CCCL.—*The Structure of Carbohydrates and their Optical Rotatory Power. Part III. 4-Galactosido-α-mannose and its Derivatives.*

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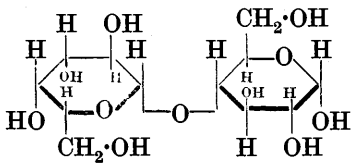
In view of the introduction in Part I it is necessary to mention here only the general significance of the present experimental results. They furnish a proof of the presence of the ordinary α-mannoside residue in derivatives of 4-galactosido-α-mannose and demonstrate that the 1 : 4-ring structure cannot apply to the normal α-methyl-mannoside ($[\alpha]_D$ + 79°). These results are diametrically opposed to the contention of C. S. Hudson.

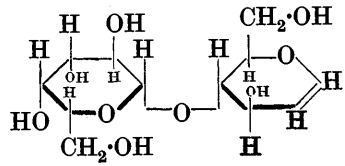
We have now isolated the α-form of 4-galactosidomannose by the adoption of methods similar to those used by Bergmann (*Annalen*,

1923, **434**, 79) for the preparation of the corresponding β-isomeride. Lactose (I) was converted through its acetobromo-derivative into lactal (II), and this by the action of perbenzoic acid in the presence of water gave 4-galactosidomannose. The α-form (III) of this biose was obtained crystalline and its physical properties and behaviour were studied.

The rotation of the α-form in water is in striking disagreement with the value required by Hudson's scheme of sugar structures. In aqueous solution the velocity of mutarotation is similar to that of α-mannose and the equilibrium value, + 27°, is slightly lower than that (30°) recorded by Bergmann. In this respect we can confirm the observations of Hudson and Waters (*J. Amer. Chem. Soc.*, 1930, **52**, 3472), who consider that the equilibrium value given by Bergmann is too high.



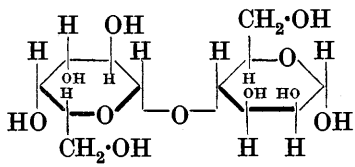
(I.) Lactose



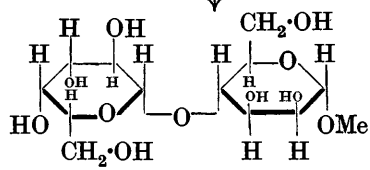
Lactal (II.)

Perbenzoic acid : water

Perbenzoic acid and methyl alcohol

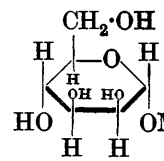


(III.) 4-Galactosido-α-mannose



4-Galactosido-α-methylmannoside (IV.)

Emulsin



and Galactose

α-Methylmannoside
(normal, $[\alpha]_D$ +79°)

We have estimated the rotation of 4-galactosido-β-mannose by the solubility method of Hudson and Yanowski : the value obtained (16°) differs but slightly from that (*ca.* 21°) given by Bergmann.

The difference between the molecular rotations of the α- and the β-form of 4-galactosidomannose is approximately 7000 units and corresponds exactly with those of the two forms of mannose (8000) and of 4-glucosidomannose (7000) but is far removed from the value (17,000) required by Hudson's scheme based on the principle of optical superposition. Indeed the optical properties of the two forms of the biose are closely parallel to those of the two varieties of ordinary mannose. The rotational difference (10,000) between 4-galactosido-α-mannose and 4-galactosido-α-methylmannoside is exactly the same as that between α-mannose and α-methylmannoside or between α-glucose and α-methylglucoside. Furthermore, the rotational difference (6500) between β-cellobiose and β-lactose is found also between 4-glucosido-α-mannose and 4-galactosido-α-mannose and between 4-glucosido-β-mannose and 4-galactosido-β-mannose.

When lactal was brought into contact with perbenzoic acid in methyl alcohol the corresponding α-glycoside of the biose, 4-galactosido-α-methylmannoside, was isolated in good yield. This crystalline substance showed an optical rotation value differing widely from that which was calculated on Hudson's scheme, wherein the mannosido-residue is considered to be that of an unknown α-methylmannoside having $[\alpha]_D + 125°$. It is evident that, the biose link being situated at position 4 of the reducing hexose unit, the 1 : 4 ring structure cannot apply to this mannosido-residue. If it should be the case, therefore, that enzyme cleavage of 4-galactosido-α-methylmannoside gives rise to the normal α-methylmannoside ($[\alpha]_D + 79°$) and not to the hypothetical variety (calc. $[\alpha]_D + 125°$), it should become evident that the known variety $[\alpha]_D + 79°$ cannot possess the 1 : 4-ring structure.

