CLXXIX.—*The Structure of Carbohydrates and their Optical Rotatory Power. Part VI.* 4-*Glucosido-mannose and its Methylated Derivatives.*

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LEST it should be thought that there might be some substance in the suggestion that the methylation of mannose derivatives, under the conditions which we have applied, is accompanied by isomeric change, we have continued our work on the above biose, which was selected by Hudson (*J. Amer. Chem. Soc.*, 1930, **52**, 1680, 1707) for his critical test of the validity of his arguments from optical superposition.

We have already shown (J., 1930, 2636) that the glycoside of this biose, namely, 4-glucosido-α-methylmannoside, yields α-methylmannopyranoside, $[\alpha]_D$ 79°, on hydrolytic cleavage by emulsin, a result which establishes the claim that our earlier views on the structure of the ordinary form of α-methylmannoside were valid. We have also shown that the optical rotation of the glycoside of the biose shows the same irregularities when compared with its diglucose analogue as does ordinary α-methylmannoside when compared with α-methylglucoside. Consequently there can be no validity in the suggestion that this irregularity in optical rotation has any structural meaning. We have considered, however, that, although it may seem unnecessary to pursue the argument further, yet it might promote a reconciliation to our point of view if it could be shown that the same structural rigidity which we have claimed for α-methylmannoside undergoing methylation treatment

is fully borne out by the behaviour on methylation of 4-glucosidomannose or its derivatives.

For this reason we have oxidised 4-glucosidomannose to 4-*glucosidomannonic acid*, a change which opens the pyranose ring and leaves the point of junction of the mannose residue with glucose entire. This compound yielded on methylation a crystalline methylated bionic ester, *methyl octamethyl-4-glucosidomannonate*. That the 4-position remained intact, after methylation, at the point of junction of the two C_6 residues, was shown by the isolation, after hydrolysis of the above ester, of crystalline 2 : 3 : 5 : 6-tetramethyl γ-mannonolactone and 2 : 3 : 4 : 6-tetramethyl glucopyranose. This result is in every way parallel to that derived from the similar investigation of cellobiose and cellobionic acid, which are the " epimerides " of 4-glucosidomannose and its corresponding bionic acid (Haworth, Long, and Plant, J., 1927, 2809). Neither in respect of a shift in the position of the biose linking, nor in the character of the ring structure of the non-reducing glucose residue, was any irregularity or isomeric change observed, inasmuch as the glucopyranose structure of the non-reducing residue remains also unimpaired as shown by the isolation of 2 : 3 : 4 : 6-tetramethyl glucopyranose.

This latter result is confirmed also by the methylation of the glycoside of the biose, namely, 4-glucosido-α-methylmannoside. This gave rise quite normally to *heptamethyl 4-glucosido-α-methylmannoside*, which underwent hydrolytic cleavage with dilute hydrochloric acid to give, again, 2 : 3 : 4 : 6-tetramethyl glucopyranose, and also a 2 : 3 : 6-trimethyl mannose which was characterised as its crystalline anilide.

A circumstance well worth noting is that the fully methylated biose shows a wide variation of specific rotation, from + 28° to + 74°, in the six solvents selected for a comparison of this physical " constant." These variations are even more marked than those recorded for tetramethyl α-methylmannoside (Part IV, J., 1930, 2654). The inference can only be drawn that it would be imprudent to attach any structural significance to so variable a property.

From a specimen of incompletely methylated 4-glucosidomannonic acid, a crystalline *trimethyl γ-mannonolactone* was isolated.

EXPERIMENTAL.

Methylation of 4-β-Glucosido-α-methylmannoside.—A solution of 4-β-glucosido-α-methylmannoside (Haworth, Hirst, Streight, Thomas, and Webb, *loc. cit.*) (7·0 g.) in water (40 c.c.) was methylated in the usual way with methyl sulphate (50 c.c.) and 30% aqueous sodium hydroxide (125 c.c.). The product was dissolved in acetone

(25 c.c.) and water (25 c.c.) and remethylated at 50—55° with methyl sulphate (25 c.c.) and 30% aqueous sodium hydroxide (63 c.c.). After a third methylation under similar conditions *heptamethyl 4-β-glucosido-α-methylmannoside* was obtained after distillation under diminished pressure as a viscid uncrystallisable syrup (7·2 g.), b. p. 177—180°/0·01 mm. (bath temperature), $n_D^{21°}$ 1·4627, $[\alpha]_D^{21°}$ + 28° in water (*c*, 1·67), + 46° in chloroform (*c*, 1·37), + 50·5° in benzene (*c*, 1·45), + 52° in ethyl alcohol (*c*, 1·78), + 74° in ether (*c*, 1·87), + 60° in acetone (*c*, 1·0) (Found: C, 52·95; H, 8·6; OMe, 53·4. $C_{20}H_{38}O_{11}$ requires C, 52·9; H, 8·4; OMe, 54·6%).

