Hydroxylation of Elaidic Acid with Hydrogen Peroxide–Acetic Acid–Sulfuric Acid.—A well-stirred solution consisting of 270 g. (0.898 mole) of 94% elaidic acid, 810 ml. of glacial acetic acid, and 20 g. of concentrated sulfuric acid was heated to 40° and oxidized at that temperature with 122.8 g. of 25.5% hydrogen peroxide added dropwise over a period of fifteen minutes. The reaction was only slightly exothermic. A granular precipitate began to form after about thirty minutes and continued to increase in bulk as the oxidation proceeded. The total reaction time was five hours. The reaction mixture was poured into hot water (95 to 100°) and stirred for several minutes at that temperature. A semi-solid, white upper layer was obtained. The mixture was cooled to room temperature; the aqueous layer, which contained a small quantity of glistening needles, was filtered by suction, and the filtrate was discarded. The main bulk of the product was remelted with water and stirred for a few minutes to remove additional acetic acid and the lower aqueous layer was discarded. No precipitate was deposited from the second aqueous layer. The product, a hard white solid, was air-dried. It weighed about 300 g. and consisted of 40% hydroxy-acetoxystearic acids, the remainder being mainly dihydroxystearic acid (iodine number, 6 to 7; acid number, 170; saponification number, 224). The product was heated at 100° for one hour with an excess of 2 *N* aqueous sodium hydroxide, and the soap solution was poured into excess hydrochloric acid with stirring. The granular precipitate which formed was filtered by suction. It weighed 280 g., and consisted of somewhat impure 9,10-dihydroxystearic acid, m. p., 122–125° (lit. 130°). Crystallization from 95% ethyl alcohol (7 ml./g.) at 0 to 5° yielded 220 g. of pure 9,10-dihydroxystearic acid as glistening plates, m. p., 130–131°. The yield was 78%.

Hydroxylation of Red Oil (Commercial Oleic Acid) with Hydrogen Peroxide–Acetic Acid–Sulfuric Acid.—As described under the hydroxylation of red oil with hydrogen peroxide and formic acid, 100 g. (0.369 mole of double bond) of commercial oleic acid (iodine number, 93.7; oleic acid, 66.8; linoleic acid, 18.6; saturated acids, 14.6%) was dissolved at 25° in 300 ml. of glacial acetic acid containing 7.9 g. of concentrated sulfuric acid, and 51.2 g. of 25.1% hydrogen peroxide was added dropwise over a five-minute period. The temperature was maintained at 40° throughout by cooling or warming as required. The total reaction time was six hours. The yield of 9,10-dihydroxystearic acid, after saponification, acidification, and crystallization, was slightly lower than the yield obtained when hydrogen peroxide and formic acid were employed to oxidize red oil.

Summary

1. A new and rapid general reaction for the quantitative hydroxylation of long-chain, monounsaturated, aliphatic compounds has been described. The oxidizing agent, performic acid, is not isolated but is prepared and utilized *in situ*. This is accomplished by dissolving the unsaturated compound in formic acid and adding hydrogen peroxide. Because of the rapidity of the reaction and the mild conditions, only one mole of hydrogen peroxide is required for each mole of monounsaturated compound.

2. This reaction has been applied to pure oleic, elaidic, and hendecenoic (undecylenic) acids, oleyl alcohol, and methyl ricinoleate to give excellent yields of the corresponding hydroxy derivatives.

3. In addition, it has been shown that substantially identical results are obtained when acetic acid containing catalytic quantities of sulfuric acid is substituted for formic acid in the mixture with hydrogen peroxide. The oxidizing agent in this case is peracetic acid.

