XXV.—*The Constitution of the Disaccharides.*
Part IV. The Structure of the Fructose Residue in
Sucrose.

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A NEW constitutional formula for sucrose (I) was proposed in a former communication in this series, where it was shown that the

Fischer's formula for sucrose.

fructose residue contained in sucrose could not possess the structure generally assigned to it (Haworth and Law, T., 1916, **109**, 1314).

The data on which this conclusion was based may be summarised as follows: (1) On hydrolysis with dilute acids, octamethyl sucrose does not show the phenomenon of "inversion," as does sucrose, whereas, in conformity with the usual experience, the cleavage of the methylated disaccharide ought to give rise to a molecular mixture of tetramethyl glucose and tetramethyl fructose which would be predominantly laevorotatory.

(2) This unexpected result was traceable to the fructose residue which, differing in structure from ordinary fructose, displayed a pronounced dextrorotation.

(3) The crystalline tetramethyl fructose (IV), isolated by Purdie and Paul (T., 1907, **91**, 296) during their study of the methylation of methyl fructoside, showed a specific rotation of $[\alpha]_D -121^\circ$ in aqueous solution, whilst the corresponding product derived from the hydrolysis of octamethyl sucrose by Haworth and Law (*loc. cit.*) was a liquid which distilled easily, but did not crystallise on nucleation with a specimen of Purdie and Paul's compound. Moreover, the new form of tetramethyl fructose was unstable towards permanganate and behaved uniformly as a sugar derivative of the γ - or supposed ethylene-oxide type.

Opportunity has again been taken to isolate this new form of tetramethyl fructose and to submit it to further constitutional study. The isolation of this compound in a state of purity from the products of hydrolysis of octamethyl sucrose was attended with much experimental difficulty. This was due to its occurrence along with the isomeric tetramethyl glucose, since the latter distils at approximately the same temperature as the methylated fructose fragment.

In the present series of experiments it has been found that the difficulty formerly experienced in separating the cleavage products vanishes if heptamethyl sucrose is substituted for the completely methylated biose in a parallel experiment. The advantage conferred by the use of heptamethyl sucrose is attributable to the nature of its scission products, which distil at temperatures sufficiently remote from each other to admit of easy separation by fractional distillation. The partly methylated biose was obtained by methylating sucrose in alkaline solution with methyl sulphate (Haworth, T., 1915, **107**, 12), and this has been shown to be a pure chemical individual, in which the fructose constituent is fully methylated, but only three methyl groups are present in the glucose component.

When heptamethyl sucrose was hydrolysed with 0.4 per cent. hydrochloric acid at 60° for six and a-half hours the specific rotation

diminished from $[\alpha]_D + 64.3^\circ$ to $+52^\circ$, and at the latter value remained constant. The products of this change consisted of a molecular mixture of a trimethyl and a tetramethyl hexose, which was easily separable by distillation at the Gaede pump. The first fraction distilled as a colourless, mobile liquid at $110\text{--}112^\circ/0.35\text{ mm.}$, having $n_D 1.4545$, and gave analytical results corresponding with a tetramethyl hexose. A second fraction was collected as a viscid syrup, boiling at $170^\circ/0.43\text{ mm.}$, showing $n_D 1.4755$, and analysis proved it to be a trimethyl hexose. The amounts of both fractions were approximately equal.

Clearly it was of importance to decide which of these individual fractions represented the glucose or fructose residue in the original sucrose. This point was established by submitting the trimethyl hexose to further methylation, when it was converted into a crystalline substance melting at 83° , showing $[\alpha]_D + 84^\circ$, and identical with tetramethyl glucose (compare Haworth and Law, *loc. cit.*, p. 1324), a result which afforded ample proof that the trimethyl hexose was a glucose derivative. Consequently, it follows that the tetramethyl hexose having the lower boiling point represented the fructose residue in sucrose.

Attention has been focussed in the course of the present investigation on this specimen of tetramethyl fructose. In the previous communication on this subject (*loc. cit.*) an approximation only could be made as to the probable specific rotation of this compound in aqueous solution, and the value was tentatively given as $[\alpha]_D + 29.3^\circ$. The correct value, determined experimentally for the first time, differs only slightly from this anticipated result, and is now recorded as $[\alpha]_D + 31.7^\circ$. The substance is very unstable towards permanganate, and conforms to the new type of hexoses which have been provisionally named ethylene-oxide sugars. Further, the constitution previously assigned to the new variety of tetramethyl fructose (III) has been subjected to close experimental scrutiny:

Tetramethyl fructose
(ethylene-oxidic).

Tetramethyl fructose
(butylene-oxidic).

