[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

# The Formation of Difructofuranose 2,1′:1,2′-Dianhydride from Polyfructosans and its Structural Significance[1,2]

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RECEIVED OCTOBER 20, 1955

The acetates of polyfructosans composed of D-fructofuranose units joined principally by 2,1′-glycosidic bonds give rise to di-D-fructofuranose 2,1′:1,2′-dianhydride (III) when treated with fuming nitric acid. It is believed that the formation of III may be taken to indicate that at least three adjacent fructofuranose units are joined by 2,1′-glycosidic bonds.

Inulin triacetate reacts smoothly with fuming nitric acid to give crystalline hexa-*O*-acetyl-difructofuranose dianhydride (III).[3-8]

Since the preponderant unit present in inulin is anhydro-D-fructofuranose, about thirty such units linked through positions 2 and 1′ making up the bulk of the polysaccharide molecule,[9-10] the mechanism of the degradation can be pictured as a proton attack on one of the glycosidic oxygen atoms of the anhydrofructofuranose chain (I) resulting in the rupture of the molecule at that point and the transient formation of a carbonium ion II.

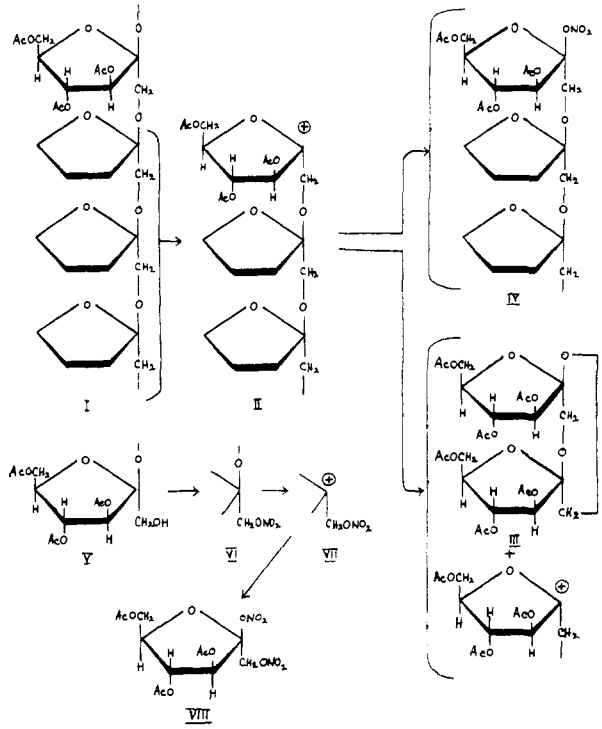


Fig. 1.—The action of fuming nitric acid on a 2,1′-linked polyfructosan acetate.

Either of two competing reactions can then follow: (A) the neutralization of the positive charge by the capture of a negatively charged nitrate ion to give IV, or (B) a ring closure to form hexa-*O*-acetyl-D-fructofuranose 2,1′:1,2′-dianhydride (III), resulting from the transfer of the positive charge to the glycosidic carbon atom of the adjoining fructose residue. Reaction B can continue as a chain reaction until it is terminated by the capture of a nitrate ion or by reaching a point in the molecule where no further ring closure to form III is possible.

Figure 1 illustrates the proposed mechanism. The conditions of the experiment should lead to the conversion to a nitrate group of the hydroxyl group (V) formed by the initial proton attack on I. This is supported by the reported presence of tri-*O*-acetyl-D-fructose di-*O*-nitrate (VIII) in the reaction mixture,[3] presumably formed *via* VI and VII.

The aqueous acid hydrolysis of inulin appears to proceed by a similar mechanism. Among the products of such a hydrolysis are found three isomeric difructose dianhydrides,[6,7,11,12] amounting to about 5% of the hydrolyzate. Under these conditions, the following competing paths are available for the stabilization of the positively charged molecular fragment: (1) the neutralization of the positive charge by the capture of a hydroxyl ion, (2) a ring closure to form the difructofuranose 2,1′:1,2′-dianhydride, resulting in the transfer of the positive charge to the glycosidic carbon atom of the adjoining fructose residue, (3) a ring closure to form difructofuranose 2,4′:1,2′-dianhydride resulting in the loss of a proton from the hydroxyl group at position 4 of the adjacent fructose residue and (4) a ring closure to form difructofuranose 2,3′:1,2′-dianhydride, resulting in the loss of a proton from the hydroxyl group at position 3 of the adjacent residue. The low yield of the dianhydrides indicates that reaction (1) predominates.