4. Either of the two hydroxylation methods described should be suitable for the industrial production of hydroxylated fatty acids and related compounds. Application of these reactions to red oil (commercial oleic acid) gave good yields of 9,10-dihydroxystearic acid, m. p., 92–94°

PHILADELPHIA, PENNSYLVANIA RECEIVED JUNE 25, 1945

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

Isolation of Constituents of Cane Juice and Blackstrap Molasses by Chromatographic Methods

BY W. W. BINKLEY,[1] MARY GRACE BLAIR[1] AND M. L. WOLFROM

In a previous communication[2] from this Laboratory, a chromatographic method for the separation of carbohydrate acetates was established. Application of this procedure has now been made to cane juice and to cane blackstrap molasses. The molasses was a typical Cuban blackstrap. The bulk of the fermentable sugars was removed from the molasses by fermentation with baker's yeast and the resultant solution was deionized by passage over ion exchange resins. The nature of the products fixed on the exchange resin columns is under further investigation. The non-fermented residue constituted 15.6%[3] of the original molasses, contained 10% of the original total sugar content (by reduction methods) and was a dark brown, hygroscopic solid with a bitter taste and a raisin-like odor. This product was acetylated with hot acetic anhydride and sodium acetate, a stringent acetylation procedure which no doubt led to the essential destruction of many of the sensitive substances contained in the mixture. The major portion of the fermentation glycerol was then removed as glycerol triacetate by distillation methods. The bulk of the residual acetylated material consisted of highly colored products which were strongly adsorbed by Magnesol, a behavior indicative of a polymeric struc-

(1) Sugar Research Foundation Research Associate (W. W. B.) and Fellow (M. G. B.) of The Ohio State University Research Foundation.

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

(3) All percentages are on the basis of the original wet products

ture. These were not further investigated. The less strongly adsorbed material was developed into several distinct zones. Inositol[4] hexaacetate was isolated from one of these zones by crystallization methods and yielded pure inositol on acetate removal. The mother liquor from the inositol crystallization was further chromatographed on Magnesol and the chromatogram was developed into three zones. From one there was isolated by crystallization methods a further quantity of inositol hexaacetate (total, 0.215% of the original molasses, as inositol) and also D-mannitol hexaacetate (0.05% of the original molasses, as D-mannitol) from which D-mannitol was obtained on acetate removal. A small quantity of β-D-glucose pentaacetate (0.07% of the original molasses as D-glucose) was obtained from the second zone while the third zone contained residual glycerol triacetate.

Application of the above procedure to the fermentation residue (containing 1% of the original sugar content by reduction methods) of cane juice led to the isolation of inositol (as the hexaacetate, 0.003% of the cane juice as inositol) and of fermentation glycerol (as the triacetate). No mannitol hexaacetate was isolable. The cane juice was collected from normal cane in Louisiana during December, 1944, and was shipped to Columbus, Ohio, under toluene. It was fermented under aseptic conditions as promptly as possible upon receipt. Fermentation of pure sucrose under identical conditions followed by the same analytical procedure led to the isolation of only glycerol triacetate. Inositol and D-mannitol were not found present.

It was of interest to investigate the inositol content of cane juice and cane blackstrap molasses by known analytical methods. To this end the bioassay procedure of Beadle[5] was employed. This method depends upon the growth response exhibited by a "mutant" of the mold *Neurospora crassa* requiring inositol for growth. The results are shown in Table I. Beadle found that the mold employed did not utilize salts of phytic acid (inositol hexaphosphate) and this finding was corroborated in our laboratory. The phosphate groups of phytic acid are difficult to remove but after acid treatment some inositol becomes available for utilization by the mold. We found that about one-third of the bound inositol in phytic acid becomes available to the mold under the hydrolytic conditions employed. Very approximately this same amount was likewise found by Beadle[5] on employing the same hydrolytic conditions although this worker was not inclined to attribute much significance to this utilization figure. The fact that an increase in the amount of apparent inositol occurs after acid hydrolysis may nevertheless be employed as a qualitative indication of the presence of phosphorylated inositol and if the figure of one-third hydrolysis by the acidic conditions employed be accepted, results are obtained that are in reasonable agreement with the amounts actually isolated.

