twelve to fourteen times with each batch. Purification of the methylated starch (obtained as a white powder) was effected by prolonged extraction with boiling water, followed by solution of the dried material in chloroform and precipitation from the filtered chloroform solution by the addition of ether and light petroleum. The flocculent precipitate was repeatedly washed with light petroleum and dried in a current of cold air. The properties of the trimethyl starch so prepared are given in the table:

Sample.	No. of methylations.	OMe, %.	$[\alpha]_D^{19°}$ in $CHCl_3$.	Weight, g.
I	13	44·4		
	14	44·8	+211°	42·5
II	12	—		
	13	45·2	+215	—
III	12	44·4		
	13	44·7	+214	42·8
IV	13	45·1		
	14	45·1	+215	37·0

The Velocity of Acetolysis of Trimethyl Starch by Acetyl Bromide.—Trimethyl starch (1·0464 g.) was dissolved in chloroform (6 c.c.), the cooled solution, after being mixed with acetyl bromide (3 c.c.) in chloroform (9 c.c.), was quickly adjusted to 20 c.c. with chloroform, and the rotation of the solution observed in a polarimeter tube (l = 1 dm.) with fused glass end-plates and provided with a calcium chloride tube. The solution was then kept at 20° and polarimetric observations were made at intervals: α_D changed from + 13·1° to + 17·7° in 9½ hours.

The acetolysis was now arrested at a number of different periods after mixing, and the products examined.

Acetolysis I. Reaction arrested after 20 minutes: The solution of trimethyl starch (10·46 g.)

and acetyl bromide (30 c.c.) in chloroform to make a total volume of 200 c.c. was prepared as described in the preliminary experiment. The solution was kept at 20° for 20 minutes and a portion of it (150 c.c.) was then poured on ice. The mixture was gently stirred for a few minutes, care being taken to avoid the formation of an emulsion, until the excess of acetyl bromide was decomposed. The chloroform layer was then separated and washed repeatedly with ice-cold sodium bicarbonate solution until free from acid. After drying over anhydrous magnesium sulphate, the chloroform solution was evaporated to dryness under diminished pressure. The residue was a pale brown syrup (10·12 g.) which contained bromine. It was readily soluble in hot water and the aqueous solution was strongly reducing to Fehling's solution and contained ionised bromine.

The syrup was dissolved in dry methyl alcohol (150 c.c.) and kept in the cold for 48 hours. Water (100 c.c.) was now added, and the acid solution neutralised with barium carbonate. After filtration, the neutral solution was evaporated to dryness, and the residue extracted with ether. Evaporation of the dried ethereal extract gave a syrup (6·9 g.) which did not reduce Fehling's solution until after it had been boiled with dilute hydrochloric acid. It contained no bromine, and alkali-titration showed the absence of acetyl groups. It was clearly a mixture of methylglycosides. The mixture was separated by fractional distillation at 0·002 mm. pressure, as follows :

Fraction.	Bath temp.	Weight, g.	$n_D^{22°}$.	$[\alpha]_D^{20°}$ in $CHCl_3$.	OMe, %.	Nature.
1	110—185°	1·56	—	− 8·48°	—	Mainly monosaccharides
2	190—210	2·35	1·4700	+75·9	46·6	Mainly disaccharides
3	220—260	1·25	1·4755	+92·7	—	Mainly trisaccharides
4	Residue	1·25	—	—	—	Mainly dextrins

Acetolysis II. Reaction arrested after 20 minutes : The procedure described under acetolysis I was repeated with a larger quantity of trimethyl starch. From 26·16 g. of trimethyl starch there were obtained 25·05 g. of the methylglycoside mixture. This was separated by distillation into the following fractions :

Fraction I (monosaccharides), 11·48 g., 46% of the glycoside mixture.
Fraction II (disaccharides), 8·42 g., 33·7% ,, ,, ,,
Fraction III (trisaccharides), 2·20 g., 8·8% ,, ,, ,,
Fraction IV (undistilled residue), 2·90 g., 11·6% ,, ,, ,,

Fraction IV was subdivided into fraction IV (ether-soluble), 2·30 g., and fraction (V) (ether-insoluble), 0·60 g.

Fraction I was subdivided as follows by a very slow distillation from a Widmer flask at 0·01 mm. pressure :

Fraction.	Bath temp.	Weight, g.	$n_D^{20°}$.	OMe, %.
Ia	105—108°	0·84	1·4450	—
Ib	107—116	2·17	1·4534	55·0
Ic	118	5·84	1·4560	52·6
Id	120—130	1·51	1·4568	52·6
Ie	140	0·80	1·4638	47·7

Fraction II (to which was added the very small undistilled residue from the refractionation of I) was hydrolysed by boiling it for 7 hours with 2% methyl-alcoholic hydrogen chloride (200 c.c.). After neutralisation with silver carbonate, the solution was filtered and evaporated, and the residue purified by extraction with ether. Removal of the ether gave a syrup (9·1 g.), which was distilled from a Widmer flask at 0·01 mm. pressure :

Fraction.	Bath temp.	Weight, g.	$n_D^{20°}$.	OMe, %.
H IIa	120°	0·77	1·4553	52·9
H IIb	120—130	5·02	1·4570	—
H IIc	130—155	1·90	1·4614	49·3
H IId	—	0·79	1·4706	—

