CXLI.—*The Structure of Normal Fructose: Crystalline Tetramethyl β-Methylfructoside and Crystalline Tetramethyl Fructose* (1 : 3 : 4 : 5).

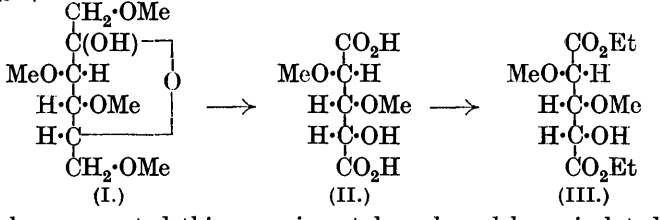
By Walter Norman Haworth, Edmund Langley Hirst, and Abraham Learner.

The stable crystalline variety of tetramethyl fructose originally prepared by Purdie and Paul (J., 1907, **91**, 289) has been the subject of a constitutional study by Irvine and Patterson (J., 1922, **121**,

2696), who reached the conclusion that the structural formula of this substance was to be represented as 1 : 3 : 4 : 6-tetramethyl fructose (I). Since it is now recognised that this methylated fructose is structurally similar to ordinary fructose or lævulose, the importance of the allocation of a final constitutional formula to this sugar is evident.

Our reason for instituting an inquiry into the constitution of tetramethyl fructose was that the structure applied to this sugar presented an anomaly when compared with the new structural formulæ we have applied to glucose and other hexoses. The experimental basis for the older formulæ has been shown to be precarious, and an entirely new and fundamental investigation has been necessary in order to place the chemistry of even the simple sugars on a secure basis. Until this was achieved, it was clearly premature to interpret results with any accuracy in the field of the polysaccharides. In the present paper, we have continued our earlier inquiry (Haworth and Hirst, J., 1926, 1858) and have deemed it advisable to scrutinise closely the experimental evidence on which Irvine and Patterson based their conclusions, which have been held to support the butylene oxide formula for fructose, and for stable tetramethyl fructose (I).

These authors reported that in deciding between various competing formulæ which may represent the stable tetramethyl fructose, " the crucial reaction is the formation of dimethoxyhydroxyglutaric acid (II) by the agency of nitric acid." " This oxidation involves the conversion of the $-CH_2 \cdot OMe$ group in the 6 position into CO_2H." It was alleged that the above acid was identified by the isolation and analysis of its diethyl ester (III), which was crystalline (m. p. 86—87°), and its combustion data agreed with the calculated formula, $C_{11}H_{20}O_7$.



(I.) (II.) (III.)

We have repeated this experimental work and have isolated the crystalline oxidation product (m. p. 87—88°) described by Irvine and Patterson. The C and H analysis of this compound agrees with the molecular formula $C_{11}H_{20}O_7$, but other analytical data as well as the properties of the compound are not consistent with its identification as diethyl dimethoxyhydroxyglutarate. Thus, the titration of this substance gives results which hardly support its

formulation as a dibasic ester, but are more in harmony with its recognition as a monobasic ester. It must, however, be recorded that the titration of this compound, inasmuch as it is sensitive to alkali, may easily give misleading results. When warmed with dilute alkali, it gave a yellow solution; it also reduced Fehling's solution readily. This behaviour is characteristic and points definitely to the presence of a reducing group in the substance.

We realised that the constitution of Irvine and Patterson's compound, $C_{11}H_{20}O_7$, could not be securely based on analytical evidence alone, although those authors were content to rely merely on combustion figures, remarking that the determination of methoxyl was "not diagnostic." Further study of the properties of the substance was necessary in order to furnish unmistakable reasons for the constitution (IV) which we have already proposed on other grounds (Haworth and Hirst, *loc. cit.*). The new evidence may now be briefly outlined, and it will be observed that we have relied only on crystalline derivatives which have been ultimately related to substances of definitely known structure.