TABLE I

INOSITOL AND PHYTIN CONTENTS OF CANE JUICE AND CANE BLACKSTRAP MOLASSES AS BIOASSAYED BY THE BEADLE PROCEDURE[a]

Substance	Free inositol, %[b]	Inositol after acid hydrolysis,[c] %	Phytin inositol,[d] %
Cane juice	0.005	0.005	0.000
Cane blackstrap molasses	.134	.196	.186

[a] Ref. 5. [b] *Cf.* ref. 3. [c] Hydrolytic conditions of Beadle employed. [d] Calcd. on basis that phytic acid releases *ca.* one-third of apparent inositol on acid hydrolysis. The phytin assay of Heubner and Stadler (ref. 6) as modified by Earley (ref. 7) was inapplicable to the molasses, due to interfering substances. The cane juice gave an initial test for iron.

The data of Table I show that the cane juice as received in Columbus contained free inositol but no phytin. Phytin must nevertheless be a constituent of the cane since it was found in the sample of Cuban blackstrap molasses. Various factors may have been responsible for this lack of phytin in our sample of cane juice: the cane may not have contained phytin at the particular stage of growth at which the sample was taken; the sample was not fresh (although it was protected against bacterial and mold spoilage by toluene) and the phytin may have become enzymically dephosphorylated; the iron container used in shipment (as well as iron from the mill rollers) may have caused a precipitation of the phytic acid as the very insoluble iron salt. The last explanation is probably the most credible since the cane juice gave initially a positive test for iron. A negative test for phytin was obtained on employing the analytical procedure of Heubner and Stadler,[6] as modified by Earley.[7] This procedure depends upon the precipitation of phytic acid as the ferric salt under conditions of mild acidity. The figure of 0.005% inositol compares with the value of 0.003% obtained on direct isolation.

The data of Table I for the molasses show that a significant increase in apparent free inositol occurred after acid hydrolysis. Therefore combined inositol was assuredly present. Accepting the value of one-third hydrolysis under the conditions employed, we obtain the following figures expressed in percentage of the original molasses: free inositol, 0.134; combined inositol, 0.186; total inositol, 0.320; phytin as magnesium salt ($C_6H_6(PO_4)_6Mg_6$), 0.820. The procedure of Earley[7] was found inapplicable to blackstrap molasses because of interfering substances. Now the total amount of free inositol obtained by direct isolation was 0.215% of the original molasses. This would appear to be too high in relation to the

(4) By inositol we denote the common, naturally occurring, meso form of m. p. 225°.

(5) G. W. Beadle, *J. Biol. Chem.*, **156**, 683 (1944).

(6) W. Heubner and H. Stadler, *Biochem. Z.*, **64**, 422 (1914).

(7) E. B. Earley, *Ind. Eng. Chem., Anal. Ed.*, **16**, 389 (1944).

amount found on bioassay. The explanation is to be found in the dephosphorylation of phytin by the yeast in the fermentation procedure employed. The fermentation was effected without added salts, depending upon a sufficient excess of yeast and any nutrients already present for suitable fermentation. A solution containing only D-glucose and calcium phytate (90 mg. per 100 cc.) produced, after fermentation with commercial baker's yeast, 68% of available inositol as determined by the bioassay of Beadle[5] (the original calcium phytate contained no available inositol). Woolley,[8] employing a pure single yeast strain, found no utilization of phytin by his yeast. On the other hand, Pitcher[9] found that phytin exhibited Bios I activity toward certain strains of yeast. These diverging results may be explained by the differing nature of the yeasts.[10] It was determined by the Beadle bioassay that a negligible amount (0.00004 g. per g. of yeast) of apparent inositol was introduced by the baker's yeast, a finding in harmony with the work of Kögl and van Hasselt.[11] This trace of inositol did not necessarily arise from the yeast cell.

