CCCLXX.—*The Constitution of Raffinose.*

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Raffinose is the most important of the trisaccharides, and its relationship to sucrose renders a determination of its constitution of peculiar interest in view of the new constitutional formula which has been assigned to sucrose by Haworth and his co-workers. It occurs in sugar beet and accumulates in the molasses, from which it is not removed by the strontium hydrate treatment. It is present, also, in eucalyptus manna, and in the leaves of the common yew (*Taxus baccata*), but the most fruitful source of raffinose is cotton seed. From decorticated cotton-seed meal it has been extracted for the purpose of the present work.

Raffinose yields on hydrolysis with dilute acids, melibiose and fructose; and with stronger acids, the hexoses galactose, glucose, and fructose are formed in equal proportions. With emulsin, it

changes into sucrose and galactose, whilst invertase converts the trisaccharide into melibiose and fructose.

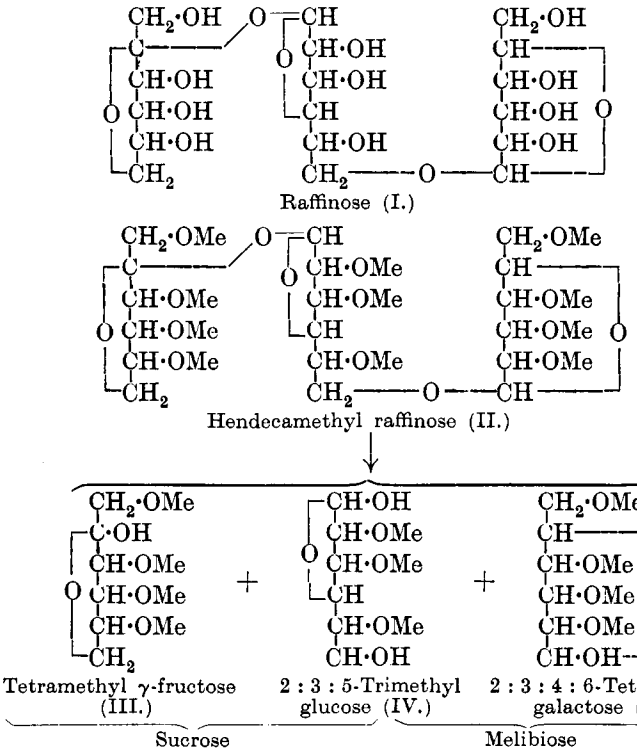
It will be clear from the above observations that raffinose is a complex containing galactose and fructose residues situated on either side of a glucose residue, and the linking between galactose and glucose must be of the β-type, since this is severed by emulsin. On the other hand, the linking which joins fructose to glucose must be similar to that in sucrose, since this union undergoes scission by means of invertase.

When raffinose was completely methylated, under the conditions described in this communication, eleven methoxyl groups were introduced, and the *hendecamethyl raffinose* was a viscid syrup distilling at 238—240°/0·02 mm., having n_D 1·4680, and $[\alpha]_D$ + 128·4° in water. During the methylation with methyl sulphate, neutral conditions were observed as far as was possible, but owing to the development of local acidity, a small proportion only of the sugar was degraded to melibiose and fructose. With use of 1 per cent. aqueous hydrochloric acid, the methylated raffinose was hydrolysed, and yielded three methylated fragments which were recognised to be tetramethyl galactose, giving a crystalline anilide identical with that derived from methylated lactose; secondly, a trimethyl glucose, which gave a crystalline glucoside, identical with that isolated from methylated amygdalin and consequently having the structure of 2 : 3 : 5-trimethyl glucose (butylene oxidic); thirdly, a tetramethyl fructose, which was dextrorotatory and identical with that obtained from methylated sucrose, so that this fructose fragment contained an amylene oxide ring structure. On the basis, therefore, of previous work with other sugars, it was easily possible to assign a structural formula to raffinose, since the degradation products could all be compared with other authentic specimens which were in our possession and for which the structural formulæ have been ascertained by oxidation methods.

The steps which have led to the allocation of the constitution I to raffinose are indicated on p. 3127.

The tetramethyl galactose formula (V) is represented as containing an amylene oxide ring, since its lactone is dextrorotatory, and must thus have its oxide ring engaging the hydroxyl group attached to the fifth carbon atom as shown by Pryde (this vol., p. 1808). It would be more correct to represent the Hudson rule as requiring that a lactone having its oxide ring on the right should show a greater dextrorotation than the acid from which it is formed, and it has been ascertained that this is the case. The usual form of tetramethyl galactose must therefore be represented as having methoxyl groups in the positions 2 : 3 : 4 : 6 and not 2 : 3 : 5 : 6

as was formerly thought to be the case, and the lactose and melibiose formulæ should now be modified to conform with this view.



