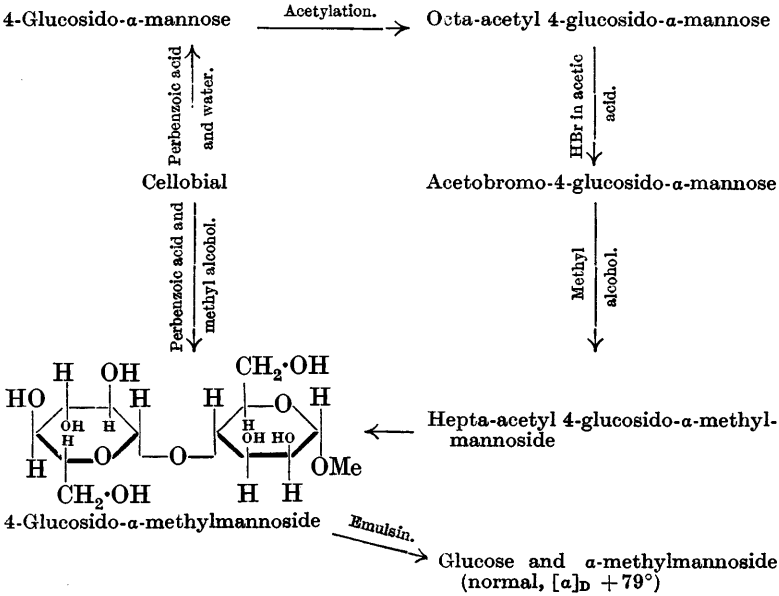
CCCXLIX.—*The Structure of Carbohydrates and their Optical Rotatory Power. Part II. 4-Glucosido-α-mannose and its Derivatives.*

By WALTER NORMAN HAWORTH, EDMUND LANGLEY HIRST, HARVEY RICHARD LYLE STREIGHT, HARRY A. THOMAS, and JOHN IVOR WEBB.

IN Part I (preceding paper) it is explained that Hudson's recent views on the ring structure of α-mannose and its derivatives require the recognition of a hypothetical form of α-mannose (calculated $[\alpha]_D$ + 77°) as a constituent unit in the disaccharide 4-glucosido-α-mannose.

During the course of our experiments, which were conducted on a considerable scale, several modifications in Bergmann and Schotte's method of preparation of this biose and its derivatives (*Ber.*, 1921, **54**, 1564) were introduced. We have prepared for the first time the corresponding bioside, namely, *4-glucosido-α-methylmannoside*, and its *hepta-acetate*. The rotational values of these and of the previously prepared substances in this series have been carefully studied. But the most important feature of this communication is the isolation, by the action of emulsin on 4-glucosido-α-methylmannoside, of the ordinary normal form of α-methylmannoside ($[\alpha]_D$ + 79°) along with glucose.



Not only do the rotational values of these new compounds, and also of the biose itself, conflict with the calculated values derived from Hudson's statistical methods, but the recognition of α-methylmannoside of the normal variety as the mannosidic residue in the bioside is an experimental disproof of his hypothesis regarding the type of α-mannose which occurs in 4-glucosidomannose.

We have obtained the 4-glucosido-α-methylmannoside by two independent methods. (1) This method is exactly analogous to that described by Bergmann and Schotte (*loc. cit.*) for the isolation of the free biose by acting on cellobial with perbenzoic acid in the presence of water. By substituting methyl alcohol for water, the 4-glucosido-α-methylmannoside is obtained in excellent yield. (2) The aceto-bromo-derivative of 4-glucosido-α-mannose (Brauns, *J. Amer. Chem. Soc.*, 1926, **48**, 2776), a substance that is prominently involved in the argument applied by Hudson in the course of his supposed proofs of the structure of sugars, combines with methyl alcohol in the presence of either silver carbonate or quinoline to give *hepta-acetyl 4-glucosido-α-methylmannoside*, identical with the acetate we have prepared by the direct acetylation of the bioside. Deacetylation of the hepta-acetate afforded 4-glucosido-α-methylmannoside, identical in physical constants with the compound prepared by method (1).

By employing Hudson and Yanowsky's solubility method a calculation of the rotation of the corresponding 4-glucosido-β-mannose has been made.

