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

Chromatographic Fractionation of Cane Blackstrap Molasses and of Its Fermentation Residue[1]

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In continuation of our studies on the composition of cane blackstrap or final molasses, we have chromatographically fractionated a Cuban sample on clay, employing a flowing type of chromatogram. To this end a preliminary rough separation was made by first adsorbing the material on a layer of clay and then developing this successively with 95% ethanol, 80% ethanol, 50% ethanol, and water to yield in the effluents the fractions designated S, B, C and D, respectively. The total return of molasses solids was nearly quantitative. Fraction S was processed to remove the main sucrose fraction by direct crystallization[3] and the fat, odor and pigment fraction by petroleum ether extraction,[4] as previously described, to leave the residual Fraction A. The use of clay as a chromatographic adsorbent for carbohydrate materials follows its establishment for this purpose by Lew, Wolfrom and Goepp.[5] Fractions A and B were then subjected to an exhaustive chromatographic fractionation on clay–Celite, employing a relatively large column, 100 × 7.4 cm. (diam.), containing 2 kg. of adsorbent.[6] Since clay is very selective but has a low capacity, a ratio of 1 part of substance to 400 parts of adsorbent was employed. The column was operated under a vacuum of 100 mm. and the rate of flow was very slow, continuous operation over a period of months being required. This undesirable feature could probably be alleviated by employing a high pressure column. Development was effected successively with 95 and 90% ethanol. Figure 1 is a diagram of the results obtained with this flowing type of chromatography. D-Glucose and sucrose were crystallized directly at the points shown. This represents the first direct isolation of crystalline D-glucose from cane blackstrap molasses.

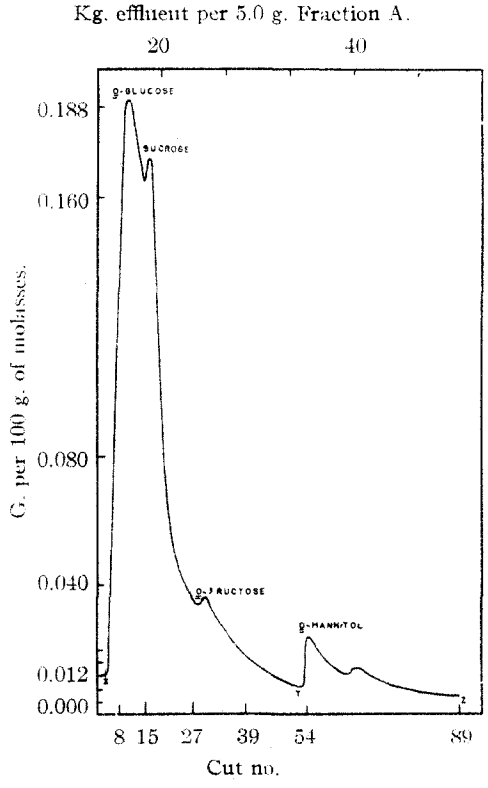

Fig. 1.—Chromatography on clay of Fraction A from Cuban blackstrap molasses: development with 95 ethanol/5 water, X to Y; with 90 ethanol/10 water, Y to Z. Each cut number represents a point on curve as determined by weighing the solvent-free residue; total material recovery, *ca.* 90%.

The fractions or cuts that did not crystallize and the residual material from the cuts containing the D-glucose and sucrose, were then acetylated with zinc chloride and acetic anhydride under relatively mild conditions. This method of acetylation was adopted since it was known to yield crystalline derivatives of D-fructose.[7–9] Previously a more vigorous acetylation procedure, hot acetic anhydride and sodium acetate, had been applied to cane blackstrap molasses fermentation residue[10] and D-glucose, D-mannitol and *meso*-inositol[11] were separated as their acetates by chromatographic procedures employing Magnesol–Celite as adsorbent.[12] The presently employed acetylation procedure is more conducive to quantitative treatment. Acetylation by this method, followed by chromatography,

(1) Reported in part in *Abstracts Papers Am. Chem. Soc.*, **112**, 13Q (1947).

