[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Chromatographic Isolation of Cane Juice Constituents

BY W. W. BINKLEY[1] AND M. L. WOLFROM

The juice expressible from the stalk of the sugar cane has long been known to contain reducing sugars,[2] whose amount decreases to maturity and then again increases.[3] Louisiana cane at harvesting normally contains 1–1.5% reducing sugars. Analytical data and the concomitant presence of sucrose would indicate that these reducing sugars are probably D-glucose and D-fructose, but to our knowledge such sugars or their derivatives have never been isolated and adequately identified from this plant source. Without such an actual separation of a pure substance, or of a characteristic, crystalline and identifiable derivative, its presence in a mixture cannot be considered as demonstrated. Prinsen Geerligs[3] separated from Javan cane juice a lime salt which on decomposition with carbon dioxide yielded a reducing sirup of large levorotation. He thus presented presumptive but not definitive evidence for the presence of D-fructose.

In the work herein described, chromatographic methods[4,5] have been applied to the problem of the identification of the reducing sugars present in cane juice. Normal Louisiana cane juice solids were prepared by ice sublimation and acetylated. The acetate mixture was then chromatographed on a commercial hydrated magnesium acid silicate and D-glucose, D-fructose and sucrose were identified as their crystalline acetates. In the course of this work it was necessary to effect a chromatographic separation of the anomeric forms of D-glucopyranose pentaacetate. Control experiments indicated that the sucrose underwent no significant acetolysis under the acetylating conditions employed.

We believe that the general type of chromatographic procedure herein described is capable of wide extension to the sugar fraction of plant products, the separation and identification of whose components have hitherto been difficult if not impossible.

Experimental

Cane Juice.—The unclarified or raw sugar cane (*Saccharum officinarum* L., variety Coimbatore 290) juice was collected and expressed in Louisiana in November, 1945. It was quick-frozen, packed in solid carbon dioxide and shipped to Columbus, Ohio, where it was still frozen on arrival.

Anal. (% original juice) solids, 12.2; ash, 0.3; sucrose, 9.4; reducing sugars (as invert sugar), 1.1; *p*H, 6.0; *d*, 1.047 g. per ml. at 25°.

Acetylation of Cane Juice Solids.—The cane juice solids were prepared by desiccation through ice sublimation ("lyophilization"). An amount of 5.8 g. of cane juice solids was added slowly, with mechanical stirring, at −10° to an acetylating mixture of 30 cc. of acetic anhydride and 0.5 g. of freshly fused zinc chloride. The temperature of the reaction mixture was maintained at −15 to −10° for three hours and was then allowed to rise to 15° over a period of eighteen hours. The unreacted solids (3.2 g.) were removed by decantation and the acetylation mixture was poured slowly into 400 g. of ice and water. This solution was adjusted to a *p*H of 6.3 with sodium bicarbonate and was then extracted with chloroform. The extract was dried and the chloroform removed below room temperature. A viscous, golden sirup resulted; yield 4.0 g. A water–ethanol solution of the unreacted solids gave crystals which melted at 180–182° (unchanged on admixture with an authentic specimen of sucrose of like melting point), $[\alpha]^{25}D$ +66.9°, (*c* 2.7, water). The crystals are thus identified as sucrose.

First Chromatography of Acetylated Cane Juice Solids.—An amount of 2.0 g. of the above-described acetylated cane juice solids was dissolved in 25 cc. of benzene and the solution added at the top of a 235 × 52 mm. column[6] of a mixture (170 g.) of 5 parts (by weight) of "Magnesol"[7] and 1 part of "Celite."[8] The chromatogram was developed with 1250 cc. of 100/1 benzene[9]/ethanol[10] (volume ratio), the column extruded and streaked with a freshly prepared aqueous solution of 1% potassium permanganate in 2.5 *N* sodium hydroxide. Six distinct zones were formed and these were cut out (the indicator streak was removed with a scalpel) and eluted with acetone. Pertinent data relative to the material in these zones are listed in Table I.