The molecular rotational difference of the α- and β-forms of the biose is similar to that of the α- and β-forms of mannose itself, although it was the supposed anomaly of this difference which led Hudson to ascribe different ring structures to the two mannoses.

It has been shown in Part I that the ordinary form of α-methylmannoside cannot be a furanoside form (1 : 4) but must, in view of the present work, combined with that published in previous papers, be α-methylmannopyranoside (1 : 5-ring) : this is in full agreement with the view which we have consistently held.

EXPERIMENTAL.

Preparation of Cellobiose Octa-acetate.—The method of Haworth and Hirst (J., 1921, **119**, 193) was used with slight modifications which facilitated large-scale experiments. Filter-paper (100 g.), with a moisture content of 6—7%, was cut into small pieces, placed in an enamelled can immersed in a freezing mixture, and incorporated by vigorous stirring with 450 c.c. of an acetolysis mixture prepared by the gradual addition of concentrated sulphuric acid (138 c.c.) to acetic anhydride (1000 c.c.) cooled in a freezing mixture. The mixture was then heated and stirred on a boiling water-bath for

about a minute. The stiff pasty mass liquefied and a vigorous exothermic reaction commenced, which was allowed to continue unaided by external heat until the contents of the can became dark mahogany in colour. The mixture was then quickly poured into cold water (2 litres) and the precipitated cellobiose octa-acetate was isolated and purified (yield, 23 g.) by the method previously described. By this method it was easily possible for two workers to carry out the acetolysis of 2·5 kilograms of cellulose in one day.

Preparation of Cellobial.—Cellobiose octa-acetate was transformed quantitatively into hepta-acetyl cellobiosidyl bromide, m. p. 187—188°, $[\alpha]_D^{22°}$ + 96° in chloroform (*c*, 1·1), by Zemplén's method (*Ber.*, 1928, **61**, 930). Hexa-acetyl cellobial was then prepared by reduction of the latter substance with zinc and acetic acid (Bergmann and Schotte, *loc. cit.*). The following conditions, which must be closely followed, were found to be the most suitable for routine working. Hepta-acetyl cellobiosidyl bromide (30 g.), dissolved in 90% acetic acid (500 c.c.) containing 5 drops of a 0·5% solution of chloroplatinic acid in 50% acetic acid, was stirred for 4 hours with zinc dust (200 g.), the temperature being kept at about 12° and never allowed to rise above 15°. The liquid was then filtered and poured into ten times its volume of ice-water. Crystallisation of hexa-acetyl cellobial soon commenced and was complete in 12 hours. After recrystallisation from ether the substance had m. p. 137°, $[\alpha]_D^{18°}$ — 20° in chloroform (*c*, 1·2). Yield, 70% (Found : C, 51·4; H, 6·1. Calc. for $C_{24}H_{32}O_{15}$: C, 51·4; H, 5·8%).

The de-acetylation of hexa-acetyl cellobial (20 g.) was carried out by a slight modification of Bergmann and Schotte's method (*Ber.*, 1921, **54**, 1564), methyl-alcoholic ammonia (250 c.c.) being used. After remaining for 12 hours at 15°, the solution was concentrated under diminished pressure until crystallisation commenced. The material (7·2 g.) which separated was pure cellobial, m. p. 177°. On addition of ether to the mother-liquor a further quantity (3·5 g.) of cellobial was obtained, m. p. 174°. After one crystallisation from methyl alcohol this was pure.

The cellobial used in the experiments described below had m. p. 177°, and $[\alpha]_D^{17°}$ + 1° in water (*c*, 1·6) (Found : C, 46·7; H, 6·9. Calc. for $C_{12}H_{20}O_9$: C, 46·7; H, 6·5%).

Hexa-acetyl Deoxycellobiose.—If the temperature rose to 30—35° during the reduction of hepta-acetyl cellobiosidyl bromide, *hexa-acetyl 2-deoxycellobiose* was obtained instead of hexa-acetyl cellobial when the filtered solution containing the reduction product was poured into water. The new substance was recrystallised from chloroform–ether, forming long needles, m. p. 196°, $[\alpha]_D^{22°}$ — 15° in chloroform (*c*, 1·5). It reduced boiling Fehling's solution, contained

no bromine, and showed no unsaturation when tested with bromine in chloroform (Found : C, 50·0; H, 6·2. $C_{24}H_{34}O_{16}$ requires C, 49·8; H, 5·9%).

