JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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VOLUME 73 AUGUST 6, 1951 NUMBER 8

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Action of Heat on D-Fructose. II.[1] Structure of Diheterolevulosan II

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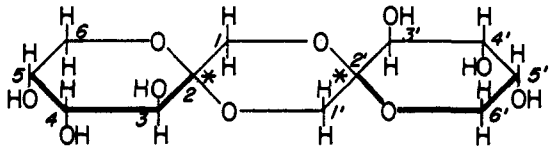
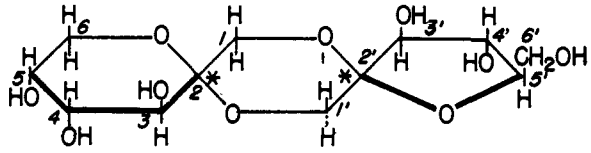
Chromatographic assays show that diheterolevulosans I and II, produced by the action of heat on D-fructose, are formed in a combined yield of 30% and in the ratio I/II : 1/2 by the action of hydrochloric acid on D-fructose. Diheterolevulosan II is dimorphous. The nitric acid oxidative hydrolysis of its crystalline hexamethyl ether, with subsequent derivatization, yields the furanose derivative V (also so prepared from trimethylinulin) and the pyranose derivative VII. This, together with the previously reported results of periodate oxidation, demonstrates that diheterolevulosan II is in all probability D-fructopyranose-D-fructofuranose 1,2′:2,1′-dianhydride (II). Polarimetric observations on the acetolysis of diheterolevulosans I and II likewise support this structure. Improvements in the chromatographic techniques employing clay are recorded and an apparatus for conveniently conducting methylations with liquid ammonia is described. Formic acid is shown to be useful in removing excess nitric acid.

In studying the action of heat on concentrated aqueous solutions of D-fructose, Wolfrom and Blair[1] isolated a reaction product which was a hitherto unknown di-D-fructose dianhydride and was designated diheterolevulosan II. The present communication is concerned with the determination of the structure of this substance.

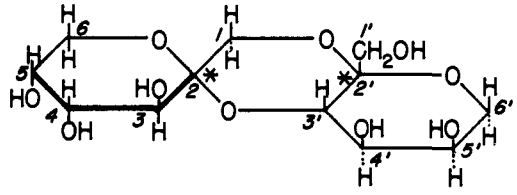
Wolfrom and Blair[1] had shown that hydrochloric acid solutions effected a facile dehydration of D-fructose to diheterolevulosan II and was the procedure of choice for its preparation. These reaction conditions were first employed on D-fructose by Pictet and Chavan[3] but they had overlooked the formation of this substance which is produced along with the compound I, designated diheterolevulosan by them and herein termed diheterolevulosan I. The structure of I is adequately established[1,4,5] as di-D-fructopyranose 1,2′:2,1′-dianhydride (I). We herein report an exhaustive and quantitative chromatographic investigation of the difructose dianhydrides produced when D-fructose is treated with four parts of concentrated hydrochloric acid at −5° for three days. Only the two are formed in significant amounts in a ratio of diheterolevulosan I/diheterolevulosan II : 1/2 and in a combined yield of *ca.* 30% from D-fructose. Our analytical data are shown in Table I and illustrate the applicability of these techniques to carbohydrate analysis. In the course of this work some new refinements in these rather difficult procedures were devised especially as regards streaking on clay and removing traces of colloidal siliceous matter from the chromatographed substances. Crystallization methods are entirely inadequate for separation of the diheterolevulosans I and II.



I. Diheterolevulosan I (di-D-fructopyranose 1,2′:2,1′-dianhydride).



II. Diheterolevulosan II (D-fructopyranose-D-fructofuranose 1,2′:2,1′-dianhydride).



III. Di-D-fructopyranose 1,2′:2,3′-dianhydride.
*Anomeric configuration unknown.

(1) Previous communication: M. L. Wolfrom and Mary Grace Blair, THIS JOURNAL, **70**, 2406 (1948).

(2) Sugar Research Foundation Research Associate (W. W. B.) and Fellow of The Ohio State University Research Foundation (Project 190).

(3) A. Pictet and J. Chavan, *Helv. Chim. Acta*, **9**, 809 (1926).

