

## Diheterolevulosan III and IV

## The Structure of Diheterolevulosan IV\*

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The anhydride mixture resulting from the treatment of D-fructose with concentrated hydrochloric acid<sup>1</sup> has been shown to contain two compounds, diheterolevulosan I and II, the structures of which have been proved to be di-D-fructopyranosido 1,2':2,1'-dianhydride and a D-fructopyranose-D-fructofuranose 1,2':2,1'-dianhydride respectively<sup>2-6</sup>. Later, during the course of the present investigation, Wolfrom and coworkers have reported the isolation of a third substance of the same type<sup>7</sup>, which they designated diheterolevulosan III, shown by periodate oxidation to be either a di-D-fructopyranose 1,2'-2,3'-dianhydride or an anomer of diheterolevulosan II. According to the same authors, application of the Hudson isorotation rules to the rotatory data of the two compounds supports the latter view.

In our work on the isolation and structure of diheterolevulosan I and II<sup>6</sup>, we used paper chromatographic methods, but in some cases there were discrepancies between the chromatographic and the optical rotatory data, and the investigation was therefore continued using other methods.

The anhydride mixture prepared as before was divided by crystallisation into separate fractions, shown in the diagram, Fig. 1, followed by further fractionation on a carbon-celite column using the gradient elution technique (linear gradient) with ethanol as eluant. The elution was followed polarimetrically and by paper chromatography. A typical run is shown in Fig. 2. The components were usually isolated as their acetates which were fairly easy to purify by crystallisation. Diheterolevulosan I and II and the two new compounds diheterolevulosan III and IV were obtained in this manner from the two mother liquors. In addition some amorphous material probably containing the fructose anhydrides described by Wolfrom *et al.* together with some compounds of reducing character were obtained. The crystalline fractions 11, 12 and 131 only yielded diheterolevulosan I, II and IV. As the resolution of diheterolevulosan I, II and IV was not complete on the columns used,

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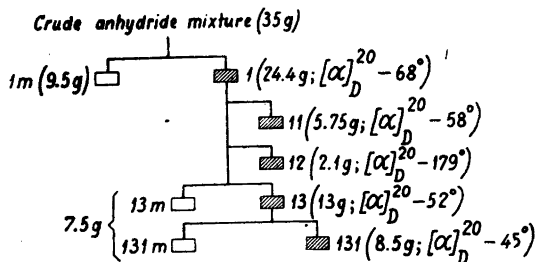


Fig. 1. Recrystallisation chart. Fractionation of the crude anhydride mixture from hydrochloric acid treated D-fructose.

a quantitative estimation of the relative amounts of these compounds must be omitted. Nevertheless the relatively large amount of diheterolevulosan IV obtained by us compared with the small amount of syrup unaccounted for in Wolfrom's experiments and which may contain the same substance is noteworthy. A possible explanation is that a higher reaction temperature was used in our experiments than in Wolfrom's. On the other hand the yield of diheterolevulosan III is of the same order in both experiments.

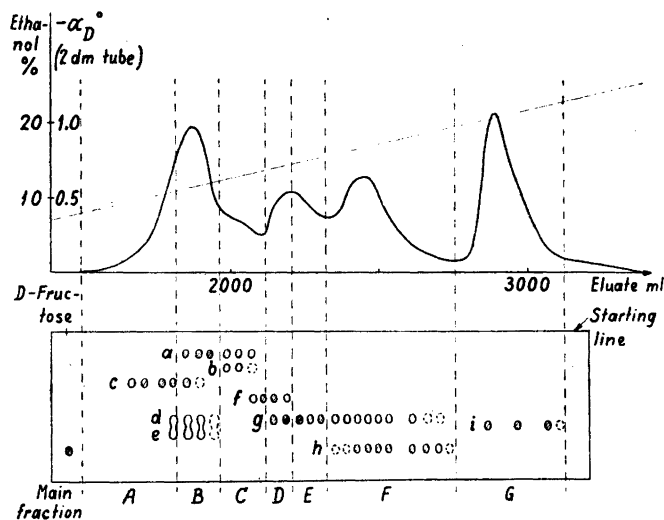


Fig. 2. Chromatographic separation on a carbon column of fructose anhydride mixture from hydrochloric acid treated D-fructose, using the gradient elution technique. Optical rotation curve and paper chromatogram of the fractions. Spot on paper chromatogram: a—e unknown substance; f diheterolevulosan I; g diheterolevulosan II and IV; h unknown substance; i diheterolevulosan III.

