STRUCTURE OF DIFRUCTOSE ANHYDRIDE III (DIFRUCTOFURANOSE 1,2', 2,3'-ANHYDRIDE)

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ABSTRACT

Difructose anhydride I, one of three isomeric difructose anhydrides derived from inulin by hydrolysis with aqueous acids, has been shown by Haworth and Streight to yield upon methylation and hydrolysis, two molecules of 3,4,6-trimethylfructose. Difructose anhydride III, upon methylation and hydrolysis, failed to yield the characteristic osazone of 3,4,6-trimethylfructose, but it was shown by oxidation to trimethylarabonolactone that this methylated fructose was one constituent of a mixture of two trimethylfructoses. Both trimethylfructoses in the mixture were shown to be furanose derivatives.

Tritylation of difructose anhydride III yielded a crystalline trityl derivative which was methylated to form tritrityltrimethyldifructose anhydride III. The latter was detritylated and hydrolyzed, and the products of hydrolysis were separated by fractional distillation of their methyl fructosides. The lower-boiling fraction was identified as the fructoside of 3,4-dimethylfructose, $[\alpha]_D^{20} = -60.66$, by oxidation to a 3,4-dimethyl dibasic lactol acid. The higher-boiling fraction was found to be the fructoside of 4-methylfructose, as indicated by the melting point of its glucosazone (156° C), identical with that of the osazone prepared from 4-methylglucose. The trityl groups were shown to be substituents of the three primary alcohol groups. The sugar was thus constituted of two furanoid fructose residues, with two oxygen bridges connecting carbon atoms 1 and 2 of the one residue with 2' and 3' of the other.

Crystalline ditritryltetra-acetyldifructose anhydride I yielded, upon methylation and hydrolysis, 3,4-dimethylfructose, and by substitution of the trityl by methyl groups, 6-methylfructose.

A mechanism is suggested by which the difructose anhydrides are formed during the hydrolysis of inulin.

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I. INTRODUCTION

In previous articles it has been shown that purified inulin yields, upon hydrolysis with aqueous acids, a mixture of sugars consisting of about 92 percent of fructose, 3 percent of glucose, and 5 percent of a group of three nonreducing difructose anhydrides [1].[2] These latter substances were found to have specific rotations of +27.0, +13.85, and +135.62, in terms of sodium light, and were designated difructose anhydrides I, II, and III, respectively. They were in each instance isolated in crystalline form.

The first of these difructose anhydrides, which was readily isolated in the form of its crystalline hexa-acetate by the procedure of Jackson and Goergen [Ia], was studied by Haworth and Streight [2], who found that its hexamethyl derivative yielded, upon hydrolysis, two molecules of 3,4,6-trimethylfructofuranose identical with that derived from fully methylated inulin.   They concluded that the two fructose units were mutually linked by two oxygen bridges connecting, respectively, carbon atoms 1 and 2 of the one fructose residue with carbon atoms 2 and 1 of the other (formula *G*, page 189).

This same sugar was prepared in more than 20-percent yield from inulin acetate in chloroform solution by the action of fuming nitric acid, a reaction discovered by Irvine and Stephenson [3].   These authors, however, identified it as a monomeric anhydrofructose. Bodycote, Haworth, and Woolvin [4] showed that it was identical with Jackson and Goergen's difructose anhydride I.   It is significant that only the first of the three difructose anhydrides is formed by this reaction.

It becomes important now to determine the structures of the two remaining difructose anhydrides in order to study the relations of the three sugars to one another and, if possible, their relation to inulin. It was our early expectation that the three sugars would differ in the respective configurations of the second carbon atom.   If such were the case, combinations $\alpha\alpha$, $\beta\beta$, and $\alpha\beta$ would yield three different disaccharides.   This view, which was also suggested by Schlubach and Knoop [5] and by Haworth and Streight [2], will be shown in the present article to be untenable.

II. HEXAMETHYLDIFRUCTOSE ANHYDRIDES AND THEIR HYDROLYSIS

Complete methylation of the three sugars caused in each case the introduction of six methyl groups.   The first and third hexamethyl sugars proved to be liquids, while the second showed striking crystalline properties.   All three were readily distillable in a high vacuum. The physical properties of the hexamethyldifructose anhydrides are given in table 1.

The hydrolysis was carried out in 0.8 *N* hydrochloric acid solution, the course of the reaction being followed by polarimetric observations. The hydrolysis curves of the three methylated sugars, as shown in figure 1, appeared to converge at a final specific rotation of +25° to 30°, which suggested that the product of hydrolysis was 3,4,6-trimethylfructose, whose rotation in water is +30.5°.

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[2] Figures in brackets indicate the literature references at the end of this article.

TABLE 1.—*Physical properties of hexamethyldifructose anhydrides*

Difructose anhydrides	Hexamethyldifructose anhydrides										
[α]$_D^{20}$ (in water)	d_4^{20}	n_D^{20}	Boiling point		Melting point, uncorrected	[α]$_D^{20}$			[α]$_{5461}^{20}$		
			Pressure	Temperature		Pure liquid	In chloroform	In water	Pure liquid	In chloroform	In water
			mm	°*C*	°*C*						
I.......... +27.0	1.2050	1.4704	0.01	170 to 175	....	+19.1	+23.7	+50.4	+22.4	+27.5	+59.8
II......... +13.85	....	a1.4673	.35	169 to 170	73	a−41.9	−28.2	+6.0	....	−33.4	+6.7
III........ +135.64	1.1918	1.4658	.42	161 to 165	....	+155.7	+157.9	+164.5	+182.7	+186.9	+194.7

a Supercooled liquid.
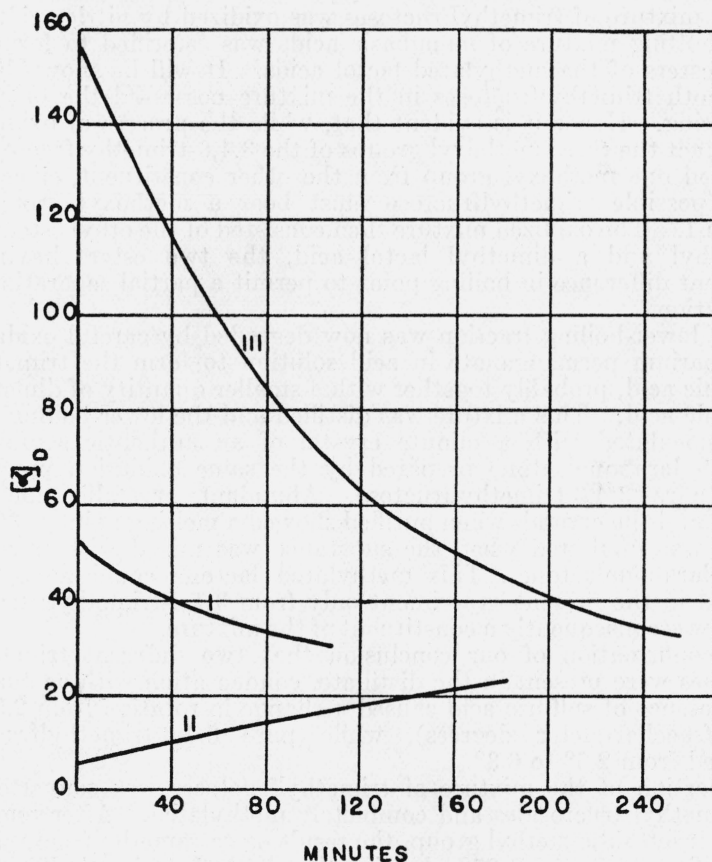
FIGURE 1.—*Hydrolysis of hexamethyldifructose anhydrides.*

The resulting samples of trimethylfructose distilled from the products of hydrolysis behaved, very differently, however, when treated with phenylhydrazine for the purpose of osazone formation. The distillate from the first sugar yielded nearly quantitatively the hydrated 3,4,6-trimethylosazone of melting point 81° C which Haworth and Learner [6] obtained from hydrolyzed trimethylinulin and Ha-

worth and Streight [2] from this same methylated disaccharide. The distillates from the second and third disaccharides, on the contrary, became discolored, and in each instance separated an oil from which but a small quantity of crystals could be separated after long standing. These crystals were so contaminated that it was found impossible to purify them sufficiently to observe their melting temperature. Our attention was thenceforth confined to the third disaccharide. The structure of difructose anhydride II will be discussed in a later article.

It seemed probable that the distillate from the third hexamethyl disaccharide consisted of two different trimethylfructoses, one of which, as suggested by the formation of osazone crystals, may have been the familiar 3,4,6. That the latter was indeed one constituent of the mixture was proved by the following experiment, in which the procedure of Haworth and Learner [6] was followed in detail.

The mixture of trimethylfructoses was oxidized by nitric acid, and the resulting mixture of monobasic acids was esterified to form the ethyl esters of the methylated lactol acids. It will be shown below that both trimethylfructoses in the mixture possessed the butylene oxide ring. Hence it is evident that, while the process of oxidation left intact the three methoxyl groups of the 3,4,6-trimethylfructose, it removed one methoxyl group from the other constituent, since any other possible trimethylfructose must bear a methoxyl group on carbon 1. The oxidized mixture then consisted of the ethyl esters of a trimethyl and a dimethyl lactol acid, the two esters having a sufficient difference in boiling point to permit a partial separation by distillation.