(4) H. H. Schlubach and C. Behre, *Ann.*, **508**, 16 (1934).

(5) Emma J. McDonald and R. F. Jackson, *J. Research Natl. Bur. Standards*, **35**, 497 (1945).

TABLE I

CHROMATOGRAPHIC ASSAY OF DIHETEROLEVULOSAN MIXTURE FROM D-FRUCTOSE AND HYDROCHLORIC ACID[a]

Fraction	Amt. chromatographed, g.	Diheterolevulosan I, g.	Diheterolevulosan I, yield,[b] %			Diheterolevulosan II, g.	Diheterolevulosan II, yield,[b] %		
A_1	2.50	1.18	7.1			0.88	5.3		
A_2	2.50	1.25		7.5		0.85		5.1	
A_3	2.50	1.23			7.4	0.79			4.7
B_1	2.50	0.33	2.0			2.22	14.5		
B_2	2.50	0.27		1.8		2.18		14.2	
B_3	2.50	0.29			1.9	2.20			14.3
C	3.20	0.65	0.3	0.3	0.3	2.92	1.5	1.5	1.5
Total, %			9.4	9.6	9.6		21.3	20.8	20.5

[a] See experimental portion for details. [b] Basis original D-fructose.

Diheterolevulosan II has been found to exist in dimorphous forms[6] which possess identical solution rotations and are indistinguishable in their decomposition points. They are sharply differentiated by their characteristic X-ray powder diffraction diagrams. The high energy, metastable form was first isolated in this laboratory by Miss Blair. In later work in our laboratory the substance crystallized only in the stable dimorph. Fortunately, the original sample of Miss Blair was at hand and was still in its original crystalline form, as attested by X-ray powder diffraction. Recrystallization then effected a complete conversion to the other dimorph exhibiting the second X-ray powder diffraction pattern. This further demonstrates the value of X-ray powder diffraction for the convenient identification and characterization of crystals.

Wolfrom and Blair[1] record that diheterolevulosan II reduces three moles (per mole) of periodate ion with the concomitant formation of one mole of formic acid and no formaldehyde. On this basis, structure II was favored although other possibilities were present. Evidence for the presence of a furanose moiety in diheterolevulosan II would strongly indicate structure II. Supporting, though not definitive, evidence of this type has now been found in acetolysis experiments. When triacetylinulin is acetolyzed with acetic anhydride, acetyl bromide and hydrogen bromide[7,8] and the product is processed with water, there is obtained a good yield of 1,3,4,6-D-fructofuranose tetraacetate. The acetolysis reaction thus contained a D-fructofuranose derivative, presumably the acetylated D-fructofuranosyl bromide, to which could be ascribed the large dextrorotation, $[\alpha]_D$ +60° (halide basis),[8] observed in the reaction mixture. A large levorotation was exhibited by β-D-fructopyranose tetraacetate dissolved in the same acetolysis mixture (Table II). It would be expected, therefore, that a compound I hydrolyzing to two pyranose units would show a large levorotation, and one (II) hydrolyzing to an equimolar mixture of pyranose and furanose units would show a significantly smaller rotation, whatever the sign. In order to test these deductions, hexaacetyldiheterolevulosan I,[4] composed of two pyranose units, was dissolved in the acetolyzing mixture and the rotation change, was, in fact, large and to the left (Table II). The rotation of hexaacetyldiheterolevulosan II[1] was then followed in the same mixture and the final rotation observed was indeed relatively small and to the left (Table II). Unfortunately, the period of observation was necessarily shorter (due to coloration of the mixture) than with the isomer but the observed change was much less during the same period. Thus structure II is indicated for diheterolevulosan II.

TABLE II

ROTATION[a] CHANGES OF D-FRUCTOSE DERIVATIVES IN ACETIC ACID CONTAINING ACETYL BROMIDE AND HYDROBROMIC ACID[b] AT 25°

Time, hr.[c]	Tetraacetyl-β-D-fructopyranose, 0.264 *M*, $[\alpha]_D$	Hexaacetyldiheterolevulosan I, 0.119 *M*, $[\alpha]_D$	Hexaacetyldiheterolevulosan II, 0.158 *M*, $[\alpha]_D$
0.5		−19.5°	−26°
1.0		−23	−28
1.5	−67°	−26	−28.5
3.0		−30	−29
10	−67.5	−51	
25		−74	
50		−80	

[a] Specific rotations are on the acetylated glycosyl bromide basis, assuming a complete conversion to this derivative. [b] Four milliliters of acetyl bromide and 4 ml. of 16% hydrobromic acid diluted to 25.0 ml. with acetic acid. [c] Observed until solution became too dark to read.