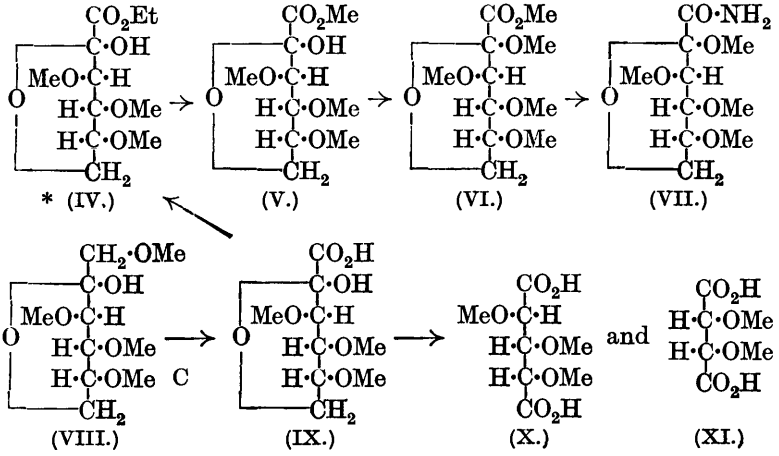
The ethyl ester, $C_{11}H_{20}O_7$, m. p. 87—88° (IV), has been converted directly into the corresponding methyl ester (V), which is crystalline (m. p. 119—120°) and has the molecular formula $C_{10}H_{18}O_7$. The latter had also previously been isolated (Haworth and Hirst, *loc. cit.*) by direct treatment of the acid described by Irvine and Patterson with methyl alcohol and hydrogen chloride. From these facts it is clear that the related acid must be $C_9H_{16}O_7$, and not the dimethoxyhydroxyglutaric acid, $C_7H_{12}O_7$. This methyl ester (m. p. 119—120°), like the ethyl ester (m. p. 87—88°), reduces Fehling's solution actively, but the latter property entirely disappears on the introduction of one additional methyl group by methylation, a procedure which gives rise to a product (VI), m. p. 102°, which is stable to alkali * and is similar in the magnitude of its specific rotation to the original methyl ester, pointing to the retention of the same structural form. This product gives on treatment with methyl-alcoholic ammonia a crystalline amide (VII), m. p. 118—119°, and the nitrogen content and other analytical data

* For this reason, it seems impossible to apply to the methyl ester and the methylated methyl ester the formula of an open-chain ketone such as (A) or (B).

(A) $CO_2Me \cdot CO \cdot [CH \cdot OMe]_3 \cdot CH_2 \cdot OH$ (B) $CO_2Me \cdot CO \cdot [CH \cdot OMe]_3 \cdot CH_2 \cdot OMe$

The cyclic formulæ (V) and (VI) which we have assigned to these products suggest their relation to the monobasic acid obtained by Ohle, Koller, and Berend (*Ber.*, 1925, 58, 2577) by oxidation of β-fructose-diacetone with permanganate.

of this derivative point to its being the amide of a (methylated) monocarboxylic acid, $C_9H_{16}O_7$. Moreover, we have degraded Irvine and Patterson's ethyl ester (m. p. 87—88°) by further oxidation with nitric acid, and the products isolated were recognised through their crystalline amides. These were (*a*) *d*-arabo-trimethoxyglutaramide, m. p. 227°, and (*b*) inactive dimethoxysuccinamide, m. p. 246°.



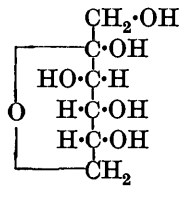
It is evident that *d*-arabo-trimethoxyglutaric acid (X) and *i*-dimethoxysuccinic acid (XI) could not be derived from a primary oxidation product formulated by Irvine and Patterson as in (II) or its ethyl ester as in (III), but the isolation of these acids is consistent with the allocation of the structural formula (IX), or the ethyl ester (IV). For reasons already given in the footnote, an open-chain ketonic formula for the ethyl ester is excluded, and the behaviour of the product both on methylation and on oxidation favours the adoption of the formula (IV). It may be added that the high lævorotation of the substance, which is almost identical with that of the original crystalline tetramethyl fructose, also supports the formulation as a cyclic compound, as does the persistence of the reducing group during treatment with an acid oxidising agent. Had there been present a $>CO$ linking or a terminal $-CH_2{\cdot}OH$ group, neither of these could have survived the treatment with nitric acid. In either event, the evidence from the further oxidation of this product supports our final conclusions ascribing a structure to tetramethyl fructose.

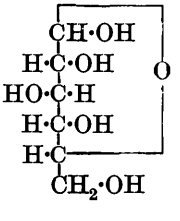
* The oxygen of the ring is not actually directed to the left as shown in these plane formulæ, but is in the same plane as the five carbon atoms of the ring; nor is it implied that the reducing group is necessarily on the right.

