

DIFRUCTOSE ANHYDRIDES FROM HYDROLYZED INULIN

THE STRUCTURE OF DIFRUCTOSE ANHYDRIDE. III

BY

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Doctor of Philosophy

1935.

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## DIFRUCTOSE ANHYDRIDES FROM HYDROLYZED INULIN

### THE STRUCTURE OF DIFRUCTOSE ANHYDRIDE III

In previous articles Jackson and Goergen<sup>1</sup> and Jackson and McDonald<sup>1</sup> have described the isolation and properties of three isomeric crystalline difructose anhydrides which were found to constitute about five percent of the products of aqueous acid hydrolysis of inulin. These sugars designated I, II, and III have specific rotations of  $[\alpha]_D^{20} = +27.0, +13.85,$  and  $+135.62,$  respectively, and are all hydrolyzable by aqueous acid into two molecules of fructose. As was announced in the articles cited the study was continued in order to determine the structures of the sugars and the nature of the isomerism. While this study was in progress Haworth and Streight<sup>2</sup> have isolated the crystalline hexacetate of the first sugar according to the method described by Jackson and Goergen and after methylation of the sugar and hydrolysis have obtained in 80% yield 3,4,6 trimethyl fructofuranose, showing that the disaccharide is symmetrically constructed and that carbon atoms 1 and 2 of each fructose residue

participate in the condensation. The isolation of three isomeric difructose anhydrides led us to the early expectation that they might be the stereoisomers,  $\alpha\alpha$ ,  $\alpha\beta$ , and  $\beta\beta$  of the same disaccharide. Haworth and Streight and Schlubach and Kncop have also suggested this. This possibility will be examined in detail in this thesis.

The present investigation includes a study of the completely methylated sugars and the determination of the structure of difructose anhydride III by identification of the products of hydrolysis of the fully methylated and of the partially methylated sugar.

The methylation was carried out in the usual manner, which consists of a partial methylation of the sugar by means of dimethyl sulfate in alkaline solution followed by treatment with methyl iodide and silver oxide. The physical properties of the three hexamethyl difructose anhydrides are given in Table I. The first and third methylated sugars proved to be distillable liquids, while the second showed striking crystalline properties. The latter appeared in crystalline form even before it was completely extracted from the silver residues and in subsequent preparations separated from solution in large well formed prisms. One crystal 2 cm. in length was obtained.

TABLE I

Diffructose Anhydrides		Methylated Diffructose Anhydrides												
[α] 20 D in H <sub>2</sub> O	d <sub>4</sub> <sup>20</sup> pure liquid	n <sub>D</sub> <sup>20</sup>	Boiling Point Pressure	Melting Point uncorr.	[α] 20 D		[α] 20 D		In water	Pure liquid	In chloroform	In water	In chloroform	In water
					Pure liquid	In chloroform	Pure liquid	In chloroform						
I +27.0	1.2050	1.4706	0.01	170-175°C	+19.1	+23.7	+50.4	+22.4	+27.5	+59.8				
II +13.85		1.4673 <sup>1</sup>	0.35	169-170°C	-41.9 <sup>1</sup>	-28.2	+6.0		-33.4	+6.7				
III +135.64	1.1918	1.4658	0.417	161-165°C	+155.7	+157.9	+164.6	+182.7	+186.9	+194.7				

1. supercooled liquid

In the case of all three compounds when water is used as solvent the specific rotation is more positive than when no solvent is used or when the methylated sugar is dissolved in chloroform. This effect of solvent is much greater in the case of the second sugar.

The hydrolysis of the methylated difructose anhydrides was carried out in 0.8N HCl at 95°C. The progress of the reaction, in each case, was followed by noting the change in specific rotation. The solutions became dark colored before the reactions were complete, thus it was impossible to obtain the final rotations. These hydrolysis data are presented in Figure 1 and Table II.

TABLE II

Hydrolysis of Hexamethyl difructose anhydrides  
in 0.8N HCl at 95°C.

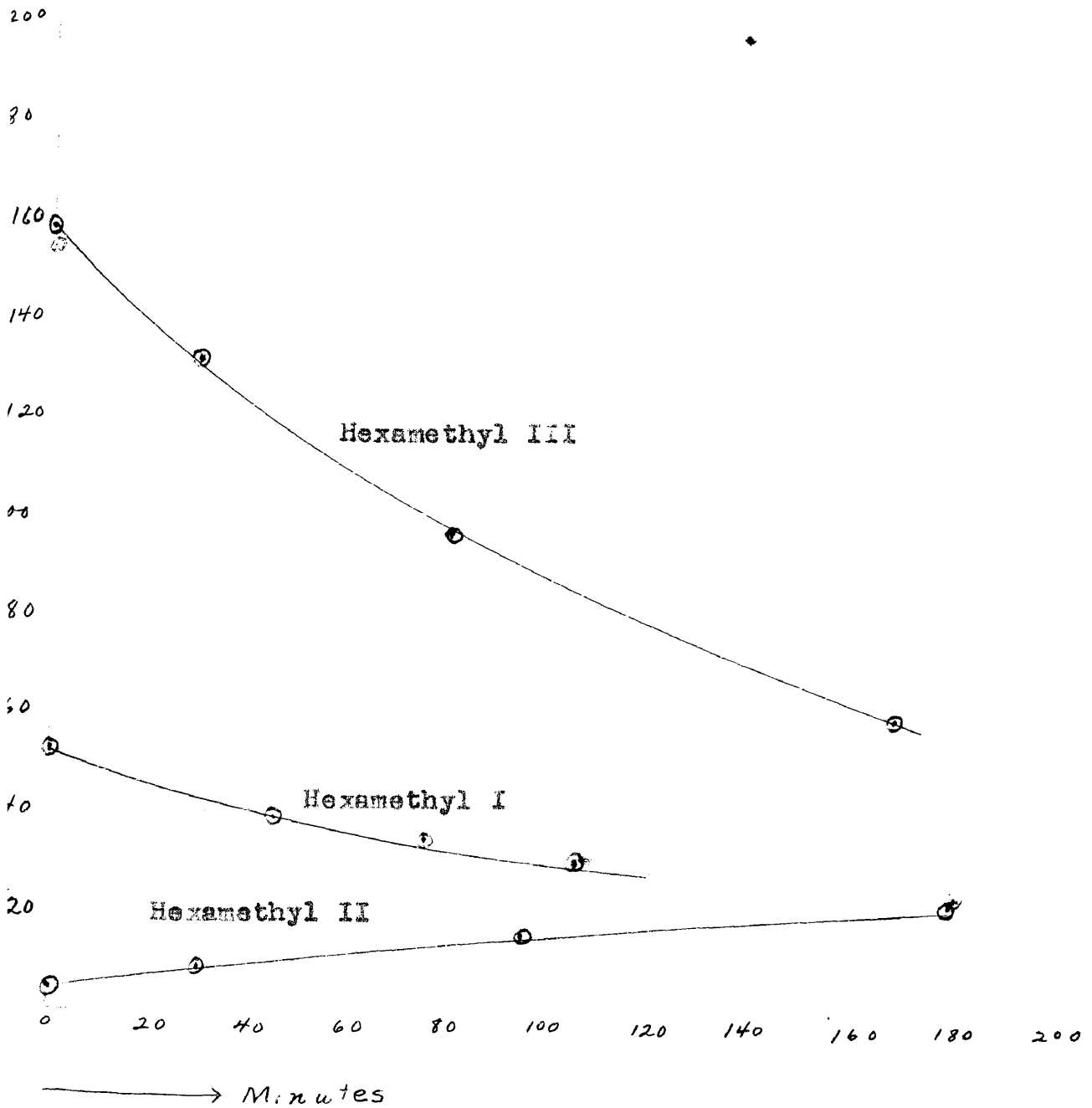
Time in min.	I 1 direct rotation	$[\alpha]_D$	II 1 direct rotation	$[\alpha]_D$	III 2 direct rotation	$[\alpha]_D$
0		+53	+1.2	+5.5	+18.2	+158.6
30			+2.05	+9.5	+15.2	+131.0
45	+7.41	+38.4				
75	+6.66	+34.5				
80					+11.1	+ 95.1
95			+3.4	+15.7		
105	+5.89	+30.5				
169					+6.95	+59.6
180			+5.0	+23.0		

1. rotations taken in 2 dm. tube  
2. rotations taken in 1 dm. tube  
All rotations observed in sugar  
degrees in a quartz-wedge saccharimeter.



