LXXXIX.—*Melezitose and Turanose.*

By Grace Cumming Leitch.

The discovery by Hudson and Sherwood (*J. Amer. Chem. Soc.*, 1918, **40**, 1456) that the manna found adhering to the twigs and needles of the Douglas fir consists largely of melezitose has made it possible to obtain this trisaccharide in a pure condition, and Harding (*Sugar*, 1923, 240) has shown how best to isolate the sugar from this source.

As the elucidation of the structure of this trisaccharide would solve simultaneously the problem of the structure of the reducing disaccharide turanose, an investigation was carried out by the methylation method. The conclusion was drawn that melezitose consists of two glucose residues of amylene-oxidic type united with one fructose residue of the type found in sucrose. While research on the nature of the fructose derivative was proceeding, two papers on the same topic were published by Zemplén and Braun (*Ber.*, 1926, **59**, 2230, 2539), who have arrived at a similar conclusion. Since the experimental method hereafter described differs from that of Zemplén, and since, in addition, proof is given that the fructose residue contains an oxide linking similar to that in sucrose, it was considered advisable to submit the following confirmatory evidence with regard to the nature of these two sugars.

Methylation of melezitose by the usual method with methyl sulphate and sodium hydroxide gave *hendecamethyl melezitose*, a thick syrup which could be distilled under a high vacuum. On hydrolysis with hydrochloric acid sufficiently concentrated to rupture completely the methylated trisaccharide, this syrup yielded amylene-oxidic tetramethyl glucose and trimethyl γ-fructose in the proportion 2 : 1.

Conditions of hydrolysis similar to, but slightly more drastic than, those used by Tanret (*Compt. rend.*, 1906, **142**, 1424) for the

partial hydrolysis of the unsubstituted sugar into turanose and glucose were also employed. A pure methylated turanose, however, could not be thus obtained, for scarcely any change in rotation took place as hydrolysis proceeded and therefore polarimetric observations were of little avail for detecting its completion. Tanret's method was therefore abandoned, since, during hydrolysis with hydrochloric acid, no such difficulty was encountered, and the products could be separated from each other in a pure condition by extraction of the solution with chloroform after neutralisation with barium carbonate.

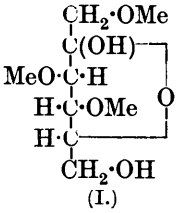
Most of the trimethyl γ-fructose remained in the aqueous solution, but some of it, together with the tetramethyl glucose, was dissolved by the chloroform. As this does not take place in the separation of tetra- and tri-methyl glucoses (Irvine and Black, J., 1926, 862; Cooper, Haworth, and Peat, *ibid.*, p. 876), the conclusion is drawn that the presence of tetramethyl glucose in an aqueous solution induces extraction of some of the trimethyl γ-fructose by chloroform, although the latter has no solvent effect on trimethyl glucose under the special conditions of the experiments. This may be due to the fact that in the one case a derivative of a γ-sugar is under investigation, whilst in the other both the tetra- and the tri-methyl glucose are of the stable or amylene-oxidic type.

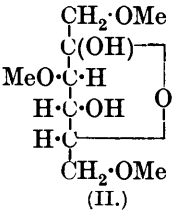
On concentration of the chloroform extract, tetramethyl glucose of the stable or amylene-oxidic type was isolated. The trimethyl fraction which remained in the aqueous solution was proved to be a ketose by the deep red coloration which it imparted to Seliwanoff's reagent (resorcinol and hydrochloric acid). It readily reduced Fehling's solution and decolorised neutral potassium permanganate in the cold. $[\alpha]_D$ in absolute ethyl alcohol, + 55·5° (Zemplén and Braun record $[\alpha]_D$ + 24°).

