

614. *Alkali-metal Derivatives of Sucrose. Part III.¹ The Composition of the Mono-O-methylsucrose prepared from a Trisodium Derivative.*

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A trisodium derivative of sucrose with methyl toluene-*p*-sulphonate gives a mixture of sucrose and its methyl ethers. A mono-*O*-methylsucrose isolated from this is a mixture of isomers, mainly those containing the substituent methyl group at position 2 of the glucose unit or position 1 of the fructose unit. Substitution also occurs at positions 3 and 6 of the fructose and the glucose moiety respectively, and is not restricted to these positions. The significance of these results on the location of the sodium atoms in the original derivative is discussed.

THE preparation of sodium derivatives of sucrose, by reaction of sodium or sodium amide with sucrose in liquid ammonia at -33° , and their condensation with organic chloro-compounds, have been previously described.^{1,2} This paper describes the structures of mono-*O*-methylsucroses prepared from a trisodium derivative.

The white crystalline monosodium derivative contains 4.47% of ammonia² and can be considered as a true alkoxide containing one mol. of ammonia ($C_{12}H_{21}O_{11}Na, NH_3$) or as a sodamide addition compound ($C_{12}H_{22}O_{11}, NaNH_2$). This could not be methylated with methyl iodide because the ammonia was methylated preferentially, with the formation of hydriodic acid which decomposed the sodium derivative. The more highly substituted derivatives, however, contain less ammonia (1.4–2.7%),² and methylation can be achieved although the reaction is not quantitative, again owing to the presence of ammonia. The trisodium compound used in this investigation (NH_3 , 2.13%) gave a product with MeO = 10.6%, whereas quantitative methylation of the trisodium alkoxide, $C_{12}H_{19}O_{11}Na_3$, should give tri-*O*-methylsucrose (MeO = 24.2%).

Of many liquids tried as methylating media, the most successful was tri-*O*-methylglycerol.^{3,4} Methyl toluene-*p*-sulphonate was found to be a satisfactory methylating agent for the trisodium derivative at the level of 1 mol. per sodium atom, so that no excess of reagent was present. Except for cellulose,⁵ toluene-*p*-sulphonic esters have not often been used for alkylation of carbohydrates. The sodium derivative in tri-*O*-methylglycerol was heated with methyl toluene-*p*-sulphonate at 110° for 5 hr. with vigorous stirring, the mixture was dissolved in water, and the solution de-ionised and concentrated to give a glassy methylated material (OMe, 10.6%). Chromatography revealed it as a mixture of sucrose, mono- and di-*O*-methylsucrose, and more highly methylated material. The components were partly separated on a cellulose column,⁶ the following fractions (total 86%) being obtained. Fraction 1 (16.3%) contained only sucrose. Fraction 2 (24.9%) was sucrose containing a small amount of mono-*O*-methylsucrose. Fraction 3 (20.0%) was chiefly mono-*O*-methylsucrose containing some di-*O*-methylsucrose. Fraction 4 (21.8%) was di-*O*-methylsucrose and more highly methylated products.

Refractionation of fraction 3 on cellulose and further purification gave a pale yellow glass, $[\alpha]_D +60.6^\circ$ in water, which gave correct analyses for a mono-*O*-methylsucrose (OMe, 8.3%). Chromatography in two solvent systems indicated that this was homogeneous. Periodate oxidation in the dark at 20° followed closely that of sucrose, 2.7 mols. of periodate being consumed and 0.86 mol. of formic acid produced after 24 hr. This suggests that there was little substitution on secondary hydroxyl groups, a result which was not confirmed by hydrolysis. Indeed, with 0.05*N*-sulphuric acid it gave a mixture

¹ Part II, Black, Dewar, Paterson, and Rutherford, *J. Appl. Chem.*, 1959, **9**, 256.

² Arni, Black, Dewar, Paterson, and Rutherford, *J. Appl. Chem.*, 1959, **9**, 186.

³ Dr. D. J. Bell, personal communication.

⁴ Fairbourne, Gibson, and Stephens, *Chem. and Ind.*, 1930, **49**, 1021.

⁵ Weaver, MacKenzie, and Shirley, *Ind. Eng. Chem.*, 1954, **46**, 1490.

