

# TWO NEW CRYSTALLINE DIFRUCTOSE ANHYDRIDES FROM HYDROLYZED INULIN

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## ABSTRACT

Hydrolyzed inulin has previously been shown by Jackson and Goergen (RP79) to contain about 5 per cent of a group of nonreducing difructoses, one of which was isolated in crystalline form and found to be a difructose anhydride of  $[\alpha]_D^{20} = +27.0$ . The mother liquors from which this substance had crystallized ultimately yielded by crystallization from alcoholic solution a second crystalline substance, designated difructose anhydride II, and a third, difructose anhydride III.

Difructose anhydride II crystallizes in truncated prisms, frequently having a hexagonal cross section, melts at  $198^\circ \text{C}$ ., and has a  $[\alpha]_D^{20}$  of  $+13.85$ . It is hydrolyzed solely to fructose.

Difructose anhydride III crystallizes from alcohol in the form of bipyramids, melts at  $162^\circ \text{C}$ ., and has a  $[\alpha]_D^{20}$  of  $+135.64$ . It is hydrolyzed to fructose by  $0.2 \text{ N H}_2\text{SO}_4$  at  $100^\circ \text{C}$ ., showing evidence of an intermediate product of hydrolysis.

From the 5 per cent of nonreducing substance in hydrolyzed inulin three crystalline difructose anhydrides have now been isolated, whose mean rotatory power ( $+58.8$ ) agrees with that of the mixture ( $+55$ ) as originally isolated. This indicates that the mixture contained the three sugars in approximately equal proportions.

The evidence accumulated in this and in previous articles indicates that inulin has a molecular weight approximating 18,000.

## CONTENTS

	Page
I. Introductory.....	709
II. Isolation of two new crystalline difructose anhydrides.....	710
III. Difructose anhydride II.....	711
IV. Difructose anhydride III.....	711
V. Discussion.....	711
VI. The molecular weight of inulin.....	712
VII. Experimental.....	713
1. Purification and analysis of difructose anhydride II.....	713
2. Hydrolysis of difructose anhydride II.....	714
3. Purification and analysis of difructose anhydride III.....	714
4. Hydrolysis of difructose anhydride III.....	715
VIII. Acknowledgment.....	715

## I. INTRODUCTORY

In a study of the hydrolysis of dahlia inulin in aqueous solution with sulphuric acid Jackson and Goergen<sup>1</sup> showed that the products of hydrolysis consisted of about 92 per cent fructose, 3 per cent aldose, and 5 per cent of a group of nonreducing difructoses. The present authors<sup>2</sup> in continuation of the study found that hydrolyzed inulin prepared from a wide variety of plants other than the dahlia

<sup>1</sup> B. S. Jour. Research, 3 (RP 79), p. 27; 1929.

<sup>2</sup> B. S. Jour. Research, 5 (RP 251), p. 1151; 1930.

also possessed approximately the same composition with respect to the analytically determinable constituents and that extensive fractional crystallization failed to alter the composition essentially. It was stated in the latter article that evidence was already at hand which indicated that the group of nonreducing disaccharides consisted of no less than three isomeric difructoses. From this fact the authors were able to compute the order of magnitude of the molecular weight of inulin. This evidence will be presented in the present article.

Jackson and Goergen isolated the nonreducing portion of hydrolyzed inulin and found that the substances in the resulting aqueous solution possessed a  $[\alpha]_D^{20} = +55$ . Upon acetylation of the mixture, a portion, about 30 per cent, crystallized and was found to be the hexa-acetate of a difructose anhydride. The acetate showed a  $[\alpha]_D^{20} = +0.54$  in chloroform and the free sugar  $+27.0$  in water. This sugar was designated difructose anhydride I.

