CCCCXVI.—*The Constitution of the Disaccharides. Part XI. Maltose.*

By Walter Norman Haworth and Stanley Peat.

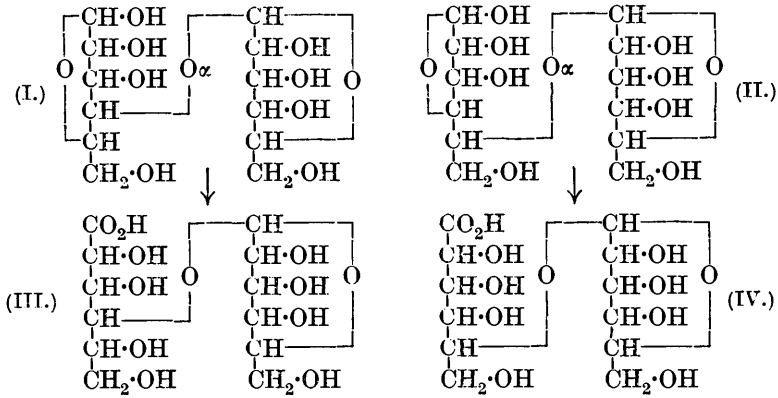
No conclusive proof of the constitution of maltose is available either from the research by Irvine and Black (this vol., p. 862) or from that by Cooper, Haworth, and Peat (*ibid.*, p. 876). In both papers, the evidence for the allocation of the two alternative formulæ (I) and (II) to maltose was based on the isolation of 2 : 3 : 6-trimethyl glucose obtained by the hydrolysis of completely methylated maltose.

As was pointed out by Charlton, Haworth, and Peat (this vol., footnote, p. 98), the isolation of 2 : 3 : 6-trimethyl glucose as a cleavage product of a methylated disaccharide does not enable a final decision to be reached as to the position occupied either by the original biose linking or by the oxide ring in the first hexose residue, since the alternative positions 4 and 5 are open for the allocation of either of these linkings. We therefore advanced, although guardedly, the suggestion that a butylene or γ-oxide (1 : 4) linking might be present in the first glucose residue in lactose and cellobiose, but we commented that this contingency was on the whole unlikely. In the later paper, we again left open this possibility in assigning a structural formula to maltose. The reason for this dubiety lay in our recognition of normal glucose as an amylene oxide and of γ-glucose derivatives as butylene oxides.

Prominence has been given by Irvine (this vol., pp. 868, 1495, 1507) to the tentative suggestion we made in the footnote above-mentioned, and in explicitly adopting this suggestion this author has asserted that in maltose and cellobiose different oxide rings occur in the first hexose residues. Thus, for maltose, Irvine and Black (this vol.,

p. 869) also proposed the two formulæ (I) and (II), adding the statement, " one of which must represent cellobiose and *iso*cellobiose, whilst the other must be reserved for maltose together with, presumably, *iso*maltose," This view has been further expanded and embodied by Irvine in the structural formulæ recently ascribed to starch (Irvine and Macdonald, this vol., p. 1508) and to cellulose (Irvine and Robertson, this vol., p. 1496). The constitutions allocated by these authors to the polysaccharides, although admittedly attractive, are based on an assertion which has, in view of the results now submitted, failed to receive experimental support. In view also of the inadequate knowledge available with regard to the constitution of *iso*maltose and *iso*cellobiose, it seemed to us to be premature at this stage to base a revision of the structural formulæ of the disaccharides and polysaccharides as a class upon the existence of these bioses.

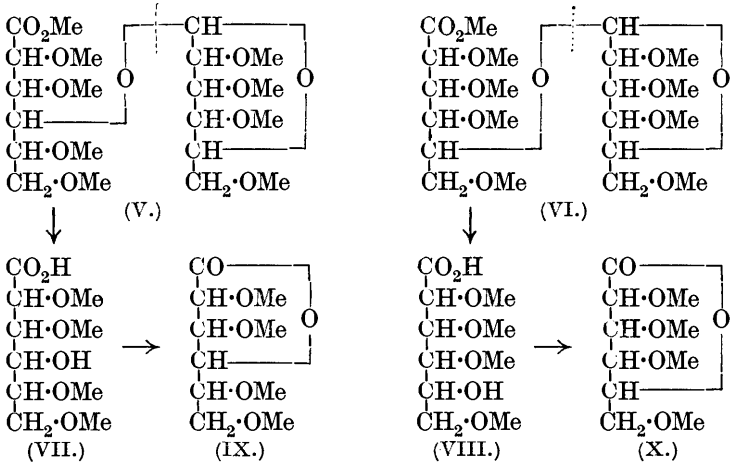
We have pursued the inquiry into the constitution of maltose and have instituted other experimental methods with the object of eliminating any dubiety in the case of this disaccharide.