TABLE I

FIRST CHROMATOGRAM OF ACETYLATED CANE JUICE SOLIDS

Zone	Approx. time, sec., for distinct zone formation in indicator streak	Zone position in mm. from top of column (232 mm. long)	Zone yield, g.	Nature of zone material
F	120	0–4	0.042	Amorphous solid
E	240	15–19	.053	Amorphous solid
D	45	34–38	.019	Partially cryst.[a]
C	75	49–53	.085	Cryst. β-D-fructose tetraacetate
B	60	90–139	1.468	Cryst. sucrose octaacetate
A	20	161–165	0.256	Cryst. α-D-glucopyranose pentaacetate; β-D-glucopyranose pentaacetate
			Total, 1.923 (96.2%)	

[a] Amount of crystalline material too small for identification.

(1) Sugar Research Foundation Research Associate of The Ohio State University Research Foundation.

(2) H. W. Wiley, *Bull. Chem. Soc. Washington*, **4**, 22 (1890); *Chem. Zentr.*, **61**, I, 354 (1890); H. C. Prinsen Geerligs, *Chem.-Zeit.*, **20**, 721 (1896).

(3) F. A. F. C. Went (and H. C. Prinsen Geerligs), *Jahrb. wiss. Botan.*, **31**, 289 (1898); H. W. Wiley, THIS JOURNAL, **25**, 855 (1903).

(4) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *ibid.*, **67**, 527 (1945).

(5) W. W. Binkley, Mary G. Blair and M. L. Wolfrom, *ibid.*, **67**, 1789 (1945).

(6) Column dimensions refer to the adsorbent.

(7) A synthetic, hydrated magnesium acid silicate manufactured by the Westvaco Chlorine Products Co., South Charleston, West Virginia. Only material passing a 200-mesh screen was employed.

(8) No. 535, a siliceous filter-aid manufactured by Johns-Manville Co., New York, N. Y.

(9) All benzene employed was free of thiophene.

(10) Absolute ethanol was employed in all chromatographic operations.

α-D-Glucopyranose Pentaacetate and β-D-Glucopyranose Pentaacetate from Zone A.—A solution of 80 mg. of the material from zone A in 2 cc. of benzene was placed on a 170 × 35 mm. column[6] of 5/1 Magnesol/Celite and developed with 650 cc. of 250/1 benzene/ethanol. Two distinct zones were obtained at a distance of 116–130 mm. and 136–153 mm. from the top of the adsorbent column. The two zones were cut out and eluted with acetone; yields 27 mg. (upper zone) and 51 mg. (lower zone).

The above experiment was repeated several times in order to obtain further material. After several recrystallizations from ethanol (95%) the material from the lower zone was identified as β-D-glucopyranose pentaacetate; m. p. 132° (mixed m. p. unchanged), $[\alpha]^{21}D$ +4° (*c* 3.9, chloroform); accepted values[11]: m. p. 132°, $[\alpha]^{20}D$ +4°. The ethanol (95%) recrystallized material from the upper zone was identified as α-D-glucopyranose pentaacetate; m. p. 112–113° (mixed m. p. unchanged), $[\alpha]^{28}D$ +101° (*c* 4.3, chloroform); accepted values[11]: m. p. 113°, $[\alpha]^{20}D$ +101.6°.

Sucrose Octaacetate from Zone B.—The material (1.47 g.) from zone B was recrystallized from 10 cc. of ethanol (95%); yield 1.26 g., m. p. 85° unchanged on admixture with an authentic specimen of sucrose octaacetate of like m. p., $[\alpha]^{28}D$ +60° (*c* 4.1, chloroform). These data identify the substance as the higher-melting dimorph of sucrose octaacetate for which the accepted constants are: m. p. 89°,[12] $[\alpha]^{20}D$ +60° (chloroform).[13]

β-D-Fructopyranose Tetraacetate from Zone C.—The material (0.085 g.) from zone C was recrystallized from 3 cc. of ethanol (95%); yield 27 mg. of prismatic crystals, m. p. 130° (mixed m. p. unchanged), $[\alpha]^{25}D$ −91.2° (*c* 3.9, chloroform). These data identify the substance as β-D-fructopyranose tetraacetate for which the accepted constants are: m. p. 131–132°, $[\alpha]^{20}D$ −91.6° (chloroform).