4-Glucosido-α-methylmannoside.—An ethereal solution of perbenzoic acid was prepared from benzoyl peroxide by following the direction of Tiffeneau (" Organic Syntheses," Vol. 8, p. 30), except that the amount of sulphuric acid required is, owing to a misprint, only one-tenth of that given in the text. When the operations were performed quickly with efficient cooling, the yield of perbenzoic acid dissolved in ether was almost quantitative. Decomposition of the per-acid invariably occurred during evaporation of the ethereal solution even under diminished pressure and the concentration of per-acid was always re-estimated after the evaporation and subsequent solution in dry ethyl acetate.

Preliminary experiments showed that perbenzoic acid did not react with methyl alcohol dissolved in ethyl acetate, the amount of per-acid being unchanged after 15 hours.

Cellobial (4 g.), which had been dried at 110° for 7 hours in a vacuum, was shaken for 2 hours at 15° with dry methyl alcohol (40 c.c.) and dry ethyl acetate (25 c.c.) containing perbenzoic acid (3 g.). The clear solution was left over-night in a dry atmosphere and then evaporated to dryness under diminished pressure. The solid which remained was boiled several times with ether to remove benzoic acid and perbenzoic acid. The resulting white powder (4·2 g.) reduced Fehling's solution slightly, but free sugar was present to the extent of less than 1%. Most of this was removed by extraction with boiling ethyl alcohol, and the remaining solid was recrystallised from methyl alcohol, giving *4-glucosido-α-methylmannoside* (3·2 g.) as characteristic roof-shaped crystals, m. p. 227—228°; $[\alpha]_D^{17°}$ + 46° in water (*c*, 1·0). Several successive crystallisations were carried out from methyl alcohol, from ethyl acetate–ether, and also from acetone–water, but no change could be effected in the m. p. or rotation. 4-Glucosido-α-methylmannoside was very soluble in water, soluble with difficulty in methyl alcohol, and almost insoluble in ethyl alcohol (Found : C, 43·7; H, 7·1; OMe, 9·1. $C_{13}H_{24}O_{11}$ requires C, 43·8; H, 6·8; OMe, 8·7%).

Hydrolysis of 4-Glucosido-α-methylmannoside.—(a) *With emulsin.* A solution of 4-glucosidomethylmannoside (2·5 g.) in water (5 c.c.) was mixed with a paste prepared by grinding under water (10 c.c.) a commercial sample of emulsin (1·3 g.) supplied by British Drug Houses Ltd. The total volume was made up to 25 c.c. by the addition of water, a few c.c. of toluene were added to maintain sterile conditions, and the mixture was placed in a thermostat at 37°. Samples of 1 c.c. were withdrawn at intervals and diluted to 50 c.c.

The reducing power was then estimated volumetrically by Fehling's solution with methylene-blue as indicator. At the beginning of the reaction there was no reduction. The reducing power steadily increased until at the end of 10 days it reached a constant value which corresponded to the presence of 0·45 g. of glucose per gram of 4-glucosido-α-methylmannoside submitted to hydrolysis. In another experiment made under similar conditions but with emulsin freshly prepared from bitter almonds (see Josephson, *Z. physiol. Chem.*, 1925, **147**, 14), hydrolysis was complete in less than 48 hours.