⁶ Hough, Jones, and Wadman, *J.*, 1949, 2511.

of sugars which was separated on a cellulose column⁶ into a mono-*O*-methylhexose fraction (9.3 parts), *D*-fructose (4.0 parts), and *D*-glucose (7.4 parts). This ether fraction was separated on thick paper into 2- (2.4 parts) and 6-*O*-methyl-*D*-glucose (1.0 part) and 1- (2.1 parts) and 3-*O*-methyl-*D*-fructose (1.5 parts), and two other unidentified ethers which were present in smaller amounts. 2- and 6-*O*-Methyl-*D*-glucose and 1-*O*-methyl-*D*-fructose were characterised by their methoxyl contents, optical rotations, chromatography with authentic samples, periodate oxidation, and osazone formation. The second mono-*O*-methylfructose component (OMe, 15.8%) was obtained crystalline and had $[\alpha]_D -54.8^\circ$ in water, which strongly suggested that it was 3-*O*-methyl-*D*-fructose ($[\alpha]_D -53.5^\circ$ in water⁷); 4-*O*-methyl-*D*-fructose ($[\alpha]_D -87.5^\circ$ in water) has not been obtained crystalline⁸ and 6-*O*-methyl-*D*-fructofuranose has a positive rotation.⁹ However, the structure of this component could not be confirmed by periodate oxidation or osazone formation, owing probably to loss of water and formation of difructose anhydride during extensive drying.¹⁰

The presence of glucose, fructose, and at least two glucose and two fructose mono-methyl ethers proves that the "chromatographically pure" mono-*O*-methylsucrose is a mixture of isomers, and this was confirmed by extensive development of the unhydrolysed material in a third solvent, by which two spots and a trace of another were identified. Because of the small quantities available and the difficulties of separating quantitatively a mixture containing six mono-*O*-methylhexoses, the proportions of the components of the hydrolysate must be considered only approximate. Nevertheless, some observations can be made. For example, from the relative proportions of unsubstituted glucose and fructose present, which are easily separated quantitatively, substitution occurs to a greater extent on the fructose than on the glucose moiety. From the distribution of mono-*O*-methylhexoses, substitution occurs chiefly at position 2 and to a smaller extent at position 6 of the glucose residue, and in the fructose residue C₍₄₎ is the most active centre closely followed by C₍₃₎. These results agree with previous views on the relative reactivities of hydroxyl groups in the glucose molecule in alkaline media.¹¹ The ratio of the rate constants for the methylation of alkali cellulose with methyl chloride was shown to be 5 : 1 : 2 for positions 2, 3, and 6 of the glucose residue,¹² and 4.5 : 1 : 2 for ethylation with ethyl chloride.¹³ Percival,¹⁴ however, found that methylation of a sucrose-tripotassium hydroxide compound gave a tri-*O*-methylsucrose substituted at the three primary alcohol groups, indicating that the potassium hydroxide was associated with the primary alcohol groups in the original addition compound.

It must be borne in mind, however, in the present study that the methoxyl content of the material was less than half that calculated from the quantitative replacement of sodium atoms. Further, the composition of the other methyl ethers formed has not yet been determined. Consequently, the location of much of the sodium present in the original trisodium derivative is still unknown, but sufficient has been done to indicate that analysis of the methylated products from sodium derivatives will be extremely complex.

EXPERIMENTAL

Analytical Methods.—Solutions were evaporated under reduced pressure below 60°. Paper-partition chromatography was on Whatman No. 1 paper at 20° with the following solvent systems (v/v): (1) butan-1-ol-ethanol-water (40 : 11 : 19); (2) (ethyl methyl ketone half saturated with water)-ammonia (99 : 1); (3) butan-1-ol-benzene-pyridine-water (5 : 1 : 3 : 3).

⁷ Anderson, Charlton, Haworth, and Nicholson, *J.*, 1929, 1337.

⁸ Barry and Honeyman, *Adv. Carbohydrate Chem.*, 1952, 7, 81.

⁹ MacDonald and Jackson, *J. Res. Nat. Bur. Stand.*, 1940, 24, 181.

¹⁰ MacDonald, *Adv. Carbohydrate Chem.*, 1946, 2, 265.

¹¹ Sugihara, *ibid.*, 1953, 8, 1.

¹² Croon and Lindberg, *Svensk Papperstidn.*, 1957, 60, 843.

¹³ Croon and Flamm, *ibid.*, 1958, 61, 963.

¹⁴ Percival, *J.*, 1935, 648.