We have carried out this crucial experiment by submitting 4-galactosido-α-methylmannoside to the action of emulsin and have isolated the ordinary form of α-methylmannoside ($[\alpha]_D + 79°$), along with galactose, as the hydrolysis products. We had previously assured ourselves that this form of α-methylmannoside was unaffected by emulsin, and the recently isolated, new variety of α-methylmannofuranoside ($[\alpha]_D + 113°$) (Haworth and Porter, this vol., p. 649; Haworth, Hirst, and Webb, *ibid.*, p. 651) was similarly shown to undergo no change in the presence of this enzyme.

The above 4-galactosido-α-methylmannoside exhibits towards *N*/100-hydrochloric acid at 95° a behaviour which is in every way similar to that of normal α-methylmannoside and widely different

from that of the new α-methylmannofuranoside or of any known variety of a γ-glycoside.

EXPERIMENTAL.

Preparation of Lactal.—(a) Lactose octa-acetate was obtained by heating lactose (100 g.) at 100° for 2 hours with acetic anhydride (400 c.c.) and fused sodium acetate. The mixture was poured into water, and the gummy precipitate washed several times with hot water. A white granular mixture of the α- and the β-form of lactose octa-acetate was soon obtained. It was used without further purification.

(b) Hepta-acetyl lactosidyl bromide (Fischer and Fischer, *Ber.*, 1910, **43**, 2530; Hudson, *J. Amer. Chem. Soc.*, 1925, **47**, 2054) was prepared by acting upon dry lactose octa-acetate (40 g.), dissolved in acetic acid (50 c.c.), with a saturated solution of hydrogen bromide in glacial acetic acid (100 c.c.). After $1\frac{3}{4}$ hours at 18° chloroform was added (100 c.c.) and the solution was poured into ice-water (1200 c.c.). The chloroform layer was washed with water, and then with aqueous sodium bicarbonate until neutral, and was dried over magnesium sulphate for 2 hours. Crystallisation of hepta-acetyl lactosidyl bromide took place after the cautious addition of light petroleum (b. p. 40—60°) to the chloroform solution (yield, 75%); m. p. 137° (decomp.), $[\alpha]_D^{25°}$ + 108° in chloroform.

Unless the exact amount of light petroleum was added with great caution, the product was usually obtained as a white amorphous powder, m. p. about 120°, $[\alpha]_D^{15°}$ + 110° in chloroform. This was stable and, contrary to the suggestions of Fröschl and Zellner (*Monatsh.*, 1930, **55**, 32), appeared to be pure hepta-acetyl lactosidyl bromide; it gave hexa-acetyl lactal on reduction.

(c) Hexa-acetyl lactal was obtained from hepta-acetyl lactosidyl bromide (100 g.) by a modification of Bergmann's method (*Annalen*, 1923, **434**, 86), the reduction being carried out in 50% aqueous acetic acid (1000 c.c.) with careful control of temperature (0—5°). The mixture of zinc dust (200 g.) and solution was mechanically stirred during the 2 hours required for the reduction. After filtration water was added until a slight turbidity appeared. Addition of a nucleus at this stage led to the rapid crystallisation of hexa-acetyl lactal. Usually the material obtained was pure and only on rare occasions was recrystallisation necessary. M. p. 114°, $[\alpha]_D^{20°}$ — 18° in chloroform (*c*, 1·0). This value we regard as more probable than that (— 8°) recorded by Bergmann for hexa-acetyl lactal (Found: C, 51·6; H, 5·8. Calc. for $C_{24}H_{32}O_{15}$: C, 51·4; H, 5·8%).

Deacetylation was carried out by ammonia in methyl alcohol. A solution of hexa-acetyl lactal in eight times its weight of

methyl alcohol was saturated at 0° with dry ammonia and left at room temperature for 12—15 hours. The ammonia and methyl alcohol were then evaporated under diminished pressure at 45°, the distillation being interrupted from time to time in order that the lactal which separated might be removed by filtration. This product was washed with methyl alcohol, triturated three times with warm ethyl acetate to remove traces of acetamide, and dried at 90° (yield, 88%).