Though X-ray crystallographic data have not been determined for our preparation of diheterolevulosan III its identity with the substance described by Wolfrom *et al.* is evident from the following facts:

	Wolfrom <i>et al.</i>	Present investigation
Diheterolevulosan	M.p. 255–258° (no dec.)	M.p. 240–242° (dec.)
III	$[\alpha]_D^{30}$ –179° (water, $c = 3.6$ )	$[\alpha]_D^{30}$ –183° (water, $c = 2$ )
Acetate	M.p. 135.5–136.5°	M.p. 130–131°
	$[\alpha]_D^{25}$ –169° (chloroform, $c = 2$ )	$[\alpha]_D^{30}$ –159° (chloroform, $c = 2$ )

On periodic acid oxidation both preparations yield one mole of formic acid with the consumption of three moles of oxidant.

When oxidized with periodic acid, diheterolevulosan IV (m.p. 279–281°,  $[\alpha]_D^{30}$  –309° in water) consumes four moles of oxidant with the formation of two moles of formic acid. The acetate (m.p. 268–269°,  $[\alpha]_D^{30}$  –199° in chloroform) shows a remarkable stability towards heat and is easily purified by sublimation at 12 mm pressure.

On the paper chromatogram, using the butanol-ethanol-water solvent, diheterolevulosan II and IV travel with approximately the same rate and only a little slower than diheterolevulosan III. Diheterolevulosan IV is only with difficulty made visible with resorcinol-hydrochloric acid spraying reagent probably owing to its resistance to hydrolysis.

The structure of diheterolevulosan IV has been shown by the periodic acid oxidation and by its complete methylation and subsequent hydrolysis and identification of the fructose trimethyl ether formed following the general procedure described in a preceding paper<sup>6</sup> to be that of a di-D-fructopyranose 1,2':2,1'-dianhydride. It therefore obviously is an anomer of diheterolevulosan I ( $[\alpha]_D^{30}$  –44° in water).

Applying the Hudson isorotation rules (*cf.* Wolfrom *et al.*<sup>7</sup>) to this pair, assuming diheterolevulosan I to be the  $\alpha, \alpha'$ - and diheterolevulosan IV the  $\beta, \beta'$ - form:

$$\begin{array}{rcl}
 [M]_D^{30} \text{ of diheterolevulosan I,} & & (-44 \times 324) = -14\,200 \\
 [M]_D^{30} \text{ } \gg \text{ } \gg \text{ IV,} & & (-309 \times 324) = -100\,100 \\
 \text{Subtracting} & & 4A = 85\,900 \\
 & & A = 21\,500
 \end{array}$$

This value is in good agreement with that calculable for the two methyl D-fructopyranosides:

$$\begin{array}{rcl}
 [M]_D^{30} \text{ of } \alpha\text{-D-form} & & (+46 \times 194) = 8\,900 \\
 [M]_D^{30} \text{ } \gg \text{ } \beta\text{-D-form} & & (-172 \times 194) = -33\,400 \\
 \text{Subtracting} & & 2A = 42\,300 \\
 & & A = 21\,200
 \end{array}$$

Thus the anomeric structure of diheterolevulosan I and IV is established with some certainty.