The fact that hexa-*O*-acetyl-difructofuranose dianhydride (III) appears to be the only difructose dianhydride derivative formed in the nitric acid degradation of inulin acetate indicates that, under the experimental conditions prevailing, positively charged acetyl ions are not eliminated in a manner analogous to the loss of protons accompanying the formation of difructose anhydrides during the aqueous acid hydrolysis of inulin. From this, it fol-

(1) This work forms part of a thesis submitted by L. A. Boggs to the University of Minnesota in partial fulfillment for the degree of Ph.D. (1951).

(2) Paper No. 2788, Scientific Journal Series, Minnesota Agricultural Experiment Station.

(3) J. C. Irvine and J. W. Stevenson, THIS JOURNAL, **51**, 2197 (1929).

(4) E. W. Bodycote, W. N. Haworth and C. S. Woolvin, *J. Chem. Soc.*, 2389 (1932).

(5) W. N. Haworth and H. R. L. Streight, *Helv. Chim. Acta*, **15**, 693 (1932).

(6) R. F. Jackson and Sylvia M. Goergen, *J. Research Natl. Bur. Standards*, **3**, 27 (1929).

(7) Emma J. McDonald and R. F. Jackson, *ibid.*, **24**, 181 (1940).

(8) Emma J. McDonald, *Advances in Carbohydrate Chem.*, **2**, 253 (1946).

(9) W. N. Haworth, E. L. Hirst and E. G. V. Percival, *J. Chem. Soc.*, 2384 (1932).

(10) E. L. Hirst, D. I. McGilvray and E. G. V. Percival, *ibid.*, 1297 (1950).

(11) R. F. Jackson and Emma J. McDonald, *J. Research Natl. Bur. Standards*, **6**, 709 (1931).

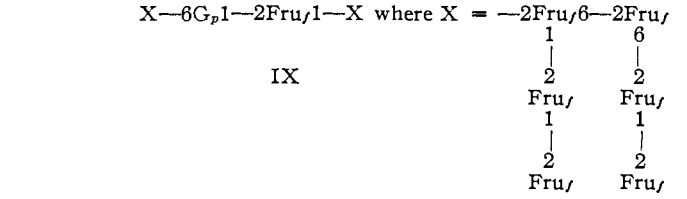
(12) Emma J. McDonald and R. F. Jackson, *ibid.*, **35**, 497 (1945).

lows that the formation of the hexa-*O*-acetyl derivative of difructofuranose 2,1′:1,2′-dianhydride (III) by degradation with fuming nitric acid would not be expected to take place with an acetylated polyfructosan having less than three adjacent anhydrofructofuranose units linked together by 2,1′-bonds.

The structure of the glucofructosan found in the tuber of the Hawaiian *ti* plant (*Cordyline terminalis*) has recently been investigated in this Laboratory.[13] The high yield (40% approx.) of 3,4,6-tri-*O*-methyl-D-fructose found in the hydrolyzate of the methylated polysaccharide indicated that one of the principal linkages is the 2,1′-type. This is the characteristic linkage found in inulin and it appeared probable that degradation of the acetylated *ti* fructosan with fuming nitric acid would lead to the formation of hexa-*O*-acetyldifructose anhydride (III). However, treatment of acetylated *ti* fructosan with fuming nitric acid, under the same conditions as those which provided approximately a 20% yield of crystalline hexa-*O*-acetyl-difructose dianhydride (III) from dahlia inulin acetate, did not form the crystalline product directly.