Hexamethyldiheterolevulosan II was prepared in crystalline form through the reaction between methyl iodide and the polyalkoxide formed with potassium in liquid ammonia. A convenient apparatus for effecting this type of methylation is described. The ether was characterized by melting point, rotation and X-ray powder diffraction data.

The di-D-fructose dianhydrides are resistant to acid hydrolysis and D-fructose, even when methylated, is susceptible to acid decomposition. Recourse was therefore had to oxidative hydrolysis with nitric acid, following the general procedures established by Hirst[9] for the monosaccharide units.

As a model experiment, and to obtain authentic material for comparative purposes, trimethylinulin (IV) was oxidatively hydrolyzed with nitric acid and the product isolated as the crystalline derivative V, designated methyl 1-C-carbamyl-2,3,5-trimethyl-D-arabinofuranoside (*syn.* methyl furanoside of trimethyl-2-keto-D-gluconamide), identical in properties with those reported by Haworth, Hirst and Nicholson[10] for the product of a similar series of reactions starting from tetramethyl-D-fructofuranose. This procedure gives a new and improved preparative method for V. It was found that the reduction of nitric acid by formic acid, used by Quartaroli[11] for the quantitative determination of nitrate ion, was well suited for removing the excess nitric acid at the end of the oxidation.

Hexamethyldiheterolevulosan II (VI) was oxidatively hydrolyzed in essentially the same fashion and the resultant mixture of acids was subjected

(6) The possibility of this was first called to our attention by Professor G. L. Clark of the University of Illinois, Urbana, Ill.

(7) J. C. Irvine and E. T. Stiller, THIS JOURNAL, **54**, 1079 (1932).

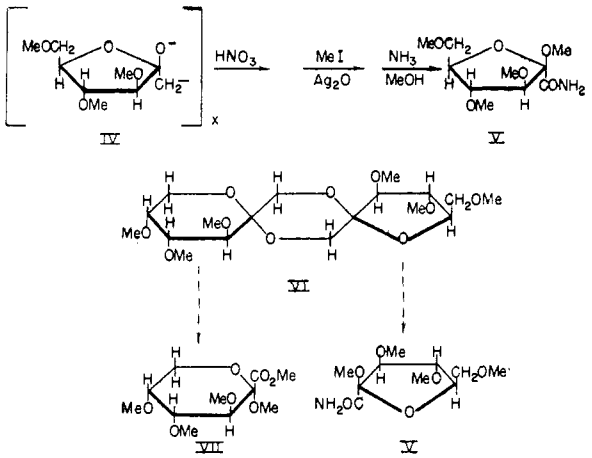
(8) W. W. Binkley and M. L. Wolfrom, *ibid.*, **68**, 2171 (1946).

(9) E. L. Hirst, *J. Chem. Soc.*, 350 (1926).

(10) W. N. Haworth, E. L. Hirst and V. S. Nicholson, *ibid.*, 1513 (1927).

(11) A. Quartaroli, *Staz. sper. agrar. ital.*, **44**, 157 (1911); **47**, 161 (1914); *Gazz. chim. ital.*, **41**, II, 64 (1911); **53**, 345 (1923).

to a preliminary fractionation by chromatography on anhydrous magnesium sulfate. The fractions, otherwise intractable, were converted to amides and any ketone groups present were stabilized through formation of their methyl glycosides by the Purdie methylation reaction.[12] This reaction



would also convert to methyl ethers any free secondary or primary hydroxyl groups present. There was isolated, in low yields, methyl 1-C-carbomethoxyl - 2,3,4 - trimethyl - D - arabino-pyranoside (VII, *syn.* methyl 2,3,4,5-tetramethyl-2-keto-D-gluconate) and V. Identification of these crystalline derivatives was made by melting point, mixed melting point, and X-ray powder diffraction data.