The alternative formulæ (I) and (II) differ fundamentally in the position occupied by the biose linking. In formula (I) this linking engages the groups on the fourth carbon atom of the first glucose residue and the oxide ring is amylene oxidic; in formula (II) the biose linking is attached to the fifth carbon atom of the hexose chain, the oxide ring then being butylene oxidic. To reach a conclusive result it appeared to us essential that we should eliminate the oxide ring from the first hexose residue in maltose in order to concentrate attention on the position of the biose linking.

Maltose, on oxidation with bromine water, gives rise to maltobionic acid which, at the present stage of our knowledge, would be formulated either as (III) or (IV). It seemed possible to decide

between these alternatives by adopting the device of methylation, followed by hydrolysis to the constituent hexose acid and sugar. We therefore oxidised maltose to maltobionic acid and methylated the calcium salt of this acid with methyl sulphate and alkali, and thereafter the silver salt of the methylated acid was methylated by methyl iodide in presence of silver oxide. The compound so obtained was essentially methyl octamethylmaltobionate (V or VI), which distilled easily in a high vacuum. The hydrolysis of this ester with dilute mineral acid was accompanied by the cleavage of the bionic linking and resulted in the isolation of a tetramethyl gluconic acid (VII or VIII) along with crystalline tetramethyl glucose. On heating, the tetramethyl gluconic acid underwent ring closure, giving the corresponding tetramethyl gluconolactone (IX or X). The exposed hydroxyl group in (VII) or (VIII) represents the position of attachment of the original biose linking to the first hexose chain in maltose, and it is seen that the solution of the problem rests upon the diagnosis of the tetramethyl gluconolactone obtained and its recognition as belonging to the γ- or δ-series (IX or X).



We were in a position to decide this problem, since our earlier work had shown that the γ- and δ-lactones of glucose differ widely in their rates of hydrolysis, as revealed by polarimetric changes and by titration during hydrolysis. The crystalline tetramethyl lactone isolated from the above experiments corresponded in these respects with 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone, m. p. 26—27° (IX). Confirmation of this result was again possible in that the crystalline phenylhydrazide of 2 : 3 : 5 : 6-tetramethyl gluconic acid (VII) has a m. p. 134—136°, whilst the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl

gluconic acid (VIII) has a m. p. 114—115°. When the tetramethyl gluconolactone isolated as described above from methylated maltobionic ester was transformed into the phenylhydrazide, this had a m.p. 134—136°, and was identical in all respects with an authentic specimen derived from 2 : 3 : 5 : 6-tetramethyl gluconic acid. A mixed melting-point determination was made with the phenylhydrazides prepared from (*a*) the above lactone from methylated maltobionic acid, (*b*) the lactone obtained by oxidation, followed by methylation, of 2 : 3 : 6-trimethyl glucose, which we have shown to be a γ-lactone (this vol., p. 89), and (*c*) the lactone obtained by oxidation of tetramethyl γ-glucose, and in each case no depression of melting point occurred. The data for the recognition of the tetramethyl gluconolactone as a γ-lactone are based on these comparisons and are supplemented by the following facts.

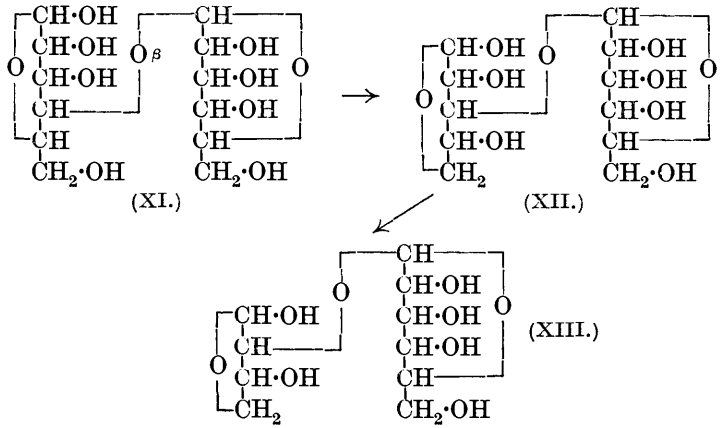
When crystalline tetramethyl glucose is oxidised to the corresponding lactone, this is identified as 2 : 3 : 4 : 6-tetramethyl δ-gluconolactone (X) (Charlton, Haworth, and Peat), and confirmation of this result has been contributed by Hirst (this vol., p. 389), who has also shown by other oxidation methods that crystalline tetramethyl glucose is substituted in positions 2, 3, 4, 6. The m. p., 114—115°, of the crystalline phenylhydrazide derived from this δ-lactone was depressed to 104—108° in a mixture with the present specimen.

