818. *The Chemical Synthesis of Polysaccharides. Part III.*[1] *Positions of Alkylation in a Partly Methylated Amylose.*

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A " mono-O-methylamylose," prepared by a method claimed to secure selective methylation of the 2-hydroxyl group, is proved to be rather randomly methylated, with only a limited excess of methyl at C-2. A new derivative of 2-*O*-methyl-D-glucose is reported.

Gaver, Tieszen, and Lasure [2] patented a process for the preparation of starch substituted selectively at the 2-hydroxyl position by various alkyl groups, including methyl, which consists in treating a " monosodio starch " with methyl iodide. As we are interested in the synthesis of modified starch-type polysaccharides for use in tests of enzyme specificity we applied this process to the methylation of potato amylose.

On acid hydrolysis, however, the product yielded a complex mixture, not simply 2-*O*-methylglucose. It was resolved by charcoal and paper chromatography, combined with paper electrophoresis, into substances behaving chromatographically as glucose, 2-, 3-, and 6-*O*-methylglucose, and di-*O*-methylglucoses. By quantitative analysis the relative amounts by weight of the products were glucose (43·5%), mono-*O*-methylglucoses (36·4%), and di-*O*-methylglucoses (14·6%), the remainder (5·5%) being incompletely hydrolysed products. The mono-*O*-methylglucoses were separated into pure 2-*O*-methylglucose and a mixture of the 3- and the 6-isomer, the relative molar amounts being 2·6 : 1 : 1·2. Thus 2-*O*-methylglucose constituted only 20% of the sugars in the hydrolysed *O*-methylamylose. The sugar was identified as its β-tetrabenzoate and a new derivative, probably the α-anomer, was also obtained.

The results refute the claim by Gaver *et al.*[2] to have made a selectively substituted starch. There is general agreement that the secondary hydroxyl groups of starch react more readily with alkali than do the primary hydroxyl groups, and hence the secondary groups can be more readily etherified.[3] The present results agree with this view but show that there is no over-riding selectivity for reaction at the 2-hydroxyl group. Lenz [4] recently reported similar results for the product of methylation of monosodiocellulose, hydrolysis of which yielded 2-, 3-, and 6-*O*-methylglucose in the molar ratio 4·9 : 1 : 4·1, as well as di- and tri-*O*-methylglucoses.

EXPERIMENTAL

*Preparation of Mono-*O*-methylamylose.*—Potato amylose [5] (7·35 g.) was stirred in a closed vessel at 85—87° for 2 hr. with anhydrous butanol (50 ml.) and sodium hydroxide (2·23 g.). The suspension was cooled, centrifuged in a stoppered bottle, and washed successively with anhydrous butanol and toluene. The sodio-amylose was transferred to a combustion tube in anhydrous toluene (20 ml.), methyl iodide (7·5 g.) was added, and the tube was sealed and heated at 100° for 4 hr. After cooling, sodium iodide was removed from the suspension by repeated washing with methanol. The sticky mass was hardened with butanol and ether and dried in a vacuum.

*Acid Hydrolysis of " Mono-*O*-methylamylose."* [2]—A small portion of the methylamylose was hydrolysed in 1·5N-sulphuric acid at 100° for 3 hr., then neutralised with ion-exchange resin. Electrophoresis on paper in borate buffer of pH 8·7 [6] revealed substances migrating with glucose, 2- and 3(or 6)-*O*-methylglucose, and paper chromatography in 2,4,6-collidine–ethyl acetate–

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[1] Part II, Haq and Whelan, *J.*, 1958, 1342.
[2] Gaver, Tieszen, and Lasure, U.S.P. 2,671,780/1954.
[3] Sugihara, *Adv. Carbohydrate Chem.*, 1953, **8**, 16.
[4] Lenz, *J. Amer. Chem. Soc.*, 1960, **82**, 182.
[5] Hobson, Pirt, Whelan, and Peat, *J.*, 1951, 801.
[6] Foster, *J.*, 1953, 982.

water (2 : 5 : 5, by volume)[7] resolved the mixture of mono-*O*-methyl glucoses into substances migrating with the 2-, 3-, and 6-*O*-isomers.