The contents of the flask were then diluted with an equal volume of ethyl alcohol. This precipitated some proteins, which were removed by centrifuging for 15 minutes at 4000 r.p.m. The solid was washed with 50% alcohol and again separated on the centrifuge. The combined liquid portions were evaporated to dryness under diminished pressure, giving a syrup (A) which was extracted five times for 30 minutes each with boiling 90% ethyl alcohol (100 c.c.). The united alcoholic extracts were concentrated to 35 c.c. and kept at 15° for several hours; pure α-methylmannoside (0·09 g.), m. p. 189°, $[\alpha]_D^{16°}$ + 79° in water (*c*, 0·6), then crystallised in characteristic columns with pointed ends. Further crops of similar material, m. p. 189°, were obtained on further concentration to 20 c.c. The total yield at this stage was 0·22 g. Renewed concentration gave a strongly reducing syrup, and accordingly the solution was evaporated to dryness, leaving a syrup which was added to the residue from syrup (A) (above). The combined syrups were dissolved in water and fermented with yeast for 15 hours at 37° to destroy the glucose present. An equal volume of ethyl alcohol was added, and the solid material separated in the centrifuge (4000 r.p.m.). The solid was washed with 50% alcohol and again centrifuged. The liquid portions were evaporated to dryness and extracted five times with boiling ethyl alcohol (100 c.c.). On concentration of the alcoholic extracts some protein matter separated. This was allowed to settle over-night and removed by filtration. The removal of alcohol by evaporation was then continued until the volume of solution was 35 c.c. At this stage 0·10 g. of α-methylmannoside, m. p. 188°, crystallised. Further concentration yielded 0·33 g. of slightly impure product, m. p. 170°, from which 0·15 g. of pure α-methylmannoside, m. p. 189—190°, was obtained by recrystallisation. The total yield of α-methylmannoside was therefore 0·47 g. This material was non-reducing. It had the characteristic crystalline form of authentic α-methylmannoside and its physical constants, m. p. 189°, $[\alpha]_D^{18°}$ + 79° in water (*c*, 1·3), were in exact agreement with he recorded values. A mixed m. p. determination with α-methylmannoside, m. p. 189—190°, showed no depression (Found : C, 43·4;

H, 7·1; OMe, 15·8. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·2; OMe, 15·9%).

Finally the whole of the material was recrystallised from ethyl alcohol in good yield without change in m. p. or rotation.

Since 6 c.c. of the original hydrolysis mixture had been withdrawn for reduction tests, the total amount of 4-glucosido-α-methylmannoside available for transformation into α-methylmannopyranoside was 1·9 g. The amount of α-methylmannopyranoside theoretically obtainable was therefore 1·03 g., and 0·47 g. of this quantity was isolated in a pure condition (yield, 46%).

Control experiments showed that α-methylmannofuranoside was unaffected by emulsin under the conditions prescribed above. Details of this work are given in the following paper on 4-galactosido-methylmannoside.

Acetylation of the α-methylmannopyranoside obtained from 4-glucosido-α-methylmannoside gave tetra-acetyl α-methylmannopyranoside. The substance (0·05 g.) was boiled for 2 minutes (see Dale, *J. Amer. Chem. Soc.*, 1924, **46**, 1046) with acetic anhydride (0·8 c.c.) and fused sodium acetate (0·04 g.). The mixture was poured into water (6 c.c.), the acid neutralised with sodium bicarbonate, the product extracted from the neutral solution by chloroform, the chloroform evaporated, and the resulting syrup crystallised from 40% alcohol, giving tetra-acetyl α-methylmannopyranoside, m. p. 63°, $[\alpha]_D^{17°}$ + 50° in chloroform (*c*, 0·5). In admixture with tetra-acetyl α-methylmannofuranoside (m. p. 63°), the m. p. was depressed by 15—20°.

(b) *Hydrolysis with* N/100-*hydrochloric acid.* The rotation of 4-glucosido-α-methylmannoside in *N*/100-hydrochloric acid ($[\alpha]_D^{18°}$ + 46°) remained unchanged after 3 hours at 95°. After 5 hours the value $[\alpha]_D^{18°}$ + 48° was observed and the solution was then slightly reducing. A volumetric estimation with Fehling's solution showed that during 5 hours hydrolysis had proceeded to the extent of 5% at most. The slight increase in rotation indicated that hydrolysis was taking place more at the biose linking than at the methylmannosidic group. The rate of hydrolysis was comparable with that of lactose and altogether different from that of α-methylmannofuranoside, which requires only 2 hours for complete hydrolysis under these conditions.