Hydrolysis of Heptamethyl 4-β-Glucosido-α-methylmannoside.—Hydrolysis of the methylated bioside (6·8 g.) was complete after about 7 hours at 95° in 5% aqueous hydrochloric acid (100 c.c.). During the reaction the specific rotation increased regularly from the value $[\alpha]_D^{21°}$ + 28° to + 49°. $[\alpha]_D^{21°}$ + 33·5° (1 hr.); + 38° (2 hrs.); + 42·5° (3 hrs.); + 45° (4 hrs.); + 49° (7 hrs., constant value). The solution was neutralised with barium carbonate and extracted ten times with chloroform. The chloroform extract (1500 c.c.) gave on evaporation under diminished pressure a pale yellow syrup (4·0 g.), which was boiled with light petroleum (b. p. 80—100°). The light petroleum was separated by decantation from the undissolved syrup and on cooling deposited tetramethyl glucopyranose as long needles, m. p. 86°, not depressed on admixture with an authentic sample; $[\alpha]_D^{21°}$ + 83°, equilibrium value in water (*c*, 1·3). The yield of crystalline tetramethyl glucopyranose was 70% of the theoretical.

The neutral aqueous solution was evaporated to dryness at 40° under diminished pressure, the last traces of water being removed by the addition and subsequent evaporation of alcohol. The dry solid was digested with boiling chloroform and on removal of the solvent 2 : 3 : 6-trimethyl mannose (2·5 g.) was obtained as a viscid syrup which failed to crystallise (Found: OMe, 37. $C_9H_{18}O_6$ requires OMe, 41·9%). When heated for 3 hours at 80° with aniline (0·1 c.c.) in dry benzene (1 c.c.), the substance (0·1 g.) gave a crystalline anilide. The benzene and aniline were removed by heating at 50—60°/0·02 mm. The resulting yellow syrup crystallised when triturated with dry ether. Recrystallisation from dry ether gave long needles of *2 : 3 : 6-trimethyl mannose anilide*, m. p. 127—128° (Found: C, 60·5; H, 7·9; N, 4·9; OMe, 31·2. $C_{15}H_{23}O_5N$ requires C, 60·6; H, 7·8; N, 4·7; OMe, 31·3%). A mixed m. p. determination with the anilide of the trimethyl mannose obtained from heptamethyl 4-β-galactosido-α-methylmannoside showed no depression. The anilide is decomposed by water and its preparation can be effected successfully only when dry reagents are used.

Methylation of 2 : 3 : 6-trimethyl mannose by silver oxide and methyl iodide gave tetramethyl (αβ)-methylmannoside as a colourless liquid, b. p. 95°/0·02 mm., $n_D^{12°}$ 1·4492, from which tetramethyl β-methylmannoside, m. p. 35—36°, separated on cooling in the refrigerator. The liquid portion was hydrolysed with 6% aqueous hydrochloric acid at 95° and gave tetramethyl mannopyranose, recognised as its characteristic anilide, m. p. 143°.

When oxidised by bromine in aqueous solution for 44 hours at 40°, 2 : 3 : 6-trimethyl mannose (1·9 g.) gave 2 : 3 : 6-trimethyl mannonic acid, which was isolated by the usual method as its lactone (1·8 g.), $[\alpha]_D^{20°}$ + 66° (initial value in water). This was a viscid syrup which gave, on further methylation with methyl iodide and silver oxide, tetramethyl γ-mannonolactone, m. p. 108—109°, alone or when mixed with an authentic sample (Goodyear and Haworth, J., 1927, 3136).

Methylation of 4-β-*Glucosidomannonic Acid.*—Pure 4-β-glucosido-α-mannose (9 g.), conforming to the standards previously recorded (*loc. cit.*), was oxidised by bromine in aqueous solution in the presence of barium benzoate (Hudson and Isbell, *J. Amer. Chem. Soc.*, 1929, **51**, 2225). The resulting 4-β-glucosidomannonic acid was isolated as the *calcium* salt, obtained as an amorphous hygroscopic powder (9·6 g.) when a concentrated aqueous solution was poured into alcohol (Found: Ca, 5·4. $C_{24}H_{42}O_{24}Ca$ requires Ca, 5·3%). The calcium salt (9·2 g.) was dissolved in water (40 c.c.) and acetone was added until a slight permanent turbidity appeared. Methylation was then carried out in the usual way by methyl sulphate (80 c.c.) and 30% aqueous sodium hydroxide (200 c.c.), the temperature being kept at 55—60° except at the beginning of the reaction when it was 35°. The mixture was cooled to 0° and acidified with sulphuric acid. The solid which had separated (sodium sulphate) was removed by filtration and extracted with boiling alcohol, the solution being rendered alkaline in order to prevent hydrolysis. The alkaline alcoholic extracts were evaporated to dryness, giving a white solid (A). The aqueous portion was extracted with chloroform (B), made alkaline, and evaporated to small bulk under diminished pressure. This material, the solid (A) and the syrup obtained from the chloroform extract were united and re-methylated under conditions similar to those of the first methylation. After a third treatment most of the product was obtained in the chloroform extract (B) (total yield, 84%). The methylated 4-β-glucosidomannonic acid (9·6 g.) was now esterified by heating it with methyl iodide (20 c.c.) and silver oxide (25 g.) for 6 hours at 45—50°. After two methylations under these conditions a pale yellow, viscid syrup (8·9 g.) was obtained which gave on distillation

