90. *Separation of Methylated Methylglycosides by Adsorption on Alumina. A New Method for End-group Determinations in Methylated Polysaccharides.*

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The quantitative separation of tetramethyl methylglucosides from trimethyl methylglucosides by adsorption on activated alumina from solution in ether–light petroleum has been achieved. This provides a new and rapid method for the determination of the end groups in methylated polysaccharides. By this means the repeating units of methylated banana and rice starch have been demonstrated to be 26 and 33 units respectively, in close agreement with determinations previously made by fractional distillation methods (Hawkins, Jones, and Young, J., 1940, 390; Hirst and Young, J., 1939, 1471). The partial separation of trimethyl methyl-*l*-arabofuranoside and trimethyl methyl-*d*-xylopyranoside has also been accomplished.

The complete separation of fully methylated sugars from partially methylated sugars has always been a troublesome operation, especially when one of the components is present in small amount only. From theoretical considerations it seems that the separation of methylated sugars by adsorption on some basic material should be a relatively simple matter. An examination of the possibility of separating tetramethyl methylglucoside and trimethyl methylglucoside by means of activated alumina (chromatographic adsorption) has demonstrated that these sugars may be separated with relative ease, and that tetramethyl methylglucoside in quantities of about 150 mg. may be recovered in a yield of 94% when admixed with 20 times the quantity of trimethyl methylglucoside. Some separation of the α- and the β-forms of the methylglucosides can also be effected, the β-form being less strongly adsorbed in both cases.

This new technique was applied to determine the nature of the repeating unit of methylated banana starch and methylated rice starch. The results, 33 glucose residues per repeating unit in rice starch and 26 in banana starch, are in close agreement with those reported previously (31 residues and 26 residues respectively).

The samples of methylated derivatives used were from material prepared by Hawkins, Jones, and Young (*loc. cit.*) and Hirst and Young (*loc. cit.*), who kindly supplied the sample of methylated rice starch. A direct comparison was, therefore, possible between the results obtained by the new method and those established by fractional distillation procedure. The use of the new technique enables the repeating unit to be determined on smaller quantities of methylated polysaccharides.

The method is useful for separating the components of constant-boiling mixtures. For example, a fraction obtained from the distillation of the glycosides from the hydrolysis of methylated damson gum (see Hirst and Jones, J., 1938, 1178; 1939, 1482; Hirst, J., 1942, 70) and which contained trimethyl methyl-*l*-arabofuranoside and trimethyl methyl-*d*-xylopyranoside resisted all attempts at fractionation by the distillation technique. Chromatographic analysis of this fraction on alumina, however, proved that it was a mixture, and some separation of arabinose and xylose derivatives was achieved, but the separation was complicated by the fact that the α- and the β-glycosides of the two sugars were also separated.

The method shows promise also for the fractionation of methylated polysaccharides (see below), but further work on the possibilities opened up by this technique as applied to carbohydrate chemistry must await a more opportune moment.

Experimental.

All the solvents used were first stirred with activated alumina, filtered, and distilled. A mixture of trimethyl methylglucoside (3·0 g.) and tetramethyl methylglucoside (150 mg., $n_D^{16°}$ 1·4430) was dissolved in a mixed solvent consisting of ether (25 c.c.) and light petroleum (b. p. 60—80°) (25 c.c.). The solution was filtered through a column of alumina (15 cm. × 2 cm.) (Peter Spence and Sons, Ltd., Widnes; Grade 100/200 Birlec type) and the chromatogram was developed with ether–light petroleum (b. p. 60—80°) (2 : 1 mixture by vol.). The filtrate was collected in 20 c.c. fractions, which were evaporated on the water-bath. The following products were collected: (1) 3 Mg., yellow impurity. (2) 142 Mg. of a syrup—tetramethyl methylglucoside (yield, 94%), $n_D^{16°}$ 1·4426 (Found: OMe, 61·4. Calc. for $C_{11}H_{22}O_6$: OMe, 62·0%). After hydrolysis with boiling 2N-hydrochloric acid, this gave crystalline tetramethyl glucopyranose (yield, 88%).

The third portion of eluate consisted of solvent only, but the following portion contained (3) 174 mg. of trimethyl β-methylglucoside, m. p. and mixed m. p. with an authentic sample, 60°, $[\alpha]_D^{20°}$ − 34° (*c*, 1·2 in water). The remainder of the trimethyl methylglucoside was eluted with methyl alcohol.