## EXPERIMENTAL

*Preparation and fractionation of the anhydrides.* D-Fructose (75 g) was dissolved in concentrated hydrochloric acid (300 ml) and allowed to stand 72 hours at 0°. After removal of the hydrochloric acid and fermentation of unchanged fructose as described earlier the resultant syrup (35 g) was dissolved in a small volume of hot methanol and allowed to stand at room temperature for three weeks yielding crystalline material (fraction 1, 24.4 g;  $[\alpha]_D^{20} - 68^\circ$  in water) and an amorphous mother liquor product (fraction 1m, 9.5 g).

A few crystals of diheterolevulosan I were added to the solution of fraction 1 in hot water (17 ml) and methanol (30 ml). After standing for ten days at 0° the product formed semispherical aggregates which were collected (fraction 11, 5.75 g;  $[\alpha]_D^{20} - 58^\circ$  in water). Paper chromatogram indicated the presence chiefly of diheterolevulosan I together with smaller amounts of one or several of the other diheterolevulosans. During the filtration a micro-crystalline precipitate started to form in the mother liquor which was then allowed to stand in the cold for a further few days yielding the crystalline fraction 12 (2.1 g;  $[\alpha]_D^{20} - 179^\circ$  in water). Two rather faint spots corresponding to diheterolevulosan I and II, the former predominating, were obtained on the paper chromatogram. After removal of the solvents from the mother liquor, the resultant syrup was dissolved in methanol (25 ml) yielding the crystalline fraction 13 (13 g;  $[\alpha]_D^{20} - 52^\circ$  in water) and an amorphous mother liquor product (fraction 13m). On the paper chromatogram fraction 13 gave a strong spot on the same level as diheterolevulosan II and a weaker due to diheterolevulosan I. It was recrystallised from methanol-ethanol-water producing crystals (fraction 131, 8.5 g) and a mother liquor (fraction 131m).

Fraction 1m (9.5 g) was dissolved in water (130 ml) and adsorbed on the top of a column (35 × 4.5 cm) of equal parts (by weight) of "carbo animalis pro analysi" (J. P. Riedel and E. de Haen) and Celite. The carbohydrates were then eluted with aqueous ethanol (4 000 ml), the concentration of which was increased linearly from 0 to 30%. The eluate was divided by means of an automatic fraction collector into fractions (ca 25 ml each), which were then investigated with respect to their optical rotation (2 dm tube) and by means of paper chromatography (Fig. 2). They were divided into main fractions (A–G) as indicated in the figure. After evaporation *in vacuo* and dissolution in a small volume of methanol, fractions A and B remained in solution while fractions C–G gave crystals which were collected and characterized by paper chromatography and optical rotation measurements.

Fractions A and B amounted to 400 and 800 mg respectively. Fraction C (430 mg) yielded diheterolevulosan I (220 mg;  $[\alpha]_D^{20} - 43^\circ$  in water), fraction D (850 mg) diheterolevulosan II (330 mg;  $[\alpha]_D^{20} - 39^\circ$ , contaminated with diheterolevulosan I), fraction E (900 mg) diheterolevulosan II (560 mg;  $[\alpha]_D^{20} - 36^\circ$ ), fraction F (1.1 g) diheterolevulosan II and IV (460 mg;  $[\alpha]_D^{20} - 108^\circ$ , single spot on paper chromatograms) and fraction G (560 mg) diheterolevulosan III (380 mg,  $[\alpha]_D^{20} - 168^\circ$ ).

Upon evaporation under reduced pressure of the eluate from the column preceding fraction A, an oil (1.0 g) was obtained which on paper chromatography appeared to contain mainly glycerol together with very small amounts of fructose. The eluate succeeding fraction G gave on evaporation a syrup (440 mg) of complex composition whilst washings from the column (2 000 ml 50% ethanol) when concentrated under reduced pressure (40°) gave a residue (600 mg) which suddenly underwent spontaneous decomposition.