The lactal so obtained was crystalline and had m. p. 191—192°, $[\alpha]_D^{23°}$ + 27·5° in water (*c*, 1·6) in agreement with the constants recorded by Bergmann. Addition of bromine took place, corresponding exactly to the presence of one double bond. All samples of lactal used in the following experiments had the above constants. A typical specimen was analysed (Found: C, 46·6; H, 6·8. Calc. for $C_{12}H_{20}O_9$: C, 46·7; H, 6·5%).

4-*Galactosido*-α-*methylmannoside*.—Preliminary experiments showed that methyl alcohol was unaffected by perbenzoic acid in ether. Lactal (10 g.) was shaken for 2 hours with dry methyl alcohol and a 15% solution of perbenzoic acid (8 g.) in dry ether, prepared as described on p. 2639. During the first half-hour most of the lactal dissolved and much heat was evolved. Excessive rise of temperature was avoided by surrounding the bottle in the shaking-machine with ice, and as the reaction proceeded a dense white crystalline precipitate was formed. This was filtered off, washed with methyl alcohol, extracted three times with boiling ether and then with chloroform to remove benzoic acid and perbenzoic acid, and dried in the steam-oven. An estimation by Willstätter's hypoiodite method showed that the product (7 g.) had a reducing power corresponding to the presence of about 5% of 4-galactosido-mannose.

Almost the whole of the reducing material was removed by extraction with boiling ethyl alcohol, and recrystallisation from methyl alcohol or from acetone–water then gave pure *4-galactosido-α-methylmannoside* as short rods, m. p. 207°, $[\alpha]_D^{19°}$ + 66° in water (*c*, 0·7). The crystallisation from methyl alcohol was repeated several times, but produced no change in m. p. or rotation; crystallisation from acetone–water was equally without effect (Found: C, 43·6; H, 7·1; OMe, 8·0. $C_{13}H_{24}O_{11}$ requires C, 43·8; H, 6·8; OMe, 8·7%).

4-Galactosido-α-methylmannoside underwent acetylation readily when it was heated for a few minutes with acetic anhydride and sodium acetate and also when it was kept for 2 days in the cold with pyridine and acetic anhydride. In the former case the reaction mixture was poured into water, and the product extracted with

chloroform. In the latter, chloroform was added at the end of 2 days, the mixture poured into water, and the pyridine removed from the chloroform layer by shaking with dilute sulphuric acid and then with water. Evaporation of the dry chloroform solution left a syrup (yield, quantitative), from which *hepta-acetyl 4-galactosido-α-methylmannoside* was obtained by the addition of light petroleum to a concentrated chloroform solution. The syrup which was precipitated was separated from the supernatant liquor and rubbed with a glass rod. It gradually hardened and after complete removal of the solvent it became a white amorphous friable powder, $[\alpha]_D^{18°}$ + 36° in chloroform (*c*, 1·5); $[\alpha]_D^{28°}$ + 19° in benzene (*c*, 1·6). Attempts to crystallise the hepta-acetate were unsuccessful (Found : $CH_3{\cdot}CO$, 46·0; OMe, 3·7. $C_{27}H_{38}O_{18}$ requires $CH_3{\cdot}CO$, 46·3; OMe, 4·8%). On de-acetylation with ammonia in methyl alcohol, hepta-acetyl 4-galactosido-α-methylmannoside gave quantitatively 4-galactosido-α-methylmannoside, identical with the material described above.

Control Experiments on the Separation of Galactose and α-Methylmannopyranoside.—Owing to unfavourable solubilities the separation of galactose from α-methylmannopyranoside by fractional crystallisation was found to be impracticable. The separation of an artificial mixture of these substances was readily achieved, however, by the following method. Lead acetate (10 g.), dissolved in water (30 c.c.) containing a few drops of aqueous ammonia (*d* 0·880), was slowly added to a solution of galactose (1 g.) and α-methylmannopyranoside (1 g.) in water (25 c.c.) until formation of the dense white precipitate ceased. The solution was filtered, the precipitate washed with dilute lead acetate solution, and the filtrate tested with lead acetate and ammonia. If no precipitation occurred, the lead was removed by means of hydrogen sulphide and the clear colourless solution was evaporated to dryness under diminished pressure. The syrup which remained was dark in colour owing to the presence of colloidal sulphides. It was dissolved in alcohol, treated with charcoal, and again evaporated to dryness. The ammonium acetate was removed either by repeated extraction with ether or by heating in a high vacuum at 100° for several hours. The syrup was then dissolved in a little hot ethyl alcohol and, on cooling, α-methylmannopyranoside, m. p. 189—190°, was obtained in a yield of about 30%.

