CCII.—*The Constitution of the Disaccharides. Part XIV. Melibiose and its Relationship to Raffinose.*

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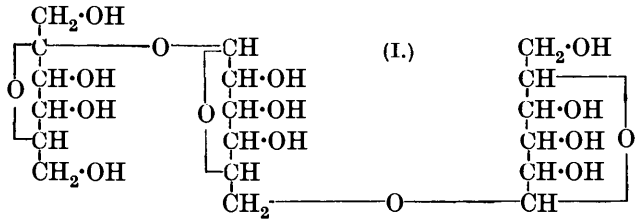
RAFFINOSE was the subject of a constitutional study in an earlier communication (Haworth, Hirst, and Ruell, J., 1923, **123**, 3125), wherein it was stated that completely methylated raffinose gives rise on hydrolysis to three methylated fragments: (1) normal tetramethyl galactose; (2) 2 : 3 : 4-trimethyl glucose*; (3) tetramethyl γ-fructose, and in the course of recent work these experimental results were confirmed. The constitution advanced for this trisaccharide, translated into terms of the revised formulæ for the constituent hexoses (Charlton, Haworth, and Peat, J., 1926, 89; Haworth and Hirst, *ibid.*, p. 1858), is formulated



(I.)

It was also stated that "from this determination of the structure of raffinose it may be inferred that the constitution of melibiose proposed by Haworth and Leitch (J., 1919, **115**, 809) receives substantial confirmation, but inasmuch as structural changes may occur in the resulting disaccharide when the fructose residue is eliminated from raffinose, it has been thought desirable to undertake a separate investigation of melibiose, which is now in progress."

The results of that investigation are now presented, and they support the view that the glucose and galactose residues occurring in raffinose are identical with those in melibiose. During the isolation of hendecamethyl raffinose in the earlier work, a small quantity of a crystalline by-product was encountered, m. p. 78°, which was considered to be a completely methylated disaccharide, probably melibiose. This had been formed by partial hydrolysis of raffinose during the process of methylation. This crystalline substance when purified (m. p. 101—103°) gave on hydrolysis 2 : 3 : 4 : 6-tetramethyl galactose, and a liquid trimethyl glucose which on conversion into the glucoside gave the characteristic crystalline 2 : 3 : 4-tri-

* This formula was indexed as 2 : 3 : 5-trimethylglucose on the basis of the older formula for glucose.

methyl β-methylglucoside, m. p. 94°, identical with that previously isolated from methylated raffinose.

For the purpose of this comparison, we have again submitted hendecamethyl raffinose to hydrolytic cleavage, and have separated the trimethyl glucose fragment from the other methylated hexoses by extraction with chloroform. The tetramethyl derivatives of galactose and γ-fructose are removed by this solvent, as is also a small proportion of the trimethyl glucose, but the greater proportion of the trimethyl glucose remains in the aqueous solvent. Isolation of this residue and conversion into the glucoside gave again the characteristic crystalline 2 : 3 : 4-trimethyl β-methylglucoside, m. p. 93—94°. Finally, we have prepared melibiose from raffinose and have transformed this sugar into heptamethyl methylmelibioside, which is a crystalline compound. It has previously been described by Schlubach and Rauchenberger (*Ber.*, 1925, **58**, 1184) as forming needles, m. p. 98·5° (after twice crystallising from light petroleum). Ordinarily, the substance is found to melt at about this temperature, but two further crystallisations raised the melting point to 101—103°, and this specimen gave no depression of m. p. when mixed with the methylated melibiose isolated as a by-product during the methylation of raffinose (see above). On repeated crystallisation, however, the m. p. was raised to 106—107°, and the specific rotation was correspondingly diminished from $[\alpha]_D + 113°$ to $[\alpha]_D + 97·8°$ (in water).