When acetylated in acetic anhydride-pyridine solution the crystalline material from fraction F yielded impure diheterolevulosan IV hexaacetate (160 mg from ethanol, m.p. 260–265°). A pure product was obtained by recrystallisation from ethanol and by sublimation under water pump pressure, m.p. 268–269° (under sublimation),  $[\alpha]_D^{20} - 199^\circ$  (chloroform,  $c = 2$ ). (Analysis: Found C 50.2; H 5.67. Calc. for  $C_{24}H_{32}O_{16}$ : C 50.0; H 5.60.)

The crystalline material from fraction G was acetylated in a similar manner yielding diheterolevulosan III hexaacetate (520 mg) from ethanol in thin plates, m.p. 129–131°. Despite repeated recrystallisations from ethanol the melting point could not be raised

above 130.5–131° (*cf.* Wolfrom *et al.*, m.p. 135.5–136.5°).  $[\alpha]_D^{20} = -159^\circ$  (chloroform,  $c = 2$ ). (Analysis: Found C 50.1; H 5.63. Calc. for  $C_{24}H_{32}O_{16}$ : C 50.0; H 5.60.)

The relatively large amount of material from fraction 1 m which remained amorphous even after separation on the carbon column is explained by the fact that the mother liquors remaining show a very complicated composition on paper chromatograms. The three dominating amorphous components were found in fractions A, B and F. They have not been investigated further.

Fraction 13 m + 131 m (7.5 g) was separated in the same manner as 1 m, the optical rotation curve obtained being very similar to that shown in Fig. 2. However, relatively more material was obtained in the crystalline state. Diheterolevulosan III hexaacetate (240 mg, m.p. 128–129°) and diheterolevulosan IV hexaacetate (600 mg, m.p. 263–267°) were obtained as well as diheterolevulosan I and II (*ca* 5 g together).

Fraction 11 (5.75 g) was acetylated without further separation, yielding diheterolevulosan I hexaacetate (6.5 g, m.p. 165–168°) and diheterolevulosan IV hexaacetate (170 mg, m.p. 267–268°).

Fraction 12 (2.1 g) was added to a carbon column (23 × 3.3 cm) and eluted with aqueous ethanol (2 000 ml) the concentration of which was raised linearly from 0 to 40 %. In the optical rotation curve there was only one peak corresponding to a partly separated mixture of diheterolevulosan I, II and IV. Unfortunately, owing to an accident with the fraction collector, the last parts of the eluate where diheterolevulosan III should eventually be found were lost. The eluate was evaporated to give two fractions the first of which contained mainly diheterolevulosan I and II and the second (1.09 g,  $[\alpha]_D^{20} = -250^\circ$ ) diheterolevulosan II and IV. The latter, when acetylated, gave diheterolevulosan IV hexaacetate (1.2 g, m.p. 265–267°).

Fraction 131 (8.5 g;  $[\alpha]_D^{20} = -45^\circ$ ) was separated on a carbon column in the same way as 1 m, the optical rotation curve of the eluate showing two peaks close together corresponding to diheterolevulosan I and II and diheterolevulosan IV respectively. Diheterolevulosan III if it had been present should have given rise to a third peak well separated from the two obtained. The first part of the eluate gave a mixture of diheterolevulosan I and II (5.8 g,  $[\alpha]_D^{20} = -39^\circ$ ) and the last a mixture (1.75 g) which upon acetylation yielded diheterolevulosan IV hexaacetate (130 mg, m.p. 260–264°).

*Diheterolevulosan III.* Diheterolevulosan III hexaacetate (200 mg) in absolute ethanol (5 ml) containing sodium ethoxide (100 mg) was allowed to stand at room temperature overnight. After dilution with water the reaction mixture was passed through the Amberlite resin, IR 120, and then evaporated under reduced pressure. The residue was dissolved in ethanol and yielded diheterolevulosan III (95 mg) which on recrystallisation from aqueous ethanol had m.p. 240–242° (dec.),  $[\alpha]_D^{20} = -183^\circ$  (water,  $c = 1.8$ ). (Analysis: Found C 44.4; H 6.14. Calc. for  $C_{12}H_{20}O_{10}$ : C 44.4; H 6.22. Periodic acid consumption (10 mg of substance in 0.1  $C$   $H_5IO_6$  (2 ml), 24 hours at room temperature) 3.12 moles. Formic acid liberated when a sample (10 mg) dissolved in 0.25  $C$  sodium metaperiodate (1 ml) and water (5 ml) was allowed to stand at room temperature overnight: 0.9 moles.)