Formulæ (V) and (VI) show that the maltobionic acid having the constitution (III) could alone give rise on complete methylation and hydrolysis to the γ-lactone. On the other hand, a maltobionic acid of the structure (IV), giving a methylated maltobionic acid of formula (VI), would lead to a δ-lactone (X) identical with that obtained on oxidising crystalline tetramethyl glucose, but this was found not to be the case.

The experimental data thus point to the allocation of formula (III) to maltobionic acid, and it therefore follows that, assuming no displacement of the biose linking either during methylation or during oxidation of maltose to maltobionic acid, the constitution of maltose should be represented by formula (I). We are at present engaged in applying the same method of proof to both lactose and cellobiose, and this work we wish to reserve.

Meanwhile, the constitution ascribed to cellobiose (Charlton, Haworth, and Peat, *loc. cit.*; Haworth and Hirst, J., 1921, **119**, 193) has received support from the work of Zemplén (*Ber.*, 1926, **59**, 1254), who has degraded cellobiose (XI) first to the hexose–pentose (XII), which gives an osazone, and finally to a hexose–tetrose (XIII), and the failure of the latter to form an osazone is attributed by this author to the presence of the biose linking on the carbon

atom adjoining the reducing group, *i.e.*, in position 4 in the original cellobiose. If this experimental work by Zemplén be accepted as a proof of the constitution of cellobiose, then it follows, from the evidence given in the present paper, that maltose and cellobiose possess the same constitution, the two disaccharides differing only in a stereochemical sense.



(XI.) (XII.) (XIII.)

A similar constitutional study of lactose by the same author (Zemplén, *Ber.*, 1926, **59**, 2402) confirms the structural formula we have applied to this disaccharide (Charlton, Haworth, and Peat, *loc. cit.*; Haworth and Leitch, 1918, **113**, 188).

The available evidence is thus opposed to the opinions expressed by Irvine (this vol., pp. 869, 1496, 1508) in his endeavour to classify the disaccharides and polysaccharides (starch and cellulose) on the basis of a difference in the positions of (*a*) the biose linking in maltose and cellobiose and (*b*) the oxide-ring linking in each of these bioses.

EXPERIMENTAL.

Methylation of Maltobionic Acid. Formation of Methyl Octamethylmaltobionate.—Pure maltose was oxidised with bromine water (Fischer and Meyer, *Ber.*, 1889, **22**, 1941; Glattfeld and Hanke, *J. Amer. Chem. Soc.*, 1918, **40**, 989), and the product isolated as calcium maltobionate. This was purified by solution in water and precipitation with alcohol, the process being repeated many times until reducing sugars were removed.

The calcium maltobionate was methylated several times in aqueous solution with methyl sulphate and alkali, the standard methods employed by one of us being adopted together with the modifications described in the researches on amygdalin (Haworth and Leitch, J., 1922, **121**, 1925; Campbell and Haworth, *ibid.*, 1924,

125, 1340). After methylation by methyl sulphate, the acid product was isolated from its salt and submitted again to a similar methylation procedure. The partly methylated maltobionic acid (Found : OMe, 41·7%) was now soluble in methyl iodide and was further methylated three times by Purdie's method.

It was essential throughout this investigation to ensure that any material which might have undergone hydrolysis during any stage of the manipulation should be completely eliminated (in order to avoid the presence of methylated gluconolactone or gluconic ester) from the product which was carried on to the final stages. The material (15 g.) was therefore fractionated at 0·03 mm. as follows, and the first fraction discarded :

Fraction I.	B. p. → 155°.	Weight 2·0 g.	n_D 1·4470.
,, II.	,, 160—165°.	,, 2·7 g.	,, 1·4610.
,, III.	,, 167—170°.	,, 10·0 g.	,, 1·4638.

The last two fractions (Found : OMe, 52·7%) were united and twice re-methylated with moist silver oxide and methyl iodide and again with the dry Purdie reagents. The product (11 g.) appeared to contain heptamethyl maltobionolactone as well as the expected methyl octamethylmaltobionate, and failure to methylate it completely was attributed to the persistence of the lactone ring in the former of these constituents.

The whole of the material was dissolved in aqueous barium hydroxide at 40° and kept over-night. Thereafter the excess of barium hydroxide was removed by admitting carbon dioxide until the solution was neutral. The filtrate was analysed to determine its barium content, and then mixed with the equivalent proportion of aqueous silver sulphate for the precipitation of the barium as sulphate, this and the subsequent operations being conducted in a dark room. The filtered solution was completely evaporated at 35—40° under diminished pressure, and the residual silver salt, dried with methyl alcohol, was treated with a large excess of methyl iodide under reflux, the initial vigour of the reaction being moderated by cooling. Dry silver oxide and methyl iodide were afterwards added and the mixture was digested at 45°; the product ($n_D^{20°}$ 1·4603) was again distilled at 0·05 mm.