Estimation of the Repeating Unit of Disaggregated Rice Starch.—Rice starch (Fraction 3, Hirst and Young, *loc. cit.*, p. 1481) (3 g.) was boiled with 1·7% methyl-alcoholic hydrogen chloride (50 c.c.) for 15 hours. The solution was neutralised with silver carbonate and worked up in the usual manner. The resulting syrup (3·4 g.) was extracted three times with ether to eliminate as much dimethyl methylglucoside as possible and the extracts were evaporated to a syrup. The syrup was dissolved in ether (2 parts) and light petroleum (b. p. 60—80°) (3 parts) (60 c.c. in all) and chromatographed on alumina, the column being washed through portionwise, with more of the ether–light petroleum mixture. This operation achieved a partial separation only and gave, after evaporation of the solvents, two fractions (A) and (B). Fraction (A) contained tetramethyl methylglucoside admixed with trimethyl methylglucoside. Fraction (B) contained no tetramethyl methylglucoside, since it had a high refractive index, $n_D^{20°}$ 1·4570, and a low methoxyl value (Found: OMe, 50·4%. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%). It was not further investigated.

Syrup (A) was dissolved in ether–light petroleum (b. p. 60—80°) (1 : 1); the solution was passed into a column of alumina (as described above) and elution was carried out with 20 c.c. portions of ether–light petroleum (1 : 1). The following fractions were obtained:

Fraction I. 7 Mg. of a yellow oil with a pungent odour; $n_D^{16°}$ 1·4900.

Fraction II. 105 Mg. of tetramethyl methylglucoside, $n_D^{16°}$ 1·4449, $[\alpha]_D^{20°}$ + 133° (*c*, 1·0 in water) (Found: OMe, 61·7. Calc. for $C_{11}H_{22}O_6$: OMe, 62·0%). On hydrolysis with 2N-hydrochloric acid, this gave tetramethylglucopyranose, isolated in 91% yield, m. p. 94° (Found: OMe, 52·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%).

Fraction III. 222 Mg. of trimethyl β-methylglucoside, m. p. 59°, $n_D^{16°}$ 1·4534 (supercooled), $[\alpha]_D^{20°}$ − 34° (*c*, 0·9 in water).

The yield of tetramethyl methylglucoside was 3·0% of the weight of mixed methylglucosides obtained on hydrolysis, corresponding to a chain length of 35 glucose residues. If, however, allowance is made for a loss of 6% of tetramethyl methylglucoside during recovery, the yield becomes 3·2%, corresponding to 33 glucose residues in the repeating unit.

Estimation of the Repeating Unit of Banana Starch.—The methylated starch was part of a fraction described by Hawkins, Jones, and Young (*loc. cit.*, p. 393) and designated fraction E by them. The methylated polysaccharide (4·0 g.) was boiled with 1% methyl-alcoholic hydrogen chloride (100 c.c.) for 16 hours. The neutralised solution was evaporated, the syrupy residue (4·55 g.) extracted twice with light petroleum (b. p. 40—60°) (25 c.c.), and the solution passed through a column of alumina which had been previously wetted with light petroleum. The column was eluted with ether and the filtrate, on evaporation, gave a syrup (1·976 g.). This syrup was dissolved in ether (25 c.c.)–light petroleum (b. p. 60—80°) (25 c.c.), and the solution passed through a column of alumina (see above). The alumina was eluted with ether–light petroleum (b. p. 60—80°) (2 : 1), and the eluate collected in 20 c.c. fractions. These on evaporation gave :

Fraction I. 14 Mg. of a yellow oil with a pungent odour, $n_D^{17°}$ 1·4985.

Fraction II. 114 Mg. of tetramethyl methylglucoside, $n_D^{16°}$ 1·4424, $[\alpha]_D^{20°}$ + 34° (*c*, 1·0 in water) (Found : OMe 62·3. Calc. for $C_{11}H_{22}O_6$: OMe, 62·0%).

Fraction III. 46 Mg. of tetramethyl methylglucoside, $n_D^{16°}$ 1·4437, $[\alpha]_D^{20}$ + 86° (*c*, 0·4 in water) (Found : OMe, 61·8%).

Fractions II and III were combined and hydrolysed with 2N-hydrochloric acid. Tetramethyl glucopyranose was isolated from the hydrolysate in the usual manner (yield, 84%), m. p. and mixed m. p. with an authentic specimen, 95° (Found : OMe, 51·8. Calc. for $C_{10}H_{20}O_6$: OMe, 52·6%).

The column was then eluted with ether and gave, after evaporation of the solvent :

Fraction IV. 120 Mg. of trimethyl β-methylglucopyranoside, m.p. 59—60°, $n_D^{16°}$ 1·4535 (supercooled) (Found : OMe, 53·4%).