The lower-boiling fraction was now degraded by careful oxidation with barium permanganate in acid solution to form the trimethyl-arabonic acid, probably together with a smaller quantity of dimethyl-arabonic acid. This mixture was distilled and the lower-boiling fraction inoculated with a minute crystal of an authentic sample of trimethylarabonolactone prepared by the same oxidation processes from pure 3,4,6-trimethylfructose. Abundant crystallization followed, and the crystals when purified showed a melting point of 30° C, which was unaltered when the substance was mixed with pure trimethylarabonolactone. This methylated lactone could have been derived in the present experiment only from 3,4,6-trimethylfructose, which was consequently a constituent of the mixture.

In confirmation of our conclusion that two different trimethyl-fructoses were present in the distillate, condensation with acetone in the presence of sulfuric acid caused a change in rotation from 2.3° to 3.5° (saccharimeter degrees), while pure 3,4,6-trimethylfructose changed from 2.5° to 6.3°.

A portion of the mixture of trimethylfructoses was converted to their methyl fructosides and completely methylated. After removal of the fructosidic methyl group, the resulting tetramethylfructose had a specific rotation of +37°, showing that both the trimethylfructoses in the distillate belonged to the furanose series.

With the oxide ring in the 2,5 position, there are four possible trimethylfructoses; namely, 1,3,4, 1,3,6, 1,4,6, and 3,4,6. The distillate from the methylated third sugar contained as one constituent 3,4,6-trimethylfructose, together with one of the remaining three. From these, 1,3,4 can be eliminated. Hibbert, Tipson, and Brauns [7] have prepared this substance by hydrolysis of fully methylated

levan produced by the action of *Bacillus mesentericus* on cane sugar solutions. The resulting 1,3,4-trimethylfructose showed in water a specific rotation of $-51.4°$. If this trimethylfructose had been produced together with 3,4,6 of specific rotation $+30.5°$ by the hydrolysis of the methylated third sugar, the rotation would have approached an end value of approximately $-10°$. As shown by curve III, figure 1, the end point of the hydrolysis was definitely positive; hence 1,3,4-trimethylfructose is excluded as a possible constituent. The products of hydrolysis of the methylated third sugar were then 3,4,6- and either 1,3,6,- or 1,4,6,-trimethylfructose.

Montgomery [8] synthesized 1,4,6-trimethylfructose by a chain of reactions starting from α-diacetonefructose. For the purified substance, he found a specific rotation in chloroform of $+29.7°$. Since 3,4,6-trimethylfructose has a rotation of $+27.7°$ in the same solvent, the rotation of a mixture of these trimethylfructoses would not differ greatly from that of either constituent. Hence the rotation of the products of hydrolysis of the methylated third sugar would be consistent with that of a mixture of 1,4,6- and 3,4,6-trimethylfructose. 1,3,6-Trimethylfructose, however, has not been prepared and thus is not excluded as a possible constituent.

Pertinent also are the respective rotations of the acetone condensation products. Montgomery found that 3,4,6-trimethylfructose underwent a large increase in rotation upon condensation with acetone, an observation verified in the present investigation, while 1,4,6 changed but slightly. The rotation of the mixed trimethylfructoses from the methylated third difructose anhydride, upon condensation with acetone, increased by an amount roughly intermediate between that of 3,4,6- and that of Montgomery's 1,4,6-trimethylfructose.

III. TRITYL COMPOUNDS OF DIFRUCTOSE ANHYDRIDE III AND THE DERIVED PARTIALLY METHYLATED FRUCTOSES

In order that a distinction might be made between 1, 4, 6- and 1, 3, 6-trimethylfructoses, a study was made of the reaction between the difructose anhydrides and triphenylchloromethane (trityl chloride) in pyridine solution with the expectation of substituting only the primary hydroxyl groups according to the observations of Helferich and his coworkers [9]. When the procedure was applied to difructose anhydride III, difficulties arose because the preference for the primary hydroxyls apparently was not greatly in excess of that for the secondary. Nevertheless, it was possible to obtain in crystalline form a trityrityl derivative of the sugar (*B*), which indicates the probable presence of three primary hydroxyls. No conclusion, however, is drawn respecting the structure of the sugar from this reaction, but the trityl groups are definitely allocated to the primary hydroxyls by the independent argument given below.

The tritylated sugar was acetylated to form a trityryltriacetyl derivative, which was methylated in acetone by dimethyl sulfate and sodium hydroxide to yield amorphous trityryltrimethyldifructose anhydride III (*C*). This substance was detritylated in chloroform solution containing hydrobromic acid, and the resulting trimethyl-

difructose anhydride, together with the hydrobromic acid, was extracted with water and hydrolyzed to form a mixture of partially methylated fructoses. The latter were converted to the methyl fructosides and fractionally distilled.

The lower-boiling fraction, consisting of a methyl dimethylfructoside, was converted to a dimethylfructose of $[\alpha]_D^{20} = -60.66$ in water. The sugar was converted to its osazone, which still retained two methyl groups. This osazone was identical with that of the 3,4-dimethylfructose prepared by other methods, as described below. Oxidation with nitric acid produced a dibasic dimethyl lactol acid (F), whose calcium salt crystallized with four molecules of water of crystallization and contained two methyl groups. This calcium salt was evidently identical with the salt prepared by Hibbert, Tipson, and Brauns [7] from 1,3,4-trimethylfructose by the same oxidative process. The foregoing reactions, together with the demonstration that only furanose sugars are present in the disaccharide, eliminate carbons 1, 5, and 6 as positions for the introduced methyl groups, which are thus allocated to positions 3 and 4. The lower-boiling fraction was consequently the fructoside of 3,4-dimethylfructose (E).

The higher-boiling fraction proved to be a monomethyl methylfructoside, which, upon hydrolysis, yielded a monomethyl fructose of $[\alpha]_D^{26} = -87.5$ (at equilibrium). It yielded a monomethylglucosazone which melted at 156° C. This same osazone has been prepared from 4-methylglucose by three other groups of investigators [10], with essential agreement in the temperature of fusion. Our high-boiling fraction was therefore the fructoside of 4-methylfructose (D). In formulas D and E, the furanose ring is retained provisionally, since no argument can be advanced for either form in preference to the alternative.

There are but three possible monomethylfructoses which could have been formed in the present instance, since positions 1 and 5 are necessarily excluded from consideration. These are 3-, 4-, and 6-monomethylfructose. In each instance the respective glucosazone has previously been prepared, 3-methylglucosazone from 3-methylfructose, and 4- and 6-methylglucosazones from the respective methylglucoses. Inasmuch as, in the present investigation, the identification of the monomethylglucosazone from our higher-boiling fraction was of first importance for the determination of the structure of the disaccharide, we have deemed it advisable to prepare the three monomethylglucosazones in order to verify the published values of the melting points and to distinguish between them by means of mixed melting points.

3-Methylglucosazone was prepared very simply from 3-methylfructose. 6-Methylglucosazone was prepared in very pure form from 6-methylfructose, whose preparation is described below. The melting-point and mixed melting-point data are given in table 2. Evidently the low-melting point of 4-methylglucosazone distinguishes it clearly from the 3- and the 6-methylglucosazones. This is further corroborated by the depressed melting temperatures of the respective mixtures.

The rotatory power in equilibrium of the free 4-methylfructose $(-87.5°)$ serves moreover to distinguish it from the 3-methylfructose $(-53°)$ and the 6-methylfructose $(+6.4°)$. Substitution on the fourth carbon atom alters the rotation of fructose much less than

substitution on the third atom, while a methyl group on carbon 6 compels the sugar to retain the furanose form.

TABLE 2.—*Melting points of substituted phenylglucosazones*

3-Methyl [11]	Melting point
	°C
Irvine and Hynd	164 to 165
Freudenberg and Hixon	178 to 179
Anderson, Charlton, and Haworth	178 to 179
McDonald and Jackson	176
4-Methyl [10]	
Pacsu	156 to 157
Munroe and Percival	158
Schinle	159
McDonald and Jackson	156
6-Methyl [12]	
Helferich and Becker	177
Ohle and Vargha	178
Kuhn and Ziese	177
McDonald and Jackson	183
Mixtures (McDonald and Jackson)	
3- and 4-Methyl	150
6- and 4-Methyl	149
3,4-Dimethyl (McDonald and Jackson)	
From inulin	126
From difructose anhydride I	126
From difructose anhydride III	ª 121

ª Compound not thoroughly purified.

IV. STRUCTURE OF DIFRUCTOSE ANHYDRIDE III

The experimental work outlined in section II reveals that the hexamethyldifructose anhydride yielded upon hydrolysis 3,4,6-tri-methylfructose and either 1,3,6- or 1,4,6-trimethylfructose. The tritylated methylated sugar yielded 3,4-dimethylfructose and 4-monomethylfructose. The 3,4-dimethylfructose was evidently derived from the same fructose residue which had yielded 3,4,6-tri-methylfructose by hydrolysis of the fully methylated disaccharide. The primary alcohol on carbon 6 was therefore substituted by the trityl group in conformity with Helferich's generalization. Similarly, in the other fructose residue, since 4-methylfructose was obtained from the tritylated methylated sugar, primary alcohols on carbons 1 and 6 were substituted by trityl groups. The hexamethyl sugar thus yielded upon hydrolysis 3,4,6- and 1,4,6-trimethylfructose.