In allocating this structural formula (III) to the fructose residue

in sucrose the only basis on which one could proceed was the valuable group of facts recorded by Irvine, Fife, and Hogg during their investigation of the new variety of tetramethyl glucose (V), described as a γ -sugar (T., 1915, 107, 524). These authors showed that a derivative of γ -glucose examined by them showed a remarkable capacity to increase its oxygen content to the extent of one atomic proportion during methylation with silver oxide and methyl iodide. Surprising though this behaviour may be, it finds a parallel in the oxidation of glucose with phenylhydrazine which gives rise to the glucosazone. It is established that in the latter case the oxidation change effects removal of the hydrogen atoms attached to the second carbon atom from the reducing end of the hexose chain. The analogy which has been drawn between the two reactions leads to the suggestion that in both cases oxidation is confined to the groups associated with the carbon atom which adjoins the reducing group:

The above formulæ are given in this simple form for the sake of clearness; it will be recognised that the two products of oxidation indicate only intermediate stages of the reactions which have been studied. The main point is, that if the analogy be admitted, the ethylene-oxide form of methyl glucoside is the only one that can hold for the new type of sugar derivative.

In order to furnish a proof of this structural arrangement, Irvine, Fife, and Hogg (*loc. cit.*) submitted a derivative of the new type of γ -sugar, namely, tetramethyl γ -glucose (V), to (a) reduction,

(b) oxidation. The product of reduction was a tetramethyl sorbitol (VI), which showed optical exaltation in the presence of boric acid, and thus, in the light of Böeseken's recent work (*Ber.*, 1913, **46**, 2612), the compound contained two hydroxyl groups attached to neighbouring carbon atoms, a result which affords strong support to the ethylene-oxide formula of the sugar.

On the other hand, oxidation of the sugar (V) with permanganate gave rise to a unimolecular lactone, the formation of which would appear to be precluded if the ethylene-oxide structure were adopted as above. Whilst, therefore, this mode of representation was provisionally proposed for the new or γ -sugars, it was recognised that other interpretations were possible.

Consequently, in the special case under review, namely, that of the fructose residue isolated from heptamethyl sucrose, it was considered of first importance to provide some additional experimental basis on which the structure assigned to this compound could rest. Apart from the butylene-oxide form of tetramethyl fructose (IV) which has been definitely characterised, there would appear to be no fewer than four possible formulæ by which the tetramethyl fructose isolated in the course of the present investigation may be represented; these are given as the following, and for the sake of comparison the oxidation products to which they may be expected to give rise are printed below in the vertical column:

The choice of oxidising agent, namely, dilute nitric acid, was determined by the fact that oxidation of a sugar of the formula (IX), if effected with bromine water, would leave a primary alcohol group exposed, whilst nitric acid would oxidise both the reducing and primary alcohol groups. On submitting the specimen of tetramethyl fructose to oxidation with nitric acid at 60° , a viscid liquid was formed which distilled at $190\text{--}200^{\circ}/1.8\text{ mm.}$, and analysis gave $C=48.17$; $H=7.43$; $OMe=43.4$. Such a result is not in agreement with any of the figures quoted above, although it approximates closely to those required for dehydration of two molecular proportions of $C_8H_{16}O_6$, namely, $C_{16}H_{30}O_{11}$, which requires $C=48.24$; $H=7.54$; $OMe=46.7$ per cent. A determination of molecular weight by the cryoscopic method gave 354; that required by the bimolecular anhydride is 398, whilst the unimolecular formulæ require approximately half this value.

Inspection of the formula (X) will indicate that this represents an α -hydroxy-acid, and may be referred to lactic acid as a type. It is well known that lactic acid undergoes conversion into an anhydride or semi-lactide, either by heating at $130\text{--}140^{\circ}$, or spontaneously at the ordinary temperature in a dry atmosphere. The formula (XII) admits of the possibility of the formation of a unimolecular β -lactone, since Nef has shown that mannono- β -lactone is easily formed, but passes into aqueous solution as regenerated mannonic acid (*Annalen*, 1914, **403**, 204). Nef also mentions the existence of bimolecular α -lactones, by which is meant substances analogous to the semi-lactide from lactic acid, which has already been quoted. On the other hand, formulæ (XI) and (XIII) do not admit of the possibility of dehydration except by the ordinary process of preparing acid anhydrides.