(2) Sugar Research Foundation Research Associate of The Ohio State University Research Foundation (Project 190).

(3) W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **69**, 664 (1947).

(4) W. W. Binkley and M. L. Wolfrom, *ibid.*, **70**, 290 (1948).

(5) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *ibid.*, **67**, 1865 (1945); **68**, 1449 (1946).

(6) For a photograph of this column see W. W. Binkley and M. L. Wolfrom, No. 10, Scientific Report Series, Sugar Research Foundation, Inc., New York, Fig. 2 (1948).

(7) D. H. Brauns, *Verslag. K. Akad. v. Wetensch., Amsterdam*, 577 (1908).

(8) M. L. Wolfrom and A. Thompson, THIS JOURNAL, **56**, 880 (1934).

(9) W. W. Binkley and M. L. Wolfrom, *ibid.*, **68**, 1720 (1946).

(10) W. W. Binkley, M. Grace Blair and M. L. Wolfrom, *ibid.*, **67**, 1789 (1945).

(11) The common, naturally occurring *meso* form of m. p. 225°.

(12) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, THIS JOURNAL, **67**, 527 (1945).

yielded with pure sucrose a nearly quantitative return of sucrose octaacetate. With D-glucose there was obtained a nearly quantitative return of approximately equal amounts of the chromatographically separable pyranoid anomers. D-Fructose yielded under these conditions D-fructopyranose tetraacetate, readily separable on Magnesol–Celite, and a mixture of β-D-fructopyranose pentaacetate and *keto*-D-fructose pentaacetate. Unfortunately, these two derivatives were found in the same zone and only that form crystallized which was present in excess. The total return as acetate derivative was then about 50% of theory for D-fructose. These results reflect again the highly tautomeric nature of this ketose.[13] Application of these techniques to the residual cuts of Fraction A led to the isolation, as acetates, of further quantities of sucrose and D-glucose, and of D-mannitol and D-fructose. This represents the first isolation of D-fructose, as a crystalline derivative, from cane blackstrap molasses.

Fraction B was investigated in the same manner (Fig. 2) and led to the isolation of further quantities of sucrose and *meso*-inositol (both acetylated and unacetylated forms). This is the first record of the direct isolation of crystalline *meso*-inositol from this source.

The nature of the other maxima present in Figs. 1 and 2 was not resolved by the isolation of crystalline products. They nevertheless represent mixtures of closely related substances or distinct components thus chromatographically

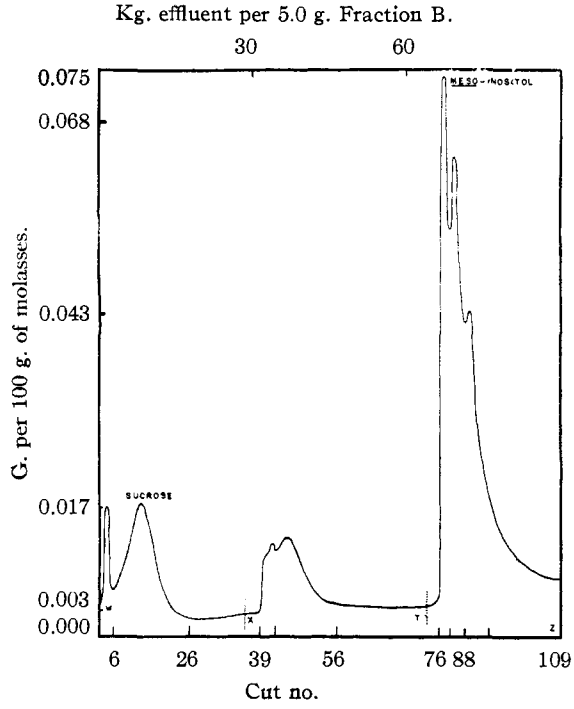

Fig. 2.—Chromatography on clay of Fraction B from Cuban blackstrap molasses: development with 95 ethanol/10 water, W to X; with 90 ethanol/10 water, X to Y; with 70 ethanol/30 water, Y to Z. Each cut number represents a point on curve as determined by weighing the solvent-free residue; total material recovery, *ca.* 90%.