In contrast to the unbranched chain of fructose units in inulin, the *ti* fructosan has been found to be highly branched.[13] Hence, on the basis of the above mechanism of reaction for the formation of hexa-*O*-acetyl-difructose 2,1′:1,2′-dianhydride (III) from 1,2′-linked polyfructofuranose acetates by means of fuming nitric acid, a possible explanation of the failure to produce III by this reaction from *ti* fructosan acetate lies in the hypothesis that the branches are so distributed that no fully acetylated difructose dianhydride III can be formed. If this were true, and the further assumption, namely, that the fructose residues present at the points of branching were removed under the conditions of the experiment also proved correct, a partially acetylated, partially nitrated derivative of the difructose dianhydride IV should be formed. Inspection of formula IX, proposed[13] as a possible structure to explain the methylation and periodate oxidation studies, shows that it conforms to these requirements

IX




In order to test this possibility, the sirupy product resulting from the degradation of the acetate of the *ti* polyfructosan was first denitrated by treatment with iron powder in acetic acid solution[14,15] and then acetylated. By this means a 4% yield of the characteristic hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride (III) was obtained. Dahlia inulin triacetate and artichoke inulin triacetate gave rise directly to 20 and 40–50% (approx.) of III when treated with fuming nitric acid.

These results indicate that the *ti* fructosan molecule includes at least one group of three fructofuranose units mutually joined by 2,1′-linkages. It is also apparent that a much smaller proportion of the *ti* polysaccharide is so constituted than is the case with dahlia and artichoke inulin.

(13) L. A. Boggs and F. Smith, THIS JOURNAL, **78**, 1880 (1956).
(14) J. W. H. Oldham, *J. Chem. Soc.*, **127**, 2840 (1925).
(15) D. J. Bell and R. L. M. Synge, *ibid.*, 836 (1938).

## Experimental

**The Action of Fuming Nitric Acid upon the Acetates of Polyfructosans. A. The Acetate of Dahlia (*Dahlia variabilis*) Inulin.**—The acetylated polyfructosan, prepared by the method of Haworth, Hirst and Percival,[9] was dissolved in ethanol-free chloroform (dried over anhydrous magnesium sulfate) and treated with fuming nitric acid (freshly distilled); see Table I. In other experiments in which no solvent was used, fuming nitric acid was added directly to the acetate. In both cases a homogeneous solution resulted. At the end of the degradation the reaction mixture was poured on to ice and water and the product was extracted with chloroform. The combined chloroform extracts were washed successively with water, dilute sodium bicarbonate solution, then water and dried ($MgSO_4$). Removal of solvent *in vacuo* gave a sirup which was dissolved in the minimum amount of ethanol from which the product crystallized. The hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride was filtered off, washed with ice-cold ethanol, ether and dried. The results of some typical experiments carried out as described above are recorded in Table I.

TABLE I

PRODUCTION OF HEXA-*O*-ACETYL-DIFRUCTOUFURANOSE 2,1′:1,2′-DIANHYDRIDE FROM DAHLIA INULIN ACETATE BY DEGRADATION WITH FUMING NITRIC ACID

| Wt. of inulin acetate, g. | $CHCl_3$, ml. | $HNO_3$, ml. | Time, min. | Temp., °C. | Yield of hexaacetyl difructosan (approx.) Wt., g. | % by wt. |
|---|---|---|---|---|---|---|
| 1.0 | 15 | 7 | 120 | 24 | 0.20 | 20 |
| 1.0 | None | 5 | 120 | 24 | .18 | 18 |
| 1.0 | None | 5 | 90 | 26 | .19 | 19 |
| 1.0 | None | 5 | 120 | 16 | .24 | 24 |
| 5.0 | None | 25 | 120 | 13 | 1.10 | 22 |

When the hexa-*O*-acetyl-difructofuranose dianhydride was recrystallized from ethanol it had m.p. 128°, $[\alpha]^{25}D$ ±0° in chloroform (*c* 2). These values are in agreement with those previously recorded.[3,5]

**B. The Acetate of *Ti* (*Cordyline terminalis*) Polyfructosan.**—When this acetate was treated with fuming nitric acid in the manner described in A above for dahlia inulin acetate no crystalline hexaacetyldifructosan was obtained from the sirupy product.

To a solution of the latter (5 g.) in glacial acetic acid (5 ml.), iron powder (0.5 g.) and a piece of magnesium turning were added and the mixture was heated for about 1 hour in a water-bath at 60°.[14,15] Removal of the acetic acid *in vacuo* followed by extraction of the residue with acetone gave a sirupy product which again failed to crystallize when it was nucleated with a specimen of hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride.