The isolation of inositol from the sugar cane and from cane blackstrap molasses adds another name to the list of constituents isolated from these commercially important sources. Inositol is widely distributed in the plant world but the fact that it is an essential nutrient (Bios I) for most yeasts makes this isolation of significance since blackstrap molasses is used in large quantities as a source of fermentation alcohol. In her original work, Eastcott[12] demonstrated by yeast growth methods that Bios I was present in "Domolco"[13] molasses. Crystalline Bios I was at the same time isolated from tea and identified as inositol. Miss Eastcott's source of molasses cannot be considered as botanically defined.

The presence of small amounts (0.05%) of D-mannitol in Cuban blackstrap molasses and its absence in cane juice adds support to the contention that D-mannitol, while indeed widely distributed in many plants, is not a normal constituent of the sugar cane. Its introduction is brought about by bacterial contamination and its amount may serve as a rough index of the amount of such contamination. Sugar house operations are not aseptic and small amounts of D-mannitol are undoubtedly present in all blackstrap molasses. There are many types of bacteria[14] that produce D-mannitol from carbohydrate sources, especially D-fructose. The occurrence of gross amounts of D-mannitol in damaged and bacterially infected cane has been described by Browne[15] and by Walton and Fort.[16] We have performed experiments to demonstrate that aseptically performed fermentations with good quality baker's yeast did not introduce D-mannitol.[17] The single fermentation procedure employed by us did not give complete fermentation, as is evidenced by the isolation of a small amount (0.07%) of D-glucose (as β-D-glucose pentaacetate) after fermentation.

In the deacetylation of inositol hexaacetate and D-mannitol hexaacetate, saponification was effected with sodium hydroxide followed by removal of the sodium and acetate ions with ion exchange resins. This procedure represents a new method of sugar acetate deacetylation which offers certain advantages over previous methods.

Experimental

Materials.—The sugar cane (*Saccharum officinarum* L., variety Coimbatore 290) juice[18] was collected from normal cane and expressed in Louisiana before December 18, 1944, and was shipped to Columbus, Ohio, under toluene. It was fermented as promptly as possible upon receipt.

Anal. Solids, 12.2; *d*, 1.047 g. per ml. at 25°; ash, 0.3.

The sugar cane blackstrap molasses was produced in Cuba by the Cunagua central of the American Sugar Refining Co., New York, N. Y.[19] It was stored at 8–10°. We were informed by the producers that the cane from which this material was made consisted of 75–80% of the Javan varieties POJ 2714 and POJ 2878, the remaining 20–25% being the original Cuban stock termed Crystalina.

Anal. Color, nearly black; taste, burnt sweet; solids, 82.3; *d*, 1.468 g. per ml. at 25°; ash, 5.5; sucrose,[20] 32; reducing sugars (as invert sugar).[21] 15.

Isolation and Acetylation of the Non-fermented Fraction of Cane Blackstrap Molasses; Removal of Fermentation Glycerol.—The cane blackstrap molasses (225 g.) was dissolved in 800 g. of sterilized distilled water in a 3-liter sterilized flask equipped with a water seal. Starch-free baker's yeast (Fleischmann, 25 g.) was added and the mixture was maintained at 30° for three days with occasional gentle agitation. The spent yeast was removed by centrifugation and the centrifugate was diluted with distilled water to a volume of 3 liters (ash content of soln., 0.41%). This solution was passed at the rate of 1 liter per thirty minutes over 2.25 kg. of cation exchange resin (Amberlite IR-100[22]) packed in a glass tube 80 mm. in diameter and 1 m. long. The effluent (ash content of soln., 0.03%) was then passed at the same rate over a like amount of anion acceptor resin (Amberlite IR-4[22]). The material adsorbed on the ion exchange columns is under further investigation. The effluent from the anion acceptor column was concentrated under reduced pressure to a thick sirup which was dried initially at room temperature by pouring on a glass plate and finally by drying in a desiccator under reduced pressure; yield 35 g. (15.6%[3] of the original

(8) D. W. Woolley, *J. Biol. Chem.*, **140**, 453 (1941).

