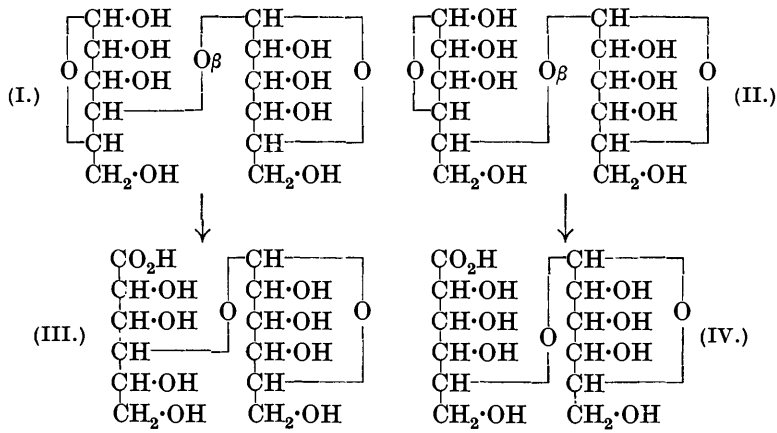
CCCLXXVI.—*The Constitution of the Disaccharides. Part XVI. Cellobiose.*

By WALTER NORMAN HAWORTH, CHARLES WILLIAM LONG, and JOHN HERBERT GEOFFREY PLANT.

A CONSTITUTIONAL study of cellobiose was undertaken in this series (Part V) six years ago (Haworth and Hirst, J., 1921, **119**, 193), and the experimental results were as follows: Methylated cellobiose ⟶ crystalline tetramethyl glucose + crystalline 2 : 3 : 6-trimethyl glucose; and a structural formula was ascribed to the disaccharide on this basis. In common with all carbohydrate formulæ, this constitution was modified by the recognition of the revised formula of glucose in its normal derivatives (Charlton, Haworth, and Peat, J., 1926, 98), and the structure represented by (I) was assigned to cellobiose by the latter authors:

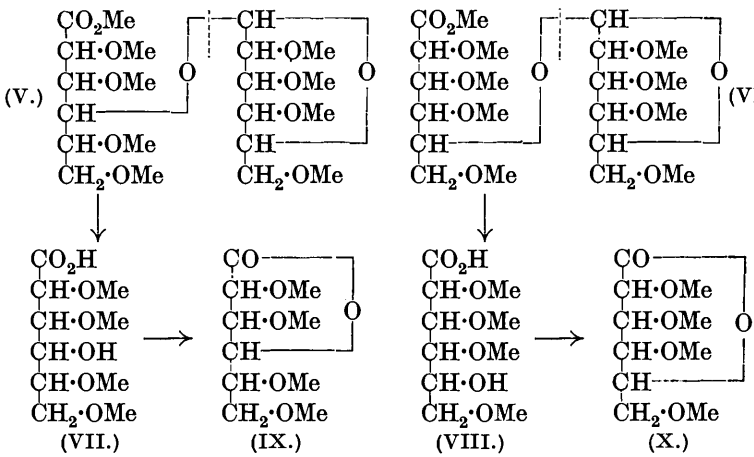


Consideration was given to an alternative formula for cellobiose (II), and it was stated that the isolation of 2 : 3 : 6-trimethyl glucose by cleavage of methylated cellobiose rendered it possible to regard the attachment of the reducing hexose component through position (5) to the other hexose residue, in which case the oxide ring of this first residue would be a butylene or γ-oxide (1 : 4) and not an amylene or δ-oxide (1 : 5). In the former case, cellobiose would be

to the extent of one residue a γ-sugar, but this was stated to be an unlikely contingency. Whilst making this reservation, therefore, the formula (I) for cellobiose was advocated, inasmuch as it correctly represented the properties of the disaccharide and was consistent with all the facts.

Meanwhile, we have instituted a series of experiments designed to eliminate any remaining doubt as to the structure of cellobiose. This disaccharide undergoes oxidation at its reducing position to give a monobasic acid designated cellobionic acid, and this compound can be formed only by opening of the ring of the reducing glucose component. The structure of cellobionic acid will thus be represented by formula (III) if (I) be accepted for the disaccharide, whilst (IV) will represent the acid if (II) be the structure of the disaccharide. It will therefore be seen that, in the two rival formulæ for the acid, the position of the oxygen link connecting the two C_6 chains differs in that in (III) this link engages the carbon in position (4), and in formula (IV) it engages the carbon in position (5).

