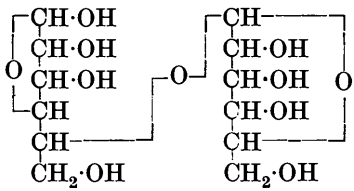
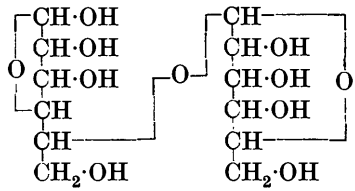
CCCCXIII.—*The Constitution of the Disaccharides. Part XVII. Maltose and Melibiose.*

By WALTER NORMAN HAWORTH, JOHN VAUGHAN LOACH, and CHARLES WILLIAM LONG.

THE problem of the structure of maltose and melibiose has excited unusual controversy and various rival formulæ appear in the literature from time to time intended to supplant those submitted by one of us. The most recent of these attempts to reconstruct the constitution of these disaccharides is that made by Irvine in the published transcript of an address to the American Chemical Society (*Chemical Reviews*, 1927, **4**, 203). Without contributingany fresh evidence, Irvine has now advocated the following scheme, wherein maltose is given formula (I) and melibiose is given formula (II) :



(I.)

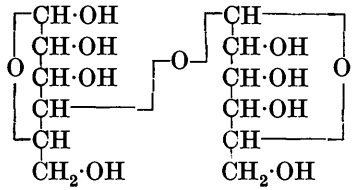


(II.)

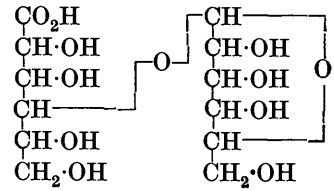
It is to be noticed that the revised structural form initially advocated by one of us for glucose in its normal derivatives is adopted for the non-reducing residue in (I), whilst the reducing glucose component in each disaccharide is given the character of a butylene oxide form which one of us applied to γ-glucose derivatives. Thus the effect of these new attempts to represent these disaccharides is to emphasise that each of them contains as its reducing hexose component a labile or γ-glucose residue. No evidence is given for the hypothesis that either maltose or melibiose is a γ-sugar, nor indeed do the properties of these disaccharides warrant that such an assumption need be entertained.

These views advanced by Irvine are indeed capable of simple and complete investigation, and in the case of maltose they have already been shown to be invalid by Haworth and Peat (J., 1926,

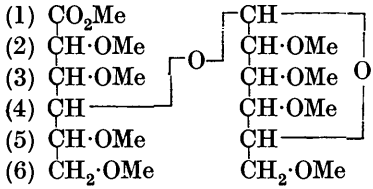
3094). The latter authors have demonstrated that maltose has the constitution (III), since it gives on oxidation a maltobionic acid which can only be formulated as in (IV). Completely methylated maltobionic ester (V) yields on hydrolysis only tetramethyl glucose and the tetramethyl gluconic acid (VI) which forms the γ-lactone (VII), and the structure of the latter has been proved by its oxidation to *d*-dimethoxysuccinic acid (VIII) and not to xylo-trimethoxyglutaric acid (Haworth, Hirst, and Miller, this vol., p. 2436), which is obtained from the corresponding tetramethyl δ-gluconolactone.



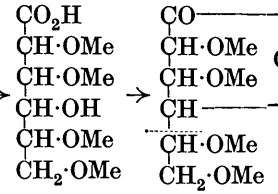
Maltose (III.)



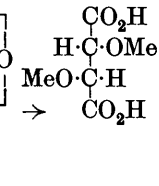
(IV.)



(V.)



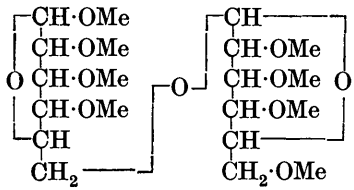
(VI.) (VII.)



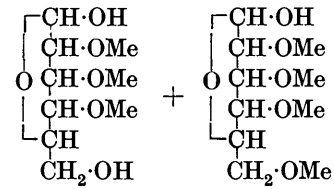
(VIII.)

These facts leave no room for doubt that the biose linking in maltose is situated at position 4 in the reducing biose. If this result is compared with that furnished by the isolation of 2 : 3 : 6-trimethyl glucose as a cleavage product of fully methylated maltose (Irvine and Black, J., 1926, 862; Cooper, Haworth, and Peat, *ibid.*, p. 876), the oxide ring of this hexose unit is seen to be present at the 5th carbon atom as shown in the maltose structure (III).