Papers were sprayed with α -naphthol-phosphoric acid,¹⁵ benzidine-trichloroacetic acid,¹⁶ 3% *p*-anisidine hydrochloride in butan-1-ol,¹⁷ and aniline hydrogen oxalate and phthalate.¹⁸ R_{Glu} , R_{Suc} , R_{Fru} , and R_{F} are the rates relative to glucose, sucrose, fructose, and the solvent front respectively. Electroionophoresis¹⁹ was carried out in borate buffer of pH 10. Whatman 3MM sheets, after preliminary extraction with hot benzene-ethanol (1:1), were used for the chromatographic separation of the mono-*O*-methylhexose fraction. Unless otherwise stated, specific rotations were measured in water at 20°.

Materials.—The trisodium derivative was prepared as described in Part I² and gave the following analysis: sodium, 16.4; sucrose (as $\text{C}_{12}\text{H}_{22}\text{O}_{11}$), 81.3; ammonia, 2.13%; sodium atoms per sucrose mol., 3.00. Commercial (Eastman Organic Chemicals) methyl toluene-*p*-sulphonate was purified before use by dissolution in chloroform, drying (Na_2SO_4), recovery, and distillation, the fraction boiling at 132°/1–2 mm. being used. Tri-*O*-methylglycerol was prepared by exhaustive methylation of epichlorohydrin.⁴

Methylation of the Trisodium Derivative.—The compound (6.082 g.) was dispersed in tri-*O*-methylglycerol (30 ml.), methyl toluene-*p*-sulphonate (1 mol. per Na atom) added, and the mixture heated at 110° for 5 hr. with stirring and exclusion of moisture. The mixture was dissolved in water (50 ml.), and the solution de-ionised (Amberlite resins IR-120- H^+ and IR-45- OH^-) and evaporated to a glass (4.962 g.) (Found: OMe, 10.6%). In a second experiment, the derivative (4.223 g.) gave a glass (3.371 g.; OMe, 10.5%).

Fractionation of the Glass.—The combined products (8.33 g.) were fractionated on a cellulose column⁶ (50 × 3.8 cm.) with butan-1-ol saturated with water as eluant, partial separation being effected:

Fraction 1 (1.356 g.), R_{F} identical with that of sucrose. The material was purified by treatment with charcoal, and the syrup crystallised from methanol {m. p. 178°; $[\alpha]_{\text{D}} + 66.4^\circ$ (*c* 2); Found: C, 41.9; H, 6.5. $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ requires C, 42.1; H, 6.5%}. Identity was confirmed by conversion with sodium acetate-acetic anhydride²⁰ into sucrose octa-acetate, m. p. 86–88° (Found: C, 49.2; H, 5.7. Calc. for $\text{C}_{28}\text{H}_{38}\text{O}_{19}$: C, 49.6; H, 5.6%).

Fraction 2 (2.074 g), sucrose containing a small amount of mono-*O*-methylsucrose.

Fraction 3 (1.661 g.), mono-*O*-methylsucrose and some di-*O*-methylsucrose.

Fraction 4 (1.819 g.), di-*O*-methylsucrose and higher methylated material.

The identification of the components of fractions 2–4 was based on the relative positions of the spots on paper chromatography (solvent 1), with di-*O*-methylsucrose and 1',4,6'-tri-*O*-methylsucrose²¹ as reference spots. Both α -naphthol-phosphoric acid¹⁵ and *p*-anisidine hydrochloride¹⁷ are extremely sensitive sprays for detecting sucrose methyl ethers.

Isolation of Mono-*O*-methylsucrose from Fraction 3.—Fraction 3 (1.66 g.) was refractionated on a tightly packed cellulose column⁶ with butan-1-ol saturated with water as the eluant, to give a mono-*O*-methyl (812 mg.) and a di-*O*-methyl fraction (241 mg.). After further purification, the mono-*O*-methylsucrose (629 mg.) was obtained as a pale yellow, non-reducing glass, $[\alpha]_{\text{D}} + 60.6^\circ$ (*c* 2.1) (Found: OMe, 8.3. $\text{C}_{13}\text{H}_{24}\text{O}_{11}$ requires OMe, 8.7%). Paper chromatography (solvent 1) over an extended period showed a single spot (R_{Glu} 1.11, R_{Suc} 1.44); solvent 2 also revealed only one spot. After the hydrolysis had proved that the material was a mixture of isomers, chromatography was repeated with solvent 3, two spots (R_{Glu} 1.01, 1.15) and a trace of another (R_{Glu} 1.27) being detected.