TABLE I

CONSTITUENTS OF CUBAN BLACKSTRAP MOLASSES[a] AS DETERMINED BY ANALYTICAL AND CHROMATOGRAPHIC PROCEDURES

Constituent	Analytical, %[b]	Chromatographic, %
Sucrose	34.8[c] 35.9[d]	30.5[e]
Apparent D-glucose by copper reduction	17.6[f] 18.9[g]	
D-Glucose		6.0[h]
D-Fructose		1.4[i]
Total		7.4
Solids	86.5	
Ash	9.2[j]	

[a] From Cunagua Central of the American Sugar Refining Co., New York, N. Y.; 1947 production. [b] Basis whole molasses; d. 1.337 (g./ml. at 25°). [c] Modified Clerget; polarimetric assay before and after inversion. [d] Copper reduction before and after inversion. [e] Total of 3 lots as sucrose and 4 lots as sucrose octaacetate; all calcd. as sucrose. [f] Lane-Eynon method after successive treatment with basic lead acetate and potassium oxalate, as standardized against pure D-glucose. [g] Lane-Eynon method after treatment with potassium oxalate alone, as standardized against pure D-glucose. [h] Total of 1 lot as D-glucose, 4 lots as α-D-glucopyranose pentaacetate and 4 lots as β-D-glucopyranose pentaacetate; all calcd. as D-glucose. [i] Total of 4 lots as β-D-fructopyranose tetraacetate, 1 lot as β-D-fructopyranose pentaacetate and 2 lots as *keto*-D-fructose pentaacetate; all calcd. as D-fructose. [j] Sulfate ash.

(13) See P. Brigl and R. Schinle, *Ber.*, **66**, 325 (1933); **67**, 127 (1934).

segregated. Their nature is under further investigation. It is of interest to note the concordance between the analytical data obtained by customary methods on this molasses (Table I) and the collected chromatographic yields. Thus the chromatographic yield of sucrose was 30.5% of the whole molasses as compared with the values of 35 and 36% found by two analytical procedures. The amount of D-glucose chromatographically determined (6%) is far above that found for D-fructose (1.4%). Even allowing for the unquantitative nature of the latter assay, which is not liable to be in error more than 50%, and correcting to 3% D-fructose, this quantity is still only one-half of the D-glucose present. It is believed that this is essentially correct and represents the greater reactivity of this ketone. The total corrected figure of 9% is again only about one-half of that required by the copper reducing value, expressed as D-glucose. Thus, while the presence of both D-glucose and D-fructose is demonstrated, there are other reducing substances present in cane molasses. This is further shown by the data of Table II. According to the chromatographic scheme of Lew, Wolfrom and Goepp, fraction C should contain no simple sugars, yet it showed a significant reducing value. The summation of reducing values of these fractions, 17.4%, agreed well

with that found, 18.9% (same method, Table II), on the whole molasses. All of these values were enhanced by acid hydrolysis, indicating the presence of condensation or hydrolyzable polymers. Erb and Zerban[14] have arrived at somewhat similar conclusions in their investigations of the reducing value of cane molasses fermentation residue before and after hydrolysis.

The non-fermented fraction from a single yeast fermentation of a similar sample of Cuban final molasses was then subjected to the same chromatographic fractionation in the hope that the greater concentration of non-sugars might facilitate their investigation. The corresponding crude chromatographic fraction weight distribution is shown in Table II; the total material return was 90%, probably reflecting the presence of material not removed from the column. The reducing values of these fractions are recorded in the same table. Fraction C′ has an unusually high value which cannot represent simple sugars.