Fig. 5

Hydrolysis of hexamethyl difructose anhydrides  
in 0.8N HCl at 95°C



All three curves appear to approach a final specific rotation value of about  $+30^{\circ}$  which is the value for pure 3,4,6 trimethyl fructose in aqueous solution. This is known to be the final product of hydrolysis in the case of the methylated first sugar. The boiling points of the hydrolyzed methylated sugars are also in agreement with that of 3,4,6 trimethyl fructose. Thus far the results suggest that 3,4,6 trimethyl fructose may be the only product of hydrolysis.

All attempts, however, to convert the distillate quantitatively into the phenyl osazone failed. A relatively small yield of osazone was obtained which upon purification showed the correct melting point of the hydrated form of 3,4,6 trimethyl fructose osazone. Pure 3,4,6 trimethyl fructose prepared by the hydrolysis of hexamethyl difructose anhydride I and also prepared by the hydrolysis of trimethyl inulin could be converted into the phenyl osazone almost quantitatively. The failure to obtain a proper yield of this osazone from the hydrolyzed hexamethyl difructose anhydride III must be ascribed either to the presence of some unsuspected impurity or to the presence of two trimethyl fructoses, one of which is not the 3,4,6 derivative.

Similar conclusions may be drawn from a comparison of the results obtained by condensing 3,4,6 trimethyl fructose and the products of hydrolysis of the hexamethyl III

with acetone in the presence of  $H_2SO_4$ . The change in direct rotation as the reaction progressed was observed in each case. These data are given in Table III and Figure 2. Although their initial specific rotations were similar, + 27.0 for 3,4,6 trimethyl fructose and +27.6 for the hydrolyzed hexamethyl III, a large difference was observed as the reaction proceeded. The 3,4,6 trimethyl fructose condensed with acetone to form a much more positively rotating substance than that obtained when the hydrolyzed hexamethyl III was reacted with acetone. Attempts to isolate the acetone derivatives were not successful.

TABLE III

Reaction of 3,4,6 trimethyl fructofuranose and of the hydrolysis products of hexamethyl difructose anhydride III in H<sub>2</sub>SO<sub>4</sub> - acetone solution

Time in min.	3,4,6 trimethyl fructofuranose		Hydrolysis product of hexamethyl III	
	direct rotation	$[\alpha]_D$	direct rotation	$[\alpha]_D$
0	2.56	27.0	2.28	27.6
10	3.8		2.6	
20			2.7	
25	4.5			
30			2.9	
40	5.2		3.0	
50			3.0	
60	5.5		3.2	
70	6.0		3.3	
85	6.1			
100	6.3		3.4	
110	6.3			
130	6.3			
145			3.5	

All rotations observed in sugar degrees in a quartz-wedge saccharimeter.

Acetone-sulphuric acid condensations with 3-4-6 trimethyl fructose  
fructose and hydrolysis products of hexamethyl difructose anhydride III

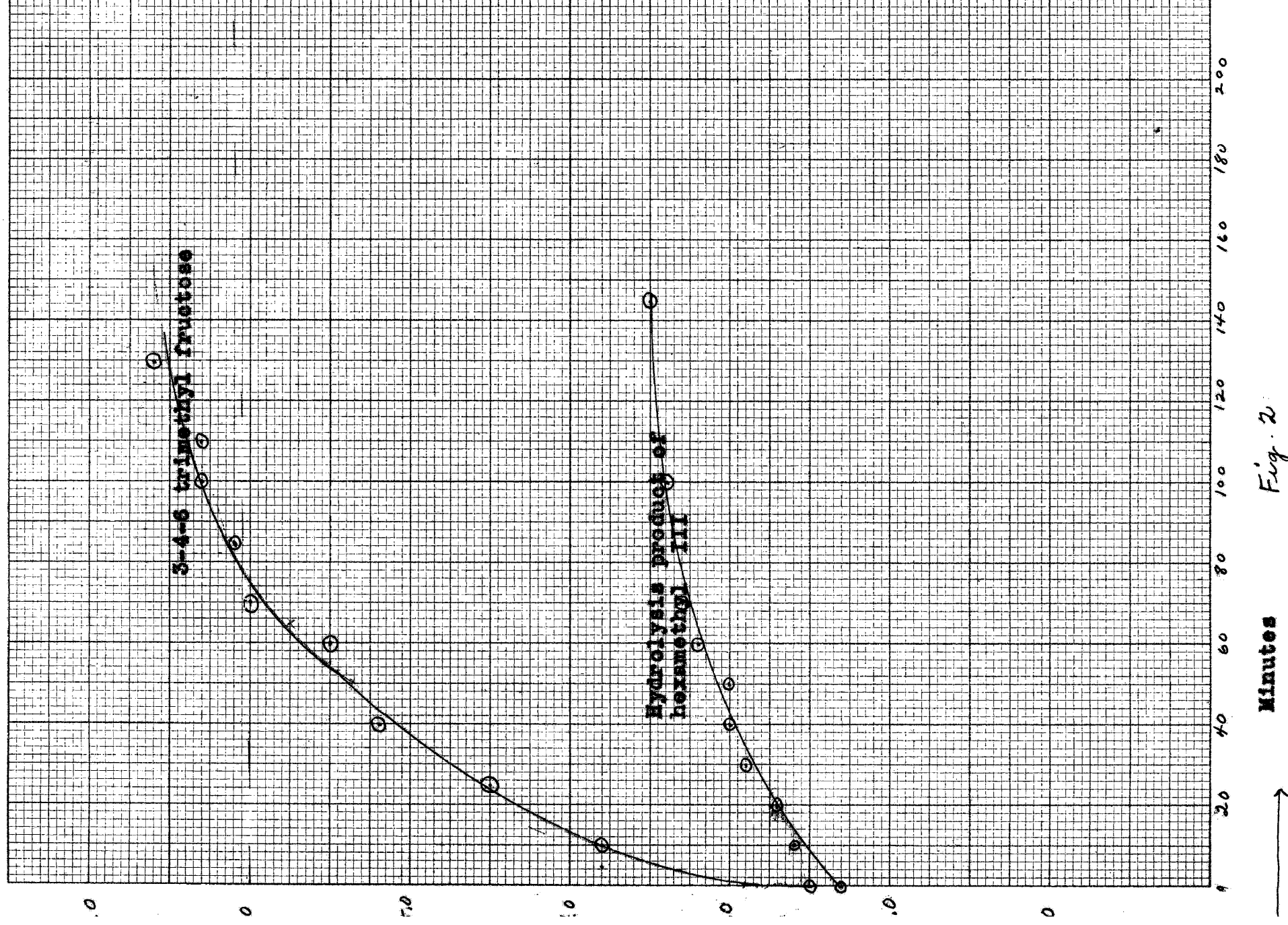


Fig. 2

Minutes



If 3,4,6 trimethyl fructose were the sole product of hydrolysis of the three hexamethyl disaccharides it would follow that the isomerism occurred on carbons 2 which conceivably might have the alpha or beta configuration. There would then be possible the combination  $\alpha\alpha$ ,  $\alpha\beta$  and  $\beta\beta$ . Following Hudson's principles of isorotation let  $F$  represent the rotatory power of carbons 3,4,5 and 6, and let  $+A$  represent the rotatory power of the  $\alpha$  configuration of carbon 2, then the  $\beta$  configuration of this second carbon would be represented by  $-A$ . The three isomeric sugars would have molecular rotations which could be expressed as follows:

(1)	$2F + 2A$	$= 2F + 2A$
(2)	$2F + A - A$	$= 2F$
(3)	$2F - 2A$	$= 2F - 2A$

The sugar represented by (2) would have a molecular rotation equal in value to that of the average of the other two sugars. The molecular weights of all three sugars would be the same, therefore, the relation above described would apply to their specific rotations. The specific rotations of the three difructose anhydrides were found to be +27.0, +13.85 and +135.64. A glance at these figures shows that the average of no two rotations is even approximately equal to the third.

This must be either because the dioxan ring\* exerts an effect such that these sugars are not comparable to the ones cited by Hudson in connection with his rules of isorotation or that they have different types of structure.

The hydrolysis of hexamethyl difructose anhydride III yielded a sirup having a rotation of approximately  $+30^\circ$  in aqueous solution. Upon conversion into the methyl fructoside followed by methylation and finally removal of the fructoside groups, tetramethyl gamma fructose alone was formed. Thus, both fructose residues must contain the furanose ring.