An exactly similar procedure was employed for the separation of galactose and α-methylmannofuranoside. Both methods were used for the removal of ammonium acetate. The final crystallisations in this case were from methyl alcohol containing ether and the yields were somewhat higher (40%). The recovered material

had m. p. 119°, alone or when mixed with an authentic sample. No conversion into α-methylmannopyranoside took place and the α-methylmannofuranoside was unaffected by the several hours' heating at 100° during the removal of ammonium acetate.

Hydrolysis of 4-Galactosido-α-methylmannoside by Emulsin.—The following control experiments were made in the first place.

(a) α-Methylmannopyranoside (1 g.) in 5% aqueous solution was kept for 48 hours at 37° under toluene in contact with a freshly prepared emulsin preparation (1 g.) (Josephson, *Z. physiol. Chem.*, 1925, **147**, 14). The emulsin was added in the form of a cream prepared by grinding the powdered preparation under water. There was no development of reducing power and after addition of alcohol, followed by separation of the solid material in the centrifugal machine, the clear liquid was evaporated to dryness. The residue was dissolved in hot absolute alcohol and, on cooling, unchanged α-methylmannopyranoside, m. p. 189°, was obtained (yield, 60%).

(b) α-Methylmannofuranoside was recovered unchanged after treatment under similar conditions, m. p. and mixed m. p. 118°. Again there was no development of reducing power. In this case the final crystallisation was from methyl alcohol containing ether (yield, 70%). A special search was made for α-methylmannopyranoside, but none was found and therefore no transformation from the furanose to the pyranose form had taken place.

To a solution of 4-galactosido-α-methylmannoside (2·4 g.) in a little water, emulsin (1·2 g.), ground to a paste with water, was added. The volume was made up to 24 c.c. and after the addition of a few c.c. of toluene the mixture was placed in a thermostat at 37°. To serve as a control for the rate of hydrolysis, a similar 10% solution of lactose was made up with the same weight of emulsin and left in the thermostat. Aliquot portions were taken from this solution from time to time and, after dilution, titrated against Fehling's solution. At the end of 7 days the reducing power of both solutions corresponded to 60% hydrolysis. The solid material was then separated in a centrifuge and washed with a little water and the washings were added to the liquid portion. Fresh emulsin (1 g.) was then added to each solution. After a further 2 days the reducing power of each solution indicated almost complete hydrolysis (95%).

The solid material of the main experiment was then separated in the centrifuge and washed as before. The united aqueous portions were treated with lead acetate (5 g.) dissolved in water (10 c.c.). A precipitation of protein matter occurred immediately and on the further addition of 10 drops of aqueous ammonia (*d* 0·880) a dense white precipitate containing galactose was thrown down. After

filtration the liquid was tested with lead acetate and ammonia but gave no precipitate. The residue on the filter was washed with dilute lead acetate solution. The lead was then removed from the combined filtrates by precipitation with hydrogen sulphide. The aqueous solution was evaporated to dryness under diminished pressure, leaving a brown viscid syrup; this was dissolved in alcohol and decolorised with charcoal, and the alcohol removed by distillation. The syrup which remained was extracted seven times with boiling ether to remove ammonium acetate and then dissolved in alcohol. The volume of solution was reduced to 3 c.c. by evaporation and, on cooling, α-methylmannopyranoside (0·11 g.) crystallised, m. p. 189—190° alone or in admixture with an authentic sample, $[\alpha]_D^{17°}$ + 79·2° in water (*c*, 0·5) (Found: C, 43·2; H, 7·4; OMe, 15·3. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·3; OMe, 15·95%). A further crop (0·13 g.) of nearly pure α-methylmannopyranoside (m. p. 176°) was obtained by extraction of the solid residues, and 0·05 g. of unchanged 4-galactosido-α-methylmannoside, m. p. 206—207°, was isolated from the mother-liquors. After allowance was made for the material used for estimations during the hydrolysis and for the 5% which remained unhydrolysed, the amount of α-methylmannoside theoretically obtainable was 1·1 g. Since the yield of pure α-methylmannopyranoside was 17% of this and the method used for the separation of galactose and α-methylmannopyranoside gave yields of about 30% in control experiments, it was clear that the amount of α-methylmannopyranoside formed during the hydrolysis must have been considerably more than 17% of the theoretical.