The formation of 3,4,6- and 1,4,6-trimethylfructoses requires that the two fructose residues be linked by oxygen bridges connecting positions 1 and 2 of the one fructose residue with 2′ and 3′ of the other. Two alternative combinations must be considered possible; namely, a 1,2′-2,3′ anhydride and a 1,3′-2,2′ anhydride. The former anhydride consists of two somewhat similar glycosidic unions, while the latter consists of one glycosidic and one ethereal linkage. While
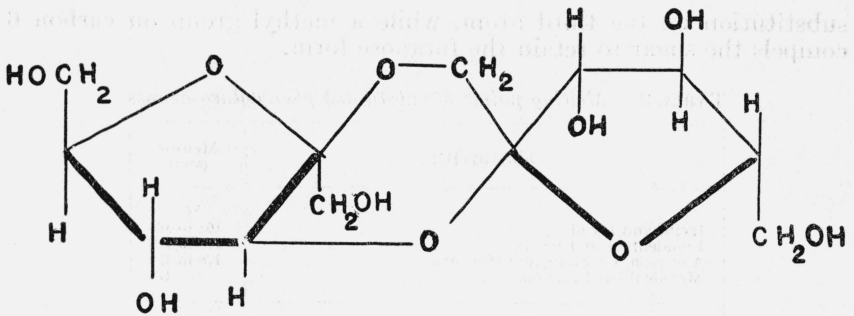
(A) Difructose anhydride III.
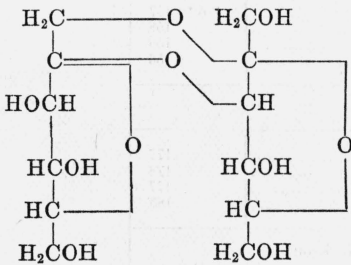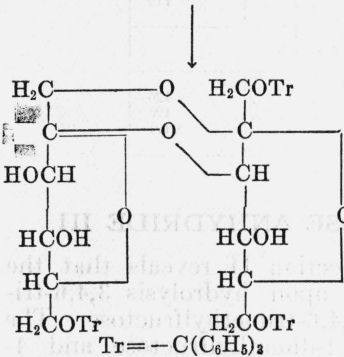
(A') Difructose anhydride III.

$Tr = -C(C_6H_5)_3$

(B) Trityldifructose anhydride III.
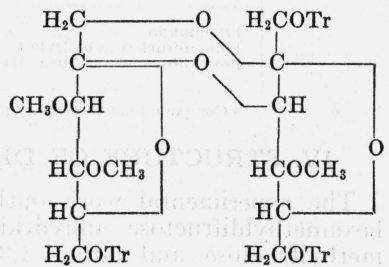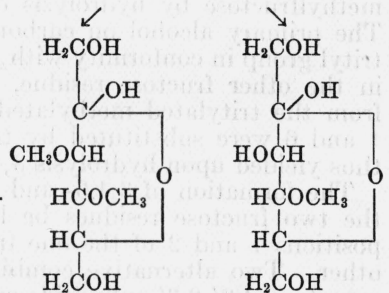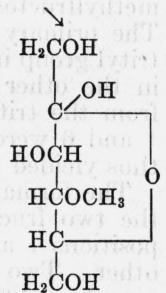
(C) Trityltrimethyldifructose anhydride III.
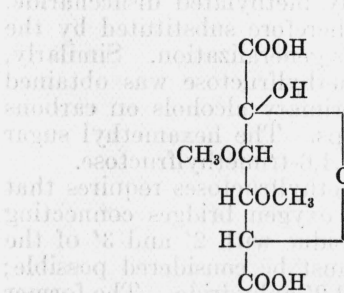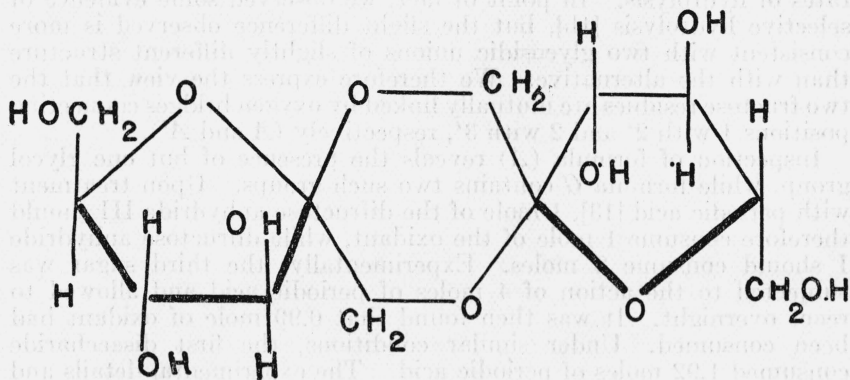

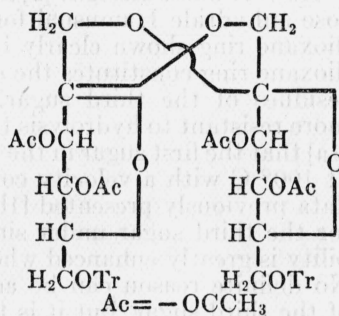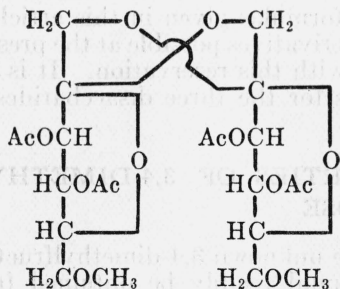(F) 3,4-Dimethyllactol acid.
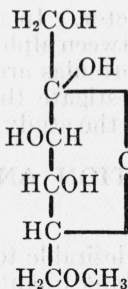
(E) 3,4-Dimethylfructose.

(D) 4-Methylfructose.

(*G*) Difructose anhydride I.

(*H*) Ditrityltetra-acetyl-
difructose anhydride I.

(*I*) 6,6′-Dimethyltetra-acetyldifructose   (*J*) 6-Methylfructose.
anhydride I.

two glycosidic unions would probably hydrolyze at approximately the same rate, two such fundamentally different modes of linkage as a glycoside and an ether would be expected to have widely divergent rates of hydrolysis. In point of fact, we observed some evidence of selective hydrolysis [1b], but the slight difference observed is more consistent with two glycosidic unions of slightly different structure than with the alternative. We therefore express the view that the two fructose residues are mutually linked by oxygen bridges connecting positions 1 with 2' and 2 with 3', respectively (A and A').

Inspection of formula (A) reveals the presence of but one glycol group, while formula G contains two such groups. Upon treatment with periodic acid [13], 1 mole of the difructose anhydride III should therefore consume 1 mole of the oxidant, while difructose anhydride I should consume 2 moles. Experimentally, the third sugar was subjected to the action of 4 moles of periodic acid and allowed to react overnight. It was then found that 0.99 mole of oxidant had been consumed. Under similar conditions, the first disaccharide consumed 1.92 moles of periodic acid. The experimental details and the many interesting conclusions which can be derived from this oxidative reaction are reserved for a later article.

Haworth and Streight [2] have ascribed the great stability of difructose anhydride I, unusual for furanoid sugars, to the presence of the dioxane ring shown clearly in the perspective formula G. A similar dioxane ring constitutes the connecting link between the two fructose residues of the third sugar. The latter is, however, considerably more resistant to hydrolysis than the first disaccharide. It was shown [1a] that the first sugar in the presence of 0.2 N sulfuric acid hydrolyzed at 100° C with a velocity constant of 0.009. A rough calculation of data previously presented [1b] shows a velocity of hydrolysis of 0.006 for the third sugar under similar conditions. The difference in stability is greatly enhanced when the two disaccharides are methylated. No definite reason can be advanced to explain the greater stability of the third sugar, but it is to be noted that the dioxane ring in this case includes one secondary alcohol group, while the anhydro ring in the first sugar includes two primary alcohol groups. Moreover, the unknown alpha-beta relations of the constituent fructose may be a contributing factor. In none of the formulas given in this article is a distinction between alpha and beta derivatives possible at the present time and the formulas are presented with this reservation. It is our purpose to investigate these relations for the three disaccharides in continuation of the study.

V. PREPARATION AND PROPERTIES OF 3,4-DIMETHYL-FRUCTOSE

It appeared desirable to prepare the unknown 3,4-dimethylfructose in more abundant quantities than could readily be obtained from difructose anhydride III, which itself was available in limited amounts. For this purpose, inulin served satisfactorily for a starting material. Under carefully determined conditions inulin was converted in pyridine solution to a monotrityl derivative and the latter acetylated by addition of acetic anhydride to the reaction mixture. The diacetylmonotritylinulin was methylated in acetone solution by addition of dimethyl sulfate and sodium hydroxide and the methylated

compound obtained in the form of an amorphous powder. The substance was detritylated, hydrolyzed, and converted to acetone-dimethylfructose in a single operation by dissolving in acetone and adding sulfuric acid. The acetonedimethylfructose was isolated by distillation, converted to methyl dimethylfructoside, which received a final purification by fractional distillation. Some uncertainty was inherent in this procedure arising from the possibility that tritylation may have been incomplete and thus partial methylation may have produced other dimethyl derivatives than the one desired.