In the earlier paper (Haworth and Hirst, *loc. cit.*), it was observed that accompanying the acid $C_9H_{16}O_7$, which we formulate as in (IX), there was also isolated from the oxidation experiments arabo-trimethoxyglutaric acid (X) and *i*-dimethoxysuccinic acid (XI), and it is now made clear that these two acids may be regarded as secondary oxidation products formed by the breakdown of the primary oxidation product (IX) which has been isolated as its ethyl ester (IV) and its methyl ester (V). It would appear that the formation of these secondary products, which alone are of service in determining the ring structure of normal crystalline tetramethyl fructose, eluded the observation of Irvine and Patterson, since they did not examine the liquid esters which were evidently absorbed on porous tile.

The position, therefore, is that had the previous workers ascribed what we now consider to be the correct molecular formula to the primary oxidation product $C_9H_{16}O_7$ or its ethyl ester (IV), this could not in itself have resolved the difficulties attending the determination of the constitution of normal tetramethyl fructose, since the positions of the methoxyl groups would have remained undetermined and also the positions of the oxygen bridge. The cyclic structure of the original hexose appears to be still retained in this oxidation product. Such a result is as unexpected as it is remarkable in the sugar group, inasmuch as during the prolonged treatment with a drastic oxidising agent such as nitric acid (either of concentration *d* 1·2 or *d* 1·42) the first grouping to suffer attack is not the reducing group, which is preserved intact in the primary stage, but the terminal group (1), namely, the $-CH_2\cdot OMe$ group. The grouping in position (6) appears to be unimpaired, the allocation of the carboxyl group to position (6) being rendered impossible by the series of transformations recorded above. These observations serve to explain why normal tetramethyl fructose resists the oxidising action of bromine water and emphasise the stability of the amylene oxide ring, which must now be regarded as a constituent part of this sugar. The parallel behaviour of tetramethyl γ-fructose will be described in a subsequent paper, but here the inter-conversions which have been traced are still more remarkable and furnish an entirely novel conception of the behaviour of sugars.

(XII.)





(XIII.)

In view of the evidence now adduced, supplementing that already furnished (Haworth and Hirst, *loc. cit.*), we consider that the stable crystalline variety of tetramethyl fructose should be indexed as 1 : 3 : 4 : 5-tetramethyl fructose (VIII) containing a 2 : 6-oxide ring. Evidently, ordinary crystalline fructose contains the same structure, and is to be regarded as having also a 2 : 6- or amylene oxide ring (XII), thus bringing normal fructose into line with the revised formula for normal glucose (XIII).

It may also be added that tetramethyl β-methylfructoside, which had previously been described as a colourless liquid, $[\alpha]_D$ — 120·1° in methyl alcohol (Steele, J., 1918, **113**, 257; Irvine and Patterson, J., 1922, **121**, 2696), has now been isolated as a crystalline compound, m. p. 33—34°, $[\alpha]_D$ — 149·1° in water. An improved method of preparation leading to the isolation of this substance in a pure state has been adopted, namely, by subjecting tetra-acetyl β-methylfructoside, as isolated by Hudson (*J. Amer. Chem. Soc.*, 1916, **38**, 1216), to the action of alkali and methyl sulphate. The replacement of acetyl groups by methyl groups is completed finally by means of Purdie's reagents.

Experimental.

Preparation of Tetra-acetyl Fructose.—The method of preparation described by Hudson and Brauns (*J. Amer. Chem. Soc.*, 1915, **37**, 2736) was adopted with slight modifications introduced by our colleague, Mr. C. F. Allpress. Finely powdered *d*-fructose (100 g.) was added with constant stirring to 500 c.c. of acetic anhydride, containing 9 g. of zinc chloride, maintained near 0°. After 2 hours, the temperature was kept at 10° for ½ hour, and thereafter at 16° for 2 hours. Then followed the usual treatment with water (500 c.c.), neutralisation with sodium bicarbonate, and extraction of the product with chloroform. The crystalline tetra-acetyl fructose was most conveniently isolated from the dried chloroform extract in the following way. After removal of the solvent by distillation under diminished pressure, an equal volume of ether was added to the warm syrupy residue and, on cooling, the crystalline material separated in a form which could readily be filtered and freed from adhering syrup by washing with ether. Alternatively, the sticky mass of crystals and syrup obtained by Hudson and Braun's method could be extracted with boiling ether, the syrup being thereby removed. Recrystallisation from alcohol gave the pure substance, m. p. 127—129°.