We have been able to decide between these two alternative formulæ by methylating cellobionic acid completely, and the product, which was *methyl octamethylcellobionate*, was then submitted to hydrolysis. The cleavage products to be expected are seen to be different according as we accept formula (V) or formula (VI) as a correct representation of the methyl octamethylcellobionate. In addition to 2 : 3 : 4 : 6-tetramethyl glucose, the cleavage products



should contain a monobasic acid of the C_6 series which, if formula (V) be correct, would be the γ-hydroxy-acid (VII); or if formula (VI)

be correct, the δ-hydroxy-acid (VIII). Since each of these acids is already known and has been fully characterised by one of us in earlier investigations, a way was opened up enabling us to reach a decision through identification of this essential product. It will be seen that an acid of formula (VII) would give on heating a 1 : 4- or γ-lactone, recognisable as 2 : 3 : 5 : 6-tetramethyl gluconolactone, and similarly an acid of formula (VIII) would yield a 1 : 5- or δ-lactone, identifiable as 2 : 3 : 4 : 6-tetramethyl gluconolactone; and since each of these lactones has been fully characterised and degraded by oxidation methods (Haworth, Hirst, and Miller, this vol., p. 2436), there can be no dubiety in assigning a final constitution to each of them, and therefore to cellobiose itself.

When methyl octamethylcellobionate was hydrolysed with mineral acid, the cleavage products were isolated in an analogous manner to that described in recent papers on maltose (Haworth and Peat, J., 1926, 3094) and lactose (Haworth and Long, this vol., p. 544). Crystalline 2 : 3 : 4 : 6-tetramethyl glucose was isolated (yield, 92·7% of the theoretical), and the acid product originally retained as the barium salt was regenerated and distilled as a lactone which crystallised, m. p. 26—27° (yield, 80% of the theoretical). This compound gave a phenylhydrazide of the corresponding acid, m. p. 135—136°, and the melting point was not depressed in admixture with the phenylhydrazide of 2 : 3 : 5 : 6-tetramethyl gluconic acid which had been prepared from (*a*) methyl octamethyl-maltobionate or methyl octamethyl-lactobionate or (*b*) 2 : 3 : 5 : 6-tetramethyl γ-glucose (1 : 4) (compare Haworth and Long, Haworth and Peat, *locc. cit.*).

Again, the above lactone was shown to be identical with 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone isolated from the sources (*a*) and (*b*). There was, therefore, no doubt that the tetramethyl gluconic acid which had been formed from methyl octamethylcellobionate was that represented by formula (VII), since the lactone referred to is indicated by formula (IX). The results furnish evidence which seems to us conclusive in favour of the formulation (V) for methyl octamethylcellobionate, and therefore, if this be accepted, and it be assumed that no displacement of the biose linking had occurred in any of these transformations, cellobionic acid should be expressed by formula (III) and cellobiose by (I). This experimental result substantiates the constitutional formula which had previously been advocated by one of us and serves to remove the need for any reservation in favour of formula (II).

It should be noted in this connexion that, as indicated by Haworth and Peat (*loc. cit.*), maltose possesses the same structural formula as

cellobiose, the essential difference between these two bioses being that maltose is a glucose-α-glucoside and cellobiose a glucose-β-glucoside.

These experimental results are opposed to the views of Irvine (*Chemical Reviews*, 1927, **4**, 216), who has perceived a difference in the positions of (*a*) the biose linking in maltose and cellobiose and (*b*) the oxide ring in each of these bioses (compare Irvine and Black, J., 1926, 869; Irvine and Macdonald, *ibid.*, 1508; Irvine and Robertson, *ibid.*, 1496).

EXPERIMENTAL.

Cellobiose octa-acetate (m. p. 223—225°), prepared as described by Haworth and Hirst (J., 1921, **119**, 193) from pure cellulose, was hydrolysed by alcoholic potash to give potassium cellobiosate, and a representative sample of the latter was converted into cellobiose having $[\alpha]_D$ +24° (c = 3·33) (equilibrium value) (Maquenne, *Bull. Soc. chim.*, 1904, **31**, 854). The remaining portion of potassium cellobiosate was dissolved in a little water, and bromine was admitted, which speedily dissolved. The solution was kept in a stoppered bottle at room temperature for 10 days; it was then almost devoid of reducing action towards Fehling's solution. The unchanged bromine was removed by aeration, followed by treatment with hydrogen sulphide. The colourless solution was shaken with finely ground litharge, filtered, then agitated in presence of silver oxide in the dark, and filtered. Hydrogen sulphide was again passed into the solution, and the excess of this gas removed by aeration. The clear solution was warmed gently for a day in presence of calcium carbonate, filtered, and evaporated to small bulk. This contained calcium cellobionate along with potassium bromide.