## II. ISOLATION OF TWO NEW CRYSTALLINE DIFRUCTOSE ANHYDRIDES

Remaining from the experimental material of Jackson and Goergen was a quantity of the mother liquors from which difructose anhydride I hexa-acetate had been separated. The substances dissolved in these mother liquors had a specific rotation of about  $+35$  and a molecular weight corresponding to the hexa-acetate of a disaccharide. All efforts to induce crystallization had proved futile. A large portion of it was consequently deacetylated in order that experiments might be made with the mixture of free sugars. The resulting sugar mixture had a specific rotation of about  $+70$ . It was considered possible that even after removal of the greater part of the first sugar there might remain two additional sugars. Efforts were accordingly made to effect a separation by some method of fractional extraction. The expedient which appeared to be most promising was a method of separation by means of insoluble compounds which, it was found, the sugars formed with lime. It was planned to carry out the precipitation fractionally in the hope of separating the respective sugars. A portion was accordingly evaporated in vacuo in order to carry out this project. However, during the evaporation a crystallization of one of the constituents unexpectedly occurred and rendered elaboration of the original plan unnecessary.

At the completion of the crystallization the crystals were collected on a filter, and although deposited from a solution having a  $[\alpha]_D$  of about  $70$  showed a  $[\alpha]_D$  of only  $+13$ . This indicated the presence of still another disaccharide in the solution. The verification of this indication came very suddenly, for the filtrate from the crystals above mentioned became turbid within a few minutes after passing the filter and within a few hours had deposited an abundant quantity of crystalline material which appeared under the microscope to have a very different character from either of the two sugars previously isolated. The crude crystals showed a  $[\alpha]_D = +133.9$ .

The sugar of lower rotatory power will be referred to as difructose anhydride II; that of higher rotatory power as difructose anhydride III.

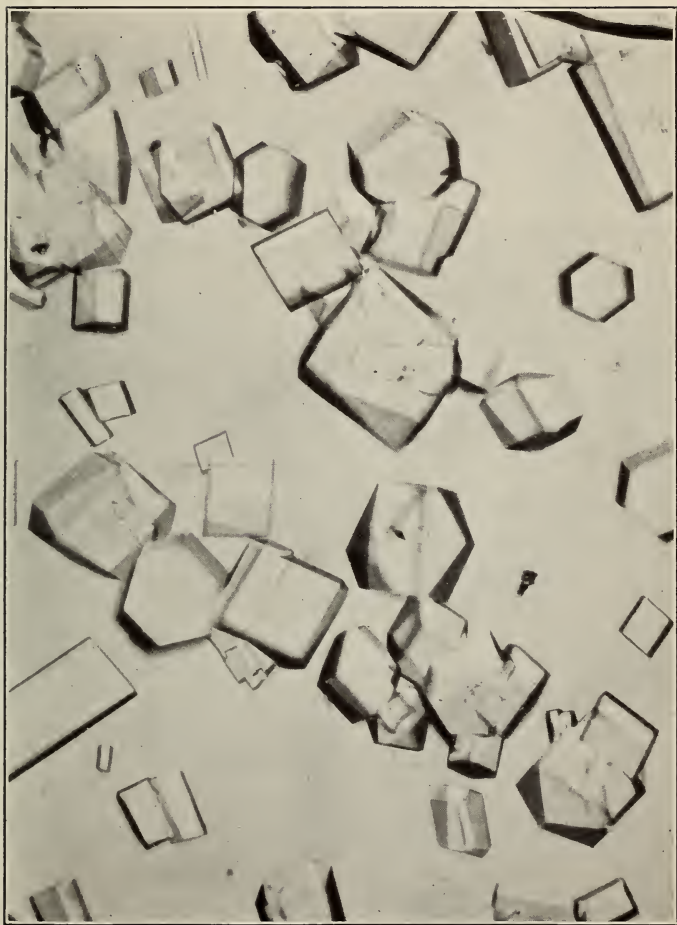


FIGURE 1.—*Diffructose anhydride II*  
(Crystallized from aqueous solution.)