This uncertainty was eliminated by utilizing difructose anhydride I as a source. This sugar, upon tritylation and acetylation, yielded a dtrityl tetra-acetyl derivative (*H*), which proved to be crystalline and lent itself to thorough purification. The tetramethyl derivative prepared by the action of dimethyl sulfate and sodium hydroxide was amorphous and was detritylated and hydrolyzed without further purification. The resulting dimethylfructose was purified by two distillations as methyl dimethylfructoside. The free sugar was obtained upon removal of the fructoside group.

The purified 3,4-dimethylfructose obtained from difructose anhydride I proved to be a viscous liquid of $[\alpha]_D^{20} = -60.66$ in water. It formed a crystalline osazone which melted at 126° C. Its structure is fairly indicated by its method of synthesis. Oxidation by nitric acid converted it to the dibasic 3,4-dimethyl lactol acid whose calcium salt has been described above. The free sugar obtained in the form of a sirup has thus far failed to crystallize. Upon removal of the solvent, the sugar showed a strong tendency to polymerize. This dimethyl sugar will be studied further.

VI. 6-METHYLFRUCTOSE

Ditrityltetra-acetyldifructose anhydride I (*H*) was detritylated in acetic acid solution by addition of hydrogen bromide, yielding a crystalline tetra-acetyl derivative, which was methylated by Purdie's reagents to form crystalline dimethyltetra-acetyldifructose anhydride I (*I*). This was deacetylated and hydrolyzed in one step, and the resulting sugar converted to its methyl fructoside and distilled. Upon removal of the fructoside group, the sugar (*J*) showed an equilibrium rotation ($[\alpha]_D^{26}$) of +6.4 and yielded an osazone melting at 183° C. This interesting derivative will receive further attention in a later article.

That the methyl group was situated on the sixth carbon atom follows from the fact that the same ditrityltetra-acetate (*H*) yielded ditrityltetramethyldifructose anhydride I from which 3,4-dimethylfructose was obtained. The trityl groups thus occupied the sixth position, and since in the formation of the dimethyltetra-acetate the methyl groups have replaced the trityl groups, they too occupy position 6. The melting point and rotation of the osazone, which are in fair agreement with the data respecting the osazone prepared from 6-methylglucose, make it probable that no migration of acetyl groups occurred during the various steps of the preparation.

VII. ORIGIN OF THE DIFRUCTOSE ANHYDRIDES

In our earlier article we expressed the view that the difructose anhydrides were integral parts of the inulin molecule, and on account of their high stability survived the hydrolysis. This idea, which was

enunciated before anything was known concerning the structures of the sugars and which indeed has encountered much adverse criticism, appears now definitely incorrect. An alternative view is suggested.

We may suppose that during the process of hydrolysis the inulin aggregate is ruptured at various points, leaving shortened chains, each fragment having a free reducing group at one end. In a relatively small number of instances the hydroxyl of the terminal reducing group apparently condenses with one of the hydroxyls of the penultimate fructose residue, thus forming a difructose anhydride entity which becomes so stable as to resist further hydrolysis. This condensation of one fructose residue with a closely contiguous residue is in keeping with the known tendency of fructose derivatives to polymerize. On the penultimate fructose residue, carbon atoms 3, 4, and 6 bear hydroxyls which are available for this condensation, and the union through an atom of oxygen of carbon atom 3 with carbon 2 of the terminal residue would lead to the formation of difructose anhydride III, whose structure has been discussed above. This condensation clearly can occur at any time before the complete resolution of the inulin fragments to individual fructose units, but not after, since we have shown in a previous article [1a] that fructose itself is not appreciably altered under the conditions of inulin hydrolysis. Difructose anhydride I, on the other hand, can only be formed by the momentary isolation of a fragment consisting of two fructose residues, followed by condensation or, obviously, by the simultaneous hydrolytic splitting of the fragment and condensation to the anhydride. This latter case is evidently what occurs during the course of Irvine and Stephenson's reaction of inulin acetate in chloroform solution with nitric acid. It is now clear why the first anhydride only is formed by this reaction, since the remaining carbon atoms are already substituted by acetyl groups.

The condensation to the anhydride in the cases of the two sugars whose structures are known occurs without the shifting of the oxide ring to the normal form. Whether mutarotation occurs is of course undetermined. If both mutarotation and ring shift can occur before condensation to the anhydride, the number of possible difructose anhydrides capable of formation during the hydrolysis of inulin becomes very large.

A considerable number of difructose anhydrides has already been reported, some prepared 5y depolymerization of inulin, some by synthesis, and some are of natural occurrance. It is interesting to note that all are anhydrousugars and all are nonreducing.

We have already learned that difructose anhydride II is, like III, unsymmetrically constructed and probably accounts for one of the remaining possible structures. Moreover, during the preparation of the three crystalline sugars we have accumulated a considerable quantity of mother liquors which show the same high resistance to hydrolysis, but which fail to crystallize, even when abundantly inoculated with crystals of those already isolated.

Schlubach and Knoop [14] have by exhaustive fractionation separated the polysaccharides occurring in the jerusalem artichoke into three main constituents; namely, inulin, a dilevan, and a positively rotating difructose anhydride. The latter substance, even after several hundred operations, was not obtained in a pure state but was methylated and isolated by fractional distillation of its hexamethyl

derivative. They suggest that this disaccharide is identical with our difructose anhydride III, and indeed the high rotatory power of their hexamethyl sugar, $+149°$ in chloroform, as compared with our value, $+158°$, for the highly purified substance makes this seem plausible. They report, however, that the properties of the products of hydrolysis agree with those of 3,4,6-trimethylfructose and that an osazone of melting point 77° to 79° C was formed. The essential question whether this osazone was formed in nearly quantitative yield as it is from methylated inulin is not discussed. We too obtained the crystalline 3,4,6-osazone, as stated above, but in such small yield that we were led to the conclusion that it was formed from but one of the fructose residues in the disaccharide. If their osazone was formed in good yield, it follows that the two disaccharides are different. If formed in poor yield, the question remains undetermined.

The difficulty of separation of their difructose anhydride from inulin is quite at variance from our experience. One gram of our third sugar was dissolved in a hot-water solution of 10 g of inulin and the solution allowed to cool. Upon crystallization of the inulin and filtration, the sugar appeared practically quantitatively in the mother liquor.

Schlubach and Knoop [14] also suggest that our difructose anhydride II is perhaps the same as the synthetic disaccharide which Schlubach and Elsner [15] obtained by the action of acetone on fructose in the presence of hydrochloric acid. Their sirupy hexamethyl sugar, however, showed a rotation of $+31°$ in chloroform, while ours, a crystalline substance, showed $-28°$. Moreover, their substance was hydrolyzed to 3,4,6-trimethylfructose by the mild action of oxalic acid, while our second sugar requires the prolonged action of hydrochloric acid at nearly boiling temperature. Indeed, it is the most stable of the three difructose anhydrides which we have described. In contrast to Schlubach and Elsner's methylated sugar, the products of hydrolysis in this case are not exclusively 3,4,6-trimethylfructose.

VIII. EXPERIMENTAL

1. HEXAMETHYLDIFRUCTOSE ANHYDRIDES

The procedure devised by Haworth [16] was employed for the methylation of the three crystalline sugars; namely, solution in the minimum quantity of water and successive additions of methyl sulfate and 30 percent sodium hydroxide at 70° C. The sirupy product was soluble in methyl iodide and the methylation was completed by use of methyl iodide and silver oxide in the usual manner. Since only the first difructose anhydride formed a crystalline acetate, additional preparations of its hexamethyl compound were made by direct methylation of the acetate in acetone solution by means of methyl sulfate and 30 percent sodium hydroxide [17].

Hexamethyldifructose anhydride I.—This substance formed a slightly viscous, nearly colorless liquid which distilled at about 170 to 175° C and 0.01-mm pressure. $d_4^{20} = 1.2050$; $n_D^{20} = 1.4704$; $[\alpha]_D^{20} = +19.1$ (pure liquid), $+23.7$ (chloroform, $c = 4.861$), $+50.4$ (water, $c = 1.614$); $[\alpha]_{5461}^{20} = +22.4$ (pure liquid), $+27.5$ (chloroform, $c = 3.13$), $+59.8$ (water, $c = 1.614$). Haworth and Streight [2] gave the values, $n_D^{16} = 1.4719$; $[\alpha]_D^{20} = +24$ (chloroform), $+53$ (water). *Analysis.* Calculated for $(C_9H_{16}O_5)_2$: OCH_3, 45.6. Found: 44.3.