Only Fraction A′ of the non-fermented residue was further investigated chromatographically and this with the results shown in Fig. 3. Glycerol and some residual D-glucose, D-fructose and sucrose were isolated directly or as acetates. The presence of D-mannitol was established and in addition erythritol and D-arabitol were isolated

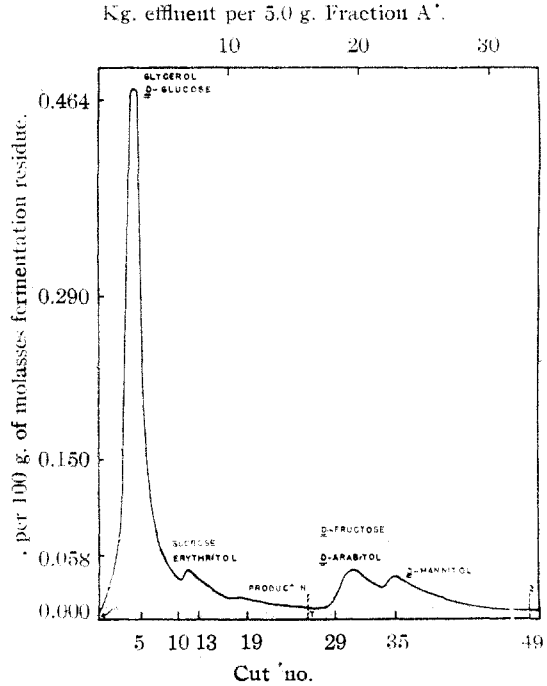

Fig. 3.—Chromatography on clay of Fraction A′ from the yeast non-fermented residue of Cuban blackstrap molasses: development with 95 ethanol/5 water, X to Y; with 90 ethanol/10 water, Y to Z. Each cut number represents a point on curve as determined by weighing the solvent-free residue; total material recovery, *ca.* 90%.

(14) C. Erb and F. W. Zerban, *Ind. Eng. Chem.*, **39**, 1537 (1947).

TABLE II

COPPER REDUCTION VALUES ON CHROMATOGRAPHICALLY FRACTIONATED CUBAN CANE BLACKSTRAP MOLASSES (BEFORE AND AFTER ACID HYDROLYSIS) AND ON SIMILAR FRACTIONS FROM CANE MOLASSES FERMENTATION RESIDUE

Fraction[a]	Wt. of fraction, as % molasses solids or % fermentation residue	Apparent D-glucose[b] Before hydrolysis, % of fraction	Before hydrolysis, %, basis whole molasses or fermentation residue	After hydrolysis[c] %, of fraction
A	39.9	45.7	15.8	75.0
B	9.1	12.2	1.1	36.2
C	11.3	4.7	0.5	6.4
D	9.3	0.0	0.0	2.9
		Total	17.4[d]	
A′	28.7	12.4	3.6	
B′	10.9	18.4	2.0	
C′	28.8	50.6	14.6	
D′	21.4	27.6	5.9	
		Total	26.1	

[a] See experimental portion for nature of these fractions from cane blackstrap molasses (A, B, C, D) and from the molasses fermentation residue (A′, B′, C′, D′). [b] All values by Lane-Eynon method after treatment with potassium oxalate and a small amount of filter-aid (Celite); as standardized against pure D-glucose. [c] Boiled two minutes with 0.25 *N* hydrochloric acid with molasses fraction concentration 0.8 g./100 ml. soln. [d] Compares with the analytical value 18.9 (same method) recorded in Table I.

as their crystalline acetates; it is probable that these two latter substances originated in the yeast. One as yet unidentified compound, designated Product N, was isolated as a crystalline acetate in low yield. This substance contained nitrogen and again it is possible that it arose from the yeast.

Further investigations on the nature of the non-crystalline fractions are underway in this Laboratory.

Experimental

Crude Fractionation on Clay of Cane Blackstrap Molasses.—The Cuban molasses employed is analytically characterized in Table I. An amount of 100 g. of this molasses was diluted with 50 ml. of distilled water. A smooth paste was prepared by the addition of 50 g. of a mixture of clay[15]/Celite[16] (5:1[17]). This paste was suspended in 2000 ml. of absolute ethanol with good agitation. The resulting suspension was poured onto a 11-12 × 7-9 (diam.) column[18] of clay/Celite (5:1), prewet with 1 liter of 95% ethanol (the azeotrope), in a 2-liter pharmaceutical percolator arranged to permit the collection of the effluent without interfering with the column operation. The chromatogram was developed successively with 5 liters each of 95 ethanol/5 water,[19] 80 ethanol/20 water, 50 ethanol/50 water, and water. The residue from the 95 ethanol/5 water column effluent (Fraction S) was treated to remove 70% (24.4 g.) of the available sucrose by crystallization[3] followed by removal of the hydrocarbon-soluble

(15) Florex XXX, a fuller's earth clay produced by the Floridin Co., Warren, Pa.