Periodate Oxidation of Mono-*O*-methylsucrose.—A 0.1% solution (10 ml.) was treated with 0.025M-sodium periodate (10 ml.) in the dark at 20°, and aliquot parts (2 ml.) were removed for estimation of periodate uptake²² and formic acid liberated.²³ The ether consumed 1.99 (1 hr.), 2.26 (3 hr.), 2.41 (6 hr.), 2.71 (24 hr.), 2.82 (48 hr.) mols. of periodate and liberated 0.38, 0.62, 0.70, 0.86, 0.81 mol. of formic acid. Under identical conditions, sucrose consumed 1.76, 2.51, 2.59, 2.96, 3.01 mols. of periodate and liberated 0.19, 0.29, 0.40, 0.63, 0.65 mol. of formic acid.

Separation of the Sugars obtained on Hydrolysis of Mono-*O*-methylsucrose.—The glass (352 mg.)

¹⁵ Albon and Gross, *Analyst*, 1952, **77**, 410.

¹⁶ Whelan, Bailey, and Roberts, *J.*, 1953, 1297.

¹⁷ Hough, Jones, and Wadman, *J.*, 1950, 1702.

¹⁸ Partridge, *Nature*, 1949, **164**, 443.

¹⁹ Foster, *Chem. and Ind.*, 1952, 1050.

²⁰ French, *J. Amer. Chem. Soc.*, 1955, **77**, 1024.

²¹ McKeown, Serenius, and Hayward, *Canad. J. Chem.*, 1957, **35**, 28.

²² Jackson, "Organic Reactions," Chapman and Hall Ltd., London, 1944, Vol. II, p. 361.

²³ Halsall, Hirst, and Jones, *J.*, 1947, 1399.

was heated with 0.05*N*-sulphuric acid (10 ml.) at 100° for 3 hr. ($[\alpha]_D - 6.5^\circ$). The cooled solution was neutralised (Phenol Red) with 0.1*N*-sodium hydroxide, ethanol (150 ml.) added to precipitate sodium sulphate, and the centrifugate concentrated to a syrup, which was fractionated on a cellulose column⁶ with butan-1-ol saturated with water as the eluant:

Fraction A (135 mg.) was a mixture of mono-*O*-methylhexoses which were separated on thick paper as described below.

Fraction B (58 mg.) was *D*-fructose. The syrup was shaken for 5 hr. with anhydrous acetone (5 ml.) containing a trace of sulphuric acid, and then treated as described by Bell²⁴ to give 1,2:4,5-di-*O*-isopropylidene-*D*-fructose (34 mg.) as needles, m. p. and mixed m. p. 117—118°, $[\alpha]_D - 143^\circ$ (*c* 1 in chloroform). Under the same conditions, 23.4 mg. of 1,2:4,5-di-*O*-isopropylidene-*D*-fructose were obtained from 40 mg. of authentic *D*-fructose.

Fraction C (107 mg.) was *D*-glucose, which was heated with sodium acetate (50 mg.) and acetic anhydride (1 ml.) at 100° for 2 hr. to give penta-*O*-acetyl- β -*D*-glucose (115 mg.). Recrystallisation from absolute ethanol gave needles, m. p. and mixed m. p. 128°, $[\alpha]_D + 4.5^\circ$ (*c* 4.9 in chloroform).

Fraction A (122 mg.) was separated on thick paper with solvent 2:

Fraction Al (13.1 mg.), $R_{Glu} 4.03$ (solvent 2), identical with authentic 6-*O*-methyl-*D*-glucose, $[\alpha]_D + 53^\circ$ (*c* 0.5) (Found: OMe, 14.9%. Calc. for $C_7H_{14}O_6$: OMe, 16.0%). The sugar consumed 2.5 (3 hr.), 2.4 (24 hr.), 2.5 (48 hr.) mols. of periodate and liberated 0.15, 0.15, 0.14 mol. of formaldehyde, the latter being estimated by the micro-colorimetric method of Hough, Powell, and Woods,²⁵ with *D*-mannitol as a source of formaldehyde for the calibration graph. 6-*O*-Methyl-*D*-glucose would be expected to give negligible amounts of formaldehyde. The structure was confirmed by the partial periodate oxidation method of Lemieux and Bauer.²⁶ Fraction Al and authentic 6-*O*-methyl-*D*-glucose gave identical yellow spots, $R_F 0.78$ (solvent 1), with fainter spots, $R_F 0.60$, when chromatograms were sprayed with 1% aniline hydrogen phthalate in water-saturated butan-1-ol. On treatment with 10% (v/v) acetic acid (0.5 ml.) and freshly distilled phenylhydrazine (0.04 ml.) at 80° for 3 hr.,²⁷ fraction Al (9.4 mg.) gave 6-*O*-methyl-*D*-glucose phenylosazone (2.9 mg.), m. p. and mixed m. p. 184° (Found: OMe, 7.7. Calc. for $C_{18}H_{24}O_4N_4$: OMe, 8.3%).