There are four possible trimethyl gamma fructoses 3-4-6, 1-3-4, 1-4-6, and 1-3-6. The properties of the first two are well known. The 1-4-6<sup>3</sup> has been prepared in a quantity too small to determine its physical properties, and 1-3-6 trimethyl gamma fructose has never been described.

In recent years Helferich<sup>4</sup> and his collaborators have shown that triphenyl chloromethane exhibits the tendency to substitute on primary hydroxyl groups in preference to secondary hydroxyl groups. Valentine<sup>5</sup> found that this reagent was selective to primary alcohol groups in

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\*Haworth (J.C.Soc. 15 pg.695 (1932) has shown that in difructose anhydride I the intermediate six atom ring joining the two sugar rings in the anhydride has the structure of a substituted dioxane.

his work on a number of polyhydroxy alcohols. In all cases of compounds containing both primary and secondary alcohol groups this reagent has been found to be selective towards the primary groups, therefore trityl condensations may be said to imply the number of primary alcohol groups present in compounds of this type.

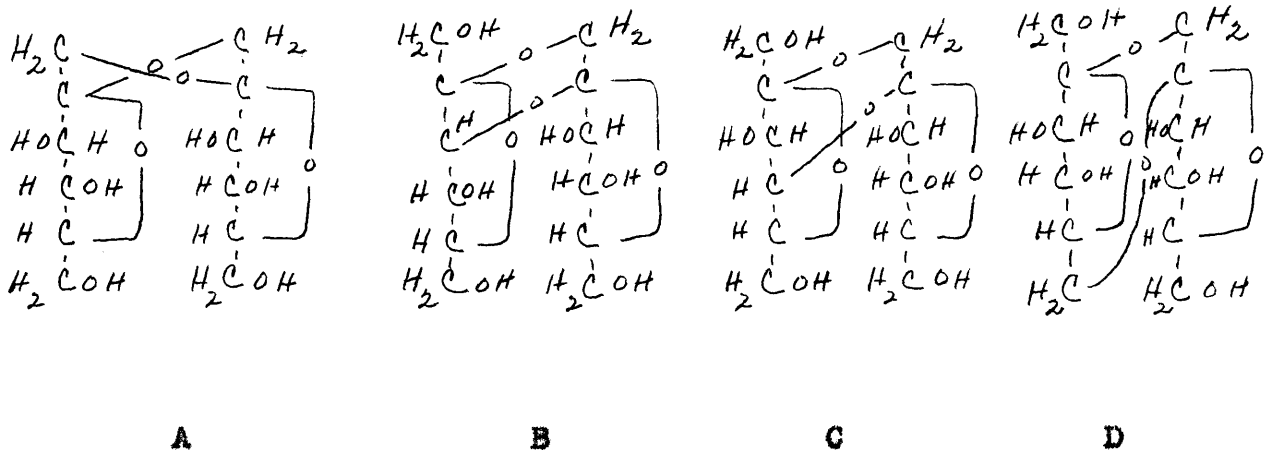
Hackett and Hudson and Hackett, Jackson, and Hudson conclude that the triphenyl chloromethane condensation is not an infallible test for primary alcohol groups since with a few compounds containing only secondary alcohol groups they have been able to make trityl derivatives. These compounds appear to be much less stable towards heat than those in which condensation has taken place between triphenyl chloromethane and a primary alcohol group.

When difructose anhydride I was treated with triphenylchloromethane in pyridine solution two trityl groups combined with the sugar in agreement with Helferich's generalization. Difructose anhydride III, in contrast, upon similar treatment formed a tri-trityl derivative. It is known that difructose anhydride I contains two primary alcohol groups and thus it is suggested that difructose anhydride III may contain three such groups.

The facts that 3,4,6 trimethyl fructose has been identified in the hydrolyzed hexamethyl difructose anhydride III and that upon conversion to the tetramethyl fructose



only the gamma form was present, require that difructose anhydride III have one of the four following structures:



Formula A represents difructose anhydride I, or its stereoisomers. Hydrolysis of a completely methylated sugar of this type would give 3-4-6 trimethyl fructose alone, and this is found to be the sole product when hexamethyl difructose anhydride I is hydrolyzed. However, when hexamethyl difructose anhydride III is hydrolyzed the low yield of phenyl osazone and the apparently different condensation with acetone indicate that another trimethyl fructose is formed together with the 3-4-6 derivative.

If formula D represented the structure of the sugar, the hexamethyl derivative upon hydrolysis would

yield a mixture of 1-3-4 trimethyl fructose and 3-4-6 trimethyl fructose. 3-4-6 trimethyl fructose has been identified as one of the products of hydrolysis by means of its phenyl osazone. 1-3-4 trimethyl fructose has been prepared in crystalline form by Hibbert<sup>7</sup>, Tipson and Brauns. It has a specific rotation of  $-51.4$  in aqueous solution. The specific rotation of 3-4-6 trimethyl fructose is  $+30^{\circ}$  in aqueous solution, hence the mixture obtained by the hydrolysis of hexamethyl III, which has a definitely positive specific rotation in the neighborhood of  $+30^{\circ}$ , cannot be a mixture of these two trimethyl fructoses. Formula D is therefore eliminated as a possible structure.

A sugar of either formula A or D would contain only two primary alcohol groups and hence only two trityl groups would be expected to combine with it readily. Difructose anhydride III forms a tritryl derivative.

The data thus far presented show that the structure of difructose anhydride III is in agreement with neither formula A nor formula D.

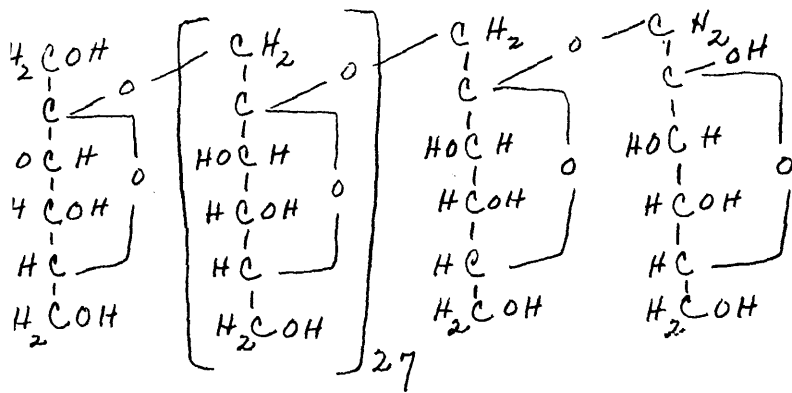
Formulas B and C represent sugars whose hexamethyl derivatives upon hydrolysis would yield 3-4-6 trimethyl fructose together with either 1-4-6 trimethyl fructose or 1-3-6 trimethyl fructose respectively. As the physical properties of these last two compounds are not known, it

is impossible to predict from these data which of the two formulas represents difructose anhydride III.

Hydrolysis of the partially methylated sugar appeared to be a means of distinguishing between these two structures and of confirming the elimination of A and D as possible formulas of difructose anhydride III. The trityl derivatives of inulin, difructose anhydrides I and III were used in preparing these partially methylated derivatives. In no case is it assumed that the condensation between triphenyl chloromethane and the sugar has taken place through a primary alcohol group. Definite proof is offered below to show the positions of the trityl groups in inulin. The procedure, by which the position of the trityl groups in the two disaccharides will be determined in the complete work, is also given. Inulin, upon tritylation and methylation, yielded a monotrityl dimethyl derivative IV. Detritylation followed by acid hydrolysis gave a dimethyl fructose. This dimethyl fructose had a specific rotation in chloroform of  $-7.0$ , thus it appears to have a 2-6 ring, and hence the hydroxyl of carbon 6 was unsubstituted. If on conversion into the tetra methyl fructose the tetramethyl normal fructose is formed, carbon 6 was unsubstituted in the dimethyl fructose. Should the tetramethyl gamma fructose result from this procedure it will be necessary to oxidize