Zemplén and Braun apparently assumed that the fructose fragment of melezitose contains the same type of oxide linking as is present in the fructose of inulin and sucrose. It was obviously necessary to prove this, and the trimethyl fructose was therefore methylated with silver oxide and methyl iodide; the mobile syrup (n_D 1·4445) thus obtained gave, as the sole product of hydrolysis, a reducing sugar which agreed in physical constants with the tetramethyl γ-fructose resulting from the hydrolysis of octa- or hepta-methyl sucrose (Haworth, J., 1920, **117**, 199). This precluded the possibility of the comparatively high specific rotation of the trimethyl fructose being due to traces of tetramethyl glucose.

Account being taken of the recent arguments of Haworth and

Hirst (J., 1926, 1858) regarding the structure of γ-fructose derivatives, the four trimethyl γ-fructoses are :


(I.)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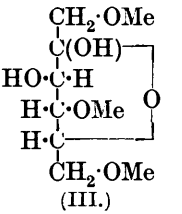
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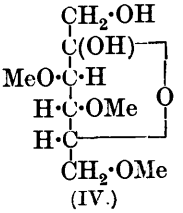
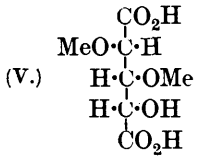

(IV.)

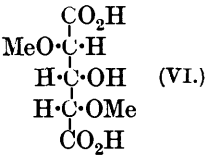
Of these, (IV) is ruled out, since turanose is capable of forming an osazone (Fischer, *Ber.*, 1894, **27**, 2486). The hydroxyl in the position next the ketose group cannot therefore be engaged in a biose linking, whence it follows that it must be methylated in the fructose fragment. In the early experiments carried out by the author, the trimethyl fructose was distilled before being analysed; the yield of trimethyl fructose which distilled was not good, and a certain proportion invariably remained as a non-volatile residue. The methoxy-content varied between 42% and 45% (Calc. for trimethyl fructose, 41·9%; for trimethoxy-anhydro-hexose, 45%). As the trimethyl fructose can be isolated in a pure condition without distillation, as seen above, this method was abandoned, but it was useful in having supplied a clue to the structure of the trimethyl fructose component. It appeared probable that distillation had resulted in the elimination of a molecule of water with consequent formation of an anhydro-fructose derivative. Anhydride formation is known to take place in 2 : 3 : 4-trimethyl glucose (Irvine and Oldham, J., 1921, **119**, 1744), which is partly converted into trimethyl glucosan on distillation. Comparison of rotation values is therefore not a trustworthy means of settling the identity of the substances under investigation, since one specimen may exist as the free sugar derivative and the other may be partly converted into an anhydro-sugar derivative.

Substances having formulæ (III) and (IV) would, on formation of anhydro-fructose derivatives, yield substances containing an ethylene-oxide linking, whilst in the product from (II) there would be present a propylene-oxide ring. No positive evidence of the existence of either of these two types of ring compounds in methylated fructose derivatives is found in the literature, whilst Haworth and Hirst (*loc. cit.*) have definitely proved that an amylene-oxide ring exists in the normal or stable type of fructose. Anhydride formation is therefore most likely where the formation of an oxide ring of this type is possible. Formula (I) admits of this, and therefore, in all probability, represents the trimethyl fructose obtained

from methylated melezitose. This deduction agrees with the experimental results of Zemplén and Braun quoted below. These authors have ruled out (III) on the ground that the reduction product of trimethyl fructose shows no alteration in rotation on addition of boric acid and therefore cannot have vicinal free hydroxy-groups. They have ascribed to trimethyl fructose formula (I), since they obtained from it by oxidation a hydroxydimethoxy-glutaric acid (V) which was not identical with authentic β-hydroxy-αα′-dimethoxyglutaric acid (VI) obtained by them by other means.

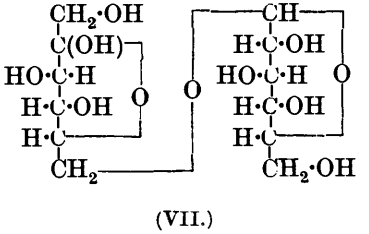


(V.)