It thus appeared that the material had been contaminated with what was probably an α-form of heptamethyl methylmelibioside; indeed a portion of the material was isolated (m. p. 122—123°).* As is usual, however, in the methylation of sugars with methyl sulphate and alkali, the major bulk of the product was isolated as the β-form. This was hydrolysed with dilute mineral acid and the mixture of tetramethyl and trimethyl hexoses was separated by taking advantage of the greater ease with which a tetramethyl hexose may be extracted from aqueous solution by chloroform. In this way this tetramethyl hexose was recovered from the solution in chloroform and was shown to contain crystalline 2 : 3 : 4 : 6-tetramethyl galactose, m. p. 71—73°; $[\alpha]_D + 149·4° \longrightarrow 116·9°$ (yield 50%). The portion remaining in the aqueous solution represented about 35% of the weight of the original methylated melibiose. The latter product was liquid, and was transformed into a mixture of the α- and β-methylglucosides by heating with methyl-alcoholic hydrogen chloride. This mixture of the two stereoisomeric

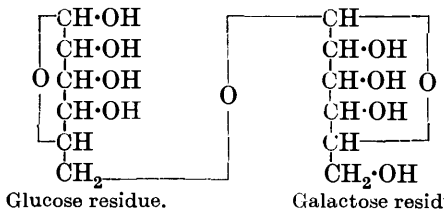
* A mixture of the α- and β-forms (m. p. 82°; $[\alpha]_D + 145°$) also gave on hydrolysis 2 : 3 : 4-trimethyl glucose, isolated as the β-methylglucoside, m. p. 94° (yield, 28%).

forms of the glucoside distilled completely and underwent spontaneous crystallisation immediately. Of the total yield of distillate, 13—15% by weight was collected as the characteristic 2 : 3 : 4-trimethyl β-methylglucoside, which on recrystallisation gave m. p. 92°. The liquid portion of the distillate doubtless contained a further quantity of this crystalline material.

Since the original distillate represented an equilibrium mixture of α- and β-forms, the former of which is liquid, it will be recognised that the whole of the product cannot be isolated as the crystalline β-variety. Proof of the identity of the glucoside was established by mixed melting-point determinations with specimens of the 2 : 3 : 4-trimethyl β-methylglucoside which had previously been isolated (*a*) from heptamethyl methylgentiobioside, (*b*) from methylated amygdalin, (*c*) from methylated raffinose, references to which preparations are given in the experimental section of this paper. Furthermore, a specimen of the crystalline 2 : 3 : 4-trimethyl β-methylglucoside has been specially prepared for the present comparison in the following way.

The 6-triphenylmethyl glucose (Helferich, Moog, and Jünger, *Ber.*, 1925, **58**, 872) was completely methylated with methyl iodide and silver oxide, and thereafter the triphenylmethyl residue was removed by hydrolysis. The specimen of 2 : 3 : 4-trimethyl β-methylglucoside obtained in this way was identical in all respects with the above material obtained from methylated melibiose.

(II.)



Glucose residue. Galactose residue.

The evidence adduced from these experimental results does not appear to be capable of any simple interpretation other than that in melibiose there is present a glucose residue which is attached through its (6) position to the reducing group of the galactose residue, and therefore the formula (II) previously advanced by Haworth and Leitch (J., 1918, **113**, 188) as modified by the introduction of the new oxide-ring formulæ for the hexoses (Charlton, Haworth, and Peat, J., 1926, 89) is fully supported.

In the interim Zemplén (*Ber.*, 1927, **60**, 923) has advanced new structural formulæ for melibiose and raffinose, differing from those which have been assigned by us to these sugars. The experimental evidence on which Zemplén bases his conclusions is the failure, which

he records, to isolate a phenylosazone from the galactoarabinose obtained by degradation of melibiose. Haworth and Long (this vol., p. 544) have already pointed out that an element of danger attaches to this method of proof, and indeed, the trimethyl hexose which would be expected on the basis of Zemplén's formula for melibiose would be 2 : 4 : 6-trimethyl glucose. Fortunately, we had a specimen of this sugar, and its glucoside, in our possession for the purpose of comparison, and it may be definitely stated that this sugar could not be isolated from the products of hydrolysis of methylated melibiose.