The two oxidation products so obtained are those expected from the methyl ether of II. Possibility III is eliminated since its hexamethyl ether could not give rise to the furanoside V under the conditions of our experiments. Formulas II and III are the only possibilities containing central dioxane rings that are consonant with the periodate oxidation data. Two other possibilities that would yield the same periodate data but would contain central rings of eight and nine members can be eliminated as highly improbable and in part contrary to the oxidation and acetolysis data. In all probability, therefore, diheterolevulosan II is represented by structure II and is a dianhydride of D-fructose containing both a pyranose and a furanose ring.

Experimental

WITH W. W. BINKLEY AND H. W. HILTON

Preparation of the Crude Diheterolevulosan Mixture from D-Fructose and Hydrochloric Acid.—Following Pictet and Chavan,[3] 200 g. of D-fructose was treated for 72 hours with 4 parts of concentrated hydrochloric acid (sp. gr. 1.19 at 15.56°) as described by Wolfrom and Blair[1] except that a temperature of −5° was maintained. The black reaction mixture was processed[1] in the same manner, Duolite A-4[13] being employed as the anion exchange resin and the excess D-fructose being largely removed by yeast fermentation. A partially crystalline residue was obtained on final solvent removal; yield 84 g. This material was dissolved in warm absolute methanol and crystals were allowed to form at 5°. The mother liquor was removed by decantation and the crystals were washed thrice with 40 ml. of absolute ethanol. The decantate and washings were combined and solvents were removed under reduced pressure. This process was repeated twice to obtain three lots (A, 30.0 g.; B, 32.6 g.; C, 3.2 g.) and a residual sirup (18.0 g.). The latter is presently under further investigation.

Chromatographic Analysis of the Crystalline Diheterolevulosan Mixture.—An amount of 2.50 g. of crystalline fraction was dissolved in 25 ml. of water. To this was added 225 ml. of absolute methanol and this solution was added immediately (before crystallization) to the top of a tapered glass column[14] containing a 23 × 7.5 cm. (diam.)[15] adsorbent column of 500 g. of Florex XXX[16]-Celite[17] (5:1 by wt.) prewet with 95/5[18]:ethanol/water and conditioned further with 50 ml. of 90/10:methanol/water. The chromatogram was developed with 900 ml. (Fraction B) or 1200 ml. (Fractions A and C) of 90/10:ethanol/water. The extruded adsorbent column was wrapped with aluminum foil to leave an exposed area 15 mm. wide running the length of the column. After drying at room temperature for 20 hours, the exposed area was streaked with alkaline permanganate (1 part of potassium permanganate in 100 parts of 2.5 *N* NaOH) indicator, employing a fine-tipped glass pipet. Three zones were indicated: a zone containing a negligible amount of material located 0–2 mm. from the column top; a 15–20 mm. interzone; an upper zone; a 20–40 mm. interzone; and a lower zone. The sectioned upper zone material was eluted with 1200–1500 ml. of 80/20:ethanol/water and the lower zone material with 2000–2500 ml. of the same solvent mixture. Solvent removal under reduced pressure yielded the crystalline zone material. Results are recorded in Table I.

To remove traces of siliceous matter from these substances, the residue from each zone was dissolved in 5 ml. of water and treated with decolorizing charcoal (50 mg. of Darco G-60) for 5 minutes at 50°. This mixture was then passed through a column (12 mm. diam.) containing consecutive layers of fibrous asbestos (2 ml.), sand (7 ml.) and Celite[17] (analytical grade, 3 ml.) arranged in that order from the column top (prewashed with water until a clear filtrate was obtained), the column being washed finally with 25 ml. of water. Pure crystalline material was obtained on solvent removal. The upper zone material was diheterolevulosan I; m.p. 261–263° (dec.),[19] $[\alpha]^{20}D$ −46° (*c* 4, water). X-Ray powder diffraction characterization[20,21]: 7.12, 5.86, 5.32 (1), 4.88, 4.65 (2), 4.42, 4.02 (3), 3.82, 3.66, 3.54, 3.37, 3.20, 3.08, 2.95, 2.86, 2.76, 2.66, 2.53, 2.36, 2.20, 2.15, 2.04, 1.94, 1.85, 1.75, 1.73, 1.68, 1.61, 1.478, 1.372, 1.316, 1.236.