a main fraction (7·8 g.), b. p. about 185—190°/0·02 mm. (bath temperature), $n_D^{20°}$ 1·4620, $[\alpha]_D^{16°}$ + 8° in water (*c*, 1·3) (Found: C, 51·9; H, 8·4; OMe, 54·4%). No material with b. p. below 155° was obtained and it follows that no hydrolysis had taken place during the methylation. The distilled material was a liquid which crystallised slowly and incompletely when kept at 50—55° for several days. The mixture was triturated with ether and filtered. To the filtrate more ether was added and then light petroleum until turbidity appeared. A further quantity of crystalline material separated after several hours. Recrystallisation from ethyl acetate–light petroleum gave *methyl octamethyl* 4-β-*glucosidomannonate* as square plates (1·2 g.), m. p. 118°, $[\alpha]_D^{21°}$ − 11° in water (*c*, 1·6), − 19·5° in chloroform (*c*, 1·2), − 3° in benzene (*c*, 1·3), − 11° in ether (*c*, 1·3) (Found: C, 52·2; H, 8·5; OMe, 57·4; *M*, cryoscopic in camphor, 480. $C_{21}H_{40}O_{12}$ requires C, 52·1; H, 8·3; OMe, 57·6%; *M*, 484). (For the subsequent treatment of this product, see the next page.)

The uncrystallisable portion of the distillate was incompletely methylated (compare the low methoxyl content of the mixed solid and liquid) and probably contained heptamethyl 4-β-glucosido-mannonolactone. During hydrolysis of the syrup (4·5 g.) with 5% hydrochloric acid at 95—100° the rotation changed in the course of 10 hours from $[\alpha]_D^{21°}$ + 12° to + 70°. The solution was neutralised with barium carbonate and extracted with chloroform. The chloroform contained tetramethyl glucopyranose and a trimethyl mannonolactone, which were separated by shaking the solution with 3*N*-aqueous sodium carbonate. The tetramethyl glucopyranose (yield, 85%) was obtained after recrystallisation from light petroleum in characteristic needles, m. p. 86°, $[\alpha]_D^{20°}$ + 83° (equilibrium value in water). The *trimethyl mannonolactone* (1·0 g.) was isolated by the method described in the following section and was recrystallised from ether, giving microscopic rods, m. p. 89° (Found: C, 49·1; H, 7·3; OMe, 42·3. $C_9H_{16}O_6$ requires C, 49·1; H, 7·3; OMe, 42·5%). In aqueous solution its behaviour resembled that of tetramethyl γ-mannonolactone. $[\alpha]_D^{20°}$ + 73° (initial value; *c*, 0·9); 72·6° (1 day); 69·5° (2 days); 67° (6 days, constant value). When the lactone was methylated with silver oxide and methyl iodide, tetramethyl γ-mannonolactone was obtained in good yield; it was identified by m. p. and mixed m. p., 108°.

The neutral aqueous portion from the hydrolysis contained the barium salt of a methylated hexonic acid. This was converted into the lactone in the usual way and it was sought to effect purification by distillation. Considerable decomposition took place, however, and no crystalline material could be isolated from the distillate,

which failed to yield a crystalline derivative when heated with phenylhydrazine. In particular, tetramethyl γ-mannonolactone appeared to be absent.

Hydrolysis of Methyl Octamethyl 4-β-*Glucosidomannonate.*—The hydrolysis of the crystalline methylated bionic ester (1·1 g.) at 95—100° with 6% hydrochloric acid was followed polarimetrically. $[\alpha]_D^{20°}$ — 11° initial value (*c*, 2·6); + 2° (30 mins.); 20° (75 mins.); 36° (135 mins.); 45° (215 mins.); 51° (250 mins.); 52° (400 mins., constant value). The solution after neutralisation with barium carbonate and extraction with chloroform was acidified with hydrochloric acid and evaporated to dryness under diminished pressure. The solid which remained was extracted with boiling ether and on removal of the ether tetramethyl γ-mannonolactone (0·25 g.) was obtained, m. p. 108° (after recrystallisation from light petroleum). A mixed m. p. determination showed no depression.

The chloroform extract, concentrated to 10 c.c., was shaken 6 times with 3*N*-sodium carbonate in order to remove tetramethyl γ-mannonolactone. After acidification the sodium carbonate solution was evaporated to dryness and the lactone was extracted with ether. M. p. 108°, after recrystallisation from light petroleum (compare Goodyear and Haworth, *loc. cit.*). The phenylhydrazide, prepared from the lactone in the usual way, had m. p. 167°, alone or in admixture with an authentic sample of the phenylhydrazide of 2 : 3 : 5 : 6-tetramethyl mannonic acid.

The chloroform now contained only tetramethyl glucopyranose (0·55 g.), which crystallised on removal of the solvent and gave, on recrystallisation from light petroleum (b. p. 80—100°), characteristic long needles, m. p. 86°, $[\alpha]_D^{20°}$ + 83°, equilibrium value in water (*c*, 1·03). A mixed m. p. determination gave no depression.

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