The case of melibiose is capable of a similar demonstration. Methylated melibiose (IX) yields on hydrolysis two cleavage products, one of which (XI) is crystalline, and the other, a liquid (X), yields a crystalline glucoside.



(IX.)



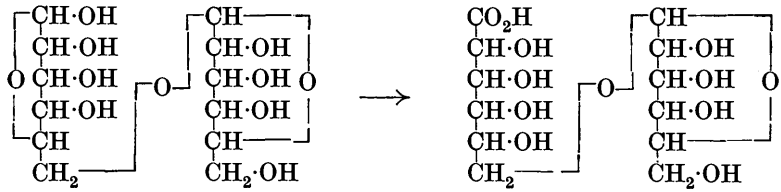
(X.) (XI.)

2 : 3 : 4-Trimethyl glucose. 2 : 3 : 4 : 6-Tetramethyl galactose.

The inference was therefore drawn by Charlton, Haworth, and Hickinbottom (this vol., p. 1527) that the constitution (XII) should be allocated to melibiose, and the same formula was also supported by the earlier work of Haworth, Hirst, and Ruell on raffinose (J., 1923, **123**, 3125), and still earlier by Haworth and Leitch (J., 1918, **113**, 188).

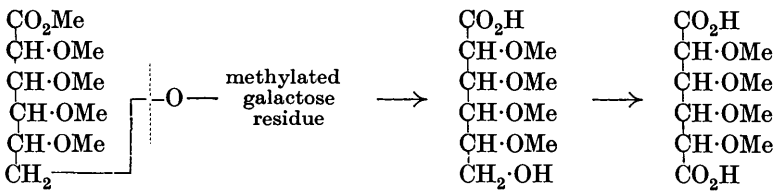
This formula for melibiose is finally established by an alternative proof wherein melibionic acid (XIII) has been methylated to give methyl octamethylmelibionate (XIV), which on hydrolysis yields, besides crystalline 2 : 3 : 4 : 6-tetramethyl galactose, the 2 : 3 : 4 : 5-tetramethyl gluconic acid (XV) which does not form a lactone. The structure of the latter product has been determined by its oxidation with nitric acid to tetramethylsaccharic acid (XVI), which gives a crystalline methyl ester identical in every respect with that obtained by methylating saccharic acid.

It is therefore demonstrated that since 2 : 3 : 4 : 5-tetramethyl gluconic acid (XV) is the only product isolated which corresponds to the glucose component of melibiose, this component is attached to the galactose residue through the hydroxyl in position 6.



Glucose residue. Galactose residue.

Melibiose (XII.) (XIII.)



(XIV.) (XV.) (XVI.)

The structural formula assigned by one of us to melibiose (XII) thus receives substantial confirmation from these new experimental results, as does also our formula for raffinose.

Another aspect of the constitution of this disaccharide is the stereochemical signification given to it in the current literature as a glucose-β-galactoside, a conclusion based on the observation that melibiose is said to undergo slow hydrolysis with emulsin (Fischer and Armstrong, *Ber.*, 1902, **35**, 3144). Helferich and

Rauch, however, have synthesised a biose of this configuration and structure, and the specific rotations both of the sugar and of its octa-acetate indicate its non-identity with melibiose (*Ber.*, 1926, **59**, 2655). Other considerations serve to show also that the stereochemical characterisation of this sugar revealing the presence of a β-biose junction between the two hexose residues is unsound. This has been argued in a previous paper (Charlton, Haworth, and Hickinbottom, *loc. cit.*) wherein it is suggested that melibiose may be a glucose-α-galactoside corresponding to maltose, which is a glucose-α-glucoside.

The argument may be supplemented by the new data now made available in the present experimental work. The methylated esters of the monobasic acids corresponding to each of the disaccharides, maltose, cellobiose, lactose, melibiose, have been prepared and their specific rotations are given, and in addition the rotation changes consequent upon their hydrolysis to the constituent hexoses and hexonic acids have been recorded. Each of these methylated esters may be regarded, for the purpose of comparison, as the equivalent of a methylglucoside or a methylgalactoside which may be recognised either as an α- or a β-form. In the case of the simple methylhexosides the magnitude of rotation alone is accepted as a sufficient indication of stereochemical homogeneity. If the analogous principle be conceded also for the methylated esters of the various bionic acids, the data now collected may be valuable evidence of the stereochemical nature of the bionic junction between the gluconic acid residue and the remaining hexose in a bionic acid, and, by inference, of the character also of the biose junction in the parent disaccharide. It may be mentioned that methylation of α-methylglucoside, etc., does not appear materially to effect interchange into the β-methylglucoside.