Discussion of Results.

The fact that melibiose is slowly attacked by the enzymes present in emulsin (Fischer and Armstrong, *Ber.*, 1902, **35**, 3144) has been widely interpreted in the literature as evidence for the classification of this sugar as a glucose β-galactoside. The specific enzyme for the hydrolysis of melibiose is, however, that occurring in bottom yeast, which is designated melibiase.

The observation of Helferich and Rauch (*Ber.*, 1926, **59**, 2655) casts doubt on the presence of a β-biose linking in this sugar, and these authors suggest that the configuration assigned may be incorrect. The following comparisons of the specific rotations may be considered in the discussion of this point.

	$[\alpha]_D$.		$[\alpha]_D$.
Maltose (β-glucose-α-glucoside)	+118°	Cellobiose (β-glucose-β-glucoside)	+16°
		Gentiobiose (β-glucose-β-glucoside)	−11
Melibiose	+124	Lactose (β-glucose-β-galactoside)	+35

Those disaccharides which possess indisputably a β-biose linking have low rotations as compared with maltose, which has an α-biose linking, and melibiose displays a rotation corresponding to that of maltose. Moreover, α-methylgalactoside has $[\alpha]_D$ + 178·8°, whilst the specific rotation of β-methylgalactoside is ± 0°. If melibiose were a glucose β-galactoside, its rotation, on the analogy of the relationship of lactose to gentiobiose, should be nearly zero, whereas if it were a glucose α-galactoside the rotation should be comparable with that of maltose, which is the case.

It has previously been remarked (Haworth and Leitch, J., 1919, **115**, 813) that there is some resemblance to maltose in the behaviour of melibiose, and this resemblance may be due to a stereochemical rather than a structural relationship.

On Hudson's method of representation (*J. Amer. Chem. Soc.*,

1916, **38**, 1566) the rotations of the disaccharides could each be divided into three parts as follows :

	$[\alpha]_D$, β-form.	$[\alpha]_D$, β-form octa-acetate.
1. Lactose β-galactose<glucose< $Ga - L_1 + R_1$ ...	+ 35°	− 4°
2. Cellobiose β-glucose<glucose< $G - L_1 + R_1$.....	+ 16	−15
3. Maltose α-glucose<glucose< $G + L_1 + R_1$	+118	+63

From (2) and (3) by subtraction L = 51 for the β-forms of the sugar, and this value is in excellent agreement with the value L = 50 for the lactonyl grouping in the simple glucosides and galactosides (re-calculated for mol. wt. 342 for disaccharides). Furthermore, Hudson has pointed out that the value + 19° for (β-lactose–β-cellobiose) agrees excellently with his calculated value + 18°.

As further confirmation of these relationships, we may consider lactose and maltose.

By subtraction
$$118 - 35 = G - Ga + 2L$$
$$83 = G - Ga + 102$$
$$\text{or } Ga - G = 19$$
(Hudson's calc. value = 18.)

In view of the above, it would appear possible to take the value L = 51 and use it in the case of melibiose, at least as a very fair approximation.

If the rotation of melibiose be compared with that of gentiobiose, we have the following possible cases :

	$[\alpha]_D$, β-form.
1. Melibiose an α-biose α-galactose<glucose< $Ga + L + R_2$ ...	+124°
2. Gentiobiose β-glucose<glucose< $G - L + R_2$	− 11
3. Melibiose a β-biose β-galactose<glucose< $Ga - L + R_2$.....	+124

(*a*) Melibiose as an α-biose.

From (1) and (2) the difference in rotation between the β-forms of melibiose and gentiobiose = 135 or Ga − G + 2L. From Hudson's observed values for the experiment, Ga − G = 19, and L calculated as above and confirmed = 51.

Hence calculated diff. = 121,
observed ,, = 135.

(*b*) If melibiose is a β-biose, from (2) and (3) the difference in rotation = Ga − G.