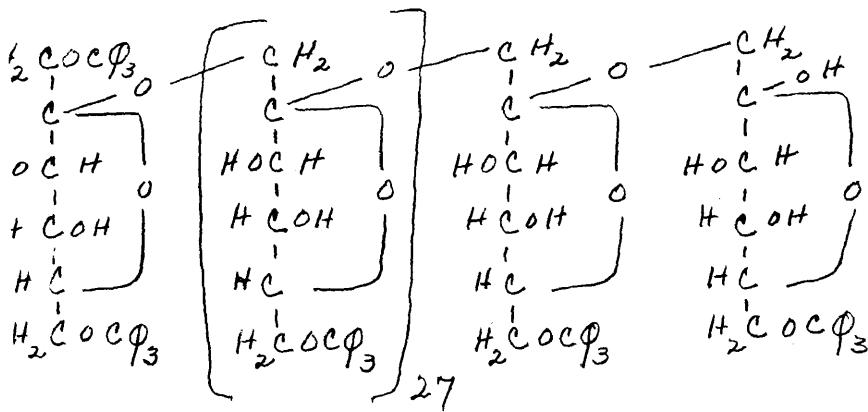
the dimethyl fructose in order to determine whether or not the hydroxyl of the 6th carbon is substituted. It is known that the hydroxyls of carbons<sup>15</sup> 1 and 2 are involved in the condensations between the fructose residues in forming the inulin molecule and that the fructose residues are gamma sugars thus involving carbon 5. The hydroxyl groups on carbons 3,4 and 6 are therefore available for substitution. Two methyl groups and one trityl group entered each fructose residue and since the hydroxyl group of carbon 6 has been shown to be unsubstituted in the dimethyl fructose, this must have been the position occupied by the trityl group.

Triphenyl chloromethane, therefore, condensed with the primary alcohol group as would be expected from Helferich's generalization.



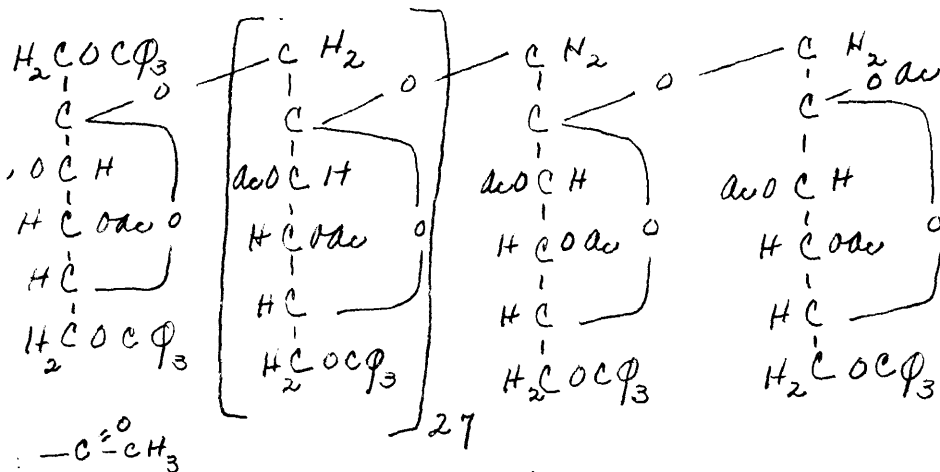
I Inulin

$\text{P}_3\text{CCl}$   
in pyridine



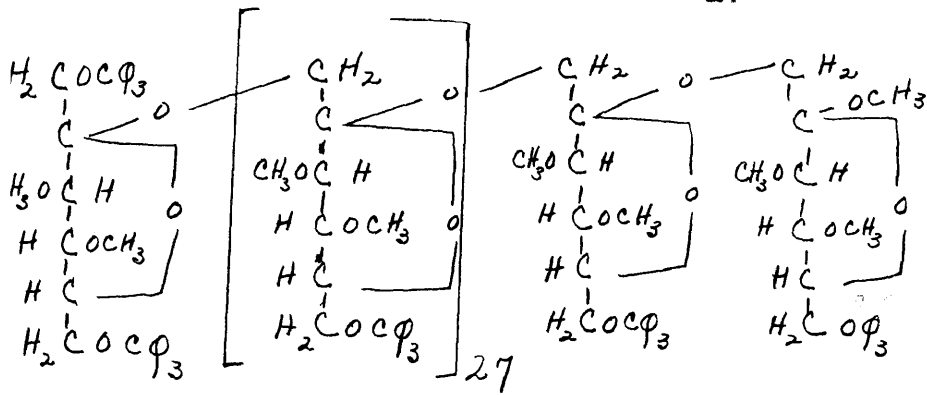
II Trityl inulin

Acetic anhydride  
in pyridine



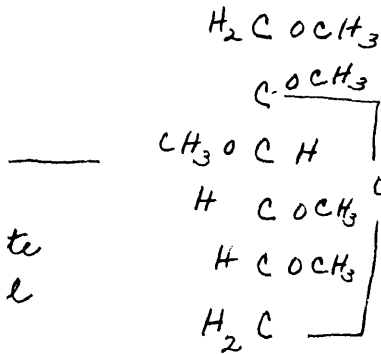
III Trityl diacetyl inulin

$(\text{CH}_3)_2\text{SO}_4 + 30\% \text{NaOH}$   
in acetone



IV *Tri-lyl-dimethyl inulin*

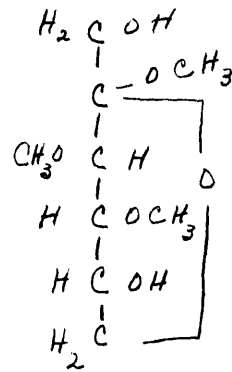
HBr in chloroform + aqueous acid hydrolysis



tel

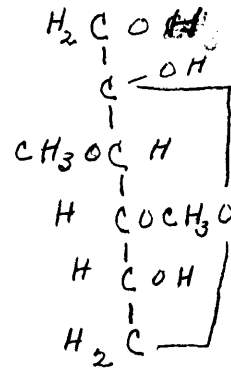
VII

1,3,4,5 tetramethyl methyl fructoside



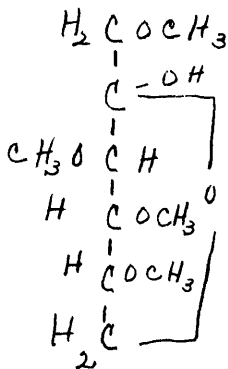
VI

3,4, dimethyl methyl fructoside



V

3,4, dimethyl-fructose



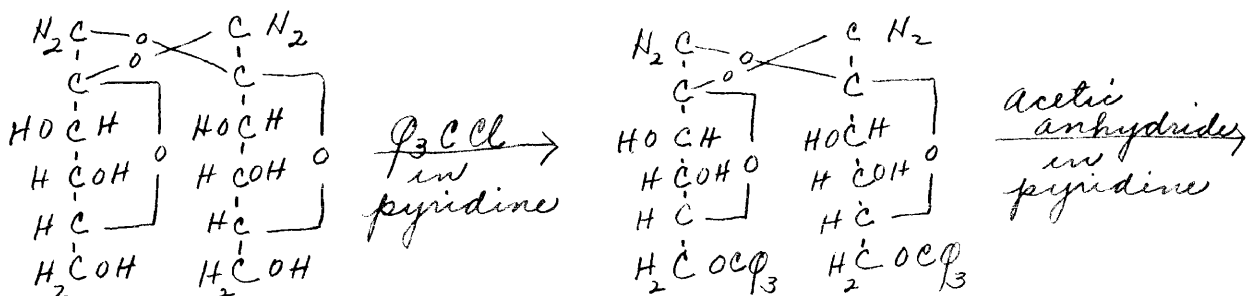
VIII

1,3,4,5 tetramethyl fructose

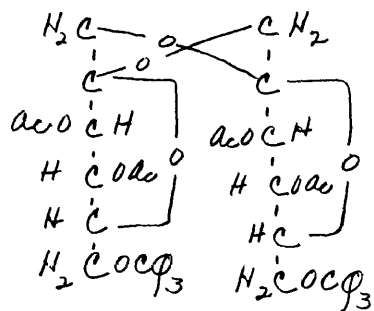
It is planned in completion of the present work to carry out the following reactions.

In difructose anhydride I the hydroxyl groups of carbons 1 and 2 of both fructose residues form the dioxan ring, hence they are not available for substitution. The fructose residues are both gamma sugars and hence carbon atom 5 is not free to react. When difructose anhydride I is tritylated and acetylated, a ditrityl tetracetyl derivative results. By replacing the acetyl groups by methoxy groups and subsequent removal of the trityl groups a tetramethyl disaccharide will result which may be hydrolyzed by aqueous acid into a dimethyl fructose. It is expected that the same 3,4 dimethyl fructose will result as that obtained from tritylated inulin.