The lower zone material was the stable dimorph (see below) of diheterolevulosan II; m.p. 257–259° (dec.),[19] $[\alpha]^{25}D$ −39° (*c* 4, water). X-Ray powder diffraction characterization[20]: 18.95, 9.36, 7.12, 6.58, 5.97, 5.40 (2), 5.07, 4.76 (1), 4.05 (4), 3.58, 3.42, 3.25, 2.93 (3), 2.46, 2.25, 2.16, 1.92, 1.76.

Dimorphism of Diheterolevulosan II.—The original filed sample of diheterolevulosan II,[1] prepared in 1947 by Mary Grace Blair, showed the following X-ray powder diffraction pattern[20]: 6.90 (4), 6.24 (3), 5.54 (1), 5.18, 4.88 (5), 4.55, 4.33 (2), 4.12, 3.92, 3.78, 3.66, 3.39, 3.22, 3.12, 2.93, 2.73, 2.64, 2.58, 2.51, 2.43, 2.34, 2.27, 2.21, 2.16, 2.08, 2.03, 2.00, 1.96, 1.92, 1.88, 1.84, 1.77, 1.71, 1.67, 1.61, 1.58, 1.53, 1.51, 1.23. A portion (100-mg.) of this material was dissolved in 5 ml. of water and 5 ml. of ethanol was added. The solvents were removed at room temperature in a desiccator under reduced pressure and the

(12) C. S. Hudson and D. H. Brauns, THIS JOURNAL, **38**, 1216 (1916).

(13) A product of the Chemical Process Co., Redwood City, California.

(14) Tapered glass tubes can be purchased from the Scientific Glass Co., Bloomfield, N. J., and the Ace Glass Co., Vineland, N. J.

(15) Adsorbent dimensions.

(16) A fuller's earth type of clay produced by the Floridin Co., Warren, Pennsylvania.

(17) No. 545, a siliceous filter-aid produced by Johns-Manville Co., New York, N. Y.

(18) All solvent ratios indicate volumes before mixing.

(19) See footnote 9, ref. 1, for melting point procedure.

(20) For these measurements we are indebted to Professor G. L. Clark of the University of Illinois, Urbana, Ill.

(21) All data were obtained with Cu K_α radiation, $\lambda = 1.5418$ Å., two hour exposure, no back reflections observed. The first figure is the interplanar spacing in Å. The second figure is relative intensity as estimated visually; if hyphenated it is expressed as % strongest line; if parenthetically expressed it denotes the most intense lines in decreasing order of intensity. We are indebted to Professor P. M. Harris for assistance in obtaining the measurements made in this laboratory.

residual sirup was crystallized by the addition of a small amount of absolute ethanol with nucleation. These crystals then exhibited the X-ray powder diffraction recorded above [main lines: 4.75 (1), 5.40 (2), 2.93 (3), 4.05 (4)]. This material is therefore the stable, lower energy form and the substance is dimorphous. The rotations of both forms are the same and the decomposition points are indistinguishable.

Anal. Calcd. for $C_{12}H_{20}O_{10}$: C, 44.44; H, 6.22. Found: C, 44.29; H, 6.13.

WITH W. L. SHILLING

Diheterolevulosan II Hexamethyl Ether.—An apparatus was assembled consisting of a three-necked round-bottomed flask connected to a jacketed lead condenser cooled with solid carbon dioxide-acetone and delivering liquid ammonia to the insulated (with cloth wrapping) reaction flask.[22] Below the ammonia delivery tube was a perforated glass cup holding potassium. This cup was supported on a glass rod connecting outside the flask through a ground joint in such a way that the cup could be rotated 180° on a vertical axis. A motor stirrer, dropping funnel and glass condenser were also attached to the reaction flask, the latter in such a manner that it could be employed for reflux or distillation and delivering into a soda-lime-guarded receiver connected to the hood through a water aspirator. The ammonia employed was bubbled through a solution of potassium in liquid ammonia before entering the top of the lead condenser.