Conversion of Tetra-acetyl Fructose into Tetramethyl β-Methylfructoside.—The tetra-acetyl fructose was treated with methyl iodide and silver oxide to give in quantitative yield tetra-acetyl

methylfructoside, the reagents being used in the proportions given by Irvine and Patterson (*loc. cit.*). The tetra-acetyl methylfructoside was then de-acetylated and methylated in one operation. To 30 g. of tetra-acetyl methylfructoside was added slowly at 50° with very efficient mechanical stirring the theoretical amount of 20% aqueous sodium hydroxide necessary to effect de-acetylation. This was completed in 3 hours. The temperature was then raised to 70° while 76 c.c. of methyl sulphate and 70 g. of sodium hydroxide, dissolved in 140 c.c. of water, were added during a period of 2¼ hours, the concentration of alkali being maintained throughout at about 10%. No darkening in colour took place and after extraction with chloroform in the usual way a pale yellow, mobile syrup was obtained (average yield 13 g.). This on distillation yielded 11 g., b. p. about 103°/0·14 mm., $n_D^{16°}$ 1·4578, $[\alpha]_{5780}$ — 120°; OMe, 55%. Methylation was completed by three successive treatments of the distillate with silver oxide and methyl iodide. This gave, in practically undiminished amount, tetramethyl methylfructoside in the form of a liquid, b. p. 105—106°/0·06 mm., $n_D^{16°}$ 1·4560. After being kept for a few hours, the material began to crystallise spontaneously, and on cooling in an ice-bath a solid mass of hard, flat prisms was obtained. The solid (m. p. 24°) was very soluble even in light petroleum, but by conducting all operations at 0° recrystallisation from this solvent to give pure tetramethyl β-methylfructoside was found to be possible. Four recrystallisations sufficed to raise the m. p. to 33—34°, further treatment being without effect on the m. p. (Found : C, 52·7; H, 8·8; OMe, 60·0. Calc. : C, 52·8; H, 8·8; OMe, 62·0%). The substance showed $[\alpha]_D^{17°}$ — 149·1° in water (c = 3·1) and behaved as a typical glucoside towards Fehling's solution; after hydrolysis with 3% aqueous hydrochloric acid at 95° for 1 hour, crystalline tetramethyl fructose was obtained in excellent yield (after recrystallisation from petroleum, m. p. 98—99°, mixed m. p. with an authentic specimen, 98—99°).

Oxidation of Normal Tetramethyl Fructose.—Crystalline tetramethyl fructose (8 g.) was oxidised with nitric acid (d 1·2) under the conditions described by Irvine and Patterson (*loc. cit.*). Removal of the nitric acid under diminished pressure was followed by esterification of the oxidation product in the usual manner, ethyl alcohol containing 4% of hydrogen chloride being used. After neutralisation with silver carbonate, the solution was evaporated to a syrup, which was dissolved in a mixture of chloroform and ether. Some solid matter was then filtered off, and after the rest of the solvent had been removed addition of light petroleum precipitated an oil which rapidly crystallised. After 15 hours, sufficient ether was added to dissolve adhering syrup and the crystals were

separated by filtration (yield 2·3 g.). Distillation of the syrup remaining in the mother-liquor gave a fraction (bath temp. 170°/10 mm.) which crystallised on cooling to give a further amount of crystalline matter, from which the adhering syrup was removed by absorption in porous tile (total yield of the crystalline product, 3·1 g.). The complete examination of this syrup has already been described in the earlier paper by Haworth and Hirst.

Properties of the Ethyl Ester, $C_{11}H_{20}O_7$.—After recrystallisation from light petroleum the substance showed m. p. 87—88°; $[\alpha]_D$ — 98° ($c = 0{\cdot}928$) in water. It reduced Fehling's solution strongly on warming and was unstable towards hot alkali, giving yellow solutions. For this reason, the titration figures are necessarily inexact and indicate a higher value for CO_2Et than is actually the case. Thus, 0·0917 g. required under standard conditions 4·3 c.c. of N/10-sodium hydroxide. This quantity of a substance of formula $C_{11}H_{20}O_7$ calculated as monobasic requires 3·5 c.c.: as dibasic (compare Irvine and Patterson) 7·0 c.c. The formulation as ester of a dibasic acid may therefore be ruled out (Found: C, 49·7; H, 7·7; OMe, 44·9. Calc.: C, 50·0; H, 7·6; OMe, 47·0%).*

Conversion of the Ethyl Ester $C_{11}H_{20}O_7$ *into the Corresponding Methyl Ester.*—The ethyl ester (0·5 g.), dissolved in 15 c.c. of methyl alcohol containing 4% of hydrogen chloride, was gently boiled for 7½ hours. The acid was then neutralised with silver carbonate, and the product isolated in the usual manner. Recrystallisation from ether yielded in all 0·33 g. of a substance identical in all respects with the methyl ester ($C_{10}H_{18}O_7$, m. p. 119°) previously isolated in the course of oxidation experiments with normal tetramethyl fructose. M. p. 119°; mixed m. p. with an authentic specimen 119° (compare Haworth and Hirst, *loc. cit.*).