(9) W. H. Pitcher, *Iowa State Coll. J. Sci.*, **16**, 120 (1941).

(10) W. Lash Miller, *J. Chem. Education*, **7**, 257 (1930).

(11) F. Kögl and W. van Hasselt, *Z. physiol. Chem.*, **242**, 74 (1936).

(12) Edna V. Eastcott, *J. Phys. Chem.*, **32**, 1094 (1928).

(13) We are informed that "Domolco" is a fancy brand of table molasses sold in small cans. Its main basis is cane molasses imported from Barbados but it is not excluded that this may have been blended with other types of sirups, including those with a plant origin other than the sugar cane.

(14) H. R. Stiles, W. H. Peterson and E. B. Fred, *J. Biol. Chem.*, **64**, 643 (1925).

(15) C. A. Browne, Jr., THIS JOURNAL, **28**, 453 (1906).

(16) C. F. Walton, Jr., and C. A. Fort, *Ind. Eng. Chem.*, **23**, 1295 (1931).

(17) *Cf.* F. W. Zerban and L. Sattler, *ibid.*, **34**, 1180 (1942).

(18) Supplied through the courtesy of Mr. A. G. Keller of Sterling Sugars, Inc., Franklin, Louisiana.

(19) We are indebted for this material to Mr. Louis A. Wills of the above company.

(20) Modified Clerget method.

(21) Munson–Walker method.

(22) A product of the Resinous Products and Chemical Co., Philadelphia, Pennsylvania.

molasses) of a dark brown, hygroscopic, glittering solid with a bitter taste and an odor reminiscent of raisins. The reducing sugar content (modified Scales method) of this residue was found to be 4.8 (calcd. as per cent. of original molasses).

The finely powdered, non-fermented fraction (35 g.) was added in small portions to a mixture of 10 g. of fused sodium acetate and 150 cc. of acetic anhydride while maintaining the reaction temperature at 95–98°. After the addition, the reaction mixture was heated for four hours at this same temperature, whereupon it was poured slowly with stirring into 1 kg. of ice and water. After standing overnight at ice-box temperature, the granular precipitate was removed by filtration and washed with water; yield 37.5 g. (Fraction A) of a dark brown solid of slight odor and taste that was nearly completely soluble in acetone, was partially soluble in chloroform and only slightly soluble in benzene.

The filtrate from Fraction A was adjusted to *p*H 6.5 with sodium bicarbonate and extracted with chloroform (five 500-cc. portions). A dark brown sirup was obtained on solvent removal from the dried chloroform extract; yield 15.3 g. Glycerol triacetate was removed from this sirup by distillation at 10^{-2} mm. with a bath temperature of 80–85°; yield 6.2 g. (Fraction B) of undistilled residue as a dark, thick sirup and 8.9 g. of distillate, b. p. 259–261° (760 mm.) (cor.), d 1.161, $n^{15}D$ 1.433 (accepted values 259°, 1.161, 1.433, respectively).

Anal. Calcd. for $C_3H_5(COCH_3)_3$: CH_3CO, 13.74 cc. 0.1 *N* NaOH per 100 mg. Found: 13.76 cc.

A portion of the above product was saponified and converted to glycerol tri-*p*-nitrobenzoate; m. p. 186–187° (cor.) (accepted value 188°) unchanged on admixture with an authentic specimen.