Control Experiment with Pure Sucrose.—The acetylation and chromatographic procedure described above for the cane juice solids was applied to a sample of pure sucrose[14] (3 g.) There was obtained 4 mg. of crystalline material in zone C (β-D-fructopyranose tetraacetate zone) and 5 mg. in zone A (D-glucopyranose pentaacetate zone).

Acknowledgment.—Acknowledgment is made to the laboratory assistance of Mr. John M. Kolbas and Miss Eloise L. Carpenter. The cane juice was kindly supplied by Dr. A. G. Keller of Louisiana State University, Baton Rouge. We are indebted to the counsel of Dr. R. C. Hockett, Scientific Director of the Sugar Research Foundation.

Summary

D-Glucose (as α-D-glucopyranose pentaacetate and β-D-glucopyranose pentaacetate), D-fructose (as β-D-fructopyranose tetraacetate) and sucrose (as sucrose octaacetate) have been isolated from sugar cane juice by chromatographic methods.

(11) C. S. Hudson and J. K. Dale, THIS JOURNAL, **37**, 1264 (1915).

(12) R. P. Linstead, A. Rutenberg, W. G. Dauben and W. L. Evans, *ibid.*, **62**, 3260 (1940); *cf.* M. Frèrejacque, *Compt. rend.*, **203**, 731 (1936).

(13) C. S. Hudson and J. M. Johnson, THIS JOURNAL, **37**, 2748 (1915).

(14) The sucrose was obtained from the Coleman and Bell Co., Norwood, Ohio; its maximum invert sugar content was stated by the manufacturers to be 0.235%.

COLUMBUS, OHIO RECEIVED MARCH 13, 1946

[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

The Stability of the Methoxyl Groups in Methylated Hydrochloric Acid Spruce Lignin[1]

BY F. E. BRAUNS

In an earlier investigation on the decomposition of fully methylated spruce wood,[1a] it was found that lignin derivatives were obtained with a considerably lower methoxyl content than was expected. This might be caused by any one of the following possibilities: (1) the lignin was not completely methylated; (2) the lignin is chemically combined with another component of the wood through an oxygen linkage, thus covering the hydroxyl group and preventing it from being methylated; or (3) methoxyl groups are split off from the methylated lignin during its isolation. To study this question, the behavior of fully methylated hydrochloric acid lignin toward a number of reagents used for the isolation of lignin was investigated.

The methylated spruce lignin used was obtained by methylation of hydrochloric acid spruce lignin which was prepared according to Kalb and Lieser[2] and was kept in a moist condition. After five methylations, a methoxyl content of 32.26% was obtained, corresponding to 9.8 methoxyl groups per lignin building unit (Table I).

To obtain lignin derivatives soluble in organic solvents for further purification, the methylated hydrochloric acid lignin was treated with reagents under conditions previously used with unmethylated hydrochloric acid lignin and also in the isolation of lignin from wood.

Extracted spruce wood and hydrochloric acid spruce lignin, when treated with acetic acid in the presence of a small amount of magnesium chloride, give acetic acid spruce lignin.[3] This lignin still contains all of its original methoxyl groups, but two of its hydroxyl groups are acetylated. Because the acetic acid hydrochloric acid lignin is soluble in dilute sodium hydroxide, the phenolic hydroxyl group must still be free. Upon methylation with diazomethane, the acetic acid hydrochloric acid lignin becomes insoluble in dilute

(1) Presented before the Division of Cellulose Chemistry at the 109th meeting of the American Chemical Society, Atlantic City, New Jersey, April 8 to 12, 1946.

(1a) F. E. Brauns and J. J. Virak, in press.

(2) L. Kalb and T. Lieser, *Ber.*, **61**, 1007 (1928).

(3) F. E. Brauns and M. A. Buchanan, THIS JOURNAL, **67**, 645 (1945).