	$[\alpha]_D$.		Equilibrium attained on hydrolytic cleavage.
(1) Methyl octamethylmaltobionate ...	+121°	→	+54·9°
(2) Methyl octamethylcellobionate ...	+ 5	→	+55
(3) Methyl octamethyl-lactobionate ...	+ 34	→	+77·2
(4) Methyl octamethylmelibionate ...	+106	→	+64

The significance of these figures is the comparatively small magnitude of rotation of the substances Nos. 2 and 3, which are the products from the authenticated β-bioses, namely, cellobiose, which is glucose-β-glucoside, and lactose, which is glucose-β-galactoside. It may be further observed that these low rotation values show an increase when the ester is hydrolysed to the constituent hexose and hexonic acid. The equilibrium rotation value of the hexose is known already from previous experiments, whilst the specific

rotations of the methylated hexonic acids are also known (Drew, Goodyear, and Haworth, this vol., p. 1237).

	$[\alpha]_D$.
Tetramethyl glucose	84° } (at equil.)
,, galactose	+117 }
2 : 3 : 5 : 6-Tetramethyl gluconic acid	+ 27
2 : 3 : 4 : 5- ,, ,, ,, ...	+ 10

Inspection of the figures available for cases Nos. 1 and 4 reveals a striking similarity between these two derivatives of maltose and melibiose, inasmuch as both the esters have initially a high specific rotation which declines to a lower value on hydrolysis. The inference which might be drawn is therefore that in each of these examples the α-linking of a glucoside is undergoing cleavage to the hexonic acid and to the α + β-equilibrated mixture of the methylated hexose.

If this evidence be considered to provide a valid argument for the case of melibiose, it will then be clear that this disaccharide is not a glucose-β-galactoside, which is the view commonly accepted on the basis of Fischer and Armstrong's observation, but is a glucose-α-galactoside.

Discussion of Results.

With the present work there is now concluded a survey of the constitution of the six commonly occurring disaccharides—sucrose, cellobiose, maltose, gentiobiose, lactose, and melibiose—and the structural classification of these may now be reviewed in the light of these new data, and of the controversy which these and the earlier results of one of us and his collaborators has excited.

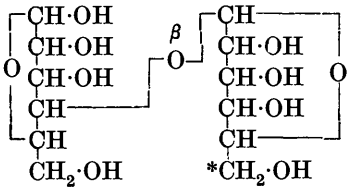
Chief among these criticisms have been those of Irvine in earlier papers (J., 1926, 867, 1506), but his more mature views appear to be concentrated in his recent article in *Chemical Reviews* (*loc. cit.*). The following extract from this article may be quoted : " In the structural classification of the disaccharides built up by Haworth " " this important issue * has been entirely overlooked," and " in addition, it is now recognised that his work involves a fundamental experimental error which renders his whole scheme valueless and that consequently, so far as the constitution of the disaccharides is concerned, we are only at the beginning of things in place of at the end."

Whilst affirming this belief, Irvine then proceeds to " turn to

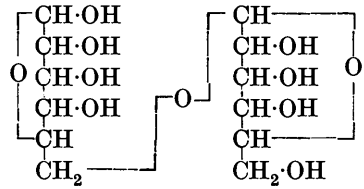
* Far from having been overlooked, this issue originated in a clear, cautious statement by one of us (J., 1926, 98, footnote) and it was there considered to be a possible though unlikely contingency, a view since justified in that it has been shown not to arise in any of the cases for which it was entertained.—W. N. HAWORTH.

the authentic results obtained," but instead of giving here only examples initiated by his own experimental work he quotes in detail, in the cases of no fewer than four of the six disaccharides, the experimental results and formulæ copied from our original papers published before he gave his address to the American Chemical Society (see *Chemical Reviews*), and in so doing he suppresses all reference and acknowledgment to the original authors.

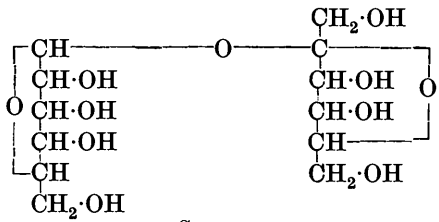
Irvine is committed to the advocacy of all but two of the constitutional formulæ for disaccharides initiated by one of us. The following are the formulæ quoted by Irvine in detail; these conclusions are identical with those previously given by Haworth and Hirst (J., 1921, **119**, 193), Haworth and Leitch (J., 1918, **113**, 188), Haworth and Wylam (J., 1923, **123**, 3120), Charlton, Haworth, and Peat (J., 1926, 89), and Haworth and Hirst (J., 1926, 1858).