Fractions III, IV, and V were separately hydrolysed with methyl-alcoholic hydrogen chloride in an identical manner. The methylhexosides formed were distilled as follows :

Fraction.	Bath temp.	Weight, g.	$n_D^{20°}$.	OMe, %.
H IIIa	120—130°	1·36	1·4576	50·1
H IIIb	140—155	0·80	1·4687	—
H IVa	130—145	1·44	1·4580	—
H IVb	145—160	0·31	1·4677	35·0
H IVc	160	0·32	1·4745	—
H V	—	0·33	1·4576	—

On the basis of the refractive indices of the fractions, taken in conjunction with their methoxyl contents, it was possible to estimate the relative proportions of tetramethyl methylglucoside, trimethyl methylglucoside and dimethyl methylglucoside. The distribution of the three glucosides is shown in the following table :

Fraction.	Weight, g.	Wt. of tetramethyl methylglucoside, g.	Wt. of trimethyl methylglucoside, g.	Wt. of dimethyl methylglucoside, g.
Ia	0·84	0·70	0·14	0
Ib	2·17	0·43	1·74	0
Ic	5·84	0	5·84	0
Id	1·51	0	1·51	0
Ie	0·80	0	0·45	0·35
H IIa	0·77	0	0·77	0
H IIb	5·02	0	5·02	0
H IIc	1·90	0	1·33	0·57
H IId	0·79	0	0·14	0·65
H IIIa	1·36	0	1·36	0
H IIIb	0·80	0	0·24	0·56
H IVa	1·44	0	1·44	0
H IVb	0·31	0	0·11	0·20
H IVc	0·32	0	0	0·32
H Va	0·33	0	0·33	0
Totals	24·20	1·13	20·42	2·65
% Weight		4·6	84·5	10·9
Molecular ratio		1	19·5	2·7

If it be assumed that the whole of the end group in the trimethyl starch (26·16 g.) has been separated and collected in the above fractions as tetramethyl methylglucoside, the amount of the latter (after adding the usual correction for experimental loss) is 4·5% of the weight of methylated starch. This value corresponds to a chain length for the starch of 27—28 glucose units, which is very close to the chain length as determined by the ordinary method of end-group assay in which the methylated starch is directly and completely hydrolysed by boiling it with 2% methyl-alcoholic hydrogen chloride.

Hydrolysis of fraction Ia (0·84 g.) with mineral acid gave 0·6 g. of tetramethyl glucopyranose, m. p. 89—90°; $[\alpha]_D^{20°}$ + 104·1° ⟶ + 81·8° in water (*c*, 0·98). Fractions Ic, Id, H IIa, H IIb, H IIIa and H IVa, when separately hydrolysed, all gave, in good yield, 2 : 3 : 6-trimethyl glucose, m. p. 118°; $[\alpha]_D^{20°}$ + 96·4° ⟶ + 72·0° in water (*c*, 1·26). No other trimethyl glucose was detected. The high proportion of dimethyl methylglucoside is noteworthy in view of the high degree of methylation of the original methylated starch.

Acetolysis III. Reaction arrested after 5 minutes : Trimethyl starch (12·30 g.) in chloroform (75 c.c.) was rapidly mixed with acetyl bromide (36 c.c.) in chloroform (120 c.c.), and the volume made up to 250 c.c. After 5 minutes at room temperature the solution was poured on ice. The working-up was conducted as previously described. The methylglycoside mixture obtained (11·52 g.) was fractionally distilled to give :

Fraction I (monosaccharides), 2·00 g.; 17·3% of the glycoside mixture.
Fraction II (disaccharides), 2·84 g.; 24·7% ,, ,, ,,
Fraction III (trisaccharides), 2·59 g.; 22·5% ,, ,, ,,
Fraction IV (undistilled residue), 4·09 g.; 35·5% ,, ,, ,,

Fraction I was subdivided as follows, by distillation from a Widmer flask in a high vacuum :

Fraction.	Weight, g.	$n_D^{20°}$.	Content of tetramethyl methylglucoside, g.
Ia	0·305	1·4440	0·281
Ib	0·850	1·4523	0·242
Ic	0·744	1·4554	0·037

The amount of tetramethyl methylglucoside in fraction I corresponds to a chain length of 27 glucose units for the methylated starch. It is concluded that the whole of the end group has been separated by acetolysis for a period of 5 minutes.

Acetolysis IV. Reaction arrested after 10 minutes : The acetolysis mixture (100 c.c.) contained 5·23 g. of methylated starch and 15 c.c. of acetyl bromide. The methylglucosides (5·0 g.) prepared from the acetolysis product were fractionally distilled :

Fraction I (monosaccharides), 0·54 g.; 10·9% of the glycoside mixture.
Fraction II (disaccharides), 1·13 g.; 22·7% ,, ,, ,,
Fraction III (trisaccharides), 0·92 g.; 18·4% ,, ,, ,,
Fraction IV (undistilled residue), 2·40 g.; 48·1% ,, ,, ,,

Acetolysis V. Reaction arrested after 260 *minutes* : The acetolysis was conducted by the standard procedure, 2·61 g. of trimethyl starch being used. Distillation of the glycosides yielded :

Fraction I (monosaccharides), 1·31 g.; 52·9% of the glycoside mixture.

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