The denitrated sirupy product was then treated with acetic anhydride (5 ml.) and pyridine (5 ml.) in the usual way. After standing overnight the mixture was poured into ice-water and the product extracted with chloroform. The pyridine was removed from the chloroform extract by washing with copper sulfate solution. The extract was then washed with sodium bicarbonate solution, water and dried ($MgSO_4$). Removal of the solvent *in vacuo* afforded a sirupy residue which readily crystallized upon being dissolved in the minimum of ethanol and nucleated with the hexa-*O*-acetyl-difructosan obtained above as in I. The crystals were filtered off and washed with ethanol (yield 4% by weight) m.p. and mixed m.p. 127°, $[\alpha]^{23}D$ ±0° in chloroform (*c* 2). The results of two experiments carried out as described above are recorded in Table II.

When the crude sirupy product, obtained as above by degradation of dahlia inulin acetate, was subjected to denitration and reacetylation no increase in yield of hexa-*O*-acetyl-difructose dianhydride resulted.

TABLE II

PRODUCTION OF HEXA-*O*-ACETYL-DIFRUCTOSE ANHYDRIDE FROM ACETYLATED *Ti* POLYFRUCTOSAN BY DEGRADATION WITH FUMING NITRIC ACID FOLLOWED BY DENITRATION AND REACETYLATION

| Wt. of *ti* fructosan acetate | $CHCl_3$, ml. | $HNO_3$, ml. | Time, min. | Temp., °C. | Yield of hexa-*O*-acetyldifructosan Wt., g. | % by wt. |
|---|---|---|---|---|---|---|
| 1.0 | 15 | 7 | 120 | 23 | 0.04 | 4.0 |
| 5.0 | None | 25 | 120 | 13 | .19 | 3.8 |

**C. The Action of Fuming Nitric Acid on Sucrose Octa-*O*-acetate.**—When sucrose octa-*O*-acetate was treated with fuming nitric acid as described above in A there was formed 1-nitro-2,3,4,6-tetra-*O*-acetyl-β-D-glucose, m.p. 150–151° (after crystallization from aqueous ethanol). A small amount of unchanged sucrose octa-*O*-acetate was also isolated, m.p. and mixed m.p. 82°, but no hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride was detected.

**D. Artichoke (*Helianthus tuberosus*) Inulin Acetate.**—(a) To an ice-cold solution of inulin tri-*O*-acetate (20 g.)[4] in chloroform (300 ml., dried over $P_2O_5$), freshly distilled fuming nitric acid (130 ml.) (prepared by two distillations of fuming nitric acid (d. 1.5) in the presence of concentrated sulfuric acid) was added slowly with vigorous stirring, moisture being excluded from the apparatus. The experiment was conducted at 0° in an atmosphere of dry carbon dioxide. Phosphorus pentoxide (80 g.) was added to the homogeneous solution in 5-g. portions during 1.25 hours. Most of the phosphorus pentoxide remained unchanged and it did not appear to become sirupy in nature. After 1.5 hours the reaction mixture was poured into ice-water and when all the phosphorus pentoxide had dissolved the colorless chloroform layer was separated and washed successively with water and sodium bicarbonate solution. After drying ($MgSO_4$) the chloroform extract removal of solvent gave a pale yellow sirupy product which was dissolved in ethanol from which the hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride crystallized. The product was filtered off, washed with ice-cold ethanol and dried (yield 9 g.[4]). Time is an important factor in this experiment and variations from 1.5 hours were found to give reduced yields of the crystalline hexa-*O*-acetyl-difructose dianhydride.

(b) To a solution of inulin acetate (10 g.) in dry chloroform (200 ml.) fuming nitric acid (100 ml.) was added with stirring. The reaction was allowed to proceed for 2 hours at 18–20°. The hexa-*O*-acetyl-difructose 2,1′:1,2′-dianhydride isolated as above amounted to 5 g. (50% of theory). Several experiments carried out for 2 hours at 0° gave lower yields of the crystalline dianhydride.

These yields of the hexa-*O*-acetyl-difructose dianhydride which are higher than those obtained from dahlia inulin acetate are believed to be due to the greater precautions taken to exclude moisture from the reaction mixture and not to any structural differences between the two polysaccharide acetates.