(16) No. 535, manufactured by the Johns-Manville Co., New York, N. Y.

(17) All adsorbent ratios are by weight.

(18) Column dimensions refer to the absorbent.

(19) All solvent ratios are by volume (before mixing).

fraction,[4] *ca.* 0.4 g., to leave a palatable, dark amber sirup; yield 34.5 g. (Fraction A). A brown, tacky residue with a bitter taste and the odor of raw cane juice was obtained from the 80 ethanol/20 water effluent after solvent removal; yield 7.9 g. (Fraction B). The 50 ethanol/50 water and water fractions were dark brown, amorphous solids with a bitter taste; yields, respectively, 9.8 g. (Fraction C) and 8.0 g. (Fraction D); total return 85.0, analytically determined solids 86.5. Fractions C and D are presently under further investigation.

Chromatography on Clay of Fraction A.—An amount of 4.9 g. of Fraction A, dissolved in 95 g. of absolute methanol, was added at the top of a 100 × 7.4 cm. (diam.) column[6] of 2 kg. of clay/Celite (5:1), prewet with 3 liters of 95% ethanol. The chromatogram was developed with 30 kg. of 95% ethanol and 25 kg. of 90 ethanol/10 water (excluding losses due to evaporation). A plot of this flowing chromatogram is shown in Fig. 1. The column was operated continuously at 100 mm. for ninety-seven days to obtain this result.

Isolation of D-Glucose from Cuts 8-14[20] of Fraction A.—Crystals of D-glucose were obtained from a methanolic solution of the individual residual sirups from cuts 8-14 and from that of their combined mother liquors; total yield 421 mg., m. p. 145-150°, $[\alpha]^{23}D$ +52.8° (*c* 3, equil., water); accepted for D-glucose: m. p. 146°, $[\alpha]^{20}D$ +52.7° (*c* 4, equil., water). An additional 457 mg. of less pure crystals was obtained on the addition of ethanol to the mother liquor. An amount of 100 mg. of these was acetylated with acetic anhydride and sodium acetate to give β-D-glucopyranose pentaacetate; yield 160 mg. (from 95% ethanol), m. p. 130-131° (mixed m. p. unchanged).

Isolation of Additional Sucrose from Cuts 15-26 of Fraction A.—Crystalline sucrose formed from the methanol-ethanol solution of the residual sirups from each of the cuts 15-26 and from their combined mother liquors; total yield 715 mg., m. p. 180-185° (dec.), $[\alpha]^{25}D$ +66.7° (*c* 4.9, water), octaacetate: m. p. 84-85° (from 95% ethanol), mixed melting point unchanged.

Acetate Chromatography of Residual Material from Fraction A after Separation of D-Glucose and Sucrose by Crystallization; Identification of D-Fructose.—The mother liquors from the above-described crystallizations of D-glucose and sucrose from Fraction A and the cuts which did not crystallize were combined (inclusively) as follows: 8-14 (Fig. 1), 15-26, 27-38, 39-53, 54-89 and each lot was acetylated and chromatographed as described below.

An amount of 1 g. (or less) of the residues from the grouped cuts was allowed to react with 10 ml. of acetic anhydride and 0.3 g. of freshly fused zinc chloride, employing good agitation, at the temperature of a salt-ice mixture for sixteen to twenty hours. If the reactants were not dissolved completely, they were heated until complete solution was obtained as follows: one to three hours at 25° and, if necessary, one to two hours at 50°. The cooled reaction mixture was poured into a mixture of 25 g. each of ice and water, employing good agitation which was continued for thirty minutes. The resulting solution was adjusted to *p*H 6 with sodium bicarbonate and was extracted with chloroform. The extracts were dried over anhydrous sodium sulfate and the residual material was obtained on solvent removal.