Cellobiose (when both hexoses are glucose).
Lactose (when the hexose * is galactose).



Gentiobiose.



Sucrose.

The allocation of the above formula to sucrose was dependent upon, and could only have been made simultaneously with, the disproof and abandonment of Irvine's butylene oxide formula for normal fructose derivatives, since it is this formula which Haworth and Hirst then assigned (July, 1926) to the γ-fructose component in sucrose (compare subsequent paper by Avery, Haworth, and Hirst, this vol., p. 2308).

For the constitutions of the remaining two disaccharides, maltose and melibiose, against which the main criticism is evidently directed, Irvine has contended for the expressions (I) and (II) quoted already on p. 3146. The purport of the present communication is to demonstrate that in these two examples Irvine's formulæ are

erroneous, as is also his formula for raffinose, and it thus appears that in none of the six cases has a disaccharide formula been initiated by Irvine which has survived. The latter author has assigned, however, structural formulæ to cello*iso*biose and to the *iso*maltose of starch, although no experimental evidence accompanies these views and most workers, including the discoverers of these bioses, would do little more at this stage than postulate the probable existence of these two disaccharides (compare Haworth, *J. Soc. Chem. Ind.*, 1927, **46**, 295).

Experimental.

Oxidation of Melibiose to Melibionic Acid.—Melibiose (28 g.) having the recognised constants and having been prepared from pure raffinose was agitated with 210 c.c. of water to which were added in portions at intervals 16 c.c. of bromine, and the mixture was kept at about 20° with frequent agitation during about 7 days; then it appeared no longer to reduce Fehling's solution. The solution was then aerated, treated with hydrogen sulphide, and again aerated. Powdered litharge (50 g.) was added, and the mixture shaken for 4 hours until all the mineral acid present had been neutralised. The filtrate and the washings from the lead salts were shaken with finely divided silver oxide and the mixture was kept in the dark for some time. It was then filtered, treated with hydrogen sulphide, and again filtered, and the filtrate was evaporated and digested with precipitated calcium carbonate (10 g.). The filtrate and washings from this treatment were treated with charcoal and evaporated to a volume of 50 c.c. This solution was poured slowly with stirring into about 2 litres of methylated spirit. The precipitated, slightly glutinous solid was placed in contact with fresh methylated spirit and stirred until a hard granular product was formed. This was collected and dried in a vacuum, appearing as an almost white powder which was calcium melibionate. A further quantity of this calcium salt was recovered from the methylated spirit by evaporation, and purified as before by pouring into a fresh lot of methylated spirit. The dried calcium salt was devoid of action towards Fehling's solution. Yield, 92%. The calcium estimation showed Ca 4·74%. It thus appeared that the solid contained either two or three mols. of ethyl alcohol.

Methylation. Calcium melibionate (20 g.) was methylated in the usual manner with methyl sulphate in presence of sodium hydroxide, and the product was submitted to two similar treatments. The methylation process was completed by the use of Purdie's reagents, which were employed initially in presence of

a little water and finally the dry reagents were employed. The crude product resulting from this treatment contained OMe, 53·9%. It was then distilled under 0·05 mm., and the small amount (1·4 g.) of distillate collected below a bath temperature of 190° was rejected. The remaining portion of the distillate (12·5 g.) was collected at a bath temperature of 200—210°, and this on redistillation had b. p. 173 –175°/0·06 mm.; $n_D^{14^\circ}$ 1·4640; $[\alpha]_D^{13^\circ}$ + 106·4° in water (c = 1·63) (Found: C, 51·9; H, 8·5; OMe, 54·5. $C_{21}H_{40}O_{12}$ requires C, 52·1; H, 8·3; OMe, 57·65%). The product thus appeared to be essentially methyl octamethylmelibionate.