Into the flask was placed 5.74 g. (0.0177 mole or 0.106 equivalents of hydroxyl) of powdered diheterolevulosan II (chromatographically pure) and approximately 5 g. (0.128 mole) of potassium was placed in the perforated cup. The cup was rotated away from the ammonia delivery tube, the lead condenser was packed with solid carbon dioxide-acetone and ammonia gas was passed through the apparatus. When 25–30 ml. of liquid ammonia had collected in the flask, the diheterolevulosan II had completely dissolved. When *ca.* 100 ml. of liquid ammonia had collected, the perforated cup was rotated so that the potassium was carried into the reaction mixture. A vigorous evolution of hydrogen ensued, and after some time the solution became turbid showing that the solution, then about 500 ml., was saturated with the potassium salt of the carbohydrate. Preliminary experiments indicated that the white turbidity appeared sooner when sodium was employed. Potassium addition was continued until the solution remained deep blue for 2 hours. The ammonia was then allowed to evaporate, 100 ml. of sodium-dried benzene was added through the dropping funnel and the condenser set for distillation. The benzene was removed at 40° under reduced pressure to effect complete removal of ammonia. The resulting practically white powder was treated with 9 ml. of methyl iodide, added through the dropping funnel, and stirred for 30 minutes. A further 15 ml. of methyl iodide (total of 55 g. or 0.385 mole) was added along with 200 ml. of dry benzene and the mixture was refluxed overnight. The condenser was again set for distillation and the solvents were removed at 45° under reduced pressure. The resultant mixture of white solid and sirup was remethylated in the same manner, after replenishing the potassium in the cup. A lesser amount of potassium was required to produce a permanent blue color during the second methylation.

The final product obtained on solvent removal was dissolved in a little water and extracted with chloroform. Solvent removal from the dried chloroform extract yielded a sirup that crystallized upon the addition of petroleum ether; yield 6.1 g. (84%) of material of good purity. Pure material was obtained on recrystallization in 90% recovery from 5 parts of cyclohexane-benzene (90:10 initial volume ratio); m.p. 101–102° (cor.), $[\alpha]^{27}D$ −22.5° (*c* 1, U. S. P. chloroform), $[\alpha]^{28}D$ −10.3° (*c* 5.9, acetic acid). X-Ray powder diffraction characterization[21]: 5.37, 4.90, 4.20 (1), 3.70 (2), 3.31 (3), 3.07 (4), 2.81, 2.52, 2.40, 2.17, 2.00, 1.89, 1.82, 1.78, 1.71, 1.62, 1.55, 1.36, 1.30.

The material was shown to be chromatographically homogeneous by the following experiments.

A solution of 1 mg. in 0.5 ml. of chloroform (ethanol-free) was chromatographed on a 100 × 10 mm. (diam.) column of 2.25 g. of acetone-washed Magnesol-Celite (5:1 by wt.) and was developed with 40 ml. of a mixture of 1 volume of absolute ethanol and 100 volumes of U. S. P. chloroform. The extruded column was streaked with the Molisch reagent and the only zone which appeared was 37–45 mm. from the top.

A solution of 2 mg. of the hexamethyl ether in 0.5 ml. of chloroform (ethanol-free) was chromatographed on a 95 × 10 mm. (diam.) column of 2.0 g. of Silene EF-[23] Celite (5:1 by wt.) and developed with 15 ml. of benzene/ethanol: 100/1. The extruded column was streaked with the Molisch reagent and the only zone detected was at 77–95 mm. from the top.

The substance was soluble in water and the common organic solvents except petroleum ether.

Anal. Calcd. for $C_{12}H_{14}O_6(OCH_3)_6$: C, 52.92; H, 7.90; OCH_3, 45.59. Found: C, 53.03; H, 7.96; OCH_3, 45.11.

Oxidative Hydrolysis of Hexamethyldiheterolevulosan II.—Hexamethyldiheterolevulosan II (1.0 g.) was dissolved in 7 ml. of concentrated nitric acid (d. 1.4) and placed in a bath whose temperature was raised to 70° in the course of 40 minutes and so maintained for 30 minutes. In the next 45 minutes the temperature was raised to 90° and held so for 90 minutes. Brown fumes began to evolve at 60° and the solution gently bubbled above 70°. At the end of the described period of heating, the gas evolution had practically ceased.