These acetate mixtures were then chromatographed by the following general procedure. An amount of 1.0 g. or less of the acetylated product was dissolved in benzene (4-5% solution). This solution was added at the top of a 175 × 45 mm. (diam.) column of a mixture (100 g.) of Magnesol[21]/Celite (5:1). The chromatogram was developed with 800 ml. of 100 benzene[22]/1 ethanol.[23] The column was extruded and streaked with a freshly prepared solution of 1% potassium permanganate in 2.5 *N* sodium hydroxide. The zones were located, isolated (the indicator streak was removed with a scalpel) and eluted with acetone. β-D-Fructopyranose tetraacetate, when present, was found in a zone about one-third from the column top and sucrose octaacetate in a zone at about the middle.

The loosely held substances (lower third of the column) were rechromatographed on 175 × 35 mm. (diam.) column of a mixture (60 g.) of Magnesol/Celite (5:1). These chromatograms were developed with 650 ml. of 250 benzene/1 ethanol. The adsorbed substances were recovered in the same manner. From this rechromatography were obtained the anomeric forms of D-glucopyranose pentaacetate in discrete zones. Another zone contained D-fructose pentaacetate in either the β-D-pyranose or *keto* forms, only one of which would crystallize.

A typical series of chromatograms is described as follows. An amount of 800 mg. of acetate from combined cuts 27-38 yielded 67 mg. of crystals from a zone 52-66 mm. from the top of the 175 mm. column; this material was identified as β-D-fructopyranose tetraacetate (m. p. 131-132°, unchanged on admixture with an authentic specimen). A zone 95-107 mm. from the top yielded 107 mg. of crystals identified as sucrose octaacetate (m. p. 85.0-85.5°, unchanged on admixture with an authentic specimen). The combined lower zones (340 mg.) were rechromatographed to yield the following zone material: *keto*-D-fructose pentaacetate at 56-77 mm. from the top, yield 69 g., m. p. 62-63° unchanged on admixture with an authentic specimen; α-D-glucopyranose pentaacetate at 125-134 mm., yield 7 mg.; β-D-glucopyranose pentaacetate at 142-148 mm., yield 10 mg.

Results of the chromatography of cuts 8-53 are summarized in Table I.

Control Experiments with Pure Sugars.—Control experiments with the pure sugars showed that on acetylation and chromatography as described above, sucrose yielded its octaacetate as isolable crystals in good purity in yields above 90%; anhydrous D-glucose produced its anomeric pyranose pentaacetates in about equal amount in yields above 90%. D-Fructose gave two main zones, one in the upper third containing the β-D-pyranose tetraacetate and the other in the lower third containing *keto*-D-fructose pentaacetate and β-D-fructopyranose pentaacetate from which that component crystallized which was in excess, the combined yield of crystalline product being *ca.* 50%.

D-Mannitol.—An amount of 656 mg. of acetate from combined cuts 54-89 was chromatographed as described above and D-mannitol hexaacetate was found in a zone 137-149 mm. from the column top in the second chromatogram; yield 25 mg., m. p. 122.5-123.0° (after recrystallization from 95% ethanol) unchanged on admixture with an authentic specimen.

Chromatography of Fraction B.—An amount of 4.8 g. of Fraction B was chromatographed on clay in the apparatus and by the procedure described above for Fraction A. The cuts obtained are shown in Fig. 2. The column was operated continuously at 100 mm. for 194 days to obtain this result. The first maximum in this curve represents sirupy material presently under further investigation. Sucrose was crystallized from the cuts 6-25 as described previously and identified in the same manner; yield 426 mg. An aqueous solution of the combined residues from cuts 76-80 yielded crystals identified as *meso*-inositol; yield 15 mg., m. p. 224-225° (cor.) (recrystallized from 95% ethanol) unchanged on admixture with an authentic specimen of *meso*-inositol, Molisch test (−), Scherer[24] inositol test (+).