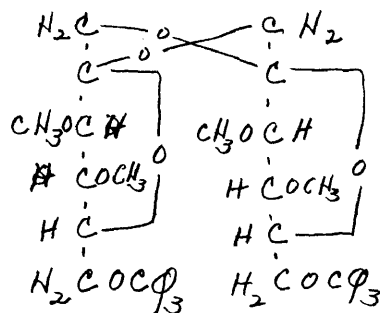
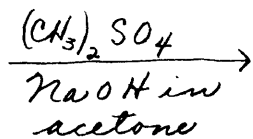
This series of reactions is represented by the following diagrams



*Difructose Anhydride I.*

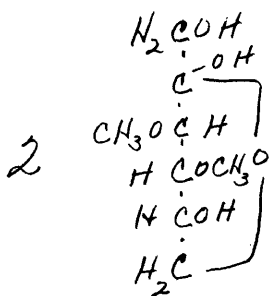


Oxitrityl tetraacetyl  
difructose anhydride  
I



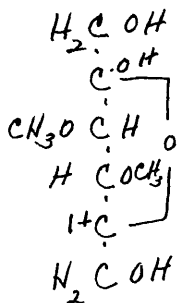
Ditrityl tetramethyl  
difructose anhydride  
I

H Br in  
chloroform

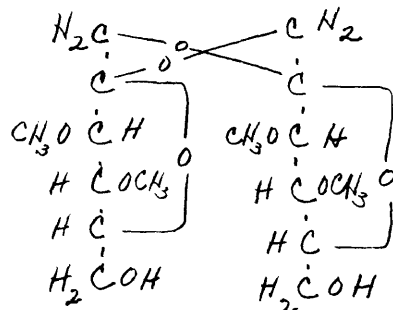
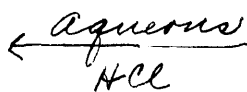


2  
3,4 dimethyl  
fructose

or



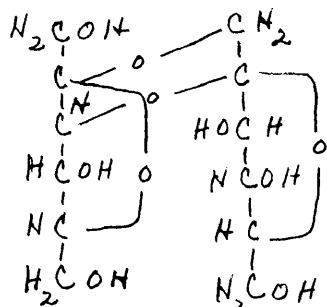
3,4 dimethyl  
δ fructose



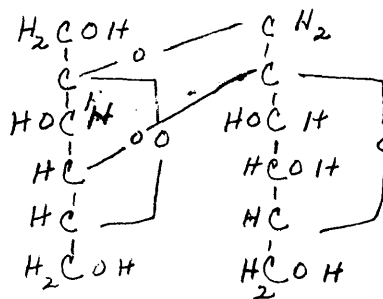
Tetramethyl  
difructose anhydride I



Difructose anhydride III has been shown to be either formula B or formula C



B



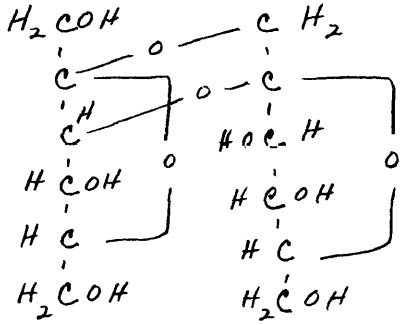
C.

In both cases trimethyl derivatives would result when the tritylated methylated sugar was detritylated. Hydrolysis of the trimethyl disaccharide would yield 3,4 dimethyl fructose, identical with that obtained by the hydrolysis to tetramethyl I or dimethyl inulin, along with 4 methyl fructose in the case of formula B and 3 methyl fructose in the case of C. 4 methyl fructose may be identified by its phenylosazone which has been prepared from 4 methyl glucose by Pascu<sup>8</sup>. 3-methyl fructose has been prepared by the methylation of alpha diacetone fructose and subsequent removal of the acetone groups. It may be identified by a mixed melting point and by its phenyl osazone. The following diagrams show this series of reactions in the case of

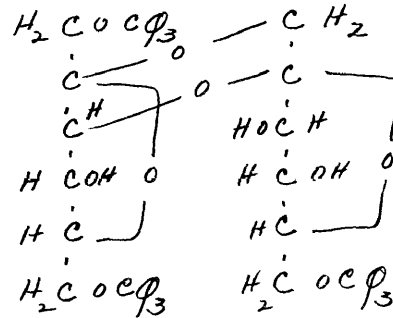
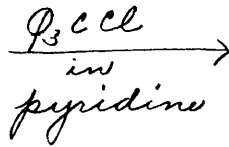
formula B. In formula C the fourth rather than the third carbon of the one fructose residue is condensed with the second carbon of the other fructose residue, and hence in this case carbon 3 is unsubstituted in the sugar and is acetylated when the tritylated derivative is treated with acetic anhydride. The acetyl groups are replaced by methyl groups and thus 3 methyl fructose would be formed along with 3,4 dimethyl fructose by the final hydrolysis rather than 4 methyl fructose, which is formed in the case of formula B.

Difructose anhydride III has been tritylated and acetylated and the resulting product by analysis is a tritryl triacetyl derivative. The replacement of the acetyl groups in this compound by methyl groups when dimethyl sulfate and sodium hydroxide in acetone solution is used as the methylating agent as well as when the tritryl derivative is methylated by means of methyl iodide and silver oxide has proved to be difficult. A large number of methylations will be required before the tritylated methylated difructose anhydride III can be isolated.

Transformations of difructose anhydride III.

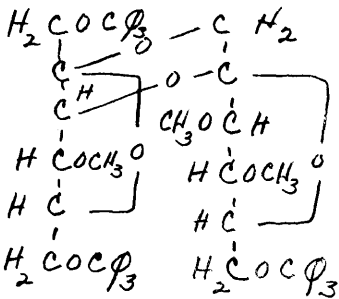


B

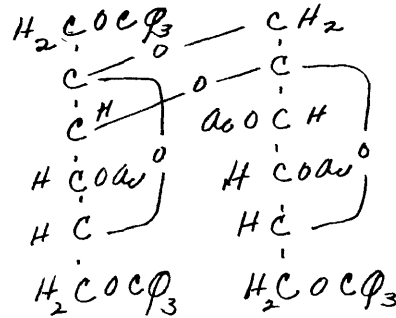
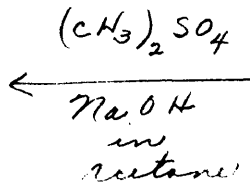


Trityl

acetic anhydride in pyridine

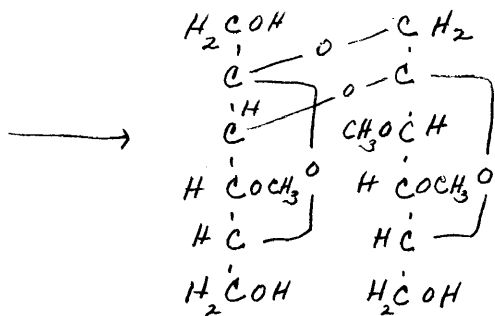


Trityl trimethyl

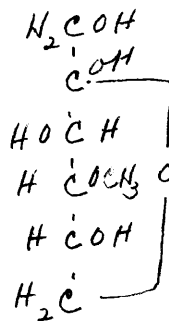
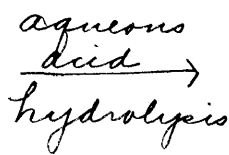


Trityl triacetyl

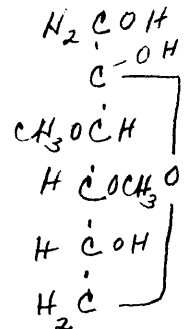
Br  
w  
chloroform



Trimethyl



4 methyl fructose



3,4 dimethyl fructose

In recapitulation it has been shown that

1. both fructose residues of difructose anhydride III are gamma sugars.