The cooled reaction mixture was diluted with 10 ml. of water and distilled at constant volume at 28 mm. and 50° for 5 hours to remove nitric acid. The water was then removed and the resultant sirup was dissolved in chloroform and the dried solution was concentrated to 35 ml. and added to 1 kg. of anhydrous magnesium sulfate[24] contained in a 2-liter pharmaceutical percolator. Development was effected with 2 liters of benzene/ethanol: 100/1. The extruded column was streaked with congo red indicator. Probably due to traces of nitric acid, the entire streak turned blue but zones could be located by their greater color intensity. The zone boundaries were as follows: zone 1, 11–62 cm. from the top of the 205 cm. column; zone 2 (very faint), 80–105 cm.; zone 3 (very faint), 132–155 cm.; zone 4, 173–205 cm.

Isolated zone 1 was eluted with methanol and ethanol and the material obtained on solvent removal was treated with an aqueous solution of barium acetate, filtered and the filtrate passed through a column of Amberlite IR-120[25] to remove cations. Water was removed from the effluent by concentration under reduced pressure and the resultant sirup was distilled under reduced pressure with toluene and ethanol to remove acetic acid as the azeotrope; yield 0.43 g. of light yellow sirup.

The above sirup was methylated in methanol (and subsequently acetone) solution with methyl iodide and silver oxide according to the general procedure of Purdie and Irvine.[26] The resultant sirup was treated with decolorizing carbon (Darco G-60) in methanol, the solvent removed and the residue extracted with boiling cyclohexane. Solvent removal from the extract left a sirup which slowly deposited crystals. These were separated from their embedding sirup by trituration with cold ether and ether-petroleum ether. The residual needles were recrystallized twice from cyclohexane, once from diisopropyl ether-petroleum ether and were then sublimed at 78° and 3 mm.; yield 7 mg., m.p. 98–99° (cor.) undepressed on admixture with an authentic specimen (m.p. 100–101°, cor.; recorded[27] 102°) of methyl 1-C-carbomethoxyl-2,3,4-trimethyl-D-arabinopyranoside (VII, *syn.* methyl 2,3,4,5-tetramethyl 2-keto-D-gluconate). Comparative material was prepared from tetramethyl-D-fructopyranose by oxidizing according to the procedure of Irvine and Patterson[28] and processing the reaction mixture according to Haworth, Hirst and Learner.[29] Both preparations gave identical X-ray powder diffraction diagrams, characterized[21] as follows: 6.73–100, 5.61–70, 4.67–70, 4.22–70, 3.88–20, 3.48–50, 3.38–50, 3.19–5, 2.98–8, 2.81–3, 2.74–3, 2.62–5, 2.47–3, 2.32–8, 2.23–8.

(22) K. W. Greenlee and A. L. Henne, *Inorg. Syntheses*, **2**, 128 (1946).

(23) L. W. Georges, R. S. Bower and M. L. Wolfrom, THIS JOURNAL, **68**, 2169 (1946).

(24) Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Missouri.

(25) A product of Rohm and Haas Co., Philadelphia, Pennsylvania.

(26) T. Purdie and J. C. Irvine, *J. Chem. Soc.*, **83**, 1021 (1903).

(27) W. N. Haworth and E. L. Hirst, *ibid.*, 1858 (1926).

(28) J. C. Irvine and Jocelyn Patterson, *ibid.*, **121**, 2696 (1922).

(29) W. N. Haworth, E. L. Hirst and A. Learner, *ibid.*, 1040 (1927).

All of the mother liquors, except the last, from the recrystallizations of the above material were combined, evaporated twice with absolute ethanol and treated for 1 week at 0° with 3 ml. of absolute ethanolic ammonia. The sirup obtained on solvent removal was extracted with several 5-ml. portions of petroleum ether (b.p. 30–60°). Long needles separated from the combined extracts. These were recrystallized from ether-petroleum ether; yield 6 mg., m.p. 99–100° (cor.) unchanged on admixture with an authentic specimen (m.p. 99–100°, recorded[10] 100–101°) of methyl 1-C-carbamyl-2,3,5-trimethyl-D-arabinofuranoside (V, *syn.* methyl furanoside of trimethyl-2-keto-D-gluconamide) prepared from trimethylinulin as described below. Both preparations gave identical X-ray powder diffraction diagrams, characterized[21] as follows: 7.31–100, 6.28–5, 5.64–5, 5.17–50, 4.73–50, 4.42–10, 3.97–80, 3.49–40, 3.24–40, 3.01–5, 2.83–5, 2.52–40, 2.39–5, 2.22–5, 2.07–10, 1.92–5, 1.83–5, 1.67–5, 1.58–5.