Preparation of Amide from the Methylated Methyl Ester, $C_{11}H_{20}O_7$ (m. p. 102°).—Owing presumably to complication following on the action of ammonia on the reducing group, it was not found possible to isolate an amide from the methyl ester, $C_{10}H_{18}O_7$. After methylation, however, the non-reducing methylated methyl ester, $C_{11}H_{20}O_7$ (the preparation and properties of which have already been described, Haworth and Hirst, *loc. cit.*), was obtained and readily gave a crystalline amide. A solution of ester in methyl alcohol

* The substance $C_{11}H_{20}O_7$ contains one OEt group and three OMe groups. In such a case, it is convenient for comparative purposes to calculate the OMe percentage for the figures of the Zeisel experiment in the usual way. This is then compared with the theoretical value calculated on the basis of reckoning four OMe groups per molecule of $C_{11}H_{20}O_7$.

(0·3 g. in 2·5 c.c.) was saturated with ammonia at 0° and allowed to remain at room temperature for 4 days. The ammonia and methyl alcohol were then removed under diminished pressure at 25°. The remaining thick syrup crystallised on being rubbed, but the crystals were contaminated with oil. Much of the oil was removed by recrystallisation from a mixture of benzene and petroleum (b. p. 60—80°). Final purification was effected by recrystallisation from light petroleum, needles or long, thin, flat plates being obtained, m. p. 118—119°; $[\alpha]_D$ (approximately) $-137°$ in water ($c = 0·94$) (Found: C, 48·3; H, 7·6; N, 5·5; OMe, 48·4. $C_{10}H_{19}O_6N$ requires C, 48·2; H, 7·6; N, 5·6; OMe, 49·8%).

Oxidation of the Ethyl Ester, $C_{11}H_{20}O_7$ (m. p. 87—88°).—The crystalline ester (1·68 g.) was treated with 22 c.c. of nitric acid (*d* 1·42) at 60°. Oxidation commenced at this temperature, and as the reaction proceeded the temperature was gradually raised to 90—95° and maintained there for 3 hours. The product was isolated as the methyl ester by the usual procedure. On removing the solvent preparatory to purification of the product by distillation under diminished pressure, partial crystallisation took place. The adhering syrup was dissolved in ether and the crystals were separated. They were found to be the methyl ester, $C_{10}H_{18}O_7$ (m. p. 119°), corresponding to the ethyl ester, $C_{11}H_{20}O_7$, used as starting material. Yield 0·53 g.; m. p. and mixed m. p. with authentic specimen 119°. This portion had therefore not undergone oxidation. The syrupy portion of the oxidation product was distilled to give 0·45 g. of colourless syrup, boiling at a bath temperature 160—165°/10 mm.; $n_D^{12°}$ 1·4446. This was dissolved in 3 c.c. of methyl alcohol saturated with ammonia. Rapid deposition of crystalline material began at 20 hours from the start of the experiment. The first crop (0·06 g.) was a mixture (m. p. about 210—215°) which was shown to contain inactive dimethoxysuccinamide by dissolving it in a slight excess of methyl alcohol, nucleating the solution with crystals of the amide in question, and allowing it to evaporate very slowly. The first crystals deposited had the characteristic shape of *i*-dimethoxysuccinamide and gave m. p. 246° (decomp.), and were identified by comparison with genuine *i*-dimethoxysuccinamide. In these comparisons of m.p.'s, it is important that the determinations should be conducted under parallel conditions. By rapid heating, the m. p. can be raised as high as 262°. After 40 hours, a crop of crystals was obtained which, after being washed on the filter with methyl alcohol and ether, proved by comparison with an authentic specimen to be arabo-trimethoxyglutaramide; m. p. 227—228° (decomp.), $[\alpha]_D$ $-48°$ in water ($c = 0·2$) (Found: OMe, 39·4. Calc.: OMe, 42·2%).

The authors express gratitude to the Department of Scientific and Industrial Research for the award of a maintenance grant to one of them (A. L.).

UNIVERSITY OF BIRMINGHAM,
EDGBASTON.

[*Received, March 28th,* 1927.]