2. When hexamethyl difructose anhydride III is hydrolyzed

(a) 3,4,6 trimethyl fructose is not the sole product of hydrolysis

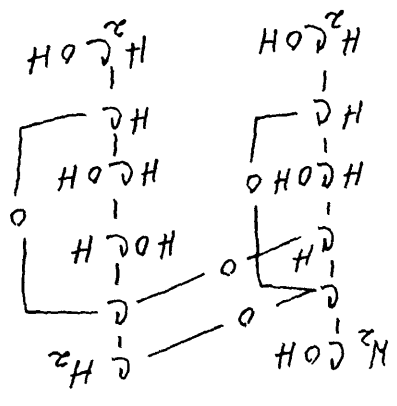
(b) 3,4,6 trimethyl fructose is one of two products of hydrolysis

(c) 1,3,4 trimethyl fructose is not formed by the hydrolysis

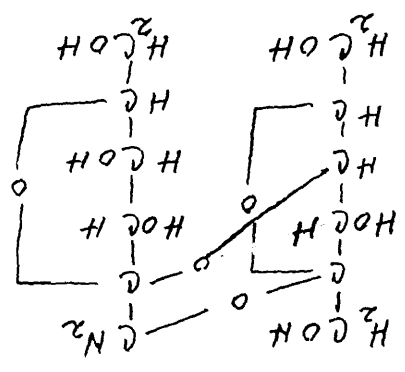
1,3,6 trimethyl fructose or 1,4,6 trimethyl must therefore be formed along with the 3,4,6 trimethyl fructose during the hydrolysis of hexamethyl difructose anhydride III and the sugar is represented by one of the two following formulas.

3. There is but little doubt that Helferich's generalization which states that trityl groups substitute selectively upon primary alcohol groups, holds for this series of compounds.

B.



C.



### Occurrence of Difructose Anhydrides in Inulin

In previous articles we suggested that the difructose anhydrides might occur as such in the inulin molecule. Other investigators have concluded that they are to be accounted for as hydrolysis products or as distinct compounds that accompany inulin and are difficult to separate from inulin. Irvine and later Haworth hydrolyzed triacetyl inulin in a chloroform-fuming nitric acid solution and thus obtained difructose anhydride I.

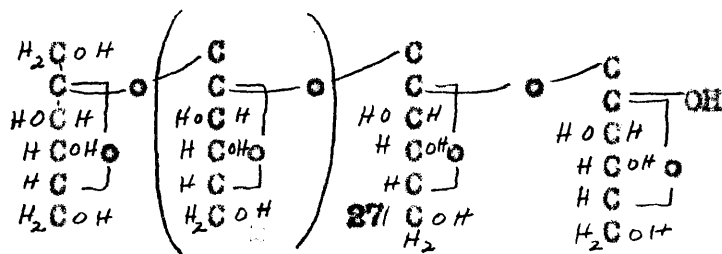
It has been shown that this disaccharide contains the same linkage between the first and second carbon atoms of adjoining fructose residues as occurs in the inulin molecule. Haworth<sup>2</sup> presents as evidence of the absence of difructose anhydride residues in inulin his failure to extract the hexacetates of these disaccharides from inulin acetate by hot water. Should these difructose anhydride acetates constitute a portion of the inulin acetate it would not be expected that they could be extracted with hot water, in which they are slightly soluble, any more than that the difructose anhydrides might be extracted from the unsubstituted inulin molecule with cold water.

Pringsheim<sup>9</sup> and Hensel hydrolyzed inulin with

aspergillus. After acetylating the unhydrolyzed portion they were unable to isolate the acetate of the first sugar. As they found that aspergillus did not hydrolyze this disaccharide, they concluded that difructose anhydride I did not occur as an integral part of inulin but must be formed as a product of aqueous acid hydrolysis.

Schlubach reports that difructose anhydride III probably occurs along with inulin (as a "Begleitstoffe") from which it is difficult to separate. However we find that one crystallization from water solution is sufficient to separate this sugar from inulin

The inulin molecule, as formulated by Haworth, is known to be composed of fructofuranoses in which the second carbon of one fructose residue is attached to the first carbon of the adjoining fructose residue. Thus Haworth's formula is written



in which the 27 is only an approximation.

Haworth states that all inulin shows some reducing power and hence he accounts for the free reducing group on the last fructose residue. We have applied this test quantitatively and from these quantitative experiments conclude that inulin does not contain

a reducing group. For this work inulin was extracted and recrystallized in solutions of pH 4.4 in order that a reducing group would not be destroyed were it present. The reducing sugar analysis was run by Munson and Walker's method for reducing sugars. A sample of 3.89 gms of inulin was found to have a reducing power equivalent to 0.0089 gms of fructose or 0.23% reducing sugar. A similar test on 5 gms of sucrose, which is known to contain no reducing groups had under the same experimental conditions a reducing power equivalent to 0.0135 gms of invert or 0.27%. If Haworth's formula is correct one out of every thirty fructose residues would contain a reducing group and inulin would have about 3% as much reducing power as fructose. The practically negligible reducing power of a carefully prepared sample of inulin shows that the reducing group incorporated in Haworth's formula is definitely absent. The possibility that substitution on carbon 1 would inhibit the reducing power of this final fructose residue, should it contain such, is small in view of the fact that Hibbert, Tipson and Brauns<sup>7</sup> find that 1,3,4 trimethyl gamma fructose "reduces Fehling's solution strongly".

Yanosky and Kingsbury<sup>10</sup> report that inulin carefully prepared contains 0.45% reducing sugar. During the preparation of their inulin they apparently did not



use any alkaline solutions that would destroy a reducing group; this again is far too small an amount of reducing sugar to be consistent with Haworth's formula.

Schlubach and Knoop<sup>11</sup> report that their purified inulin has no reducing power.

Reasonable explanations of this lack of reducing power appear to be that either the last fructose residue has condensed with a hydroxyl of the same residue to form an anhydro fructose or that condensation has taken place with a hydroxyl on the adjacent fructose residue to form a difructose anhydride. Anhydro fructoses show a great tendency to condense to the difructose form. Lävulosan<sup>12</sup> condenses to form dilävulosan, hetero-lävulosan<sup>13</sup> forms hetero-dilävulosan and gamma fructosan<sup>14</sup> forms di-gamma fructosan. When a slight acidity exists 3,4,6 trimethyl fructose shows a tendency to polymerize. It, therefore, seems more probable that the reducing group of the last fructose residue would form a difructose anhydride by condensing with a hydroxyl on the adjoining fructose residue. Such a condensation might involve a hydroxyl on carbons 3,4, or 6. Diffructose anhydride III has been shown to have a structure such that it would be formed by a dehydration

between the reducing group of the final fructose residue and the hydroxyl group of either the third or fourth carbon atom on the penultimate fructose residue.

The non-reducing portion of hydrolyzed inulin has been found to contain the three previously described difructose anhydrides. After exhaustive treatment in extracting these sugars there remains a considerable quantity of uncrystallizable sirup which may ultimately prove to contain a fourth difructose anhydride. The composition and structure of this residue as well as the structure of difructose anhydride II take on new interest in view of this theory.

EXPERIMENTAL

(1) Hexamethyl difructose anhydride III

According to the procedure described by Haworth and Learner in their methylation of inulin 10 gms of sugar, dissolved in 25 cc of  $H_2O$  was placed in a 500 cc flask fitted with a mechanical stirrer. During a period of one hour 29½ cc of dimethyl sulfate and 65 cc of 30% NaOH solution were added in such a way that the solution was always alkaline. The temperature of the bath was kept at 70°C during this time. The bath temperature was then raised to 100°C and stirring was continued for one half hour in order to decompose the unreacted dimethyl sulfate. The partially methylated sugar separated as an oil and floated on the surface. After the addition of ice the reaction mixture was made nearly neutral by the addition of  $H_2SO_4$ . 200 cc of methyl alcohol was then added.

A large volume of  $Na_2SO_4$  separated. The filtrate from this salt was evaporated under diminished pressure to 25-30 cc. The salt was well washed and the

filtrate extracted with 175-200 cc of chloroform. This chloroform solution, after drying over  $\text{Na}_2\text{SO}_4$ , was evaporated.

The partially methylated sugar was then treated with  $\text{Ag}_2\text{O}$  and  $\text{CH}_3\text{I}$  in order to complete the methylation. This reaction was carried out in a 3-necked flask fitted with a mechanical stirrer and a reflux condenser. The partially methylated sugar was dissolved in 70 gms of  $\text{CH}_3\text{I}$  and ten 4.4 gm portions of  $\text{Ag}_2\text{O}$  were added over a period of five hours. The  $\text{Ag}_2\text{O}$  and  $\text{AgI}$  were then removed by filtration. After evaporation of the  $\text{CH}_3\text{I}$  the hexamethyl difructose anhydride III was distilled under reduced pressure.