Hydrolysis. The above product was digested in a bath at 90° with a 7% solution of hydrochloric acid, and polarimetric readings were taken during the process of hydrolysis. The observations were taken by interrupting the course of the hydrolysis, removing a small sample and cooling, and these observations were continued until a constant rotation was obtained. $[\alpha]_D^{20^\circ}$ + 106·1° (initial), 99·6° (after 30 mins.), 89·6° (60 mins.), 81° (90 mins.), 73·4° (120 mins.), 64·0° (180 mins.). After the solution had been kept overnight, the reading was + 64·3°, and it was ascertained that this reading remained constant after further heating. The mineral acid was then neutralised with barium carbonate, air being drawn through the solution at 50° to assist the neutralisation. The faintly coloured filtrate was treated with charcoal until quite colourless and evaporated at 40° in a vacuum. The residue was thoroughly dried by mixing it with acetone and removal of solvent, and finally by heating in a vacuum; the white residue thus obtained was extracted many times with boiling ether. The collected extracts yielded a syrup which crystallised on keeping. It was ascertained that this was 2 : 3 : 4 : 6-tetramethyl galactose (yield, 96·2% of the theoretical). The crystals were drained on porous tile and purified from light petroleum; m. p. 71—72°; $[\alpha]_D^{21^\circ}$ + 117·8° (equilibrium). A mixed m. p. determination with an authentic specimen of 2 : 3 : 4 : 6-tetramethyl galactose proved its identity.

Again, 1 g. of the crude sugar was heated with 3 c.c. of aniline and 30 c.c. of ethyl alcohol during 5 hours. The crystalline tetramethyl galactose anilide separated and was recrystallised from ethyl acetate (yield, 92%); m. p. 192—193°, mixed m. p. determination with an authentic specimen prepared from lactose, 192—193°.

The residue remaining after the ether extraction of the tetramethyl galactose contained a barium salt of an organic acid. This was dissolved in water and treated with the equivalent of dilute hydrochloric acid to liberate the whole of the organic acid. The solution was completely evaporated at 30° in a vacuum and finally

at 35—40°. The residue was thoroughly dried by adding acetone and evaporating the solvent and finally by heating in a high vacuum at 70°. It was then extracted repeatedly with ether and the collected extracts were evaporated, yielding 4·2 g. of a tetramethyl gluconic acid (yield, 86·8% of the theoretical). The latter distilled under 0·05 mm. very slowly from a bath at 164°, giving a colourless, highly viscid syrup, which gave analytical figures pointing to its having been converted during this operation into an anhydro-form by condensation of two molecules (Found: C, 49·5; H, 8·0; OMe, 48·6. $C_{20}H_{38}O_{13}$ requires C, 49·4; H, 7·8; OMe, 51·0%).

The above undistilled acid (1 g.) was now oxidised in contact with 10 c.c. of nitric acid (*d* 1·2). Nitrous fumes began to be evolved at 75°. The temperature was gradually raised to 100°, and the solution maintained at this temperature for 5 hours. On removal of the nitric acid in a vacuum by repeated evaporation, accompanied by the intermittent addition of water, a liquid acid product was obtained. This was esterified with methyl-alcoholic hydrogen chloride (3%). Removal of the mineral acid was effected with silver carbonate and the filtrate was treated with charcoal to eliminate colloidal silver. A colourless liquid product (0·95 g.) was obtained on evaporation of this solvent. This was distilled under 0·09 mm. from a bath heated at 116—119°, and gave 0·8 g. of a colourless mobile liquid which crystallised during the distillation. This solid was recrystallised from dry ether, in which it was very soluble, giving hexagonal prisms, m. p. 77—78°. These were identical with the crystalline methyl tetramethylsaccharate which was prepared for the purpose of comparison by the methylation of saccharic acid. Karrer and Peyer (*Helv. Chim. Acta*, 1922, **5**, 577) also have prepared methyl tetramethylsaccharate from saccharic acid and they give m. p. 68°. By repeating the preparation of these authors, we also observed m. p. 68—71° for the partly purified material, but after three recrystallisations from ether the m. p. was raised to 76—77°. This preparation of the ester and of its derivatives will be described in a subsequent paper by Haworth and Jones. Mixed m. p. determination with the specimen isolated *via* melibiose, 77—78° (Found: C, 49·1; H, 7·2; OMe, 63·5. $C_{12}H_{22}O_8$ requires C, 49·0; H, 7·5; OMe, 63·3%). The diamide was also prepared: hexagonal plates (from water), m. p. 237—239° (Found: C, 45·4; H, 7·7; N, 10·7. Calc.: C, 45·4; H, 7·65; N, 10·6%).

The tetramethyl gluconic acid isolated as a cleavage product of methyl octamethylmelibionate is thus seen to be 2 : 3 : 4 : 5-tetramethyl gluconic acid, since it gives the tetramethylsaccharic acid on oxidation of a terminal $-CH_2\cdot OH$ group.

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