The residual sirups were then acetylated and chromatographed as described above for Fraction A. A further amount of sucrose was isolated as the octaacetate from cuts 6-25 (Table I). In the first chromatogram of the acetylated material from combined cuts 76-80, *meso*-inositol hexaacetate was crystallized in a zone 144-158 mm. from the column top; yield 65 mg., m. p. 216° (cor.) (mixed unchanged); Molisch test (−); Scherer[24] test (+).

(20) Inclusive.

(21) A synthetic, hydrated magnesium acid silicate manufactured by the Westvaco Chlorine Products Corp., South Charleston, West Virginia. Only material passing an 80-mesh screen was employed.

(22) All benzene employed was free of thiophene.

(23) Absolute ethanol was employed in the chromatography of acetates.

(24) J. Scherer, *Ann.*, **81**, 375 (1852).

Chromatography of the Non-fermented Fraction from the Yeast Fermentation of Cane Blackstrap Molasses.—The non-fermented residue from Cuban molasses employed in this work was that previously reported.[10] An amount of 100 g. of this material was fractionated crudely on clay as described above for whole molasses and the fractions were isolated in the same manner; Fraction A′ (corresponding to Fraction S), 28.7 g., was a viscous, amber sirup; Fraction B′, 10.9 g., was a reddish-brown, amorphous solid; Fraction C′, 28.8 g., and Fraction D′, 21.4 g. (total return, 89.8%), were air-dried on glass plates to form glossy, nearly black, brittle films. Only Fraction A′ was further chromatographed on clay as described above for Fraction A of whole molasses. Operating time was twenty-four days. Cuts 10-12 yielded crystals identified as sucrose; yield 12 mg., m. p. 180-182° (after recrystallization from 95% ethanol), mixed unchanged, Molisch (+), Benedict (−) but (+) after acid hydrolysis. Cuts 35-49 showed crystals which were combined and identified as D-mannitol; yield 75 mg. (from methanol), m. p. 166.5° (after recrystallization from 90% methanol), mixed unchanged, $[\alpha]^{22}D$ +30° (*c* 1.2, satd. borax).

Anal. Calcd. for $C_6H_{14}O_6$: C, 39.56; H, 7.74. Found: C, 39.60; H, 7.89.

The cuts that did not crystallize and the residual material from cuts 36 to 43 were combined between the cut numbers shown in Fig. 3, as 1-4, 5-9, etc. The combined lots were acetylated as described above for the corresponding fraction of whole molasses and the acetate mixtures were chromatographed and rechromatographed (except cuts 1-4 and 4-11) as described above. Glycerol triacetate was found in the bottom zone of cuts 1-4 and 4-11 and was separated by distillation at 50-80° at 0.03 mm.; yield 850 mg. from 1.000 g. of chromatographed material, b. p. 259-261°$_{760}$ (cor.), d^{20}_4 1.161, $n^{20}D$ 1.433. β-D-Glucose pentaacetate was identified in the residue from the above distillation; yield 7 mg. (from 95% ethanol), m. p. 125-128°, m. p. 130-131° on recrystallization from 95% ethanol, mixed unchanged.

Combined cuts 13-18 yielded on acetylation and rechromatography a zone near the middle of the column containing sucrose octaacetate; yield 73 mg. from 580 mg. of combined cuts 13-18, m. p. 87° (from 95% ethanol), mixed unchanged, $[\alpha]^{22}D$ +59° (*c* 1, chloroform). In a zone from the acetylated material of cuts 13-18 below this was obtained a crystalline product identified as erythritol tetraacetate; yield 7 mg. (from ethanol), m. p. 83-84°, mixed unchanged (accepted value 85°), Molisch (−), Benedict (−).

Anal. Calcd. for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25; mol. wt., 290.3. Found: C, 49.35; H, 6.40; mol. wt. (Rast), 292.