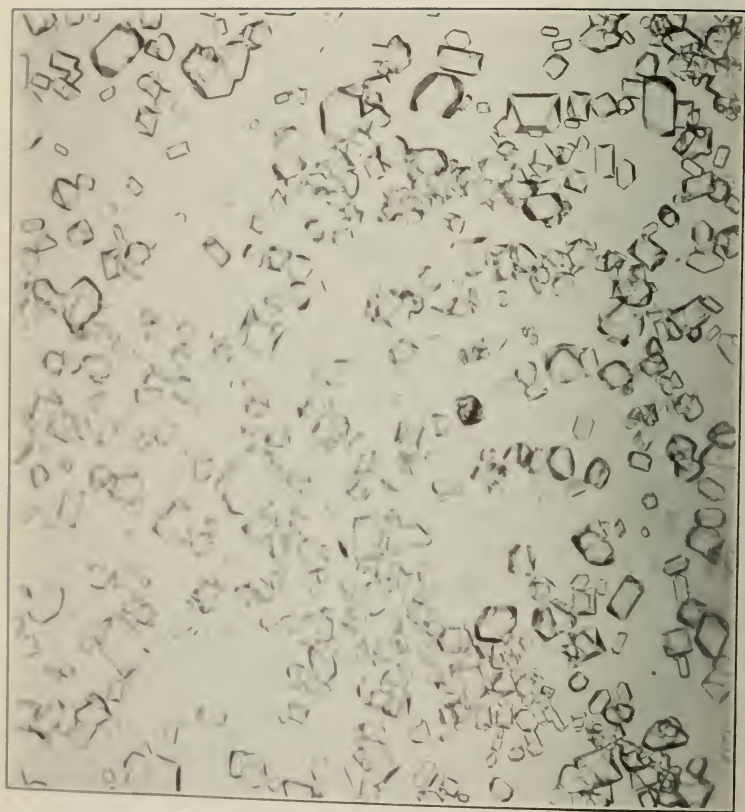


FIGURE 2.—*Difructose anhydride III*  
(Crystallized from alcoholic solution.)



## III. DIFRUCTOSE ANHYDRIDE II

This sugar crystallizes from alcoholic or aqueous solution in the form of truncated prisms (fig. 1) frequently having a hexagonal cross section. It is only slightly soluble in hot absolute alcohol, but may be conveniently crystallized from 95 per cent alcohol by dissolving in a large volume of solvent and evaporating the excess. In water it has an approximate solubility of but 32 per cent. The purified substance exhibited a  $[\alpha]_D^{20} = +13.85$  in aqueous solution and a melting point of  $198^\circ \text{C}$ . Its ultimate analysis and its molecular weight, determined by the depression of the freezing point of water, indicated that it was the anhydride of a disaccharide and had the empirical formula,  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ . Upon hydrolysis the sugar yielded only fructose. It was, therefore, a difructose anhydride isomeric with the difructose anhydride I described by Jackson and Goergen. Acetylation with acetic anhydride and sodium acetate produced a sirup, but all efforts to induce crystallization proved unsuccessful.

## IV. DIFRUCTOSE ANHYDRIDE III

The third difructose anhydride crystallizes from absolute alcohol usually in well-formed bipyramids (fig. 2), but occasionally in elongated prisms. Despite the fact that it was the last of the group to make its appearance in crystalline form, it proved to be the most readily crystallized one of the three, once crystals for seeding had become available. After repeated recrystallization the purified substance showed a  $[\alpha]_D^{20} = +135.64$  in water and a melting point of  $162^\circ \text{C}$ . The combustion analysis and a molecular weight determination by the freezing-point method indicated a disaccharide of the formula  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ .

When treated with  $0.20 \text{ N H}_2\text{SO}_4$  for 110 minutes at  $100^\circ \text{C}$ , it was 80 per cent hydrolyzed, the final product of hydrolysis consisting solely of fructose. It was, therefore, a third difructose anhydride isomeric with the two previously described. The polariscopic evidence seemed to indicate that an intermediate product of lower rotatory power than the original sugar was formed during hydrolysis. This could conceivably be ascribed to the addition of a single molecule of water. The resulting product would not necessarily acquire reducing properties if, for example, one of the hexose residues in the original substance were an anhydrofructose. The explanation, however, must await further experiments on the structure of the disaccharide.

The sugar crystallizes readily from aqueous solution in which it has a solubility of but 38 per cent.

Acetylation with acetic anhydride and sodium acetate yielded a positively rotating sirup from which we have as yet been unable to obtain crystalline material.

## V. DISCUSSION

From the nonreducing material which constitutes about 5 per cent of the products of hydrolysis of inulin we have now isolated in crystalline form the three difructose anhydrides whose properties are summarized in Table 1.