*Diheterolevulosan IV.* Deacetylation of the acetate (200 mg), as described above, yielded diheterolevulosan IV (97 mg), which on recrystallisation from aqueous ethanol formed heavy prisms, m.p. 279–281° (dec.),  $[\alpha]_D^{20} = -309^\circ$  (water,  $c = 2$ ). (Analysis: Found C 44.5; H 6.12. Calc. for  $C_{12}H_{20}O_{10}$ : C 44.4; H 6.22. Periodic acid consumption: 4.12 moles. Formic acid liberated on sodium metaperiodate oxidation: 1.7 moles.)

*Diheterolevulosan IV hexamethyl ether.* Dioxane (5 ml) was added to diheterolevulosan IV hexaacetate (290 mg = 0.5 mmol) and powdered sodium hydroxide (600 mg = 15 mmol) in a flask with an effective mechanical stirrer. Methyl sulphate (0.6 ml = 6 mmol) was added slowly (0.5 hour) at 60–65° under vigorous stirring and the temperature was then raised to 70° for 2 hours. The mixture was diluted with dioxane (5 ml) and allowed to stand at room temperature overnight. It was filtered, the filter cake being carefully washed with dry benzene, and the filtrate evaporated to dryness. The residue (dried over silica gel) was dissolved in liquid ammonia (*ca* 5 ml) and potassium metal added till a stable blue colour was obtained. After evaporation of the ammonia the residue was dissolved in dry methyl iodide (1 ml) and left overnight. The excess of methyl iodide was removed, the reaction product extracted with chloroform and the solution passed through

a short column of alumina. On the addition of light petrol to the strongly concentrated chloroform solution diheterolevulosan IV hexamethyl ether crystallised in hard aggregates (156 mg = 77 %, m.p. 119–122°). Distillation (b.p.<sub>0.1</sub> ca 170° bath temperature) and recrystallisation from chloroform-light petrol yielded prisms, m.p. 122–122.5°,  $[\alpha]_D^{20} - 243^\circ$  (chloroform,  $c = 2$ ), properties unchanged after two recrystallisations from isopropyl ether. (Analysis: Found  $\text{OCH}_3$ , 44.6. Calc. for  $\text{C}_{12}\text{H}_{14}\text{O}_4(\text{OCH}_3)_6$ : 45.6.)

*Hydrolysis of diheterolevulosan IV hexamethyl ether.* A preliminary experiment showed that the hydrolysis of the methyl ether reached ca 30 % completion when run under the same conditions as used for hydrolysis of diheterolevulosan I hexamethyl ether (1.5 *N*  $\text{H}_2\text{SO}_4$ , 80° and 18.5 hours). Paper chromatograms indicated the formation of fructose 3,4,5-trimethyl ether as the only hydrolysis product. It was found that the methyl ether of diheterolevulosan IV could be separated from the fructose trimethyl ether formed by partition between water and chloroform analogous to the corresponding methyl ethers of diheterolevulosan I and II.