From this determination of the structure of raffinose, it may be inferred that the constitution of melibiose proposed by Haworth and Leitch (T., 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.

EXPERIMENTAL.

Preparation of Raffinose.—The method of preparation adopted resembled that described by Hudson and Harding (*J. Amer. Chem. Soc.*, 1914, **36**, 2110), using decorticated cotton-seed meal as the source of the sugar. The yield of crystalline raffinose hydrate from 14·5 kilos. of cotton-seed meal was 341 grams, which melted at 77—78° and showed $[\alpha]_D$ + 101·5°. The ash content was 1·4 per cent. The figures for pure raffinose are m. p. 78°, $[\alpha]_D$ + 104°.

A sample of these crystals was purified by recrystallisation and gave pure anhydrous raffinose melting at 118°. This contained 0·41 per cent. of ash and showed $[\alpha]_D$ + 123° (c = 2).

Methylation of Raffinose.—Raffinose hydrate, in quantities of 30 grams, was dissolved in a little water, and methyl sulphate (100 c.c.) was added very slowly, along with the equivalent quantity of alkali (100 grams of sodium hydroxide in 30 per cent. solution). During the procedure, which occupied about four hours, the temperature was maintained at 70°, and the mixture was thoroughly agitated by mechanical stirring. When the whole of the reagents had been added, the solution was heated for an hour at 100°. On cooling, sodium sulphate separated, and the supernatant liquor was decanted and extracted thrice with chloroform. The separated solid was also extracted with this solvent. The united chloroform extracts were dried and evaporated, and yielded only 20 grams of a syrup. The poor yield at this stage showed that much of the product remained in the aqueous solution and this was verified by a polarimetric reading. Consequently, the aqueous portion was evaporated and the residue, after powdering, was extracted with absolute alcohol under reflux. This treatment yielded a yellow syrup weighing 15·3 grams and containing only little mineral matter. The collected chloroform extracts were subjected to further methylation with methyl sulphate, and likewise also the syrup, extracted with alcohol, from the aqueous portions. In all, 105 grams of raffinose hydrate were methylated, corresponding to 94 grams of raffinose, and the total amount of syrup collected after two methylations was 80 grams. After one methylation with silver oxide, the product contained 46·7 per cent. of methoxyl. In order to ensure complete methylation, this material was again treated three times with Purdie's reagent.

Isolation of Hendecamethyl Raffinose.—The product arising from the repeated methylation of raffinose was purified by fractional distillation, and this operation was conducted several times with methylated products prepared on separate occasions. The following example serves to illustrate the general procedure. The distillate from 15 grams was collected in three fractions, which are indicated below along with their physical properties.

	Boiling point.	Weight.	n_D.
Fraction I	76—150°/0·15 mm.	0·65 gram	1·4594
Fraction II	179—226°/0·1 mm.	3·2 grams	1·4660
Fraction III	238—240°/0·02 mm.	7·7 ,,	1·4680

The first two fractions distilled as colourless liquids from an oil-bath, but the third fraction required the use of a metal-bath, by the aid of which the distillation proceeded steadily, yielding

what proved to be the hendecamethyl raffinose which, on cooling, changed from a faintly coloured liquid to a viscid, amber syrup. There remained in the distilling flask a brown residue weighing about 3 grams and this was collected with other similar residues left from a corresponding distillation, when together they yielded a further quantity of the material represented by fraction III. Fraction I gave rise on further rectification to a mobile liquid distilling at 82°/0·07 mm. and having n_D 1·4628; it gave analytical figures agreeing with those of a tetramethyl hexose. Fraction II crystallised on keeping, and, after purification from light petroleum, melted at 78° with previous sintering at 72·5°. Analysis showed that this crystalline compound was a completely methylated disaccharide (melibiose).

A more detailed examination of the main fraction, III, was undertaken, and the analytical evidence indicated that this was a completely methylated specimen of raffinose containing eleven methoxyl groups (Found: C = 52·69; H = 8·18; OMe = 49·9. $C_{29}H_{54}O_{16}$ requires C = 52·9; H = 8·2; OMe = 51·8 per cent.). The optical rotations showed appreciable variation under different temperature and concentration conditions, a circumstance which is to be attributed to the presence of fructose and galactose residues in the compound.

	$[\alpha]_D$.	Temp.	Solvent.	c.
+	126·1°	18·5°	Water	1·838
	128·4	16·5	Water	1·005
	112·1	18	Ethyl alcohol	1·05
	112·7	16	Ethyl alcohol	1·05

The completely methylated raffinose had no reducing action on Fehling's solution and showed no tendency to crystallise. It remained over a long period without darkening as an amber-yellow, viscid syrup. Its behaviour towards hydrolytic agents indicated that it was less sensitive than methylated sucrose. Hydrochloric acid of 0·5 per cent. concentration had little effect at 80°. On the other hand, it was hydrolysed with considerably greater ease than methylated reducing disaccharides like maltose or lactose. Using 1 per cent. hydrochloric acid, no change was observed in the polarimetric readings until a temperature of 85° was reached.

