LXXXI.—*The Constitution of the Disaccharides. Part XII. Lactose.*

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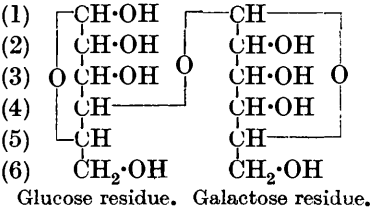
THE constitutional study of lactose (Haworth and Leitch, J., 1918, **113**, 188) showed that from completely methylated lactose the products of hydrolytic cleavage were crystalline 2 : 3 : 6-trimethyl glucose and normal tetramethyl galactose, and a structural formula was applied to lactose on this experimental basis. These results have since been experimentally verified by Irvine and Hirst (J., 1922, **121**, 1213) and by Schlubach and Moog (*Ber.*, 1923, **56**, 1957).

In the subsequent communication in which the evidence for a complete revision of the structure of normal glucose was outlined—a revision which extended also to all carbohydrates containing glucose residues—it was shown that derivatives of normal glucose should be represented by an amylene-oxide ring structure (Charlton, Haworth, and Peat, J., 1926, 89). In the latter paper, therefore, the constitutional formula previously applied to lactose and cellobiose was revised by the introduction of the above emendation. This involved the change from a butylene-oxide structure to the new amylene-oxide formulation in each of the hexose residues, and also a corresponding adjustment in the point of attachment of the biose linking from position 5 to position 4.

(I.)



Glucose residue. Galactose residue.

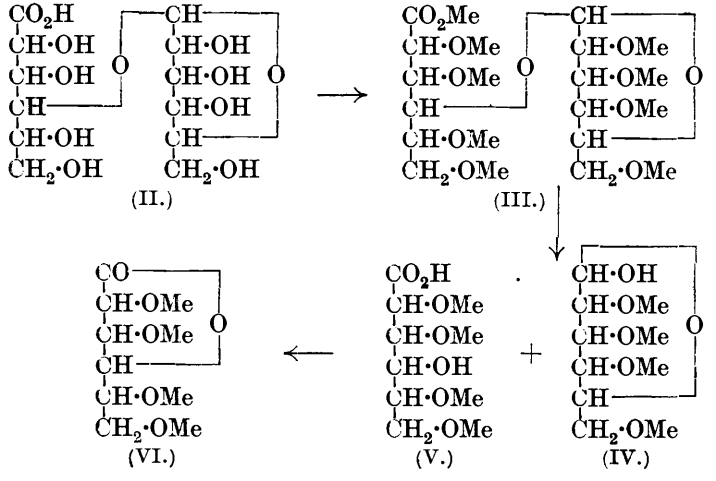
At the same time, it was recognised that the isolation of 2 : 3 : 6-trimethyl glucose as a cleavage fragment of methylated lactose left open the unlikely contingency of the presence of a butylene-oxide ring or γ-sugar residue in the reducing half of the disaccharide, since the positions 4 and 5 were available either for the allocation of an oxide-ring attachment or for the position of the biose linking. The reason for suspending final judgment in regard to this contingency was fully explained. It was pointed out that crystalline 2 : 3 : 6-trimethyl glucose, although itself an amylene-oxide sugar, could conceivably have originated as a labile butylene-oxide form at the moment of its separation from methylated lactose, and had thereafter undergone

isomeric change into the normal amylene-oxide variety which was isolated. It was recognised, however, that lactose did not itself display the behaviour of a labile or γ-sugar, and consequently we preferred the structural formula (I) which was definitely advanced.

In a subsequent paper, Irvine and Black (J., 1926, 862) took occasion to criticise the formulæ we had applied to the reducing disaccharides, including lactose and cellobiose, the constitutions of which they regarded as conjectural. Following this criticism, two papers have since appeared (G. Zemplén, *Ber.*, 1926, **59**, 1254, 2402) describing the constitutional study of lactose and cellobiose by degradation to the hexose-tetroses, and the experimental results entirely confirm the structural formulæ we had applied to lactose and cellobiose. Inasmuch as exception might be taken, however, to the method of Zemplén, whose proof depends upon the non-formation of phenylosazones by the hexose-tetroses, we have continued our work on the constitutional study of lactose, and have adopted a scheme, herein described, which furnishes, in our view, quite definite proof of the position occupied by the biose linking in this disaccharide; and consequently the nature of the oxide ring also is fully established.

In this experimental work, we oxidised lactose to lactobionic acid, the barium salt of which was methylated, thus giving rise to methyl octamethyl-lactobionate. In this compound it will be seen that the oxide-ring in the reducing hexose residue of the parent disaccharide has been eliminated, and the hydroxyl position thus exposed has been protected by a methyl residue, whilst the biose linking has presumably remained intact. Hydrolysis of this methylated lactobionate should give cleavage products which leave no doubt as to the position of the biose linking, since only one free hydroxyl group would be exposed in the hexonic acid residue resulting from the hydrolysis. On digesting the methyl octamethyl-lactobionate with dilute hydrochloric acid, we were able to isolate crystalline 2 : 3 : 4 : 6-tetramethyl galactose (IV), which had already been recognised in the course of previous work, and also crystalline 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone (VI), identical in every respect with the specimen we had recently isolated in the course of a similar investigation on maltose (Haworth and Peat, J., 1926, 3094). This crystalline lactone gave rise to a crystalline phenylhydrazide of the corresponding 2 : 3 : 5 : 6-tetramethyl gluconic acid (V), and consequently the diagnosis of these two products arising from the cleavage of the methylated lactobionic acid enables us to ascribe a constitutional formula (III) to methyl octamethyl-lactobionate and the formula (II) to lactobionic acid. It therefore follows that the constitution (I) which we had already advanced

and supported in the course of our earlier work receives further experimental verification.