Hepta-acetyl 4-Glucosido-α-methylmannoside.—A solution of 4-glucosido-α-methylmannoside (1 g.) in pyridine (10 c.c.) and acetic anhydride (8 c.c.) was kept at 16° for 2 days and then poured into ice-water. The colourless crystalline product (1·6 g.) was recrystallised from hot ethyl alcohol (5 c.c.), giving *hepta-acetyl 4-glucosido-α-methylmannoside* as rectangular plates, m. p. 184°, $[\alpha]_D^{18°}$ + 30° in

chloroform (*c*, 1·0); + 36° in acetone (*c*, 1·4); + 21·5° in benzene (*c*, 1·5). Successive crystallisations from alcohol, from acetone–water, and from ethyl acetate–ether failed to alter either the m. p. or the rotation (Found : C, 49·8; H, 6·2; OMe, 5·1. $C_{27}H_{38}O_{18}$ requires C, 49·8; H, 5·9; OMe, 4·8%). The hepta-acetate was prepared also by heating 4-glucosido-α-methylmannoside with acetic anhydride in the presence of sodium acetate (yield, quantitative).

The hepta-acetate (1·7 g.) was de-acetylated by methyl-alcoholic ammonia (25 c.c.). After 15 hours, the ammonia and methyl alcohol were removed under diminished pressure and the acetamide was eliminated by extraction with boiling ethyl acetate. The remaining solid was crystallised from methyl alcohol and also from acetone–water, giving 4-glucosido-α-methylmannoside, m. p. 227—228°, $[\alpha]_D^{17°}$ + 46° in water (*c*, 1·2) (yield, quantitative) (Found : C, 43·8; H, 7·2; OMe, 8·5%).

Rotation of 4-Glucosido-α-mannose.—This substance (compare Bergmann and Schotte, *loc. cit.*; Brauns, *loc. cit.*) was prepared by shaking for 3 hours at 18° a solution of cellobial (5 g.) in water (50 c.c.) with a solution of perbenzoic acid (4 g.) in ether (30 c.c.). The aqueous layer was extracted with ether and concentrated under diminished pressure to a thick syrup. Absolute alcohol was added until the solution was faintly turbid and after some days 4-glucosido-α-mannose crystallised in rectangular prisms with pyramidal ends containing one molecule of water of crystallisation. Further crops were obtained from the mother-liquor (yield, 5 g.). Recrystallisation was easily performed by the addition of a nucleus to a concentrated aqueous solution of the sugar which had been brought almost to the point of turbidity by the addition of alcohol. The sugar was dried in a desiccator containing soda-lime. More vigorous drying agents tended to remove slowly the water of crystallisation. M. p. (monohydrate) 139°, with effervescence. M. p. (anhydrous) 175—176°. $[\alpha]_D^{18°}$ + 20° in water (initial value calculated for anhydrous sugar), $[\alpha]_D^{18°}$ + 12·5° (equilibrium value) (Found : C, 42·2; H, 6·5. Calc. for $C_{12}H_{22}O_{11}$: C, 42·1; H, 6·4%). An estimation of the reducing power by titration with Fehling's solution showed that 1·55 g. of sugar were equivalent to 1 g. of glucose. The rate of hydrolysis in *N*-hydrochloric acid at 95° was near to that of lactose under similar conditions.

Particular attention was paid to the mutarotation of 4-glucosido-α-mannose in water, a sufficient quantity of the sugar being prepared to permit of eight successive crystallisations. At each stage the mutarotation was studied, but from the second onwards no change could be effected in either the initial or the equilibrium value. The following figures refer to a typical experiment in which 0·30 g.

of substance was dissolved in 15 c.c. of water and observed in a 2-dm. tube. The rotation values refer to anhydrous sugar. $[\alpha]_D^{19°}$ + 19·3° (3 mins. from dissolution), 19·1° (5 mins.), 19·0° (6 mins.), 18·6° (10 mins.), 17·8° (16 mins.), 16·9° (27 mins.), 15·8° (37 mins.), 14·8° (60 mins.), 14·0° (76 mins.), 13·4° (106 mins.), 12·9° (150 mins.), 12·5° (equilibrium value). From these figures the initial value was found to be + 20°. The rate of mutarotation was of the same order as that of α-mannose in water, but rather slower.

