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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[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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(hydroxymethyl)-2-furaldehyde formed in a yield of *ca.* 0.6%.

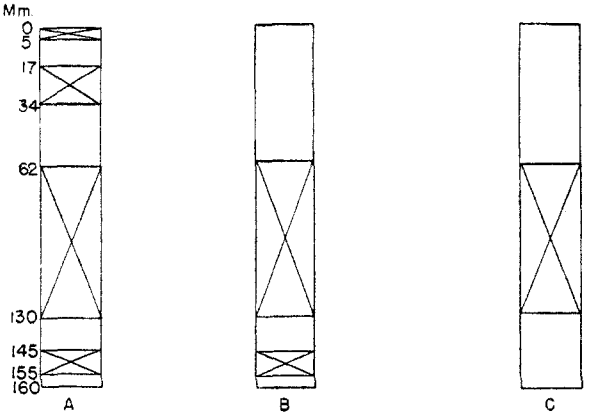

Fig. 1.—Parallel chromatograms on Florex XXX-Celite of heated D-fructose (A), D-fructose:D-glucose, 25:1 mixture (B), D-fructose before heating (C).

Enolization mechanisms account for the interconversion of D-fructose and D-glucose.[11] Such a prototropic change is catalyzed by both acids[12] and bases and thus the formation of some D-glucose in these slightly acid solutions is predictable. Some D-mannose would also be expected but its quantity should be much lower.[11] The isolation, as a crystalline derivative, of such a small amount of D-glucose from a large excess of partially decomposed D-fructose, illustrates again the value of column chromatographic techniques in sugar separations. Chromatographic innovations herein elaborated involve the employment of aluminum tubes to improve extrusion properties and the use of the Molisch reagent as a streak indicator.

Experimental

Preliminary Separation of D-Glucose from a Heated D-Fructose Aqueous Solution.—Pure D-fructose (80 g.) was dissolved in water (20 g.) with gentle warming; *p*H 6.9 at 25°, d_{29} 1.39 g./ml. This solution was refluxed (113°) for 16 hours. The cooled solution was very dark brown and had a strong burnt sugar odor; *p*H 2.7 at 25°. It was concentrated, to a water content of 2%, under reduced pressure at room temperature in a desiccator over calcium chloride and sodium hydroxide.

Five grams of the above product was dissolved in 500 ml. of 95% ethanol and 15 ml. of this solution was added at the top of a 160 x 27 (diam.) mm. column of Florex XXX[3]–Celite[3] (5:1 by wt.; 51 g.) held in aluminum tubes[13] 215 mm. long. The internal surface of these tubes was scoured thoroughly with pumice. In packing the tubes it was necessary to settle the adsorbent to prevent channeling by striking the tubes with a wooden stick although too close packing hindered extrusion. Development was effected with 190 ml. of 95% ethanol. The extruded column was air-dried overnight and streaked with alkaline permanganate (1% in 2.5 *N* NaOH) using a fine tipped pipet. Figure 1 shows the zones (A) so formed in comparison with those (B) from a known D-fructose:D-glucose (25:1 mixture) and with (C) from the original sample of D-fructose, all chromatographic conditions being identical. The two top zones were di-D-fructose dianhydrides,[5] the large central zone was unreacted D-fructose; the bottom zone was further investigated as described below. Nineteen such chromatograms were run in banks of 3 or less and their sectioned bottom zones were combined and eluted with 3 liters of 80% (by vol.) ethanol. Solvent removal under reduced pressure yielded a residue that was treated with decolorizing charcoal (Darco G-60) in water and then (after solvent removal under reduced pressure) in methanol; yield 125 mg. of final sirup.

Isolation of β-D-Glucopyranose Pentaacetate.—The above final product (125 mg.) was acetylated at 80° for 3 hours with 5 ml. of acetic anhydride and 0.1 g. of fused sodium acetate. The reaction mixture was poured onto 15 g. of ice and after several hours the pH was adjusted to *ca.* 6 with sodium bicarbonate (2 g.). The mixture was then extracted with 5 10-ml. portions of chloroform. Solvent removal under reduced pressure from the dried extract yielded a residue from which acetic acid was removed by several distillations with toluene effected in the same manner; yield 150 mg. This material was dissolved in 8 ml. of benzene and placed at the top of a 186 × 34 mm. (diam) column of Magnesol[4]-Celite (5:1 by wt.; 58 g.) and developed with 1200 ml. of benzene:ethanol = 250:1 (by vol.). The extruded column was streaked with the Molisch reagent contained in a double chambered pipet containing concentrated sulfuric acid in one chamber and a 5% solution in 95% ethanol of α-naphthol in the other. The tips of the pipet were of such a size that the rates of flow of the two solutions were about equal and the pipet was held so that the acid streak was applied first and the α-naphthol solution fell on top of it. Zones were indicated by intense purple bands. The column showed a main zone at 80–90 mm. from the top surmounted by several minor zones. Elution of this zone by acetone yielded a crude crystalline substance (9.5 mg. or 0.16% of original D-fructose, m.p. 112–115°, cor.), which was purified by sublimation at 90–105° and 0.03 mm.; yield 3 mg. or 0.05% of original D-fructose, m.p. 125–127° (cor.), m.p. 128–129° (cor.) on admixture with an authentic specimen (m.p. 130–131° (cor.)) of β-D-glucopyranose pentaacetate, X-ray powder diffraction diagram identical with that recorded[14] for this substance.

Identification of 5-(Hydroxymethyl)-2-furaldehyde.—The combined effluents from the above first chromatograms on clay were concentrated under reduced pressure and the residue was treated with decolorizing charcoal (Darco G-60) in water; yield of final sirup after solvent removal, 224 mg. This material gave positive Molisch, Selivanov and Benedict tests and showed the cherry red color with sulfuric acid and phloroglucinol that is characteristic of 5-(hydroxymethyl)-2-furaldehyde. A portion (10.5 mg.) of this residue was dissolved in water (100 ml.) and the ultraviolet absorption spectrum was measured in a 1-cm. cell (Beckman spectrophotometer, model DU). The molecular extinction coefficients found were 1,020 (max. 2,835 Å., major peak) and 375 (max. 2,280 Å., minor peak); the corresponding reported[15] values for 5-(hydroxymethyl)-2-furaldehyde are 16,500 (max. 2,850 Å.) and 3,620 (max. 2,280 Å.); yield 0.6% on basis of the original D-fructose.

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