Fraction Ia.	B. p. → 166°.	Weight 0·6 g.	$n_D^{17°}$ 1·4596.
,, IIa.	,, 170—173°.	,, 8·7 g.	,, 1·4609.

The latter fraction (Found : OMe, 55·1%) was again submitted to three methylations, without any apparent change in boiling point or methoxyl content resulting, the final analytical figures being C, 51·5; H, 8·4; OMe, 55·4 (methyl octamethylmaltobionate, $C_{21}H_{40}O_{12}$, requires C, 52·1; H, 8·3; OMe, 57·8%). The compound was a pale yellow, viscid liquid, having $n_D^{14°}$ 1·4620.

The slightly low carbon value appeared to be due to analytical difficulties in the combustion, since by mixing the compound intimately with fine copper oxide in the combustion tube and repeating the analysis, the values C, 52·15; H, 8·3% were obtained.

Hydrolysis.—The above methyl octamethylmaltobionate was hydrolysed by heating at 80—90° with 7% hydrochloric acid ($c = 4·2$), and showed the following polarimetric changes: $[\alpha]_D$ + 120·8° (initial); 110·8° (after ½ hour); 88·1° (1½ hours); 70·5° (2·75 hours); 62·4° (3·75 hours); 57·5° (5 hours); 55·9° (6 hours); 54·9° (7 hours, remained constant). Heating for ¼ hour at 100° resulted in no further change, and there was little alteration in the colour of the solution during hydrolysis.

Isolation of Hydrolytic Products.—The acids, both inorganic and organic, present in the hydrolysed product were converted into the barium salts, and water was removed from the neutralised solution at 30°. The residue, dried with alcohol, was digested with boiling ether, which removed crystalline tetramethyl glucose, this being definitely characterised by the usual procedure (yield, quantitative). The saline residue was titrated with dilute hydrochloric acid to liberate the organic acid from its barium salt, and after this solution had been evaporated at 30°/15 mm. the residual mixture of barium chloride and organic acid was dried by heating at 60° in a vacuum. Ether extraction removed the organic portion, which was now chiefly a lactone (yield, 70% of the theoretical), and a further extraction of the barium residues with chloroform gave a further yield of the undecomposed barium salt (about 20%) of the acid corresponding to the above lactone.

Following a preliminary heating at 100° for ½ hour, the distillation of the lactone gave a colourless, mobile liquid, boiling constantly at a bath temperature of 110—115°/0·02 mm. (b. p. about 90°) and showing $n_D^{13°}$ 1·4490 and $n_D^{20°}$ 1·4470, which crystallised completely (m. p. 26—27°).

The weight of pure distilled material showing the above constants represented 85% of the total crude lactone, to which was added later the portion (approximately 10%) from the above-mentioned residual barium salt.

The lactone showed the following polarimetric changes in water ($c = 2$): $[\alpha]_D^{14°}$ + 60·2° (after ¼ hour); 57·3° (2 days); 55·2° (3 days); 54·2° (4 days); 51·2° (6 days); 43·1° (13 days) (Found: C, 51·0; H, 7·7; OMe, 52·3. $C_{10}H_{18}O_6$ requires C, 51·3; H, 7·7; OMe, 53·0%).

Phenylhydrazide of 2 : 3 : 5 : 6-Tetramethyl Gluconic Acid.—The above lactone was mixed with the equivalent weight of phenylhydrazine in ether, the ether evaporated, and the residue heated at

100° for 5 minutes; it then solidified completely to a hard cake of colourless crystals. These were washed with ether, weighed (yield, quantitative), and purified from ether: m. p. 134—136° and re-melted at 135° after solidifying (Found: C, 55·8; H, 7·7; N, 8·2; OMe, 35·0. Calc.: C, 56·1; H, 7·6; N, 8·2; OMe, 36·3%).

The crystals of the phenylhydrazide were identified by m. p. determinations in admixture with each of the specimens of tetramethyl γ-gluconolactone prepared from (*a*) tetramethyl γ-glucose by oxidation with bromine water; (*b*) 2 : 3 : 6-trimethyl glucose by oxidation to 2 : 3 : 6-trimethyl γ-gluconolactone, which on methylation gave the 2 : 3 : 5 : 6-tetramethyl lactone (Charlton, Haworth, and Peat, this vol., p. 100). In each case there was no depression. For the purposes of this comparison the specimens prepared by the above authors were recrystallised several times (m. p. 134—136°). Among the residual portions of acid or lactonic products a search was made for the presence of a lactone which might give the phenylhydrazide of 2 : 3 : 4 : 6-tetramethyl gluconic acid (m. p. 114—115°), but no trace of this was discovered. A minute quantity of what appeared to be a trimethyl gluconolactone was, however, detected. There appeared to be no possibility of the presence of any tetramethyl lactone other than that described as 2 : 3 : 5 : 6-tetramethyl γ-gluconolactone.

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