Hexamethyldifructose anhydride II.—This derivative crystallized spontaneously even before it was completely extracted from the silver compounds. Upon recrystallization, it took the form of large polyhedra. One preparation formed a single crystal 2 cm in length. Melting point, 73° C (uncor.); $n_D^{20} = 1.4673$ (supercooled liquid); $[\alpha]_D^{20} = -41.9$ (pure supercooled liquid), -28.2 (chloroform, $c = 3.555$), $+6.0$ (water, $c = 3.735$); $[\alpha]_{5461}^{20} = -33.4$ (chloroform, $c = 3.555$), $+6.7$ (water, $c = 3.735$). It distilled at 169° to 170° C at 0.43 mm, bath temperature, 190° C. *Analysis.* Calculated for $(C_9H_{16}O_5)_2$: OCH_3, 45.6. Found: 45.6.

Hexamethyldifructose anhydride III.—The methylated third sugar formed a fairly mobile liquid which distilled at 161° to 165° C at 0.417 mm from a bath at 183° to 193° C. $d_4^{20} = 1.1918$; $n_D^{20} = 1.4658$; $[\alpha]_D^{20} = +155.7$ (pure liquid), $+157.9$ (chloroform, $c = 3.95$), $+164.5$ (water, $c = 2.232$); $[\alpha]_{5461}^{20} = +182.7$ (pure liquid), $+186.9$ (chloroform, $c = 3.95$), $+194.7$ (water, $c = 2.232$). *Analysis.* Calculated for $(C_9H_{16}O_5)_2$: OCH_3, 45.6. Found: 45.0.

2. HYDROLYSIS OF THE HEXAMETHYLDIFRUCTOSE ANHYDRIDES

Hexamethyldifructose anhydride I was hydrolyzed in the manner described by Haworth and Streight [2]; namely, by digestion with 0.8 N hydrochloric acid at 95° C to constant rotation. The course of the hydrolysis is shown in figure 1. The solution was neutralized with barium carbonate, evaporated to dryness in the presence of barium carbonate, extracted with ether, and distilled under 0.2-mm pressure. The 3,4,6-trimethylfructose, upon treatment with phenylhydrazine, formed the hydrated osazone (melting point, 81° C) in nearly quantitative yield.

Hexamethyldifructose anhydride III presented greater difficulty than the first sugar because of its greater resistance to hydrolytic action. In exploratory experiments it was ascertained that under the conditions necessary to approach complete hydrolysis furfural derivatives accumulated to such an extent as to interfere seriously with the purification of the trimethylfructose. Indeed, it was at first considered probable that the failure to obtain the characteristic osazone was due to excessive contamination by furfural derivatives.

The rate of destruction of 3,4,6-trimethylfructose under the conditions of hydrolysis was measured by subjecting a sample, prepared from trimethylinulin, to 0.8 N hydrochloric acid at 95° C. By a calculation based upon its initial and final rotations, it was determined that the substance had decomposed 13 percent in 1 hour. Hexamethyldifructose anhydride III was 50 percent hydrolyzed in 91 minutes and 70 percent in 157 minutes.

From these data it was roughly calculated that if the hexamethyl sugar were hydrolyzed for three periods of 91 minutes each and the trimethylfructose were removed by distillation after each period of hydrolysis, about 88 percent of the sugar would be hydrolyzed and that about 10 percent of the trimethylfructose thus formed would be destroyed. Should the acid hydrolysis consist of two consecutive treatments of 157 minutes each, 91 percent of the hexamethyl sugar would be hydrolyzed and about 20 percent of the trimethylfructose would be destroyed. It was, therefore, decided to hydrolyze the methylated sugar in three periods of 91 minutes each and to remove the trimethylfructose by distillation after each period. In order to

keep the still residue undamaged, the bath temperature was held as low as possible. This necessitated the use of an ineffective fractionating column, and the unhydrolyzed substance tended to distill over in appreciable quantities.

A sample (7.23 g) of the third hexamethyl sugar was hydrolyzed for 91 minutes at 95° C. The acid was neutralized with barium carbonate, and the filtrate was evaporated and extracted with chloroform. From the products of hydrolysis there were obtained a distillate, A, (3.42 g) of $[\alpha]_D = +50.4$, and a still residue. The latter was hydrolyzed and yielded a distillate, B, (1.72 g) of $[\alpha]_D = +30.0$. Distillates A and B were combined and redistilled, yielding distillate C (3.87 g) of $[\alpha]_D = +26.6$, and a still residue which was combined with that from B and again hydrolyzed and distilled, yielding a distillate, D, (0.71 g) of $[\alpha]_D = +29.1$. Recovered as trimethylfructose, 4.58 g. The combined distillates were dissolved in water and titrated at 10° C with neutral dilute permanganate until a spot test with a potassium iodide-starch solution showed that furfural derivatives had been completely oxidized. The solution was treated with carbon, filtered, evaporated, and the trimethylfructose redistilled.

When a small sample of the trimethylfructose was treated with phenylhydrazine in dilute acetic acid solution, the reaction mixture turned brown and deposited an oil, which was washed and dissolved in alcohol. After long standing, the solution deposited a relatively small amount of crystals, which were so highly contaminated with oily material that it was found impossible to purify them.

The mixture of trimethylfructoses was oxidized in two stages by nitric acid and by permanganate, respectively, and thus degraded to trimethylarabonolactone (melting point, 31° C), the procedure described by Haworth and Learner [6] being followed in detail.

3. TRITYL DERIVATIVES OF DIFRUCTOSE ANHYDRIDE III

Trityldifructose anhydride III.—The tritylation of the third sugar proved to be difficult, and the yields of tritrtylated sugar were small. The difficulty arose from the fact that, except under a narrow range of conditions, the desired tritrityl sugar was merely one stage in the succession of reactions and that this product was accompanied by under- and over-tritylated sugar. This appears to be one of the few cases in which the preference of the trityl group for the primary alcohol is not greatly in excess of that for the secondary. Fortunately the tritrityl sugar was the only definitely crystalline product and could thus be purified and accepted as homogeneous. The course of the condensation was followed by polariscopic observation, the rotation reaching a maximum at about 1.7 times the initial value and thereafter diminishing, probably as a result of substitution of secondary alcohol groups.

Yields of 40 to 45 percent of crystalline tritrityl sugar were obtained by the following procedure. Any considerable departure from that described caused the formation of over- or under-tritylated products. Two and one-half grams of the dried sugar was dissolved in 32 ml of redistilled pyridine and 7.2 g of trityl chloride was added (11 percent in excess of theory). The solution was heated in a glycerin bath at 80° C for ½ hour and allowed to stand overnight at room temperature. Water was added to slight turbidity, and the carbinol-pyridine complex which crystallized was removed by filtration. The

filtrate was poured into water and the separated substance collected on a filter, washed, and air-dried. The dried substance retained a considerable amount of pyridine, which it was found necessary to volatilize completely.

The final product was recrystallized in the form of fine needles to constant properties from methyl alcohol containing 5 to 10 percent of acetone. Melting point, 127° C (uncor.); $[\alpha]_D = +64.2$ (chloroform, $c=0.76$). Triphenylcarbinol [18], calculated; 74.3. Found: 73.1.

Tritrityltriacetyldifructose anhydride III.—The acetylation of the tritrityl sugar was effected very simply by the usual procedure. In 5 ml of pyridine was dissolved 0.73 g of tritrityldifructose anhydride III, 5 ml of acetic anhydride was added, and the mixture was heated on the steam bath for 1 hour. After standing overnight at room temperature, it was poured into a large volume of water from which it was recovered quantitatively. For purification, it was dissolved in warm absolute alcohol and allowed to separate slowly during partial evaporation of the solvent. The substance proved to be amorphous. $[\alpha]_D = +65.2$ (chloroform, $c=0.71$).

The analysis of the product proved difficult and unsatisfactory. Determination of acetyl groups [19] was made by refluxing the sample with standard alcoholic potash, followed by back titration with standard hydrochloric acid. Calculated for three acetyl groups: $OCCH_3$, 10.97. Found: 10.95, 10.65, 10.51. Duplicate determinations varied, however, frequently by as much as 10 percent of the quantity measured. The analytical data, although no criterion of the purity of the substance, proved definitely the presence of three acetyl groups, since four acetyl and two trityl groups would have required 17.62 percent of acetyl, while two acetyl with four trityl groups would have required 6.25 percent of acetyl.

Tritrityltrimethyldifructose anhydride III.—In 100 ml of acetone, 3.68 g of tritrityl triacetyl sugar was dissolved, and the mixture was agitated vigorously at 55° C with a motor-driven stirrer. In 10 equal portions total volumes of 36 ml of dimethyl sulfate and 96 ml of a 30-percent sodium hydroxide solution were added, together with additional quantities of acetone, the methylation requiring about 2½ hours. The reaction mixture was poured into a large volume of violently agitated water and the precipitated substance filtered and air-dried. Crude yield, 3.42 g, or 100 percent of theory. The substance was dissolved in acetone and fractionally precipitated by slow addition of absolute alcohol. Yield, 2.315 g. $[\alpha]_D^{24} = +70.2$ (chloroform, $c=1.05$). Calculated: OCH_3, 8.51. Found: 8.49.