TABLE 1.—*Properties of the isomeric difructose anhydrides*

	I	II	III
$[\alpha]_D^{20}$ (water).....	+27.0	+13.85	+135.64
Melting point.....	164	198	162
Crystalline form.....	Thin plates.	Truncated prisms.	Bipyramids.
Molecular weight (calculated 324).....	307	310	308
Approximate solubility in water <sup>1</sup> .....	32	32	38

<sup>1</sup> Expressed as grams of sugar in 100 gs. of solution at 20°C.

The three sugars resemble each other in respect of their great resistance to hydrolytic action and their strikingly low solubility in water. They differ in rotatory power, melting point, and crystalline structure. It seems probable that they are closely related in chemical structure, a problem which will engage our attention in continuation of the study of inulin.

Jackson and Goergen after isolating the nonreducing portion of hydrolyzed inulin determined its specific rotation with a fair degree of approximation. The value which they found, namely, +55, was probably slightly lower than the true value, since the product was inevitably contaminated by impurities introduced during the processes of isolation. The mean value of the measured specific rotations of the three respective difructose anhydrides (that is, +27.0, +13.8, and +135.6) which have subsequently been isolated and purified is +58.8. This relatively close agreement with the rotation of the mixture makes it appear probable that these three sugars occur in the mixture in equal proportions and that their summation accounts for the total nonreducing residue. This conclusion is further corroborated by the fact that the mother liquors after the removal of the greater part of the first disaccharide in the form of its acetate contained a mixture of the second and third disaccharides showing a specific rotation of +69.3. The complete removal of the first disaccharide would yield a mixture of mean rotation +74.7. Moreover, Jackson and Goergen obtained the acetate of the first disaccharide in about 30 per cent yield, which suggests that it is one-third of the total substance. Thus the preponderance of evidence seems to indicate that the 5 per cent of nonreducing residue from hydrolyzed inulin consists solely of these three sugars occurring in equal proportions. We hope further to establish this conclusion by effecting a more nearly quantitative separation of the constituents.

## VI. THE MOLECULAR WEIGHT OF INULIN

The molecular weight of inulin was discussed by us in a previous article.<sup>1</sup> Basing our conclusions on a clear indication of the existence of these three difructose anhydrides we showed by simple arithmetical reasoning that a 5.2 per cent nonreducing portion of hydrolyzed inulin,

<sup>1</sup> *Ann. Entomol. Soc. Am.*, 2, p. 709.

which consisted of three disaccharides, predicated a whole molecule composed in average of no less than 111 hexose residues and having a molecular weight of approximately 18,000. The fundamental basis upon which that calculation rests, namely, the existence of the three isomeric disaccharides, is now definitely established.

Our conclusion was, however, not entirely free from assumptions, since it was suggested as a possibility that the three disaccharides might have existed in the original inulin molecule as a single difructose group and that the isomerism might have been introduced by different modes of cleavage from the rest of the inulin molecule. This possibility can now probably be eliminated, for, in general, when competitive reactions, such as the hypothetical one which we have suggested, occur, one of the possible reactions usually predominates. Since there is apparently no predominance of any one of the three isomers, it is probable that the reactions by which the disaccharides are released are not competitive. It is our view, therefore, that the difructose groupings present in the inulin molecule are the same as in the sugars which we have isolated, except for the readily hydrolyzable points of contact. Probably a knowledge of the structure of the disaccharides will at some later period contribute to a final solution of the question.

The value calculated in the previous article is merely the average molecular weight of inulin prepared in the usual way by crystallization from aqueous solution. It is not our conception that inulin is a single polysaccharide of definite composition. It is rather a group of polysaccharides of very similar properties, but varying in number of hexose residues. Our analyses show extreme variations in the disaccharide content of nearly 20 per cent from the mean. While much of this variation may be ascribed to the uncertainties of a difficult analysis, there can be no doubt that real variation in composition occurs. It will be of interest to examine the more soluble polysaccharides and to determine at what point in the scale of complexity the difructose anhydrides appear.