B.P. = 161-165 .4 mm pressure

Yield 10.6 gms. 84.1% theoretical yield

$[\alpha]_D^{20} = +155.7^\circ$  (pure liquid)

$[\alpha]_D^{20} = +157.9$  (in chloroform)  $c = 3.95$  gms/100 cc

$[\alpha]_D^{20} = +164.5$  (in water)  $c = 2.2316$  gm/100 cc

$\text{OCH}_3 = 45.0\%$  theory = 45.6%

0.0471 gm sample = 0.1602 gms.  $\text{AgI}$

(2) Hexamethyl difructose anhydride II

10 gms. of difructose anhydride II was methylated by the method described above. After methylation with  $\text{Ag}_2\text{O}$  and  $\text{CH}_3\text{I}$  it separated from ether solution in large well formed crystals. The product was purified by recrystallization from ether.

Yield 8.72 gms.                      69.2% theoretical yield.

M.P. =  $73^\circ$  (unc.)

B.P. =  $169 - 170^\circ \text{C}$ .      .35 mm. pressure.

$[\alpha]_D^{20} = -41.9$  (supercooled liquid).

$[\alpha]_D^{19.3} = -28.2$  (in chloroform)       $C = 3.555$  gms/100 cc

$[\alpha]_D^{20} = +6.0$  (in water)       $C = 3.735$  gms/100 cc

$\text{OCH}_3 = 44.5\%$       Theory 45.6%

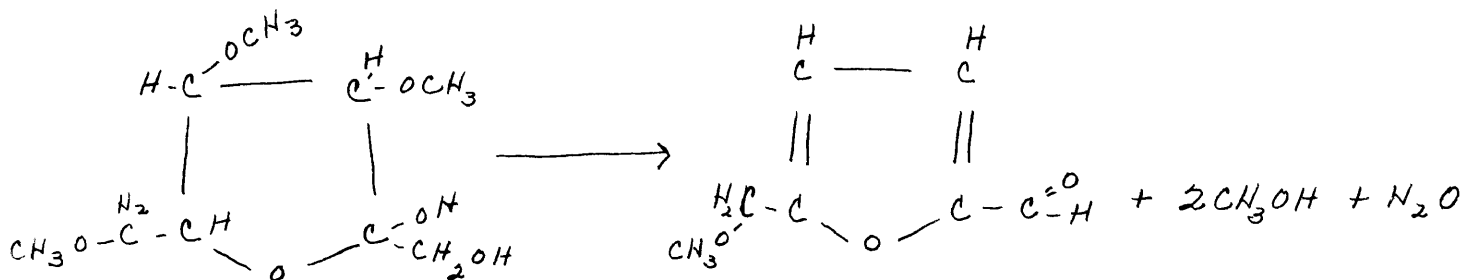
0.0506 gm sample = 0.1704 gm AgI.

(3) The Hydrolysis of Hexamethyl difructose  
anhydride III

The hydrolysis of hexamethyl difructose  
anhydride III required considerable preliminary work  
for the following reasons:

1. The methylated disaccharide is relatively  
stable to acid hydrolysis.

2. Acids have a dehydrating effect upon the  
methylated gamma fructoses which constitute the products  
of hydrolysis. 3-4-6 trimethyl fructose in acid  
solution is converted to ω-methoxy-5-methyl furfural  
according to the following reaction



3. The fractionation of mixtures of unhydrolyzed  
methylated disaccharide, trimethyl fructose, and  
methylated furfural is difficult. 3-4-6 trimethyl  
fructose was treated with 0.8N HCl at 95°C for one hour.  
Calculations based upon its initial and final rotations

showed that it had decomposed 13% during this period. By following the change in rotation when hexamethyl difructose anhydride III was hydrolyzed in 0.8N HCl at 95°C, it was found to be 50% hydrolyzed in 91 mins. and 70% hydrolyzed in 157 mins.

From these data it was calculated that if the hexamethyl derivative were treated with .8N HCl at 95°C for three periods of 91 mins. each and the trimethyl fructose were removed by distillation after each hydrolysis reaction that 87½% of the methylated disaccharide would be hydrolyzed and that about 10% of the trimethyl fructose thus formed would be destroyed. Should the acid hydrolysis consist of two consecutive treatments with 0.8N HCl at 95°C of 157 min, 91% of the hexamethyl difructose anhydride would be hydrolyzed and about 20% of the trimethyl fructose formed by the hydrolysis would be destroyed. Therefore, it was decided to carry out three hydrolyses of 91 min. each.

Accordingly 7.229 gms. of hexamethyl difructose anhydride III was treated with .8N HCl at 95°C for 91 min. The cooled reaction mixture was neutralized with BaCO<sub>3</sub> and evaporated to dryness. The solid residue was extracted with seven 15-20 cc portions of chloroform. The methylated sugar derivatives of both the disaccharide





Fraction	Bath Temp.	Boiling Point	Pressure in mm	D	chloroform
(1) <sub>A</sub>	164-184	107-133	.36	+50.4	
(1) <sub>B</sub>	166-176	107-	.28	+30.0	
(1) <sub>AB</sub>	107-156	107-111½	.38	+26.6	final
(1) <sub>C</sub>	146-172	88-114	.33	+29.1	distillate

A portion of the methyl furfural derivative, formed during the hydrolysis, remained the distillate. This gave a yellow color and the characteristic furfural odor to all of the fractions although  $\omega$ -methoxy-5-methyl furfural boils at about 90°C under these pressures. The method of Dox and Plaisante proved to be a satisfactory means of removing and determining the amount of furfural derivative present. These authors use thiobarbituric for the quantitative determination of furfural.

2.7438 gms. of the hydrolyzed product was dissolved in 5 cc of 12% HCl, to this 50 cc of thiobarbituric acid solution\* was added. After one or two minutes stirring, a

\* The thiobarbituric acid solution was prepared by grinding 0.9 gms. of thiobarbituric acid in an agate mortar with just enough water to moisten it. This paste was then washed into a beaker and diluted to about 150 cc with H<sub>2</sub>O. As soon as the acid dissolved 75 cc of hydrochloric acid of sp. gr. 1.06 was slowly added. This solution was then filtered through asbestos.

yellow precipitate formed. The reaction mixture was cooled to 10°C. over night and the coagulated precipitate collected on a weighed gooch crucible, washed with small portions of 12% HCl and dried to constant weight. 0.1604 gms. of thiobarbituric acid furfural derivative was found. Assuming that ω-methoxy-5-methyl furfural was the form of the furfural derivative there were 0.0844 gms. present and the original sample contained 3.08% of this compound. The filtrate from the thiobarbituric acid-furfural precipitate was neutralized with BaCO<sub>3</sub> and recovered by the same procedure as that used after the acid hydrolysis. 2.14 gms. of methylated fructose having a rotation of about 21° was recovered. After this treatment the products of hydrolysis distilled at a more constant temperature as shown by the following table.

Hydrolysis products after thiobarbituric acid treatment

Fraction	Bath Temp.	Boiling Point	Pressure in mm.	Grams	D
1	157-162	100-113½	.43	0.4074	+22.08
2	166-170	115-116	.40	1.1906	+20.87
3	174-176	117-109½		0.4629	+21.9

This hydrolysis of hexamethyl difructose anhydride III may thus be summarized:

7.229 gms. of hexamethyl III after three successive hydrolysis gave

4.4112 gms. of trimethyl fructose

.4757 gms. -methoxy-5-methyl furfural which is equivalent to .7544 gms. of trimethyl fructose

.4662 gms. final residue

55.5% of the hexamethyl disaccharide was recovered as a trimethyl fructose derivative. When a portion of this trimethyl fructose was treated with phenyl hydrazine in dilute acetic acid, a relatively small yield of 3-4-6 trimethyl fructose osazone could be obtained.

M.P. phenyl osazone from hydrolysis product = 80.0°C uncorr.

M.P. 3-4-6 phenyl osazone = 81°C.

Mixed M.P. = 80°C uncorr.

(4) Ditrityl difructose anhydride I

According to the method of Helferich and Brederick one gram of difructose anhydride I and 3 grams of triphenyl chloromethane were dissolved in 15 cc of dry freshly distilled pyridine. The reaction mixture was allowed to stand at room temperature for 3 days, at which time water was added drop by drop until the solution became turbid. This was cooled to 0°C for one hour. A small amount of carbinol separated in crystalline form. The filtrate from the carbinol was poured into 600 cc of ice water. A white gummy precipitate formed. When dissolved in warm methyl alcohol the tritylated derivative separated as white needles. These were crystallized from methyl alcohol to constant properties.