Hence calculated diff. = 19,
observed ,, = 135.

Therefore presumably melibiose must be an α-biose.

Similar calculations based on the observed figures for the octa-acetates of the disaccharides, gentiobiose, melibiose, and the synthetic 6-glucosido-β-galactose of Helferich and Rauch (*loc. cit.*),

also favour the presence of an α- rather than a β-biose linking in melibiose, and the agreement between the observed and the calculated value for the expression Ga′ — G′ (107 and 113) is also fairly close, whereas the calculated figure for melibiose as a β-biose is 11.

Occasion may here be taken to correct a misprint in the paper by Haworth, Hirst, and Ruell (*loc. cit.*), where the m. p. of 2 : 3 : 4-trimethyl β-methylglucoside is given as 74° instead of 94°. That this is a typographical error is seen from the context, since this product was described as being identified by a mixed m. p. determination with the same product from amygdalin biose (gentiobiose), described in the paper immediately preceding it in the Journal (Haworth and Wylam, *loc. cit.*), where the m. p. is given as 94·5°. Again, the m. p. 94°, and not 74°, is given in the abstract of Haworth, Hirst, and Ruell's paper published about the same date (*J. Soc. Chem. Ind.*, 1923, **42**, 1139); and is also given as 94° in the preliminary notice of papers to be read at the meeting on November 15th, 1923. These facts appear to have escaped the knowledge of Irvine and Macdonald (J., 1926, 1507), who on the basis of this misprint criticise the formula we have applied to raffinose.

Experimental.

Melibiose was obtained from raffinose by fermentation of the latter with top yeast containing a small quantity of malt sprouts as recommended by Hudson (*J. Amer. Chem. Soc.*, 1915, **37**, 2734), and the resulting disaccharide was identified as melibiose by the melting point and specific rotation of the octa-acetate.

Methylation.—To melibiose (22 g.), dissolved in 100 c.c. of water, 56 c.c. of sodium hydroxide solution (30%) and 38 c.c. of methyl sulphate were added at 30° over a period of 3 hours. By cautious and simultaneous addition of these reagents the solution was not permitted to become alkaline until the sugar had been deprived of its reducing activity. Thereafter 28 c.c. of the alkali were added, the temperature was raised to 70°, and the remaining portions of the reagents (making in all 196 c.c. of 30% sodium hydroxide and 107 c.c. of methyl sulphate) were admitted slowly. For example, lots of 14 c.c. of alkali and 7·6 c.c. of methyl sulphate were simultaneously admitted during a period of 25 minutes, with vigorous stirring. Finally, the solution was heated at 100° for an hour, then cooled, stirring being continuous during the separation of sodium sulphate. Over-night, more sodium sulphate separated. This was filtered off and washed with chloroform, and the filtrate was extracted four times with more chloroform. The latter extracts yielded 15 g. of syrupy product. The aqueous residues were neutralised, evaporated,

and extracted with alcohol, and the portion of partly methylated melibiose which was more soluble in water was thus recovered (10 g.).

The combined products were again submitted to the action of methyl sulphate and alkali, and to ensure complete methylation the products from this treatment were twice methylated with Purdie's reagents.

The material isolated by following this procedure was a viscid syrup which crystallised almost completely on nucleation with a specimen of heptamethyl methylmelibioside which had been isolated during the earlier experiments on the methylation of raffinose. The crude solid (yield, over 20 g.) was recrystallised from light petroleum several times (Found: OMe, 54·3. Calc., 54·7%). The initial m. p. of the crude methylated melibiose, 85—90°, was raised considerably by repeated crystallisation from light petroleum, and after four or six such recrystallisations the crystals had m. p. 101—103° and showed $[\alpha]_D$ + 113·8° in water (c = 1·22). Ordinarily, this might have been regarded as a pure specimen of heptamethyl β-methylmelibioside, and Schlubach and Rauchenberger (*Ber.*, 1925, **58**, 1184) describe the substance as melting at 98·5°. This material gives correct analyses, but it seemed to us doubtful whether it was stereochemically pure. By recrystallising this five times, the crystals showed a higher melting point, 106—107°, and a diminished rotation, $[\alpha]_D$ + 97·8° in water (c = 1), + 85·0° in ethyl alcohol (c = 1·1) (Found: C, 52·8; H, 8·5; OMe, 53·5. Calc.: C, 52·9; H, 8·4; OMe, 54·7%). It was thus evident that the purification had resulted in the removal of the α-stereoisomeride, and elsewhere we describe what appears to be the α-variety, m. p. 122—123°.