4. DETRITYLATION AND HYDROLYSIS OF TRITRITYLTRIMETHYL-DIFRUCTOSE ANHYDRIDE III

The isolation of the partially methylated fructoses from the tritrityltrimethyldifructose anhydride III was accomplished and repeated several times from different preparations of the substance with uniform results. Typically, 6.9 g of substance in a small Claissen flask was dissolved at 0° C in 130 ml of chloroform containing 2.66 g of anhydrous hydrogen bromide. The solution was allowed to remain at this temperature for about 1 hour, after which 20 ml of water was added and the chloroform distilled off under diminished pressure, leaving the methylated sugar and acid in water solution and the triphenylcarbinol in suspension. The latter was removed by filtra-

tion and extracted with 3-ml portions of water until 41 ml of filtrate had accumulated. The solution was at this point about 0.8 N with respect to hydrobromic acid. The methylated sugar was hydrolyzed for 2½ hours at 94° C, during which time the rotation (2-dm column) changed from +36.9 to −12.6 (saccharimeter degrees). The acid was removed with silver carbonate and the filtrate treated with hydrogen sulfide. The filtered solution was freed from hydrogen sulfide and treated at 10° C with neutral dilute potassium permanganate until a starch-iodide test showed the oxidation of oxidizable impurities to be complete, the equivalent of 15 ml of 0.1 N potassium permanganate being required. The solution was treated with a small amount of carbon, filtered, and evaporated to dryness, the residual solvents being removed by evacuation with a mercury-vapor pump.

The sirup was dissolved in 100 ml of dry methyl alcohol containing 0.5 g of hydrochloric acid and allowed to stand overnight. The acid was removed with silver carbonate and the filtrate evaporated.

The mixed fructosides were distilled from a small flask with a 70-mm still head. The lower-boiling fraction (0.578 g) distilled (<0.01 mm) at a bath temperature of 147° to 156° C. The higher-boiling fraction (0.651 g) was recovered at 156° to 170° C by immersing the entire still head in the bath. The still residue was transferred to a smaller flask and yielded an additional distillate of 0.244 g. Total distillate recovered, 1.474 g, or 54 percent of the theory.

The bath temperatures given above do not reveal the difference in boiling point of the two fractions. In another experiment the mixed fructosides were distilled from a small retort, the condenser arm of which was but 3 mm above the bath. The lower-boiling fraction distilled at a bath temperature of 134° to 140° C and the second fraction at 164° to 170° C at less than 0.01-mm pressure. There was thus about 30° C difference in boiling point.

The loss of nearly 50 percent of the hydrolysis products is readily explained. The methylated disaccharide was necessarily incompletely hydrolyzed, the partially methylated fructoses were severely attacked under the conditions of hydrolysis, the sugars were to some extent adsorbed by carbon, and an inevitable retention in the distilling vessel occurred.

Both fractions were redistilled, a considerable intermediate portion being discarded in order to insure the purities of the remaining fractions.

3,4-Dimethylfructose.—The lower-boiling fraction was a methyl dimethylfructoside. Calculated: OCH_3, 41.9. Found: 40.7.

A sample (0.5486 g) was dissolved in 11.4 ml of 0.1 N hydrochloric acid and hydrolyzed at 60° C for 1 hour. A parallel experiment had shown that hydrolysis was complete under these conditions and that the rotation changed from a positive value to an approximately equal negative value. To the cooled solution silver carbonate from 0.4 g of silver nitrate was added and the volume made up to 25.413 ml. A calculated volume of 0.052 ml for the precipitated silver chloride and excess silver carbonate was deducted. The filtered solution containing 0.5140 g of dimethylfructose showed $[\alpha]_D^{27} = -53.64$ ($\alpha = -6.28$, 2-dm column, saccharimeter degrees). For a sample of pure 3,4-dimethylfructose prepared as described below, $[\alpha]_D^{27} = -53.59$.

The solution, treated with phenylhydrazine in dilute acetic acid solution, deposited crystals of dimethylglucosazone, but difficulty was encountered in purification. The observed melting point, 121° C,

after recrystallization was somewhat lower than that, 126° C, of the more highly purified substance obtained by other methods of preparation. Its identity was proved beyond question by examination of the product of oxidation by nitric acid, the procedure described by Hibbert Tipson, and Brauns [7] being followed in detail.

Oxidation of 3,4-dimethylfructose.—One gram of methyl 3,4-dimethylfructoside was treated with 6 ml of concentrated nitric acid (d, 1.42) in a cool water bath, the temperature of which was gradually increased. At about 65° C gas evolution became vigorous, and the solution was kept at this temperature until the reaction had subsided. The temperature was then slowly raised to 95° C during a period of 1½ hours from the beginning of the reaction. The solution was cooled, diluted with 5 volumes of water, and evaporated under diminished pressure with continuous additions of water until eventually the final portion of distillate was approximately free from nitric acid. The thick yellow liquid consisting of dimethyl lactol acid was diluted with water, and the solution boiled with an excess of calcium carbonate. The filtered solution which was allowed to evaporate spontaneously slowly deposited polyhedral crystals. These were recrystallized from hot water and were evidently identical with the substance which Hibbert, Tipson, and Brauns derived from 1,3,4-trimethylfructose. Calculated for $C_8H_{10}O_8Ca.4H_2O$: H_2O, 20.8. Found: 19.9. Calculated for $C_8H_{10}O_8Ca$: OCH_3, 22.5; Ca, 14.57. Found 22.44, 14.79.

4-Methylfructose.—A portion of the higher-boiling fraction containing methyl monomethylfructoside equivalent to 65.6 mg of methylfructose was dissolved in 5.8 ml of 0.1 N hydrochloric acid and hydrolyzed for 1 hour at 60° C. Silver carbonate from 0.2 g of silver nitrate was added and the solution made to a volume of 17.228 ml, from which a computed volume of 0.026 ml for the silver salts was deducted. The filtered solution rotated $-1.93°$ S (2-dm column, saccharimeter degrees); whence $[\alpha]_D^{26} = -87.5$ ($c=0.387$). The same solution, after treatment with hydrogen sulfide and evaporation yielded a crystalline phenylosazone, which after recrystallization melted sharply at 156° C. Calculated for $C_{19}H_{24}O_4N_4$: OCH_3, 8.4; N, 15.05. Found: 8.6, 15.05.

Mixture of the 4-methyl with 3-methylglucosazone depressed the melting point to 150° C, and with 6-methylglucosazone to 149° C.

5. 3,4-DIMETHYLFRUCTOSE

Trityldiacetylinulin.—Inulin (10 g), previously dried overnight at 80° C and 2 hours at 120° C, was swollen in 200 ml of freshly distilled pyridine. Thirty grams of trityl chloride was added in small portions, causing usually the precipitation of inulin, which redissolved upon gentle warming. The mixture was agitated for 4 days at room temperature and then heated to 90° C for 1 hour. After it was cooled, 80 ml of acetic anhydride was added, and the mixture was heated at 65° C for 1 hour and at 110° C for ½ hour. To the cooled solution water was added dropwise to slight turbidity, and the crystals, which formed slowly, were separated by filtration. The filtrate was poured into a large volume of water with vigorous agitation. Trityldiacetyl-inulin, together with triphenylcarbinol, separated as a white precipitate. This was purified by separation as an amorphous powder from absolute alcohol. Yield, 8 g. $[\alpha]_D^{21} = +13.1$ ($c=1.22$, chloroform). Triphenylcarbinol, calculated: 53.4. Found: 53.3.

Trityldimethylinulin.—Seven grams of trityldiacetylinulin was dissolved in 250 ml of acetone. In 10 portions at 10-minute intervals total volumes of 120 ml of dimethyl sulfate and 320 ml of 30-percent sodium hydroxide were added. During the reaction, the solution was vigorously stirred and maintained at a temperature of 55° C. The reaction mixture was poured into a large volume of water which was vigorously agitated. The precipitated trityldimethylinulin was purified by separation as an amorphous powder from a methyl alcohol solution. Yield, 6.5 g, or 82 percent of theory. Calculated: OCH_3, 14.4. Found: 15.3.

No high degree of purity can be claimed for the tritylated derivatives of inulin, but they have the advantage of yielding abundant quantities of partially methylated fructose from readily available materials.

3,4-Dimethylfructose from trityldimethylinulin.—Ten grams of trityldimethylinulin was dissolved in 250 ml of acetone. Five milliliters of concentrated sulfuric acid was added, and the mixture was allowed to stand overnight at room temperature. Dry ammonia gas was passed into the solution and the precipitated ammonium sulfate separated by filtration and washed with acetone. The acetone was evaporated in the presence of barium carbonate, and the residue was extracted repeatedly with warm water. The water was evaporated, leaving a sirupy residue, which was subjected to fractional distillation at a pressure of 0.2 mm. At a bath temperature of about 80° C, β-diacetonefructose sublimed and crystallized. This was followed by acetone condensation products at about 100° C. The main fraction boiling at a bath temperature of about 115° to 130° C consisted principally of monoacetonedimethylfructose. This fraction was redistilled, yielding three approximately equal fractions, A (bath, 117° to 121° C; inside, 99° to 101° C; OCH_3, 24.4); B (bath, 122° to 126° C; inside 101° to 106° C; OCH_3, 23.1); and C (bath, 125° to 131° C; inside, 105° to 110° C; OCH_3, 21.6). Theory for monoacetonedimethylfructose: OCH_3, 25.0. The fractions were converted separately to the methyl fructosides.