Fraction A2 (31.8 mg.), $R_{Glu} 5.00$ (solvent 2) (authentic 2-*O*-methyl-*D*-glucose gave $R_{Glu} 4.83$), $[\alpha]_D + 58^\circ$ (*c* 1.5) (Found: OMe, 15.5%). The sugar crystallised but the quantity was insufficient to afford an accurate m. p. It consumed 3.8 (3 hr.), 3.9 (24 hr.), 4.5 (48 hr.) mols. of periodate and liberated 0.10, 0.28, 0.36 mol. of formaldehyde. Authentic 2-*O*-methyl-*D*-glucose consumed 4.4 (3 hr.), 4.5 (24 hr.), 4.8 (48 hr.) mols. of periodate and liberated 0.17, 0.43, 0.58 mol. of formaldehyde. Partial periodate oxidation at 0° for 1 hr.²⁸ gave a strong lemon-yellow spot in the cold ($R_F 0.28$, solvent 1) for both fraction A2 and authentic 2-*O*-methyl-*D*-glucose. It gave a negative formazan test with triphenyltetrazolium hydroxide,²⁸ indicating that position 2 was substituted. Fraction A2 (24.8 mg.), on treatment with phenylhydrazine as before,²⁷ gave *D*-glucose phenylosazone (8.6 mg.), m. p. and mixed m. p. 205°, with a negligible methoxyl content.

Fraction A3 (27.7 mg.), $R_{Fru} 3.62$ (solvent 2), identical with authentic 1-*O*-methyl-*D*-fructose, $[\alpha]_D + 64.5^\circ$ (*c* 1.1) (Found: OMe, 15.1. Calc. for $C_7H_{14}O_6$: OMe, 16.0%). The sugar consumed 3.1 (3 hr.), 3.2 (24 hr.), 3.2 (48 hr.), 3.4 (72 hr.) mols. of periodate and liberated 0.18, 0.33, 0.52, 0.49 mol. of formaldehyde. Authentic 1-*O*-methyl-*D*-fructose consumed 3.5, 3.7, 3.6, 3.5 mols. of periodate and liberated 0.28, 0.74, 0.75, 0.74 mol. of formaldehyde. The above, slightly low results for the specific rotation, methoxyl content, periodate consumption, and formaldehyde production compared with those for the authentic, crystalline sugar can be attributed to the extreme difficulty in drying syrupy fructose methyl ethers.

Fraction A3 (12.1 mg.) was treated with phenylhydrazine²⁷ to give *D*-glucose phenylosazone (4.4 mg.), m. p. and mixed m. p. 207—208°, with a negligible methoxyl content.

Fraction A4 (19.2 mg.), $R_{Fru} 4.02$ (solvent 2), identical with authentic 3-*O*-methyl-*D*-fructose, $[\alpha]_D - 54.8^\circ$ (*c* 1.82) (Found: OMe, 15.8%). The sugar crystallised but the quantity available was insufficient to afford an accurate m. p. 4-*O*-Methyl-*D*-fructose ($[\alpha]_D - 87.5^\circ$) has not been obtained crystalline.⁸ After extensive drying over phosphoric oxide (60°/0.5 mm.), fraction A4

²⁴ Bell, *J.*, 1947, 1461.

²⁵ Hough, Powell, and Woods, *J.*, 1956, 4799.

²⁶ Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

²⁷ Smith, *J.*, 1951, 2646.

²⁸ Wallenfels, *Naturwiss.*, 1950, **37**, 491.

consumed 0.8 (48 hr.), 0.8 (72 hr.) mol. of periodate and liberated 0.30, 0.37 mol. of formaldehyde. 3-*O*-Methyl-D-fructose consumed 3.4, 3.5 mols. of periodate and liberated 0.98, 1.13 mol. of formaldehyde; for 4-*O*-methyl-D-fructose the results were 1.8, 2.7, and 1.16, 1.18 respectively. It is suggested that the anomalous results for fraction A4 are due to anhydride formation¹⁰ during drying. Attempts to form an osazone were also unsuccessful. Electroionophoresis gave no appreciable mobility.

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