The hexa-*O*-acetyl-difructofuranose 2,1′:1,2′-dianhydride prepared as above and recrystallized from ethanol showed $[\alpha]^{20}D$ −0.5° (approx.) in chloroform (*c* 5), m.p. 128° when rapid heating was applied and m.p. 138° when the heating was slow. From cooling curves the true m.p. was shown to be 128°.

*Anal.* Calcd. for $C_{24}H_{32}O_{16}$: C, 50.0; H, 5.6; Ac, 44.8. Found: C, 49.7; H, 5.8; Ac, 44.4.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

# The Constitution of the Glucofructan of the Tuber of the Hawaiian "*TI*" Plant (*Cordyline Terminalis*)[1,2]

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RECEIVED OCTOBER 17, 1955

The constitution of the glucofructan present in the tuber of the Hawaiian *ti* plant has been shown to be composed of 1 unit of D-glucose and about 13 of D-fructose. The methylated polysaccharide gives upon hydrolysis 1,3,4,6-tetra-*O*-methyl-D-fructose (4 moles), 1,3,4-tri-*O*-methyl-D-fructose (2 moles), 3,4,6-tri-*O*-methyl-D-fructose (5 moles), 3,4-di-*O*-methyl-D-fructose (2 moles) and 2,3,4-tri-*O*-methyl-D-glucose (1 mole). The structural significance of these findings is discussed.

Extraction of the dried tubers of the Hawaiian *ti* plant (*Cordyline terminalis*) with water yields a glucofructan.[3] The latter, obtained in the form of an amorphous white powder, showed $[\alpha]D$ −37° (water) and was non-reducing to Fehling solution.

Upon hydrolysis it gave D-glucose and D-fructose in the approximate ratio of 1:14. The ease with which the polysaccharide underwent hydrolysis indicated that the fructose residues were present in the furanose form.

The *ti* glucofructan, which appeared to be essentially homogeneous from fractionation studies, was transformed into a triacetate with acetic anhydride and pyridine[4] and this was methylated[4,5] with methyl sulfate and sodium hydroxide to give the corresponding trimethyl derivative as a viscous liquid which also appeared to be homogeneous from fractionation studies.

Hydrolysis of the tri-*O*-methyl *ti* glucofructan with oxalic acid under conditions which have been found[5] to hydrolyze 1,2′-linked fructans composed of furanose units failed to effect complete hydrolysis. This observation indicated that *ti* fructan might contain some linkage other than the 1,2′-type.

After completing the hydrolysis by using a higher concentration of oxalic acid and a higher temperature than those required for inulin,[5] the mixture of methylated sugars was resolved by cellulose column chromatography[6] into three fractions,[7] namely, 1,3,4,6-tetra-*O*-methyl-D-fructose, 3,4-di-*O*-methyl-D-fructose and a mixture of trimethylhexoses. This mixture consisted of 1,3,4-tri-*O*-methyl-D-fructose, 3,4,6-tri-*O*-methyl-D-fructose and 2,3,4-tri-*O*-methyl-D-glucose (see Table II).

The 1,3,4,6-tetra-*O*-methyl-D-fructose was iden-

(1) Paper No. 3439 Scientific Journal Series, Minnesota Agricultural Experiment Station.

(2) This work forms part of a thesis submitted by L. A. Boggs to the Graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Ph.D. (August, 1951). Presented at the 117th National Meeting of the A.C.S. in Detroit, April, 1950.

(3) T. T. Tanimoto, *Proc. Hawaiian Sugar Planters Assoc.*, **59**, 119 (1939); *C. A.*, **34**, 6473 (1940).

(4) W. N. Haworth and H. R. L. Streight, *Helv. Chim. Acta*, **15**, 609 (1932).

(5) W. N. Haworth, E. L. Hirst and E. G. V. Percival, *J. Chem. Soc.*, 2384 (1932).

(6) L. Hough, J. K. N. Jones and W. H. Wadman, *ibid.*, 2511 (1949).

(7) L. A. Boggs, L. S. Cuendet, M. Dubois and F. Smith, *Anal. Chem.*, **24**, 1148 (1952).