Monoacetonedimethylfructose (6.2 g) was dissolved in 100 ml of 95-percent alcohol and made to 150 ml with water containing 0.18 g of hydrochloric acid, according to the procedure of Irvine and Hynd [11]. Hydrolysis of the acetone group was effected by heating in a boiling-water bath for about 1½ hours, during which time the rotation changed from −5.25° to −7.90° (2 dm, saccharimeter degrees). The acid was removed by means of silver carbonate followed by hydrogen sulfide in the usual manner. The filtrate was evaporated to dryness and dissolved in dry methyl alcohol containing 5 mg of hydrochloric acid per milliliter. During the formation of the fructoside, the initial negative rotation changed to a positive value slightly more than twice as great in the course of 3 hours. The reducing power became practically zero.

The dimethylfructose prepared by this method was invariably contaminated by monomethylfructose, which probably consisted mainly of 4-methylfructose, since 3-methylfructose, if present, was largely eliminated in the form of the low-boiling 3-methyldiacetonefructose. The separation of methyl dimethylfructoside from methyl monomethylfructoside by fractional distillation was not difficult, since there was a difference of about 30° C in boiling point. The dimethyl

derivative distilled at 0.01 mm at a bath temperature of 132° C. The substance, after hydrolysis, showed the same properties as the dimethylfructose prepared from the more reliable ditrityltetramethyldifructose anhydride I and yielded the same osazone melting at 126° C.

In one experiment, trityldimethylinulin was detritylated by dissolving it in chloroform and adding a chloroform solution of hydrogen bromide. The substance was detritylated effectively, but the dimethylinulin was apparently depolymerized in great part to difructose anhydrides, which subsequently required vigorous conditions of acidity and temperature for hydrolysis. This depolymerization to difructose anhydride is evidently analogous to Irvine and Stevenson's depolymerization with nitric acid.

Ditrityldifructose anhydride I.—One gram of sugar and 3 g of trityl chloride were dissolved in 15 ml of redistilled pyridine and allowed to remain at room temperature for 3 days. Water was added to slight turbidity, and, after the mixture was cooled to 0° C, the carbinol-pyridine complex was removed by filtration. The filtrate was poured into a large volume of ice water and the resulting white gummy precipitate washed and air-dried. From warm methyl alcohol the substance crystallized as fine needles, which were recrystallized to constant properties. Yield, 1.05 g, or 42 percent of theory. Melting point, 195° C (uncor.); $[\alpha]_D^{22}=+20.35$ (chloroform, $c=2.186$). Triphenylcarbinol, calculated, 64.4. Found: 64.4.

Ditrityltetra-acetyldifructose anhydride I.—In 120 ml of pyridine were dissolved 6.3 g of sugar and 18 g of trityl chloride. The mixture was allowed to remain at room temperature for 3 days and was then heated to 85° to 90° C for 1 hour. The solution was cooled and 60 ml of acetic anhydride added. The mixture was heated at 70°C for 1 hour and finally at 110° C for ½ hour. When the mixture was cool, water was added to slight turbidity and the carbinol-pyridine crystals separated by filtration. The filtrate was poured into a large volume of ice water. The precipitate which formed was dried and dissolved in warm absolute alcohol, from which the substance separated as transparent spheroids. Upon repeated recrystallization from absolute alcohol, ditrityltetra-acetyldifructose anhydride I appeared as needle-like crystals. Yield, 14.7 g, or 92 percent of theory. Melting point, 194° C (uncor.); $[\alpha]_D^{20}=+21.06$ (chloroform, $c=3.731$). Triphenylcarbinol, calculated, 53.3. Found: 52.5. Acetyl ($OCCH_3$), calculated, 17.6. Found: 18.0.

A portion of this compound was used for the preparation of 3,4-dimethylfructose and the remainder for 6-methylfructose.

Ditrityltetramethyldifructose anhydride I.—In 150 ml of acetone was dissolved 5.29 g of ditrityltetra-acetyldifructose anhydride I, and the solution was heated to 55° C. In 10 equal portions at intervals of 15 minutes, total volumes of 54 ml of dimethyl sulfate and 144 ml of 30-percent sodium hydroxide were added with vigorous agitation by a motor-driven stirrer. The reaction mixture was poured into water and the precipitate collected and dried. The substance was amorphous. OCH_3, calculated, 14.35. Found: 14.65.

3,4-Dimethylfructose.—Ditrityltetramethyldifructose anhydride I (6.8 g) was dissolved in chloroform and cooled to 0° C. A solution of 2.5 g of hydrogen bromide in chloroform was added and the mixture allowed to stand for about 1 hour. There was added 25 ml of water

and the chloroform was evaporated at diminished pressure, leaving the tetramethyl disaccharide and hydrobromic acid in water solution with the insoluble triphenylcarbinol in suspension. The soluble materials were filtered, and the precipitate was washed. The combined filtrate and washings (65 ml) were made 0.8 N with hydrogen bromide and some additional hydrochloric acid, and the sugar was hydrolyzed for 2 hours at 95° C, during which time the rotation changed from $+6.5$ to $-8.5°$ (2-dm column, saccharimeter degrees). The acids were neutralized with silver carbonate and the filtrate was treated with hydrogen sulfide. The filtered solution was evaporated and the substance converted to the methyl fructoside by dissolving in dry methyl alcohol and adding a methyl alcohol-hydrogen chloride solution, the final concentration of acid being 5 mg per milliliter. During fructoside formation, the rotation changed in 3½ hours from $-40.1°$ to $+8.27°$ (2-dm column, saccharimeter degrees), passing through zero in 7 minutes. Another preparation of this fructoside from monotrityldimethylinulin showed the same relative change of rotation, but on standing for a longer period the positive rotation diminished considerably. However, unlike that of unsubstituted fructose, its final rotation remained definitely positive even after several days.

The fructoside distilled at 0.01 mm at 100° C; bath temperature, 131° C. Calculated: OCH_3, 41.9. Found: 40.0. The substance was dissolved in water at 10° C and treated with neutral dilute potassium permanganate until a starch-iodide test showed the oxidation of impurities to be complete. It was filtered through carbon and redistilled at 0.01-mm pressure. The substance distilled completely at a bath temperature of 129° to 130° C. Calculated: OCH_3, 41.9. Found; 41.5.

The purified fructoside was dissolved in about 30 parts of 0.1 N hydrochloric acid. Hydrolysis was complete in 1 hour at 60° C, during which time the rotation changed from $+12.75°$ to $-11.58°$ (saccharimeter degrees). The acid was removed with silver carbonate in the usual manner. An effort was made to determine the properties of the dimethyl sugar, but during the drying the substance apparently polymerized. In another experiment 0.2047 g of fructoside, equivalent to 0.1918 g of dimethylfructose, was hydrolyzed in a small volumetric flask in 11.4 ml of 0.1 N hydrochloric acid, and the acid was neutralized with silver carbonate derived from 0.4 g of silver nitrate. The solution containing the precipitate was made to a volume of 25.396 ml at 20° C, and the volume of silver chloride and excess silver carbonate was computed to be 0.052 ml. The rotation of the filtered solution was carefully measured at several temperatures, the expansion being calculated by assuming a coefficient of 0.00025. $[\alpha]_D^t = -60.66 + 1.01(t - 20° \text{ C})$; whence $[\alpha]_D^{20} = -60.66$ (water, $c = 0.757$).

The 3,4-dimethylfructose was converted into a crystalline phenylosazone which was free from water of crystallization and melted at 126° C after recrystallization from ether.

Oxidation with nitric acid by the procedure previously described produced the same dibasic dimethyl lactol acid which was obtained from the lower-boiling fraction of the hydrolyzed, partially methylated difructose anhydride III. Its calcium salt showed the following composition: Calculated for $C_8H_{10}O_8Ca.4H_2O$: H_2O, 20.8. Found:

20.77. Calculated for $C_8H_{10}O_8Ca$: OCH_3, 22.5; Ca, 14.57. Found: 22.6, 14.43.

6. 6-METHYLFRUCTOSE

3,4,3′,4′-Tetra-acetyldifructose anhydride I.—Five grams of ditrityl-tetra-acetyldifructose anhydride I was dissolved in 20 ml of glacial acetic acid. To the solution, cooled to 5° C, was added 2 ml of acetic acid saturated with hydrogen bromide at 0° C, causing the precipitation of yellow trityl bromide. The filtrate was poured into an ice and water mixture, from which the tetra-acetyl derivative was extracted with chloroform. The chloroform solution was washed with sodium bicarbonate, dried over anhydrous sodium sulfate, and evaporated, leaving a sirupy residue. The latter was dissolved in absolute alcohol, from which the tetra-acetyldifructose anhydride crystallized in the form of rectangular plates. Yield, 1.8 g, or 53 percent of theory. Melting point, 173° C (uncor.). $[\alpha]_D^{20} = -9.9$ (chloroform, $c = 3.73$); +23.0 (water, $c = 2.96$). $OCCH_3$, calculated, 35.0. Found: 35.1.

A small portion upon acetylation yielded the hexa-acetyldifructose anhydride I described by Jackson and Goergen.