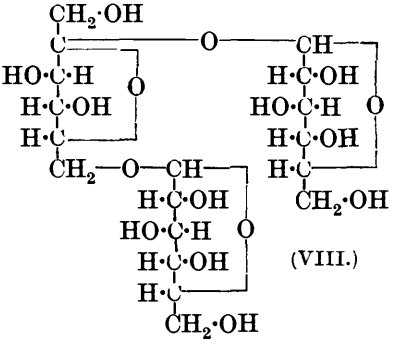


(VI.)

Turanose must therefore be represented by (VII) and melezitose by (VIII).



(VII.)



(VIII.)

Experimental.

Methylation of Melezitose. Isolation of Hendecamethyl Melezitose. —(The melezitose used in the following operations was supplied by Messrs. Baird and Tatlock, agents for The Digestive Ferments Co., Detroit, Mich., U.S.A.) Melezitose (25 g.), dissolved in the minimum volume of water, was methylated at 70° in the usual way (Haworth, J., 1915, **107**, 11) with 80 c.c. of methyl sulphate and 80 g. of 30% aqueous sodium hydroxide, care being taken to avoid acidity. The mixture was finally raised to 100° during the last half-hour of the methylation. Extraction with chloroform yielded a partly methylated syrup, which was again methylated, half-quantities of the methylating reagents being used. The chloroform extract (24 g.) was remethylated four times by Purdie's method, with excess of silver oxide and methyl iodide, 24 g. of

syrup being again obtained. This material was distilled in the vacuum of a Gaede pump; 19·5 g., b. p. 236°/0·01 mm., were then obtained [Found: OMe, 49·7; C, 52·7; H, 8·2. $C_{18}H_{21}O_5(OMe)_{11}$ requires OMe, 51·8; C, 52·9; H, 8·2%]. The distillate had n_D 1·4680, and $[\alpha]_D$ + 114° in methyl alcohol (*c*, 0·85) and + 108·6° in ethyl alcohol (*c*, 2·2). These results agree with Zemplén and Braun's $[\alpha]_D$ + 113·4° in alcohol.

Hydrolysis of Hendecamethyl Melezitose.—Method 1. This method, which was afterwards abandoned in favour of method (2) for the reason already given, consisted in applying conditions of hydrolysis similar to those employed by Tanret (*loc. cit.*) for the graded rupture of the hendecamethyl melezitose into a substituted hexose fraction and a methylated disaccharide fraction.

8 G. of an 8% solution of hendecamethyl melezitose in 20% acetic acid were hydrolysed by heating for 2 hours at 95°, the specific rotation changing from + 101·2° to + 102°. Further heating for 1 hour at 110° resulted in a further change in rotation to $[\alpha]_D$ + 103·6°. Chloroform then extracted from the mixture, neutralised by barium carbonate, 7·25 g. of a reducing syrup (n_D 1·4650). Evaporation of the aqueous solution to dryness and subsequent digestion of the barium acetate with chloroform gave no further syrup. From solubility considerations, the syrup was therefore not expected to contain any trimethyl hexose. (Tetramethyl glucose and heptamethyl turanose would both be extracted from aqueous solution by chloroform.) Distillation of the syrup remaining after removal of the chloroform gave two fractions: (1) b. p. 115°/0·3 mm. This fraction crystallised at once and was identified as tetramethyl glucose; (2) b. p. 185—190°/0·2 mm., n_D 1·4652 (Found: OMe, 46·7; C, 50·4, 50·9; H, 8·4, 8·4. Calc.: OMe, 49·3; C, 51·8; H, 8·2%).