A solution of diheterolevulosan IV hexamethyl ether (110 mg) in 1.5 *N* sulphuric acid (1.1 ml) was kept at 80° for 15.5 hours. It was then extracted with chloroform (4 × 0.5 ml) and the combined extracts were washed with water (1.5 ml) which in its turn was extracted with chloroform (2 × 0.5 ml). All chloroform phases were then combined and evaporated, and the residue crystallised from isopropyl ether, yielding unchanged hexamethyl ether (60 mg). The mother liquor was evaporated to dryness, the residue dissolved in water (0.4 ml) and this solution extracted with chloroform (3 × 0.15 ml) to recover the last traces of unchanged hexamethyl ether. The two aqueous solutions were combined with the hydrolysate, forming the fructose trimethyl ether fraction, whilst the remaining chloroform phase after evaporation was combined with the recovered hexamethyl ether (60 mg) and the combined material thus obtained was subjected, after the addition of fresh methyl ether (30 mg), to repeated hydrolysis following the scheme given above.

After the second hydrolysis the chloroform soluble material recovered weighed 75 mg (largely crystalline). Starting from this lot the hydrolysis was repeated twice, yielding in the end 30 mg of unchanged hexamethyl ether. The amount of material consumed was then 140–30 = 110 mg.

The combined extracted hydrolysates were neutralised with barium carbonate, filtered, evaporated and the residue extracted with chloroform. The chloroform solution was filtered and evaporated yielding crude fructose trimethyl ether as a pale yellow oil (122 mg). For further purification this was dissolved in a benzene-ethanol (520 : 85) solvent saturated with water (2.5 ml), added to the top of a hydrocellulose column (20 × 2 cm), and eluted with the same solvent. The eluate was collected in 3 ml fractions and the fructose methyl ether located by putting a few drops of each fraction on a filter paper and then spraying with aniline hydrogen phthalate reagent. Evaporation of the fractions (36–42) containing the bulk of the fructose methyl ether yielded an oil (93 mg which after distillation (b.p.<sub>0.8</sub> 130° bath temperature) amounted to 88 mg (73 % of theory) and had  $[\alpha]_D^{20} - 134^\circ$  (water,  $c = 2$ ), which compares favourably with  $[\alpha]_D^{20} - 128^\circ$  (water,  $c = 2$ ) observed for an authentic sample of *D*-fructose 3,4,5-trimethyl ether.

*D-Arabonamide 2,3,4-trimethyl ether from D-fructose 3,4,5-trimethyl ether.* To a solution of fructose trimethyl ether (66 mg) in acetic acid (1 ml) and water (0.5 ml) the stoichiometric amount (135 mg) of lead tetraacetate was added. After standing at room temperature for two hours, the solution was diluted with water (1 ml) and acetic acid (0.5 ml), freed from lead dioxide by centrifuging, and the supernatant liquid saturated with hydrogen sulphide and centrifuged. Evaporation of the clear solution gave the crude lactone derivative, which was distilled at water pump pressure (bath temperature 150–160°) and then dissolved in liquid ammonia. After the ammonia had evaporated, the crystalline residue was dissolved in acetone and the solution after filtration evaporated to 0.5 ml. When isopropyl ether (0.5 ml) was added, *D*-arabonamide 2,3,4-trimethyl ether separated as needles (40 mg). From the mother liquor a further quantity (10 mg) of substance was obtained making a total yield of 80 % of theory. M.p. 102.5–103°, unchanged after recrystallisation from the same solvents. When mixed with an authentic specimen m.p. 102.5–103° the melting point was again unchanged.

The overall yield of arabonamide derivative calculated upon diheterolevulosan IV hexamethyl ether consumed is 58 % of theory, proving that diheterolevulosan IV is built up from 1,2-linked D-fructopyranose units only.

#### SUMMARY

The anhydride mixture resulting from treatment of D-fructose with concentrated hydrochloric acid has been separated on carbon columns using the gradient elution technique. Two new compounds, diheterolevulosan III and IV were isolated, the former evidently being identical with diheterolevulosan III reported by Wolfrom and coworkers. Diheterolevulosan IV has been proved to be di-D-fructopyranose 1,2'-2,1'-dianhydride. Thus diheterolevulosan I and IV are anomers, and from their optical rotation it can be concluded that the former is the  $\alpha,\alpha'$ - and the latter the  $\beta,\beta'$ -form.

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