6,6′-Dimethyl 3,4,3′,4′-tetra-acetyldifructose anhydride I.—Tetra-acetyldifructose anhydride I proved to be completely soluble in methyl iodide and was methylated with Purdie's reagents in the usual manner, three methylations being required to supply the full methoxyl content. At each stage of the methylation, however incomplete, the reaction product crystallized completely, giving a false indication of homogeneity. The fully methylated substance crystallized from aqueous alcohol in the form of prismatic needles. Melting point, 127° to 128° C (uncor.). $[\alpha]_D = +10.8$ (chloroform, $c = 1.23$). OCH_3, calculated, 11.9. Found: 11.6.

6-Methylfructose and its osazone.—Dimethyltetraacetyldifructose anhydride I (0.51 g) was suspended in 17.5 ml of 0.8 N hydrochloric acid and heated at 95° C for a total of 45 minutes, which sufficed to hydrolyze the disaccharide and saponify the acetyl groups, as indicated by the constancy of rotation. The acid was removed as silver chloride and the filtrate treated with hydrogen sulfide. The filtrate was evaporated to dryness, dissolved in dry methyl alcohol containing 5 mg of hydrochloric acid per milliliter, and allowed to stand overnight. Hydrochloric acid was removed with silver carbonate and the filtrate treated at 10° C with dilute neutral potassium permanganate until a starch-iodide test showed a minute excess. The mixture was treated with a small amount of carbon and the filtrate evaporated, leaving a sirupy residue which distilled below 160° C at 0.01-mm pressure.

The distilled fructoside (0.2296 g), equivalent to 0.2116 g of 6-methylfructose, was hydrolyzed in 0.1 N hydrochloric acid at 60° C for 1 hour and was then diluted to 17.10 ml. The rotation in a 2-dm column was 0.46° (saccharimeter degrees); whence $[\alpha]_D^{26} = +6.4$ (0.06 N hydrochloric acid, $c = 1.24$).

To the solution, after polarization, was added 0.2 g of hydrated sodium acetate, 4 ml of acetic acid, and 2 ml of redistilled phenylhydrazine. The mixture was heated for 40 minutes at 62° C, during which time abundant crystallization of the osazone occurred. The crystals, recrystallized from 95-percent alcohol, took the form of very long, fine, yellow needles. Melting point, 183° to 184° C (uncor.). $[\alpha]_D^{29} = -70.9$ (95-percent alcohol, $c = 0.88$), diminishing to -55.6 in

18 days. *Analysis.* Calculated for $C_{19}H_{24}N_4O_4$: C, 61.27; H, 6.50; N, 15.04. Found: C, 61.22; H, 6.60; N, 15.00.

IX. SUMMARY

Difructose anhydrides I, II, and III, derived in about 5-percent yield from inulin which has been hydrolyzed by aqueous acids, are converted by methylation into hexamethyl derivatives which are purified by distillation. The derivatives of the first and third sugar are liquids, while that of the second is crystalline. Upon hydrolysis and distillation, I yields two molecules of 3,4,6-trimethylfructose, while III yields a mixture of two different trimethylfructoses, one of which is 3,4,6, as shown by its graded oxidation to trimethylarabono-lactone.

Tritylation of III produces crystalline tritrityldifructose anhydride III which acquires, upon methylation, three methoxyl groups. Detritylation and hydrolysis cause the formation of a dimethyl and a monomethyl fructose, which are separated by fractional distillation of their fructosides. The dimethylfructose has a $[\alpha]_D^{20} = -60.66$ and forms an osazone of melting point 126° C. Its structure is shown by its oxidation to dibasic 3,4-dimethyl lactol acid, whose calcium salt is crystalline. The dimethyl fraction is therefore 3,4-dimethylfructose.

The monomethylfructose yields a monomethylglucosazone, which melts at 156° C and is identical with that prepared from 4-methylglucose. It is shown that when mixed with 3- or 6-methylglucosazone, the only other possible monomethyl compounds which could be formed, the melting point is depressed. The monomethyl derivative is thus 4-methylfructose.

Difructose anhydride III is therefore constituted of two fructose residues with oxygen bridges connecting, respectively, carbons 1 and 2 of the one residue with 2 and 3 of the other. Its great stability is due to the presence of a dioxane ring, which serves as the connecting link between the two fructose groups.

The new derivatives of difructose anhydrides I and III which have been prepared in pure form are given in table 3. A system of nomenclature is tentatively suggested.

TABLE 3.—*Derivatives of difructose anhydrides*

DIFRUCTOSE ANHYDRIDE I	Melting point	$[\alpha]_D$	Solvent	Concentration
	°C			
Difructofuranose 1,2',2,1'-anhydride	164	+27.0	Water	4.08
3,4,6,3',4',6'-Hexa-acetyldifructofuranose 1,2',2,1'-anhydride.	137	+0.54	Chloroform	2.49
3,4,6,3',4',6'-Hexamethyldifructofuranose 1,2',2,1'-anhydride.		+23.7	____do	4.86
6,6'-Ditrityldifructofuranose 1,2',2,1'-anhydride	195	+20.3	____do	2.18
6,6'-Ditrityl 3,4,3',4'-tetra-acetyldifructofuranose 1,2',2,1'-anhydride.	194	+21.06	____do	3.73
3,4,3',4'-Tetra-acetyldifructofuranose 1,2',2,1'-anhydride.	173	-9.9	____do	3.73
6,6'-Dimethyl 3,4,3',4'-tetra-acetyldifructofuranose 1,2',2,1'-anhydride.	127-8	+10.8	____do	1.23

DIFRUCTOSE ANHYDRIDE III				
Difructofuranose 1,2', 2,3'-anhydride	162	+135.64	Water	7.85
3,4,6,1',4',6'-Hexamethyldifructofuranose 1,2',2,3'-anhydride.		+157.9	Chloroform	3.95
6,1',6'-Tritrityldifructofuranose 1,2', 2,3'-anhydride	127	+64.2	____do	0.76
6,1',6'-Tritrityl 3,4,4'-trimethyldifructofuranose 1,2',2,3'-anhydride.		+70.2	____do	1.05

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X. REFERENCES

[1a] R. F. Jackson and S. M. Goergen, BS J. Research **3**, 27 (1929) RP79.
[1b] R. F. Jackson and E. J. McDonald, BS J. Research **6**, 709 (1931) RP299.
[2] W. N. Haworth and H. R. L. Streight, Helv. Chim. Acta **15**, 693 (1932).
[3] J. C. Irvine and J. W. Stevenson, J. Am. Chem. Soc. **51**, 2197 (1929).
[4] E. W. Bodycote, W. N. Haworth, and C. S. Woolvin, J. Chem. Soc. **1932**, 2390.
[5] H. H. Schlubach and H. Knoop, Liebigs Ann. Chem. **504**, 25 (1933).
[6] W. N. Haworth and A. Learner, J. Chem. Soc. **1928**, 619.
[7] H. Hibbert, R. S. Tipson, and F. Brauns, Canadian J. Research **4**, 221 (1931).
[8] T. N. Montgomery, J. Am. Chem. Soc. **56**, 419 (1934).
[9] B. Helferich, Liebigs Ann. Chem. **440**, 1 (1924); Ber. deut. chem. Ges. **58**, 1605 (1925); **59**, 600 (1926); **62**, 1549 (1929); **63**, 959, 989 (1930).
[10] E. Pacsu, Ber. deut. chem. Ges. **58**, 1455 (1925).
R. Schinle, Ber. deut. chem. Ges. **65**, 319 (1932).
J. Munroe and E. G. V. Percival, J. Chem. Soc. **1935**, 874.
[11] J. C. Irvine and A. Hynd, J. Chem. Soc. **95**, 1220 (1909).
K. Freudenberg and R. M. Hixon, Ber. deut. chem. Ges. **56**, 2126 (1923).
C. G. Anderson, W. Charlton, and W. N. Haworth, J. Chem. Soc. **1929**, 1329.
[12] H. Ohle and L. Vargha, Ber. deut. chem. Ges. **62**, 2434 (1929).
R. Kuhn and W. Ziese, Ber. deut. chem. Ges. **59**, 2314 (1926).
B. Helferich and J. Becker, Liebigs Ann. Chem. **440**, 13 (1924).
[13] H. Herrissey, P. Fleury, and M. Joly, J. pharm. chim. [8] **20**, 149 (1934); cf. E. L. Jackson and C. S. Hudson, J. Am. Chem. Soc. **58**, 378 (1936), and many subsequent articles.
[14] H. H. Schlubach and H. Knoop, Liebigs Ann. Chem. **504**, 19 (1933).
[15] H. H. Schlubach and H. Elsner, Ber. deut. chem. Ges. **61**, 2363 (1928).
[16] W. N. Haworth, J. Chem. Soc. **107**, 8 (1915).
[17] W. N. Haworth and H. R. L. Streight, Helv. Chim. Acta **15**, 614 (1932); **15**, 693 (1932).
[18] F. Valentin, Collection Czechoslov. Chem. Communications **3**, 499 (1931). Method is given in detail by E. L. Jackson, R. C. Hockett, and C. S. Hudson, J. Am. Chem. Soc. **56**, 948 (1934).
[19] H. Meyer, Lehrbuch der Organisch-Chemischen Methodik, Vierte Auflage **1**, 671 (Julius Springer, Berlin, 1922).

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