## VII. EXPERIMENTAL

### 1. PURIFICATION AND ANALYSIS OF DIFRUCTOSE ANHYDRIDE II

The material which served as a starting point for the present series of experiments was a mixture of sirupy acetates prepared by Jackson and Goergen<sup>4</sup> from the nonreducing portion of hydrolyzed inulin. The crystalline hexa-acetate of difructose anhydride I had been in great part removed by crystallization from alcoholic solution, but even after two years' standing no other substance appeared in crystalline form. It had been previously shown by Jackson and Goergen that the substances in this sirup had a molecular weight corresponding to the hexa-acetate of a disaccharide and a specific rotation of about +31.

A portion of this sirup was deacetylated by warming gently with an aqueous solution of  $\text{Ba}(\text{OH})_2$  until the sirup was completely dissolved and allowing it to remain in alkaline solution over night.

<sup>4</sup> See footnote 1, p. 709.



The barium was then removed by adding the calculated amount of  $\text{H}_2\text{SO}_4$ . The aqueous solution was evaporated and the solvent displaced by absolute alcohol. The resulting solution was evaporated to a thick sirup for the purpose of attempting a fractional separation of the respective lime compounds, but during the evaporation an abundant crystallization of one of the constituents of the mixture occurred. This was collected and was found to have a specific rotation of  $+14.8$ . After three recrystallizations from absolute alcohol the rotation was  $13.85$  and remained unaltered by further treatment. The purified crystals were dried in a vacuum chamber heated to about  $70^\circ$  to constant weight. *Anal.* Calcd. for  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ : C,  $44.42$ ; H,  $6.22$ . Found: C,  $44.7 \pm 0.5$ ; H,  $6.0 \pm 0.1$ . (If calculated for  $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ : C,  $42.1$ ; H,  $6.5$ .) *Mol. wt.* Subs.,  $0.4744$ ,  $0.8398$ : water,  $21.9989$ ,  $25.4810$ ;  $\Delta T$ ,  $0.127^\circ$ ,  $0.201^\circ$ . Calcd.:  $324$ . Found:  $316$ ,  $305$ . (A pure cane sugar solution gave approximately the same percentage error as the mean of these determinations under the experimental conditions. Calcd.,  $342$ . Found,  $328$ .) *Rotation.*  $[\alpha]_D^{20}$  in  $\text{H}_2\text{O} = +13.85$  ( $c = 8.993$ ,  $\alpha = +7.20^\circ$ , saccharimeter, 2-dm tube),  $[\alpha]_{5.461}^{20} = +16.30$  ( $\alpha = +2.931^\circ$ , circular), and  $[\alpha]_{5.780}^{20} = +14.46$  ( $\alpha = +2.600^\circ$ , circular). *Melting point*,  $198^\circ \text{C}$ . (uncorrected).

## 2. HYDROLYSIS OF DIFRUCTOSE ANHYDRIDE II

A 5.0 ml solution, containing 201.2 mg of the second difructose anhydride ( $=40.24$  mg per ml) and sufficient  $\text{H}_2\text{SO}_4$  to make it  $0.20 \text{ N}$ , was maintained at  $100^\circ \text{C}$ . for 100 minutes. Upon analysis it was found to contain 28.1 mg of total reducing sugar per ml (Scales' method) and 28.9 mg of fructose per ml (Nyns' method). The hydrolyzed solution in a micropolariscope tube (calculated to a 2 dm length) gave a saccharimeter reading at  $20^\circ \text{C}$ . of  $-14.1^\circ$ . By correcting the observed rotation for the rotation of the disaccharide which remained unhydrolyzed we obtained the rotation of the hydrolytic products. This, calculated for fructose, yielded the value 28.7 mg per ml. In the period of 100 minutes' digestion the disaccharide was 64 per cent hydrolyzed. The agreement between total reducing sugar and the fructose calculated in two independent ways indicates that fructose is the sole product of hydrolysis.