Fraction V. 268 Mg. of trimethyl β-methylglucopyranoside, m. p. 58°, $n_D^{16°}$ 1·4537 (supercooled), $[\alpha]_D^{20°}$ − 39° (*c*, 1·1 in chloroform).

The total yield of tetramethyl methylglucoside was 160 mg., corresponding to 3·52% of the weight of methylglucosides obtained on hydrolysis of the methylated starch (repeating unit 28 residues, or after correction for experimental loss as described above, 26 units).

*Separation of a Constant-boiling Fraction containing Trimethyl Methyl-*l*-arabofuranoside and Trimethyl Methyl-*d*-xylopyranoside.*—Methylated damson gum on hydrolysis with methyl-alcoholic hydrogen chloride gave a mixture of glycosides. The most volatile portion was a mixture which could not be separated by fractional distillation, $n_D^{18°}$ 1·4374 (Found : OMe, 57·4%). Further separation was, however, effected in the following way. The material (3·644 g.) was dissolved in ether (10 c.c.) and light petroleum (b. p. 60—80°) (30 c.c.), the solution passed through a column of activated alumina (15 cm. × 2 cm.), and the column then developed with light petroleum (b. p. 60—80°). The following fractions were finally obtained :

Fraction.	Weight, g.	$n_D^{18°}$.	$[\alpha]_D^{20°}$ in methyl alcohol.	$[\alpha]_D^{20°}$ (equilibrium) after hydrolysis with 0·5N-HCl.	OMe, %.
I	1·49	1·4350	−119°	−27°	58·9
II	0·37	1·4328	− 63	−17	59·7
III	1·30	1·4330	− 26	−44	59·9
IV	0·21	1·4372	+100	−14	59·3
V	0·05	1·4510	—	—	—
VI	0·10	1·4584	—	—	—
	3·52 *g., i.e.*, 97% recovery.				

The fractions (I to IV) were each hydrolysed with 0·5N-hydrochloric acid, neutralised with silver carbonate, filtered, and evaporated to a syrup. Since 2 : 3 : 4-trimethyl *d*-xylose has an equilibrium rotation in water of + 17°, and 2 : 3 : 5-trimethyl *l*-arabofuranose an equilibrium rotation in water of − 39°, it was considered that fractions II and IV were likely to be the richest in 2 : 3 : 4-trimethyl *d*-xylose. These two fractions were therefore combined and nucleated with 2 : 3 : 4-trimethyl *d*-xylose; crystallisation then occurred. After three days the crystalline solid was removed by tiling. After recrystallisation, 2 : 3 : 4-trimethyl *d*-xylose was obtained in 25% yield (calc. on the original glycosides), m. p. and mixed m. p. with an authentic specimen, 91°. The syrup remaining absorbed in the tile still contained some 2 : 3 : 4-trimethyl *d*-xylose. From the yield of crystalline 2 : 3 : 4-trimethyl *d*-xylose (25%) and from the equilibrium values of the free sugars it was calculated that fractions II and IV contained some 50% of 2 : 3 : 4-trimethyl methyl-*d*-xylopyranoside, *i.e.*, 0·29 g.

Attempted Separation of a Fraction of Methylated Cherry Gum.—Methylated cherry gum (3 g.) which had been purified be precipitation from chloroform with light petroleum (b. p. 60—80°) (Hirst, J., 1942, 76) was dissolved in a mixture of benzene (40 c.c.) and ether (20 c.c.) and passed into a column of alumina (see above). The column was eluted with benzene (50 c.c. portions) and by this means three fractions were obtained : *Fraction I*, 0·8 g., $[\alpha]_D^{20°}$ + 14·6° (*c*, 4·0 in methyl alcohol) (Found : OMe, 37·8%), *fraction II*, 1·2 g. $[\alpha]_D^{20°}$ + 10·7° (*c*, 1·1 in methyl alcohol) (Found : OMe, 40·2%), and *fraction III*, 0·4 g., $[\alpha]_D^{20°}$ + 9·5° (*c*, 2·1 in methyl alcohol) (Found : OMe, 38·2%). All these fractions were white powders, whereas the original methylated gum was yellow. Benzene failed to remove any further material from the column, which was therefore eluted with methyl alcohol until no further extraction occurred. Evaporation of the solvent gave *fraction IV*, 0·5 g., $[\alpha]_D^{20°}$ + 2·2° (*c*, 2·1 in methyl alcohol) (Found : OMe, 41·8%). The data for these fractions indicate that some fractionation had occurred.

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