Hydrolysis.—A solution of 2 g. of heptamethyl methylmelibioside in 150 c.c. of 5% hydrochloric acid was heated at 95—100°. The rotations in a 1 dm. tube showed $[\alpha]_D$ 1·34° (initial); 1·22° (1 hr.); 1·21° (2½ hrs.); 1·18° (4 hours); 1·18° (5½ hours). The solution was neutralised with silver carbonate, filtered, and treated with charcoal to remove traces of silver, and the concentrated filtrate was extracted five times with chloroform. This extract on evaporation gave 1 g. of a syrup which crystallised spontaneously after 2 weeks; m. p. 71—73° (after crystallisation from light petroleum). It was recognised as 2 : 3 : 4 : 6-tetramethyl galactose by direct comparison and mixed melting-point determination with the specimen prepared from lactose by Haworth and Long (this vol., p. 544), $[\alpha]_D$ + 149·4°⟶116·9°.

The anilide also was prepared; m. p. 192—193°, unchanged in admixture with an authentic specimen of tetramethyl galactose

anilide. The portion of the hydrolysed product which was not extracted by the chloroform was recovered on evaporation of the aqueous solution under diminished pressure. This was a liquid (0·65 g.) representing the trimethyl hexose fragment of the hydrolysed product. A small quantity of this material was also present in the chloroform extract already mentioned, containing principally the tetramethyl galactose fragment, so that approximately equal weights of the latter and of the trimethyl hexose comprised the total products of hydrolysis (weighing 1·65 g.).

The syrupy trimethyl hexose was dissolved in dry methyl alcohol containing 0·5% of hydrogen chloride and heated at 100—110° for 10 hours. Neutralisation of the solution with freshly precipitated silver carbonate and removal of the solvent led to the isolation of a glucosidic substance, having no action on Fehling's solution and distilling under 0·01 mm. almost completely from a bath heated to 110—130°. The colourless distillate crystallised spontaneously in the receiver, but the crystals were contaminated with liquid, which was drained on porous tile. Evidently both a liquid α-form and a crystalline β-form of the glucoside were present. The crystalline product was purified by means of light petroleum and obtained as a mat of interlaced needles, m. p. 92°, and this m. p. was not depressed by admixture with 2 : 3 : 4-trimethyl β-methylglucoside which had previously been isolated from (*a*) methylated gentiobiose (Haworth and Wylam, J., 1923, **123**, 3120), (*b*) methylated amygdalin (Haworth and Leitch, J., 1922, **121**, 1921), (*c*) methylated raffinose (Haworth, Hirst, and Ruell, *loc. cit.*), (*d*) a specimen obtained from 6-triphenylmethyl glucose (compare Helferich, Moog, and Jünger, *loc. cit.*) which had been methylated with methyl iodide and silver oxide and hydrolysed to remove the triphenylmethyl residue.