A larger amount (4·49 g.) of the *O*-methylamylose was hydrolysed in 1·5N-sulphuric acid (450 ml.) at 100° for 2 hr., cooled, neutralised with sodium hydroxide, and adsorbed on a charcoal–Celite column (75 × 5 cm.).[8] Glucose was removed by elution with water and its amount estimated by optical rotation (1·852 g.). The column was then washed with 0·05M-sodium borate buffer (pH 8·7; 1·3 l.), and a gradient of aqueous ethanol was applied by attaching a reservoir of borate buffer (20 l.) to the column, the reservoir being kept at constant level by addition of 15% ethanol in buffer.[9] The progress of elution of sugar was followed by measurement of optical rotation. Two zones of optical activity were noted and a third fraction was subsequently removed by washing of the column with 50% ethanol. The appropriate fractions were combined and freed from borate by separate passage through another charcoal–Celite column which was washed with water until free from borate. The sugars were then desorbed with 50% aqueous ethanol and weighed. Paper chromatography showed the three fractions to consist of mono-*O*-methylglucoses (1·537 g.), di-*O*-methylglucoses (0·588 g.), and products of partial hydrolysis (0·213 g.), which with the free glucose already recovered represented 85·3% of the polysaccharide.

*Fractionation of Mono-*O*-methylglucoses.*—A portion (about 800 mg.) of the mono-*O*-methylglucoses was fractionated by electrophoresis[10] on sheets of Whatman No. 3 paper in 0·2M-sodium borate buffer (pH 10·0) at 10 v/cm. for 17 hr. The sugars were eluted with water and freed from borate by removal of sodium ions on Biodeminrolit resin (Permutit Co. Ltd.) in the carbonate form,[11] and boric acid by distillation with methanol. The mixture was thus resolved into 2-*O*-methylglucose and 3- plus 6-*O*-methylglucose. The sugars were dissolved in water (50 ml.) and their concentrations estimated by oxidation of portions (2 ml.) with 0·1N-iodine in 0·2N-potassium iodide (4 ml.), to which was added 0·05M-sodium phosphate buffer (pH 11·4;[12] 25 ml.). The solutions were stored for 20 hr. in the dark in stoppered vessels, acidified with 3N-sulphuric acid, and titrated with 0·01N-sodium thiosulphate. The respective total weights of methylglucoses present were 388 and 320 mg. The relative amounts of 3- and 6-*O*-methylglucose were determined by periodate oxidation and measurement of formaldehyde. The former sugar yields 1 molecular proportion of formaldehyde; the latter gives none. Oxidation was carried out in 0·06M-sodium metaperiodate (pH 1·9) at 50° for 20 hr. and formaldehyde was estimated as by Parrish and Whelan.[13] For each mole of monomethylglucose, 0·46 mole of formaldehyde was formed. The identity of the 2-*O*-methylglucose was confirmed by similar oxidation before and after reduction with sodium borohydride, 0·95 and 0·99 mol. of formaldehyde being formed respectively (calc., 1 mol. in each case).

The 2-*O*-methylglucose (270 mg.) was benzoylated as described by Oldham,[14] and the β-tetrabenzoate was obtained. The four-times crystallised derivative had m. p. 176—177°, $[\alpha]_D$ −7·0° (*c* 2·2 in chloroform) (lit.,[14] m. p. 169—170°, $[\alpha]_D$ −6·2°) (Found: C, 69·1; H, 4·6. Calc. for $C_{35}H_{30}O_{10}$: C, 68·9; H, 4·9%). From the methanolic mother-liquors was obtained a different substance, presumed to be the α-*tetrabenzoate*, which after four crystallisations had m. p. 157—158°, $[\alpha]_D$ +55·9° (*c* 0·26 in chloroform) (Found: C, 69·1; H, 4·6%).

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[7] Lenz and Holmberg, *Analyt. Chem.*, 1956, **28**, 7.
[8] Whelan, Bailey, and Roberts, *J.*, 1953, 1293.
[9] Barker, Bourne, and Theander, *J.*, 1955, 4276.
[10] Peat, Whelan, and Roberts, *J.*, 1957, 3916.
[11] Woolf, *Nature*, 1953, **171**, 841.
[12] Ingles and Israel, *J.*, 1948, 810.
[13] Parrish and Whelan, *Stärke*, 1961, **13**, 231.
[14] Oldham, *J. Amer. Chem. Soc.*, 1934, **56**, 1360.