Rotation of 4-*Glucosido*-β-*mannose.*—The β-form of the free sugar could not be obtained and only indirect methods were available for the determination of its rotation. The solubility method introduced by Hudson and Yanowsky (*J. Amer. Chem. Soc.*, 1917, **39**, 1035) and used successfully by these authors for α-mannose gave the approximate value $[\alpha]_D^{18°}$ − 1° for 4-glucosido-β-mannose. In 72% alcohol the initial value for 4-glucosido-α-mannose was + 21°, and the equilibrium value after mutarotation catalysed by a drop of ammonia was + 8°. A saturated solution of the α-form in 72% alcohol showed α_D + 0·90° and had therefore $c = 2·14$. The saturated solution was then shaken with excess of the sugar, in the presence of a trace of ammonia, until the constant rotation α_D + 0·86° was observed. Since the specific rotation at this stage was $[\alpha]_D$ + 8°, the concentration was now 5·4, from which it followed that at equilibrium the α- and β-forms were present in the ratio 2·14 : 3·3. Hence the value $[\alpha]_D^{18°}$ − 1° was calculated for the β-form in 72% alcohol. It was shown by Hudson and Yanowsky's method that the rotation in water had approximately the same value.

The Reaction between Methyl Alcohol and Hepta-acetyl 3-*Glucosido*-α-*mannosidyl Bromide.*—Octa-acetyl 4-glucosido-α-mannose was prepared by the action of pyridine and acetic anhydride on the sugar (Bergmann and Schotte, *loc. cit.*). Its constants, m. p. 202—203°, $[\alpha]_D$ + 35° in chloroform, were in agreement with those recorded by Brauns (*loc. cit.*) and could not be altered by continued crystallisations (Found: C, 49·7; H, 5·8. Calc. for $C_{28}H_{38}O_{19}$: C, 49·6; H, 5·6%). Attempts to obtain the β-isomeride have up to the present been unsuccessful.

The α-octa-acetate was next converted into hepta-acetyl 4-glucosido-α-mannosidyl bromide by hydrogen bromide in glacial acetic acid. The m. p., 169°, and rotation, $[\alpha]_D^{18°}$ + 77° in chloroform, agreed excellently with the constants recorded by Brauns.

When hepta-acetyl 4-glucosido-α-mannosidyl bromide (1 g.) was boiled for 3 hours with dry methyl alcohol (70 c.c.) in the presence of freshly prepared silver carbonate (1 g.), the bromine was eliminated as silver bromide, and on concentration crystalline hepta-acetyl 4-glucosido-α-methylmannoside (0·15 g.) was obtained. After

recrystallisation from alcohol this had m. p. 184°, alone or mixed with an authentic sample; $[\alpha]_D^{18°}$ + 30° in chloroform (*c*, 2·5). In addition to this crystalline material an intractable, slightly reducing syrup was obtained which failed to give a crystalline β-isomeride, but apparently contained some hepta-acetyl 4-glucosidomannose, since after acetylation it gave a little octa-acetyl 4-glucosido-α-mannose, m. p. 202°.

The reaction between hepta-acetyl 4-glucosido-α-mannosidyl bromide (3·5 g.) and dry methyl alcohol (35 c.c.) was also carried out in the presence of quinoline (4 g.). After 15 hours at 18° the mixture was diluted with chloroform, shaken with very dilute sulphuric acid to remove quinoline and again with aqueous sodium bicarbonate and water, and dried over magnesium sulphate. After evaporation to dryness a brittle amorphous glass was left, which was extracted with hot alcohol. When cold, the alcoholic extract deposited crystals (0·4 g.) of hepta-acetyl 4-glucosido-α-methylmannoside, which after recrystallisation from alcohol had m. p. 184°, alone or in admixture with an authentic sample. $[\alpha]_D^{17°}$ + 30° in chloroform (*c*, 1·0). On de-acetylation this material gave quantitatively 4-glucosido-α-methylmannoside, m. p. 227—228°, $[\alpha]_D^{17°}$ + 46° in water (*c*, 1·5).

The authors gratefully acknowledge the award to one of them (H. R. L. S.) of an 1851 Exhibition Scholarship.

UNIVERSITY OF BIRMINGHAM,
EDGBASTON. [*Received, October 15th*, 1930.]