It was ascertained by analysis that, in the particular experiment under review, when the product derived from the oxidation of the tetramethyl fructose was isolated from aqueous solution by evaporation of the solvent, anhydride formation had already occurred, and a consideration of all the possible formulæ leads to the conclusion that this could only take place with an α -hydroxy-acid. It will be remembered that the oxidation product was found to possess the formula $C_{16}H_{30}O_{11}$, and is thus bimolecular. Moreover, the compound was acid towards litmus, and when titrated with alkali it behaved as an acid until about half the total alkali required for complete neutralisation had been added. From this point onward the substance exhibited all the properties of a lactone, absorption of additional alkali taking place with extreme slowness. The final result corresponded exactly with the requirements for a semi-

lactide (XIV) of the formula $C_{16}H_{30}O_{11}$, and full details will be found in the experimental part.

From these considerations it will be clear that the oxidation product must have been formed from an acid of formula (X), which in turn can only represent a product of degradation of a tetramethyl fructose having the formula (III):

This result furnishes valuable evidence in support of the constitutional formula (I) for sucrose, advanced in an earlier communication by Haworth and Law (this series, Part I), and the hydrolysis of octamethyl sucrose therein described may now be formulated:

A similar representation would apply to the hydrolytic cleavage of heptamethyl sucrose, except that in this case a butylene-oxide form of trimethyl glucose is obtained as the glucose constituent.

An explanation of the observed facts that, when free sucrose is hydrolysed, glucose and ordinary laevorotatory fructose are found as the products, is given in Part I. of this series. It is there stated that although the ethylene-oxide form of fructose must first be produced, the hydrolysis is accompanied by a supplementary structural change involving the transformation of the fructose residue at the moment of cleavage into a butylene-oxide form. Such supple-

mentary changes are inhibited if the hydroxyl groups in sucrose are protected by methyl groups.

Although the additional evidence here contributed as to the structure of sucrose appears capable of no simple explanation other than that now offered, the constitution of the new form of tetramethyl fructose is being further investigated in view of the importance of the issues.

EXPERIMENTAL.

Hydrolysis of Heptamethyl Sucrose.

Heptamethyl sucrose (28 grams), prepared by the method already described (Haworth, T., 1915, **107**, 12), was dissolved in 450 c.c. of 0.4 per cent. aqueous hydrochloric acid and the mixture heated in a water-bath maintained at 60°. Polarimetric readings were taken from time to time, and hydrolysis was complete in six and a-half hours:

Time.	<i>l.</i>	α_D .	$[\alpha]_D$.
5 minutes	2-dcm.	+8.0°	+64.3°
1 hour		7.0	
1½ hours		6.46	
3 " 		6.76	
6½ " 		6.74	+54.0

At this stage the acid was neutralised with barium carbonate, and the solution filtered and evaporated under diminished pressure in presence of a trace of barium carbonate. A syrupy residue remained, which was contaminated with barium salts. The product was dissolved in absolute alcohol, filtered to remove mineral matter, and the solvent distilled. A further treatment with ether served to remove the whole of the suspended salts. Thereafter the residue was submitted to fractional distillation at the Gaede pump.

Nearly half the total product distilled as a colourless, mobile liquid at 110—120°/0.35 mm., whilst the remainder was collected at 154—170°/0.6 mm. On repeated refractionation of each portion two main fractions were obtained:

1. . . . b. p. 110—112°/0.32 mm. n_D 1.4545.
2. . . . b. p. 168—170°/0.43 mm. n_D 1.4755.

The latter substance was a viscid syrup, which failed to give an osazone on treatment with phenylhydrazine. On analysis it was shown to be a trimethyl hexose:

0.1206 gave 0.2149 CO₂ and 0.0888 H₂O. C=48.60; H=8.19.

0.1284 gave, by Zeisel's method, 0.3988 AgI. OMe=41.0.

C₆H₉O₃(OMe)₃ requires C=48.65; H=8.11; OMe=41.9 per cent.

This trimethyl hexose was submitted to further methylation by

methyl iodide and silver oxide. The product was thereafter hydrolysed with 8 per cent. hydrochloric acid at 100° for three hours in order to remove the glucosidic group and obtain the tetramethyl hexose. The latter was isolated in the usual way, and was found to consist of a liquid which quickly crystallised. Recrystallisation of the solid from light petroleum yielded characteristic prismatic needles, which were easily identified as tetramethyl glucose, melting at 83° , and showing no depression in melting point when mixed with a specimen of this sugar isolated on a previous occasion (T., 1916, 109, 1324). The specific rotation was $[\alpha]_D +84^{\circ}$ in equilibrium in water.

Since tetramethyl glucose of the butylene-oxide form was isolated as the result of this experiment, there could be no doubt that the trimethyl hexose distilling at the higher temperature in fraction (2) was a trimethyl glucose of the normal type. This showed $[\alpha]_D +55.7^{\circ}$ in methyl alcohol and $+56.0^{\circ}$ in aqueous solution, and its further study will form the subject of a future communication.