Preparation of Methyl 1-C-Carbamyl-2,3,5-trimethyl-D-arabinofuranoside (V).—Trimethylinulin[30] (44 g.) was oxidized with nitric acid as described above for diheterolevulosan II. At the end of the reaction, formic acid (87%) was added dropwise to the hot (85–90°) reaction mixture at such a rate that the vigorous evolution of nitrogen oxides and carbon dioxide was kept under control, a total of 270 ml. being added over a period of 3 hours. At the end of this time the gas evolution had stopped and the straw colored solution showed the presence of only traces of nitric acid by the ring test. Solvent was removed by concentration under reduced pressure and the resultant sirup was dried by repeatedly adding chloroform and removing by distillation under reduced pressure.

One-fourth of the sirupy product was methylated and converted to the amide as described above. Crystals (mainly dibasic acid amides) separated from the reaction mixture and, after 24 hours at 5°, were removed by filtration. The filtrate was again nearly saturated with ammonia and kept at 5° for 6 days. The sirup then obtained on solvent removal was partially crystallized from methanol-ether and further purified from hot petroleum ether (b.p. 30–60°); yield 1.6 g. of material of good purity. Pure methyl 1-C-carbamyl-2,3,5-trimethyl-D-arabinofuranoside (V) was obtained on further crystallization from cyclohexane; m.p. 99–100° (cor.). The X-ray powder diffraction characteristics of this substance are recorded above.

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COLUMBUS 10, OHIO RECEIVED JANUARY 12, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Action of Heat on D-Fructose. III.[1] Interconversion to D-Glucose

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Refluxing a concentrated (80%) aqueous solution of D-fructose for sixteen hours leads to the formation of 0.1% D-glucose and 0.6% 5-(hydroxymethyl)-2-furaldehyde. Column chromatographic methods were employed to separate these products. The former was characterized as a crystalline derivative (β-D-pyranose pentaacetate) and the latter by ultraviolet spectroscopy.

Employing column chromatographic methods[3,4] involving the isolation and characterization of crystalline substances, we have demonstrated[5] that the di-D-fructose dianhydrides designated diheterolevulosan I[5] and II[1] are formed on refluxing a concentrated (80%) aqueous solution of D-fructose. The work herein reported is concerned with the identification of two other substances formed in much lower amounts under the same conditions. The products generated in D-fructose solutions under thermal treatment are of interest in interpreting molasses formation occurring in cane sugar house processing.

It has long been known that aqueous solutions of D-fructose are thermally unstable and darken in color, become acid and decrease in rotation and reducing value on protracted heating.[6] The *p*H dependency of these changes was studied by Mathews and Jackson,[7] 3.3 being found to be the *p*H of maximum stability. Small amounts of steam-distillable three-carbon fragments are formed.[8] The ether from 5-(hydroxymethyl)-2-furaldehyde, 5,5′-(oxydimethylene)-difuraldehyde, has been isolated in 0.2% yield on autoclaving D-fructose at 160°.[9] Any 5-(hydroxymethyl)-2-furaldehyde present can decompose further to formic and levulinic acids.[10]

In the work herein reported, the solids from the heated D-fructose solution were chromatographed directly on clay with the results shown in A of Fig. 1. Herein the two top zones are di-D-fructose dianhydrides,[5] the large zone is D-fructose and the bottom zone is in the position for D-glucose (*cf.* B of Fig. 1). A separate chromatogram (C of Fig. 1) of the D-fructose employed demonstrated the absence of such a zone in the starting substance. The bottom zone material from a number (19) of such chromatograms was combined, acetylated with acetic anhydride and sodium acetate, and the resultant acetate was chromatographed on Magnesol–Celite. From this a small amount (*ca.* 0.1% of the initial D-fructose) of β-D-glucopyranose pentaacetate was isolated in crystalline form and adequately identified by melting point, mixed melting point and X-ray powder diffraction diagram. The combined effluent material from the heated D-fructose chromatograms was examined by ultraviolet spectroscopy and found to contain 5-

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