EXPERIMENTAL.

Methylation of Lactobionic Acid. Isolation of Methyl Octamethyllactobionate.—Lactobionic acid was prepared by the oxidation of lactose with bromine water, and the procedure described by Fischer and Meyer (*Ber.*, 1889, **22**, 1941) was adopted as a means of obtaining the calcium and the barium salt. An aqueous solution of the latter salt was gradually added to a large volume of alcohol which was well stirred. Barium lactobionate was precipitated as a colourless, micro-crystalline solid which contained 14·0% of barium. Calcium lactobionate, purified by the same method, contained 4·6% of calcium. It would thus appear that each salt is associated with three molecules of ethyl alcohol, and this seems to be true also of calcium maltobionate. The salts were neutral and devoid of action towards Fehling's solution.

Barium lactobionate was methylated in a manner similar to that already mentioned in the case of calcium maltobionate (Haworth and Peat, *loc. cit.*). After eight treatments with the methylating agents, a preliminary distillation at 0·09 mm. pressure showed little evidence of hydrolytic cleavage. Thus from a total distillate of 16·5 g., only 1·7 g. were collected at a bath temperature of 170—180°, and this first fraction showed $n_D^{13°}$ 1·4561. The main distillate (14·8 g.), a pale yellow, viscid liquid, showed b. p. 178—210°/0·09 mm. and $n_D^{13°}$ 1·4625—1·4689. This was subjected to one methylation with moist silver oxide and methyl iodide, followed by two further treatments with the dry reagents, and at this stage the

material was again distilled. A small first fraction (0·5 g.), which distilled at a bath temperature of 160—180° under 0·08 mm. and had $n_D^{13°}$ 1·4612, having been rejected, the main portion was collected at a bath temperature of 180—185° and distilled at 157—164°/0·05 mm. This specimen of methyl octamethyl-lactobionate, which was devoid of action towards Fehling's solution, showed $n_D^{13°}$ 1·4632 (Found: C, 51·8; H, 8·1; OMe, 54·8. Calc. for $C_{21}H_{40}O_{12}$: C, 52·1; H, 8·3; OMe, 57·6%).

Hydrolysis. The above specimen was hydrolysed in contact with 7·0% hydrochloric acid at 80—90° during 6½ hours. Portions of the solution were examined polarimetrically at intervals: $[\alpha]_D$ (initial), + 34·0°; 72·6° (after 1 hour); 74·5° (2 hours); 76·8° (3 hours); 77·6° (4 hours); 77·6° (5½ hours); 77·2° (6½ hours). The hydrolysis was probably complete after 4 hours, and certainly after 6½ hours; no appreciable change had occurred in the appearance of the solution, which now reduced Fehling's solution.

At this stage, the solution was gently heated in contact with barium carbonate until the whole of the acid product was neutralised. The colourless filtrate was evaporated under diminished pressure, and the dried residue was repeatedly extracted with ether until it no longer gave a positive test with Fehling's solution. From this ethereal extract, colourless crystals of tetramethyl galactose were isolated, showing $[\alpha]_D$ + 118·1° in equilibrium in water (yield, 95%). A mixed melting-point determination of this substance with an authentic specimen of crystalline 2 : 3 : 4 : 6-tetramethyl galactose (compare Haworth, Ruell, and Westgarth, J., 1924, **125**, 2473) gave m. p. 71—72°. Schlubach and Moog (*loc. cit.*) gave m. p. 71·5—72° for the sugar they then described as 2 : 3 : 5 : 6-tetramethyl galactose.

The crystalline anilide was prepared (m. p. 190—191°), and this m. p. was not depressed in admixture with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl galactose anilide (Found: C, 61·7; H, 7·9. Calc.: C, 61·7; H, 8·0%).

Separation of 2 : 3 : 5 : 6-Tetramethyl γ-Gluconolactone.—The colourless residue of barium salts was dissolved in the minimum of water and the free organic acid was liberated by the cautious addition of *N*/2-hydrochloric acid. Evaporation at 30°/14 mm., followed by a period of heating at 60°/0·09 mm., gave a dried residue containing barium chloride and the organic lactone, from which the latter was extracted by means of dry ether. Evaporation of the ethereal solution furnished a liquid product which was distilled under 0·03 mm. at a bath temperature of 117—135°. The cooled distillate was nucleated with a specimen of crystalline 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone prepared by oxidation of 2 : 3 : 5 : 6-tetramethyl γ-glucose, and thereafter it crystallised completely

(m. p. 26—28°), and a mixture of this substance with an authentic specimen obtained in the course of recent work on the hydrolysis of methyl octamethylmaltobionate (Haworth and Peat, *loc. cit.*) melted at 26—27·5° (yield, 90% of the theoretical).

The phenylhydrazide was prepared as colourless, silky needles, m. p. 136° (remelted at 135—136°). Also the m. p. of a mixture of this specimen with that prepared by Haworth and Peat (*loc. cit.*) from methyl octamethylmaltobionate showed no depression (Found by micro-analysis : C, 56·35; H, 7·6; OMe, 34·1. Calc. : C, 56·1; H, 7·6; OMe, 36·3%).

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