A New Form of Tetramethyl Fructose (III).

The first fraction distilling at $110\text{--}112^{\circ}/0.32$ mm. was also found to boil at $154^{\circ}/13$ mm. It decolorised permanganate readily, and behaved generally as a γ -sugar. The following analytical results were obtained:

0.2220 gave 0.4141 CO_2 and 0.1700 H_2O . $\text{C}=50.87$; $\text{H}=8.51$.

0.1420 „ 0.5430 AgI . $\text{OMe}=50.3$.

$\text{C}_6\text{H}_8\text{O}_2(\text{OMe})_4$ requires $\text{C}=50.85$; $\text{H}=8.47$; $\text{OMe}=52.5$ per cent.

The specific rotation of the sugar was determined.

Solvent.	$[\alpha]_D$.
Water.....	$+31.7^{\circ}$ (final reading)
Methyl alcohol	$+21.3^{\circ}$

When the aqueous solution was catalysed with a trace of hydrochloric acid, the specific rotation increased to $+32.6^{\circ}$.

Oxidation with Nitric Acid.—The tetramethyl fructose (4.75 grams) was oxidised by heating with dilute nitric acid (D 1.2) at 60° for five hours. The water and most of the nitric acid were removed by vacuum distillation at low temperature, and the residue was treated successively with absolute alcohol and ether, followed by distillation of the solvent and continued heating in the vacuum of the Gaede pump until the whole of the nitric acid was removed.

In a preliminary attempt to purify the residual syrup it was dissolved in ether and shaken in a separating funnel with small quantities of water. There remained in the ethereal layer about one-fifth of the original amount of the syrup, and after drying and

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removal of solvent this small residue was dried in a stream of air in a vacuum, when it gave the following analytical results:

0.1550 gave 0.2754 CO_2 and 0.1076 H_2O . $\text{C}=48.45$; $\text{H}=7.71$.

$\text{C}_{16}\text{H}_{30}\text{O}_{11}$ requires $\text{C}=48.24$; $\text{H}=7.54$ per cent.

The portion remaining in the aqueous layer was now extracted with much ether and the solvent removed by distillation. The residue was dried by heating at 100° in the vacuum of the Gaede pump, and at this stage a sample was removed for analysis:

0.1803 gave 0.3154 CO_2 and 0.1233 H_2O . $\text{C}=47.71$; $\text{H}=7.59$.

The syrup was then distilled, when it was observed that very little decomposition occurred. A small first fraction was collected as an amber-coloured syrup distilling at $185\text{--}190^\circ/1.8$ mm., and a large second fraction was obtained, boiling at $190\text{--}200^\circ/1.8$ mm. and having $n_D=1.4575$. The first fraction was analysed as follows

0.1236 gave 0.2210 CO_2 and 0.0829 H_2O . $\text{C}=48.76$; $\text{H}=7.45$.

The second fraction gave the figures:

0.1042 gave 0.1841 CO_2 and 0.0697 H_2O . $\text{C}=48.17$; $\text{H}=7.43$.

0.1092 „ 0.3590 AgI . $\text{OMe}=43.4$.

$\text{C}_{10}\text{H}_{12}\text{O}_5(\text{OMe})_6$ requires $\text{C}=48.24$; $\text{H}=7.54$; $\text{OMe}=46.7$ per cent.

This corresponds with an anhydro-acid (XIV) or semi-lactide, $\text{C}_{16}\text{H}_{30}\text{O}_{11}$. The substance appeared to be hygroscopic, and quickly absorbed water from a moist atmosphere.

During the titration with $N/10$ -sodium hydroxide the syrupy oxidation product behaved at first as an acid, and finally as a lactone. Phenolphthalein was used as indicator. 0.1192 Gram was dissolved in water and titrated with $N/10$ -sodium hydroxide. The alkali was rapidly absorbed until 2.9 c.c. had been added; thereafter additional alkali was absorbed with extreme slowness at the ordinary temperature. On adding in all 10 c.c. and heating under reflux for a quarter of an hour, the cooled solution was neutralised with $N/10$ -sulphuric acid and required 3.85 c.c. The total alkali which had combined with the compound was therefore 6.15 c.c. The theoretical amount required for an anhydro-acid, $\text{C}_{16}\text{H}_{30}\text{O}_{11}$, is 5.98 c.c.

A determination of molecular weight in benzene solution was carried out by the cryoscopic method, when the values found were 354.1 and 343.5. The anhydro-acid, $\text{C}_{16}\text{H}_{30}\text{O}_{11}$, requires $\text{M.W.}=398$.

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