The identity of the α-methylmannopyranoside obtained after hydrolysis was confirmed by its transformation into tetra-acetyl α-methylmannopyranoside. The substance (0·05 g.) was boiled for 3 minutes with acetic anhydride (0·8 c.c.) containing sodium acetate (0·04 g.). The solution was poured into water and after neutralisation with sodium bicarbonate the product was extracted with chloroform. Evaporation of the chloroform solution left a syrup which was crystallised from dilute alcohol, giving tetra-acetyl α-methylmannopyranoside (0·04 g.), m. p. 63° alone or when mixed with an authentic sample, $[\alpha]_D^{18°}$ + 49·5° in chloroform (*c*, 0·6). In admixture with tetra-acetyl α-methylmannofuranoside the m. p. was depressed by 15—20°.

Hydrolysis of 4-*Galactosido-α-methylmannoside with* N/100-*Hydrochloric Acid.*—When 4-galactosido-α-methylmannoside was heated at 95° in *N*/100-hydrochloric acid, the rotation remained unchanged for 4 hours. After 5 hours the initial value, $[\alpha]_D^{18°}$ + 65°, had increased to 66° and the solution was slightly reducing. Estimation

by Fehling's solution showed that hydrolysis had proceeded to the extent of 10% at most. The slight increase in rotation indicated that hydrolysis had taken place more at the biose linking than at the methylmannosidic group. The rate of hydrolysis was very similar to that of lactose and of a different order from that of α-methylmannofuranoside, which is complete under these conditions within 2½ hours.

4-Galactosido-α-mannose.—A 10% aqueous solution of lactal was shaken for 2 hours (in a bottle surrounded by ice) with an ethereal solution of perbenzoic acid, the weight of per-acid being slightly greater than that of lactal. The mixture was kept over-night at 15° and the aqueous layer was extracted three times with ether and evaporated under diminished pressure until the weight of syrup was three times that of the lactal used. Methyl alcohol (3 vols.) was added, and the mixture warmed. By cooling and inoculation, *4-galactosido-α-mannose* crystallised as stellar aggregates of long needles (yield, 70%). The first crystals were obtained by the addition of ether to an aqueous methyl-alcoholic solution of the sugar. A syrup was precipitated which crystallised slowly when rubbed with a glass rod.

Recrystallisation was effected by dissolving the sugar in its own weight of water and adding methyl alcohol (5 vols.). After inoculation, deposition of the characteristic tufts of needles soon commenced. This material contained one molecule of water of crystallisation and had an indefinite m. p., about 150—160°, with previous softening, $[\alpha]_D^{20°}$ + 38° in water (initial value calculated as anhydrous sugar); $[\alpha]_D^{20°}$ + 27° (equilibrium value) (Found: C, 40·2; H, 6·9. $C_{12}H_{22}O_{11},H_2O$ requires C, 40·0; H, 6·7%). Titration with Fehling's solution showed that in reducing power 162 parts of 4-galactosido-α-mannose were equivalent to 100 parts of glucose.

A special study was made of the mutarotation of 4-galactosido-α-mannose. Several successive crystallisations were carried out without alteration of m. p., initial and equilibrium rotation values. The following observations were made during a typical mutarotation: $[\alpha]_D^{18°}$ + 35° in water, *c*, 1·5 (5 mins. after dissolution), 33·5° (7·5 mins.), 33° (10 mins.), 31·5° (15 mins.), 30·5° (20 mins.), 30° (25 mins.), 29·5° (30 mins.), 28·5° (40 mins.), 28° (50 mins.), 27° (80 mins., constant value). The rate of mutarotation was of the same order as that of α-mannose. From these figures the initial rotation was, by extrapolation, 38°.

Rotation of 4-Galactosido-β-mannose.—The rotation of the β-form of the biose was estimated by Hudson and Yanowsky's solubility method (*J. Amer. Chem. Soc.*, 1917, **39**, 1013). In 76% alcohol 4-galactosido-α-mannose had $[\alpha]_D$ + 39° (initial value), + 30·5°

(equilibrium value after mutarotation). A saturated solution of the α-form in water showed α_D + 2·03° and had therefore $c = 5{\cdot}2$. The saturated solution was then shaken with excess of the sugar until the constant rotation α_D + 2·52° was observed. Since the specific rotation at this stage was + 30·5°, the concentration was now 8·27, from which the specific rotation of the β-form of 4-galacto-sido-mannose was calculated to be $[\alpha]_D$ + 16° in 76% alcohol. This value, which holds also for water, is slightly lower than that (about + 21°) recorded by Bergmann for the β-form (compare Waters and Hudson, *loc. cit.*).

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