## 3. PURIFICATION AND ANALYSIS OF DIFRUCTOSE ANHYDRIDE III

Previous to the preparation of the second difructose in quantity a number of small portions had been filtered from the mixture of sugars. The substances in the mother liquor showed a specific rotation of about  $+81$ , indicating the presence of another sugar of high rotatory power. Immediately after the filtration of one of the larger portions this substance crystallized spontaneously in the filtrate while the filtration was still in progress. When the seed crystals became available it proved to be a simple matter to induce crystallization from any of the fractions in which it was present. It is the most readily crystallizable member of the group. Even from an alcoholic solution of the mixture of sugars in the original nonreducing residue from hydrolyzed inulin it crystallizes slowly. The crude crystals showed a specific rotation of  $+133.90$ . After four recrystallizations it acquired a rotation of  $+135.64^\circ$  which remained unaltered upon further treatment. (*Anal.* Calcd. for  $\text{C}_{12}\text{H}_{20}\text{O}_{10}$ : C,  $44.42$ ; H,  $6.22$ .)



Found: C,  $44.9 \pm 0.5$ ; H,  $6.3 \pm 0.1$ . *Mol. wt.*, Subs., 0.4004: water, 24.9018;  $\Delta T$ , 0.097. Calcd.: 324. Found: 309. (Under the same experimental conditions a solution of pure cane sugar showed the same divergence from the calculated value.) *Rotation*.  $[\alpha]_D^{20}$  in  $H_2O$  =  $+135.64$  ( $c = 7.847$ ,  $\alpha = +61.49^\circ$ , saccharimeter, 2 dm tube),  $[\alpha]_{5.461}^{20} = +160.51$  ( $\alpha = +25.191^\circ$ , circular), and  $[\alpha]_{5.780}^{20} = +142.23$  ( $\alpha = +22.322^\circ$ , circular). *Melting point*,  $162^\circ C$ . (uncorrected.)

#### 4. HYDROLYSIS OF DIFRUCTOSE ANHYDRIDE III

A 5.0 milliliter solution containing 308 mg of the third difructose anhydride (=61.6 mg per milliliter and sufficient  $H_2SO_4$  to make it 0.20 *N* was hydrolyzed at  $100^\circ C$ . for 110 minutes. Upon analysis it was found to contain 54.8 mg total reducing sugar per milliliter (Scales' method) and 56.2 mg fructose per milliliter (Nyns' method). Hydrolysis estimated from reducing sugar analysis was, therefore, about 81 per cent complete. The hydrolyzed solution in a 2 dm column gave a saccharimeter reading at  $20^\circ C$ . of  $-25.1^\circ$ . Upon correcting for the rotation of the unhydrolyzed sugar and converting the resulting polarization to weight of fructose the very discordant value of 64.8 mg was obtained. The experiment was repeated with 61.14 mg per milliliter and a period of 135 minutes digestion at  $100^\circ C$ . Total reducing sugar per milliliter, 57.0 mg; fructose (Nyns), 56.7 mg; fructose by corrected polarization, 64.2 mg. Hydrolysis was computed from the reducing sugar analysis to be 84 per cent complete. The concordance between the total reducing sugar and the fructose by the very selective Nyns' method shows that fructose is the only product of complete hydrolysis. The discordance between the true fructose and the fructose by corrected polarization is probably to be ascribed to an intermediate stage of hydrolysis. The correction for the unhydrolyzed sugar involved the assumption that its rotation was unaltered. On account of its high rotatory power the correction amounted to nearly 40 per cent of the observed rotation. If the calculation is reversed and the specific rotation of the nonreducing portion is calculated the values  $+60$  and  $+66$  are obtained, respectively, in the two experiments. There are many other indications of the presence of an intermediate product of hydrolysis which at present are too vague to merit description but which will engage our attention in continuation of the present study.

A portion of the hydrolyzed solution was neutralized with  $Ba(OH)_2$  and treated with phenylhydrazine acetate. The resulting crystals after recrystallization gave the correct melting point ( $204^\circ C$ .) for glucosazone.

Considering the molecular weight determination, the carbon analysis, and the fact that fructose is the only reducing sugar formed after 80 per cent hydrolysis, we conclude that the substance under discussion is a third difructose anhydride.

#### VIII. ACKNOWLEDGMENT

The authors desire to express their appreciation of the assistance rendered by F. P. Phelps, who prepared the photographs of the crystals of the disaccharides and by M. J. Proffitt and J. A. Bogan, who extracted the large quantity of inulin which was required.

WASHINGTON, January 6, 1931.