Yield 1.05 gms.

42.2% theory

Carbinol = 64.37% method of Valentin<sup>6</sup>

0.1249 gm. sample = .0804 gm. carbinol

64.36% carbinol - theory for ditrityl difructose  
anhydride

[ $\alpha$ ] <sub>D</sub><sup>20</sup> = +20.35 (chloroform)

C = 2.186 gms/100 cc  
C = 2.186 gms/100 cc

M.P. = 195°C (uncorr).

(5) Ditrityl tetracetyl difructose anhydride I

6.3 gms. of sugar and 18 gms. of triphenyl-chloromethane were dissolved in 120 cc of freshly distilled pyridine. After remaining 3 days at room temperature the reaction mixture was heated to 85-90°C for one hour. 60 cc of acetic anhydride was then added to the cooled solution. The reaction mixture was then heated to 70°C for one hour after which the temperature was raised to 110°C for one half hour. Upon cooling ice water was added to turbidity and the reaction mixture was placed in an ice bath for one hour. The triphenyl carbinol which separated was filtered and the filtrate poured into a large volume of ice water. The precipitate which formed was dissolved in absolute alcohol. Ditrityl tetracetyl difructose anhydride I separated as transparent spheroids, which upon repeated recrystallization from absolute alcohol formed needles.

Yield 14.7 gms.                      91.8% theory

M.P. = 194°C uncorr.

$[\alpha]_D^{20} = +21.06$                        $C = 3.731$  gms./100 cc

Carbinol = 51.1%                      theory = 53.3%

0.0417 gm sample = .0213 gm. carbinol

Acetyl found 18.0%  $\text{OCCH}_3$       Calc. 17.6%  $\text{OCCH}_3$

0.0991 gm. sample = 4.14 cc      0.1004 N NaOH

(6) Tetracetyl difructose anhydride I\*

The trityl groups were removed from the ditrityl tetracetyl derivative described above by the method of Helferich and co-workers.

5 gms. of ditrityl tetracetyl difructose anhydride I was dissolved in 20 cc. of glacial acetic acid. The solution was cooled to 5°C and 2 cc. of acetic acid saturated with HBr at 0°C was added. A yellow precipitate of tritylbromide separated. The filtrate was poured into an ice and water mixture. The tetracetyl derivative was extracted with chloroform and the chloroform solution thus formed treated with NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. The sirupy residue was dissolved in absolute alcohol. Tetracetyl difructose anhydride separated from the alcohol solution in the form of rectangular plates.

Yield 1.8 gms.

5.3% theoretical

$[\alpha]_D^{20} = -9.9$  chloroform

$[\alpha]_D^{20} = +23.0$  water

M.P. = 173°C uncorr.

Acetyl found 35.1%  $\text{O}^{\text{C}}\text{CH}_3$  calc. 35.0%  $\text{O}^{\text{C}}\text{CH}_3$

.1731 gm. sample = 1410 cc. 0.1004 N NaOH.

\* This preparation was made in pursuance of a plan which was later abandoned.

The tetracetyl derivative upon acetylation gave hexacetyl difructose anhydride I, which has been described by Jackson and Goergen<sup>1</sup>.

(7) Trityl diacetyl inulin

10 gms. of inulin and 30 gms. of triphenylchloromethane were placed in 200 cc of freshly distilled pyridine. The inulin swells slowly in pyridine and hence the reaction takes place slowly. The reaction mixture was rotated for four days at room temperature and then heated to 85-90°C for one hour. Upon cooling, 80 cc of acetic anhydride was added. This solution was heated to 60-70°C for one hour and then at 110°C for one half hour. After cooling to room temperature, cold water was added dropwise to a permanent turbidity. The crystals that separated from this solution were filtered and the filtrate was poured into a large volume of water with constant stirring. Tritylated acetylated inulin along with some triphenyl carbinol separated as a white precipitate. This was filtered, washed with water and air dried. The trityl diacetyl inulin was purified by separation from absolute alcohol. It was isolated as an amorphous powder. Yield 8 gms.

Carbinol = 53.44%      theory = 53.28%

0.1568 gms. = 0.0938 gms. carbinol

$[\alpha]_D^{21} = +13.14$  in chloroform       $C = 1.224$  gms/100 cc

(8) Trityldimethyl inulin

7 gms. of trityl diacetyl inulin was dissolved in 250 cc. of acetone. 120 cc of dimethyl sulfate and 320 cc of 30% sodium hydroxide were added in ten portions at ten minute intervals. During the reaction the solution was rapidly stirred and the temperature was kept at 55°C. Upon addition of the last portion of reagents 100 cc. of water was added and the temperature was raised to 75°C for fifteen minutes in order to remove the acetone. The tritylated methylated inulin separated as a precipitate. The pure compound was isolated as an amorphous powder by separation from a methyl alcohol solution.

Yield 6.5 gms.

82% theoretical yield

Methoxy found 15.28%  $OCH_3$       Calc. 14.36%  $OCH_3$

33.7 mg. sample = 9.958 cc N/10 thiosulfate

Carbinol found 55.0%      Calc. 60.2%

.1210 gm. sample = 0665 gms. carbinol



(9) Tritrityl difructose anhydride III

5 gms. of difructose anhydride III was dissolved in 60 cc of dry pyridine. To the solution 19 gms. of triphenylchloromethane was added. The reaction mixture was allowed to stand at room temperature for two days. Water was then added until the solution became turbid. The crystals that separated were filtered and the filtrate was poured into a large volume of cold water.

The white precipitate that separated was air dried and recrystallized from methyl alcohol to constant properties.

Yield 1.08 gms. This is 7.0% the theoretical yield.

M.P. = 125-127°C uncorr.

$[\alpha]_D = +65.2$  in chloroform

Carbinol = 73.1%

Theory for tritrityl difructose anhydride III = 74.29

.0635 gm. sample = .0464 gm. carbinol.

(10) Tritrityl-triacetyl difructose anhydride III

20.2 gms. of difructose anhydride III was dissolved in 250 cc. of freshly distilled pyridine. To this solution 80 gms. of triphenylchloromethane was added. The reaction mixture was allowed to stand one week at room temperature. At this time it was heated to 70°C for one hour. After cooling to room temperature 180 cc of acetic anhydride was added and the solution was warmed to 60°C for one hour. The reaction mixture was then heated one half hour at 110°C. Upon cooling a small portion of water was added and the turbid solution was cooled over night. A large portion of the unreacted triphenylchloromethane separated as the tritylcarbinol pyridine compound and was filtered. The filtrate was poured into a large volume of water. The precipitate that was thus formed was washed with water and air dried. The tritylated acetylated sugar separated in an amorphous state from alcohol solution.

Yield 24 gms.

% theoretical yield 34.3%

Acetyl found 8.9%

.1185 gm sample = 2.45 cc 0.1004 N  
NaOH

Calc. 10.9%

Carbinol found 68.7%

.1070 gm sample = .0735 gm  
carbinol

Calc. 66.5%

(11) Dimethyl fructose

Dimethyl fructose was prepared by the detritylation and hydrolysis of trityldimethyl inulin. The detritylation was carried out by a method which previously has not been described.

An almost saturated solution of HBr in chloroform was prepared at 0°C. By analysis, 1 cc. of this solution contained 0.042 gms. HBr.

3 gms. trityl dimethyl inulin was treated with 18 cc of the HBr-chloroform solution at 0°C for thirty to forty minutes. The acid was then neutralized with a  $PbCO_3$  suspension and the chloroform was evaporated. The remaining solution was filtered and evaporated to dryness. The inorganic salts remained in the residue when the methylated inulin and any partially hydrolyzed methylated inulin were extracted with absolute alcohol. The hydrolysis was completed in .05N  $H_2SO_4$  at 60° for 80 min.

The  $H_2SO_4$  was removed by means of  $BaCO_3$  and the filtrate from this reaction was evaporated to dryness. The dimethyl fructose was extracted from this residue by repeated treatment with chloroform. Upon distillation of the chloroform the dimethyl fructose remained as a sirup.

$\frac{20}{D} = -7.8$  in chloroform.

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