Isolation of the Cleavage Products of Hendecamethyl Raffinose.

After a series of preliminary experiments designed to effect complete hydrolysis of hendecamethyl raffinose under conditions of temperature and of acid concentration which would not impair the yield or purity of the cleavage products, the following conditions were finally adopted. The hydrolysing medium was 1 per cent. aqueous hydrochloric acid containing 2 per cent. of the

methylated sugar in solution. This mixture was heated under reflux on a water-bath at 90°, and polarimetric readings were taken during a period of twenty-two and a half hours. From these records, which are reproduced below, it appeared that the rotation reached a constant value after eighteen and a half hours, when the specific value showed a diminution from + 116° to 82°. If the latter figure be corrected for the weight of hexoses generated, it appears as + 77·5°.

Hours	0	5	9	10	15½	16½	18½	20½	22½
$[\alpha]_D$	+ 116·2°	102·8°	94·4°	93·0°	86·4°	84·2°	82·1°	82·1°	82·1°

On the completion of this hydrolysis, the solution was neutralised with barium carbonate, and the filtrate from the mineral matter was concentrated at low temperature and pressure until the barium chloride could be conveniently removed by the addition of alcohol. Repeated treatment with absolute alcohol produced a filtrate which was free from mineral salts, and evaporation of the alcohol left a syrup which was completely soluble in dry ether. Distillation of the latter solvent yielded a straw-yellow, mobile syrup which represented 80 per cent. of the original weight of methylated raffinose. This was divided into three fractions on distillation; the first of these was collected at 117—120°/0·035 mm. and showed n_D 1·4567; the second distilled at 132—135°/0·4 mm. and showed n_D 1·4676, and there remained as residue an approximately equal amount, which was not distilled but was purified by glucoside formation. Redistillation of the first fraction gave a pure, colourless liquid, boiling at 110°/0·2 mm., having n_D 1·4558, and a specific rotation of + 31·7° (final value). The combined analytical data and general behaviour proved the identity of this cleavage product with tetramethyl γ-fructose (III) (Found: OMe = 49·0 per cent.). It reduced neutral permanganate freely, and behaved in other respects in exactly the same manner as a specimen of this compound prepared from methylated sucrose (Haworth, T., 1920, **117**, 199).

The second fraction and the still residue, indicated above, evidently contained both the galactose and the glucose fragments and, although the fractional distillation led to a rough separation of these two products, the second fraction mentioned above was evidently not quite homogeneous. It consisted largely of a tetramethyl hexose, and on digesting for three hours with five times its weight of aniline and sufficient absolute alcohol to make a 5 per cent. solution of the sugar, a crystalline anilide separated as long, slender needles, which were purified from ethyl acetate. The anilide melted at 192° and showed $[\alpha]_D^{21°}$ − 83·0° in acetone (c = 0·606). After two hundred hours, the specific rotation had changed to the value + 40·0°. These data corresponded exactly with those

of the anilide of tetramethyl galactose (V) (Haworth and Leitch, T., 1918, **113**, 188), which had been prepared from lactose, and a mixed melting point determination with an authentic specimen of the anilide, prepared from galactose, showed no depression.

The still residue, remaining from the previous distillation, was a yellow, viscid syrup. This was dissolved in 50 c.c. of 0·5 per cent. methyl alcoholic hydrogen chloride and heated under pressure at 110° for ten hours. At the end of this time it no longer reduced Fehling's solution, and had been converted into a methyl glucoside. On neutralisation of the acid with silver carbonate and evaporation under diminished pressure, a liquid was obtained which distilled at 109°/0·05 mm. and showed n_D 1·4562, and this partly crystallised on keeping, yielding slender, colourless needles, which could be easily separated from the liquid portion. On purification from light petroleum, they melted at 74° and showed $[\alpha]_D^{17°}$ — 24·8° in methyl alcohol (Found: OMe = 51·5 per cent.). The crystalline product showed the behaviour of a trimethyl methylglucoside and resembled very closely the product isolated by Haworth and Leitch from methylated amygdalin. The specimen of 2 : 3 : 5-trimethyl β-methylglucoside obtained by these authors was mixed with a portion of the crystals isolated as described above and a melting-point determination showed no depression. It was therefore clear that the product consisted of a methyl derivative of 2 : 3 : 5-trimethyl glucose (IV), and consequently this sugar represents the glucose fragment from the original methylated raffinose. The uncrystallised portion of the glucoside was evidently the α-form of the glucoside of this sugar.

The authors are grateful to the Carnegie Trust for a scholarship and to the Armstrong College Council for a fellowship which enabled two of the authors to engage in this work.

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[*Received, November 5th*, 1923.]