The above figures are not in agreement with those for a heptamethyl turanose, although the two analyses agree with each other. In order to discover whether this syrup would yield tetramethyl glucose and trimethyl fructose on hydrolysis, it was heated for 1 hour at 95° with 5% hydrochloric acid. The solution had then darkened considerably and the initial rotation of + 127° had changed to + 78° and was constant. Isolation of the products followed, by the method described below, and tetramethyl glucose and a trimethyl fructose were identified.

Method 2. *Isolation of tetramethyl glucose and trimethyl γ-fructose.* Hendecamethyl melezitose (10 g.) was dissolved in 5% hydrochloric acid to give an 8% solution. After 2 hours' heating at 95°, no undue darkening of the solution had taken place and the rotation had changed from the initial value + 104° to + 70° and was constant.

The solution was diluted with water, neutralised with barium carbonate, and shaken with an excess of chloroform, whereby the syrupy product was separated into a chloroform-soluble portion (A) and a water-soluble portion (B).

Treatment of A. On removal of the solvent from solution A a syrup weighing just over 6 g. was obtained. To ensure that this was a pure tetramethyl glucose uncontaminated by trimethyl hexose, the above process was repeated, by redissolving the syrup in water and shaking the solution with small volumes of chloroform. A separation into two portions was again effected, as some of the syrup (A1) was removed from the aqueous solution under these conditions whilst the rest of the syrup (A2) was extracted only when large volumes of chloroform were used.

The portion A1 (trimethyl γ-fructose) weighed 1·1 g., reduced Fehling's solution, gave Seliwanoff's reaction, and had n_D 1·4670 (Found : OMe, 43·0. Calc. : 41·9%). It was therefore regarded as, in the main, trimethyl fructose.

Treatment of A2 (tetramethyl glucose). A2 crystallised after removal of the solvent chloroform. It was drained on porous tile, and thereafter crystallised from light petroleum in long needles, m. p. 89°; mixed m. p. with authentic tetramethyl glucose, 88° (Found : OMe, 50·7. Calc. : 52·5%). It had $[\alpha]_D$ in water (*c*, 1·7) + 91·7°, falling to + 83° (equilibrium value). The substance was therefore stable amylene-oxidic tetramethyl glucose. The yield represented approximately two-thirds of the total hydrolysed material (10 g. of hendecamethyl melezitose gave, after hydrolysis, 8·7 g. of syrup, from which 4·6 g. of tetramethyl glucose and 2·3 g. of trimethyl fructose were obtained in the pure state).

Treatment of B (trimethyl γ-fructose). The aqueous solution from the hydrolysis was carefully evaporated to dryness under diminished pressure, and the residue refluxed with chloroform. This extracted a syrup (1·2 g.) (Found : OMe, 40·0. Calc. : 41·9%), n_D 1·4660, which reduced Fehling's solution very readily and instantly decolorised neutral potassium permanganate solution. On solution in cold aqueous sodium hydroxide, it immediately developed a yellow colour; on heating, this colour deepened to dark brown. Comparative tests with fructose gave the same deepening in colour, while solutions of glucose derivatives remained pale yellow even after prolonged boiling. $[\alpha]_D$ in ethyl alcohol (*c*, 6), + 55·5°.

Methylation of Trimethyl Fructose. Isolation of Tetramethyl Methylfructoside and Tetramethyl γ-Fructose.—The trimethyl fructose was methylated with an excess of Purdie's reagents. After one methylation a non-reducing syrup (n_D 1·4445) was isolated. This was hydrolysed completely by treatment with 3% hydrochloric acid

at 95° for ½ hour. From the solution, neutralised with barium carbonate, chloroform extracted a syrup which reduced Fehling's solution strongly, had n_D 1·4540, and showed the following rotations : $[\alpha]_D$ + 24·4° in methyl alcohol (*c*, 0·9), + 29·1° in ethyl alcohol (*c*, 4·2), + 32·8° (permanent) in water (*c*, 3). These results are in agreement with the standard values for tetramethyl γ-fructose (Haworth, *loc. cit.*).

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