Methyl Octamethylcellobionate.—The methylation of calcium cellobionate was carried out in a similar manner to that described in the case of the corresponding salt of maltobionic acid (Haworth and Peat, *loc. cit.*), methyl sulphate and sodium hydroxide being used initially, followed by several treatments with moist silver oxide and methyl iodide, and finally by dry silver oxide and methyl iodide. The crude product at this stage was a yellow, mobile liquid (7·1 g.; OMe, 54·6%). This distilled under 0·05 mm. from a bath heated to 203°, and the first fraction (1·5 g.) of low boiling point was rejected. The major portion of the distillate (5·1 g.) was a pale yellow, viscid syrup, $n_D^{13°}$ 1·4604 (Found: OMe, 54·3%). The latter was again distilled under 0·05 mm., the first fraction (0·3 g.) being collected on heating the bath to 176°, and the main fraction (4·6 g.) distilled at 169—171°/0·05 mm. from a bath heated

to 192°. This product did not reduce Fehling's solution and had $n_D^{14^\circ}$ 1·4609, and the analytical data agreed with the formula of *methyl octamethylcellobionate* (Found : C, 52·0; H, 8·3; OMe, 54·3. $C_{21}H_{40}O_{12}$ requires C, 52·1; H, 8·3; OMe, 57·6%).

Hydrolysis. The above product (3·1 g.) was dissolved in 100 c.c. of 7% hydrochloric acid, and heated at 80—90° until the specific rotation of the solution appeared to show no further changes. $[\alpha]_D^{19^\circ}$ + 5·4° (initial), + 32·1° (after 1 hour), 50·2° (2 hours), 54·6° (3 hours), 55·0° (4 hours; constant). At this stage finely divided barium carbonate was gradually added, and the solution aerated at 50° to assist the neutralisation. After being kept over-night, the solution was filtered and evaporated at 40°/16 mm. To the white residue, alcohol was added and distilled off under 16 mm. The dry solid was digested with boiling ether many times and the collected extracts were evaporated, leaving a syrup which crystallised in colourless needles. These were purified from light petroleum (weight, 1·5 g.; yield, 92·7%) and identified as 2 : 3 : 4 : 6-tetramethyl glucose by the following properties : m. p. 93—94°; mixed m. p. determination with a specimen prepared from methylated maltose, 93—94°; $[\alpha]_D^{\cdot}$ in water (c = 2·02) + 99·9° (5 mins.), 88·0° (30 mins.), 85·5° (1 hour), 83·0° (2 hours; constant).

The original white residue of barium salts from which the tetramethyl glucose had been extracted was warmed to eliminate traces of ether, dissolved in water, and treated with a slight deficiency of dilute hydrochloric acid. The water was evaporated at 50°/15 mm. and finally the residue was heated at 60—80°/0·09 mm. for 2 hours. Repeated digestion of this dried residue with boiling ether yielded 1·2 g. (80% of the theoretical) of a pale yellow syrup which distilled under 0·09 mm. from a bath heated to 119°, leaving only 0·1 g. of residue.

Nucleation of the cooled distillate with 2 : 3 : 5 : 6-tetramethyl gluconolactone (from methyl octamethyl-lactobionate; Haworth and Long, this vol., p. 547) resulted in immediate crystallisation; m. p. 26—27°, mixed m. p. determination 26—27° (with the specimen from which the nucleus had been taken) (Found : C, 51·2; H, 7·8; OMe, 51·4. Calc. : C, 51·3; H, 7·7; OMe, 53·0%).

Phenylhydrazide of 2 : 3 : 5 : 6-Tetramethyl Gluconic Acid.—A specimen of the above lactone (0·16 g.) in contact with phenylhydrazine (1 mol.) dissolved in ether was heated on a water-bath. Crystals of the phenylhydrazide separated, m. p 135—136° (from benzene), mixed m. p. 135—136° [with specimens prepared from the 2 : 3 : 5 : 6-tetramethyl gluconolactone obtained from methyl octamethylmaltobionate (Haworth and Peat, *loc. cit.*) and from 2 : 3 : 5 : 6-tetramethyl (γ-)glucose (Charlton, Haworth, and Peat, *loc. cit.*)]

(Found : C, 56·1 ; H, 7·65 ; OMe, 35·0 ; N, 8·45. Calc. : C, 56·1 ; H, 7·6 ; OMe, 36·3 ; N, 8·2%).

The authors are indebted to the Department of Scientific and Industrial Research for grants to two of them.

UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

[*Received, October 13th*, 1927.]