A zone near the middle of the column from the first chromatogram of the acetylated material of cuts 19-28 yielded crystals of a nitrogenous substance, as yet unidentified and designated Product N; yield 54 mg. (from 95% ethanol), m. p. 70-72° with resolidification and remelting at 103-104°, $[\alpha]^{19}D$ +183° (*c* 0.9, chloroform), colorless elongated prisms, Molisch (−), Benedict (−) before and after acid hydrolysis. The substance was not soluble in water but was soluble in benzene, ether, ethanol, chloroform and warm 0.2 *N* sodium hydroxide (without coloration).

Anal. Found: C, 49.45; H, 5.83; N, 7.76; mol. wt. (Rast), 700, 680; P, absent; S, absent.

The material in the lower zones of the above chromatogram was rechromatographed as before and from a zone near the middle of the column was obtained a further quantity of erythritol tetraacetate (from a zone below this was obtained a small amount of glycerol triacetate); yield 9 mg. (from 95% ethanol), m. p. 79-80°, m. p. 82-83° on admixture with pure material of m. p. 84-85°.

Anal. Calcd. for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25; mol. wt., 290.3. Found: C, 49.94; H, 6.25; mol. wt. (Rast), 346.

Crystals of β-D-fructopyranose tetraacetate appeared in a zone located one-third from the column top of the first chromatogram of the acetylated material from the combined cuts 29-34; yield 12 mg. (from ether), m. p. 132° (after recrystallization from ether), mixed unchanged, Molisch (+), Benedict (+), Seliwanoff (+).

The material in the lower zone of the above chromatogram was rechromatographed as specified above and crystals of D-arabitol pentaacetate appeared in a zone near the middle of the column (from a zone below this was obtained a further small amount of glycerol triacetate); yield 9 mg. (from 95% ethanol), m. p. 69-70°. Pure material was obtained on further crystallization from the same solvent; m. p. 73-74°, mixed unchanged (accepted value 74-75°), Molisch (−), Benedict (−).

Anal. Calcd. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12; mol. wt., 362.3. Found: C, 49.81; H, 6.20; mol. wt. (Rast), 403.

The combined material from cuts 35-49, after the crystallization of D-mannitol, was acetylated and a crystalline product was obtained directly; yield 107 mg. (from 95% ethanol), m. p. 117-121°. An amount of 50 mg. of these crystals was rechromatographed on 50 g. of 5 Magnesol/1 Celite using 350 ml. of 500 benzene/1 ethanol as developer. The material in the principal zone was crystalline and was identified as D-mannitol hexaacetate; yield 37 mg. (from 95% ethanol), m. p. 120-121° (after two recrystallizations from 95% ethanol), mixed unchanged.

The residual acetylated material (661 mg.), after removal of the crystalline product described above, was chromatographed by the previously designated procedure. A further quantity of D-mannitol hexaacetate appeared in the bottom zone of the first chromatogram; yield 40 mg. (from 95% ethanol), m. p. 119-120°, mixed unchanged. Three other distinct zones on this chromatogram yielded sirupy products which were not identified.

Acknowledgment.—We are pleased to acknowledge the laboratory assistance of Miss Eloise Carpenter and Messrs. John M. Kolbas and Rees B. Davis. The Cuban molasses was kindly furnished by the American Sugar Refining Co., New York, N. Y.

Summary

1. Cuban blackstrap molasses was chromatographically fractionated on clay to yield four crude fractions which were characterized by copper reduction value (before and after hydrolysis). Two of these fractions were rechromatographed on clay to yield the flowing chromatograms shown in Figs. 1 and 2. The nature of these fractions was further investigated by acetate chromatography on Magnesol. Sucrose, D-glucose and *meso*-inositol were so isolated in crystalline form (and in the form of crystalline acetates) and D-fructose and D-mannitol were isolated as crystalline acetates.

2. The chromatographic assays demonstrate that D-glucose is present in molasses in excess over D-fructose and that other substances than these hexoses are present in the reducing fraction.

3. Similar procedures applied to a molasses fermentation residue (Fig. 3) led to the isolation of crystalline sucrose and D-mannitol (and of their crystalline acetates) and of D-glucose, D-fructose, D-erythritol, D-arabitol, and an unidentified substance, as crystalline acetate derivatives.

4. The reducing values were determined for chromatographically separated fractions of the molasses fermentation residue.

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