Hydrolysis of Hendecamethyl Raffinose.—A specimen of this derivative of raffinose remained over from the previous research and it was considered desirable to obtain confirmation of the identity of the trimethyl glucose fragment occurring in the mixture of hydrolytic products obtained by digesting hendecamethyl raffinose with dilute mineral acid. Accordingly, 3·7 g. were hydrolysed at 85° with 150 c.c. of 5% hydrochloric acid. The subsequent treatment of the products followed the usual procedure, but instead of separating the mixture of partly methylated hexoses by distillation, the neutralised aqueous solution of these was extracted several times with chloroform. This treatment completely removed the fructose and galactose fragments, and the aqueous solution was now evaporated under diminished pressure, giving rise to the trimethyl hexose, which appeared as a syrup. Digestion of this syrup with methyl-alcoholic hydrogen chloride (containing 0·53% HCl) during

10 hours at 110° gave, on neutralisation followed by evaporation of the solvent, a glucoside which distilled completely, and the distillate afterwards crystallised, giving long, colourless needles, m. p. 92·5°. This was identified as 2 : 3 : 4-trimethyl β-methylglucoside by admixture with the authentic specimens previously referred to (Found : C, 50·7; H, 8·5. Calc. : C, 50·8; H, 8·5%).

Isolation of Heptamethyl Methylmelibioside from the Products of the Methylation of Raffinose [with D. A. RUELL].—On fractional distillation of crude hendecamethyl raffinose prepared by repeated methylation of raffinose (Haworth, Hirst, and Ruell, J., 1923, **123**, 3125), a syrupy product (b. p. 185—190°/0·03 mm.,; n_D 1·4660) was obtained. This solidified after some days and purification by means of light petroleum yielded crystals, m. p. 78°, which after further recrystallisation melted at 101—103° and had $[\alpha]_D$ + 113·8° in water (c = 1·22). Analytical data indicated that this crystalline substance was a completely methylated disaccharide (Found : C, 53·3; H, 8·4; OMe, 51·8. Calc. : C, 52·9; H, 8·4; OMe, 54·6%). On admixture of this compound with an authentic specimen of heptamethyl methylmelibioside, m. p. 101°, the melting point showed no depression.

Hydrolysis and Identification of Cleavage Products.—Heptamethyl methylmelibioside was hydrolysed under conditions identical with those applied in the case of methylated raffinose (Haworth, Hirst, and Ruell, *loc. cit.*) and the process was complete after 24 hours :

Time in hours	0	6	13	18	24	30
$[\alpha]_D$	+106°	93·9°	88·3°	82·3°	73·6°	74·0°

A syrupy, amber-coloured product was obtained which was soluble in dry ether. The syrup was digested in ethyl-alcoholic solution with excess of aniline for 3 hours, and on removal of the solvent and of free aniline, a semi-solid mass remained. Drainage on porous tile gave crystals which, purified by means of ethyl alcohol, appeared as silky needles, m. p. 192—193°, identical in all respects with a specimen of tetramethyl galactose anilide previously prepared from methylated raffinose or lactose, in admixture with which the m. p. was not depressed.

The syrup separating after removal of the crystals of tetramethyl galactose anilide was dissolved in alcohol, and the solution clarified with charcoal; on concentration a further small quantity of crystals separated, which were identical with the anilide mentioned above. Complete evaporation of the solution gave a viscid syrup, which was dissolved in a little aqueous alcohol and rendered faintly acid to Congo-paper with hydrochloric acid. The solution was steam-distilled in order to remove traces of aniline, and the aqueous residue

was again clarified and evaporated to dryness in a vacuum. The product obtained from this treatment was dissolved in methyl alcohol containing 0·5% of hydrogen chloride, and heated in sealed tubes at 110° for 10 hours. Thereafter the solution was neutralised with silver carbonate, and the filtrate evaporated. Extraction of this residue with dry ether yielded a viscid liquid, which distilled at 0·01 mm. from an oil-bath heated at about 140°. The distillate was a colourless liquid which partly crystallised on nucleation with 2 : 3 : 4-trimethyl β-methylglucoside. The crystals were collected and washed in light petroleum; they then melted at 90—91°. Recrystallisation from light petroleum gave m. p. 93—94°. On admixture with a specimen of 2 : 3 : 4-trimethyl β-methylglucoside obtained from a previous research on the constitution of gentiobiose, no depression of melting point was observed.

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