First Chromatography of Fractions A and B of Cane Blackstrap Molasses; Isolation of Fraction D.—Fraction A (37.5 g.) was suspended in 200 cc. of acetone and the mixture poured on a bed of 200 g. of "Magnesol"[23] (*ca.* 2.5 cm. thick and 15 cm. diameter) under moderate suction and the bed washed with 800 cc. of acetone. A dark brown, viscous sirup resulted on solvent removal; yield 12.1 g. This sirup was dissolved in 100 cc. of benzene[24] and 30 cc. of this solution was added at the top of a column (35 mm. in diameter and 45 cm. long) containing 85 g. of a mixture of 5 parts (by wt.) of "Magnesol"[23] and 1 part of "Celite."[25] The chromatogram was developed with 500 cc. of 100/1: benzene/ethanol[26] (volume ratio). The column was extruded and streaked with a freshly prepared aqueous solution of 1% potassium permanganate in 2.5 *N* sodium hydroxide. A well-defined zone near the bottom of the column was cut out (the indicator streak was removed with a scalpel), eluted with 125 cc. of acetone to yield, after solvent removal under reduced pressure, an amber-colored sirup; yield 0.97 g. (Fraction C).

An amount of 3.1 g. of Fraction B was dissolved in 25 cc. of benzene and chromatographed in the manner described for Fraction A; yield 1.30 g. of golden yellow sirup which was combined with several lots of Fraction C above to yield Fraction D. The total yield of Fraction D was 5.83 g. from the original 225 g. of molasses. The nature of the other zones on the columns from both Fraction A and Fraction B is under investigation.

Isolation of Inositol[4] from Fraction D.—An amount of 2.21 g. of Fraction D was dissolved in 50 cc. of ethanol (abs.) and crystals formed on cooling; yield 406 mg. (0.215% of original cane blackstrap molasses, calcd. as inositol), m. p. 212–214° (cor.). Pure material was obtained on one further crystallization from the same solvent; m. p. 216° (cor.), spec. rot. 0°, Molisch (−). The substance was identified as inositol hexaacetate (accepted m. p. 216°, cor., mixed m. p. with an authentic sample unchanged).

Anal. Calcd. for $C_6H_6O_6(COCH_3)_6$: C, 49.98; H, 5.60; CH_3CO, 13.9 cc. 0.1 *N* NaOH per 100 mg. Found: C, 50.05; H, 5.60; CH_3CO, 13.8 cc.

An amount of 100 mg. of the above material was saponified by standing overnight at room temperature in ethanol (25 cc.) solution to which had been added an equal volume of 0.1 *N* sodium hydroxide. The solution was passed slowly over a column (16 mm. diameter and 70 cm. long) containing 90 g. of cation exchange resin (Amberlite IR-100[22]) and the effluent was passed slowly over a like amount of anion acceptor resin (Amberlite IR-4[22]). The deionized solution was then evaporated to dryness under reduced pressure; yield 30 mg., m. p. 218–220°. Pure inositol was obtained on further crystallization from ethanol–water (1:1); m. p. 225° (cor.), mixed m. p. with an authentic sample unchanged, Tollens reduction (+), Fehling reduction (−), Scherer[27] inositol test (+). This behavior identifies the substance as inositol for which the accepted m. p. is 225° (cor.).

A blank (employing D-glucose in place of the molasses) on the fermentation procedure showed a negligible amount of apparent inositol (0.00004 g. per g. of yeast; determined by the Beadle[5] procedure) after fermentation.

Identification of D-Mannitol and D-Glucose in Fraction D.—The ethanolic solution of Fraction D, from which the bulk of the inositol had been removed by crystallization, was concentrated to a sirup and the ethanol removed by repeated reduced pressure distillations with benzene; yield 4.62 g. from the original 225 g. of molasses. An amount of 540 mg. of this sirup was dissolved in 15 cc. of benzene and added at the top of a column (35 mm. in diameter and 230 mm. long) containing 50 g. of "Magnesol"[23] and "Celite"[25] (5:1). The chromatogram was developed with 1350 cc. of 500/1 (by vol.): benzene/ethanol. Three sharp zones were obtained on streaking with the alkaline permanganate indicator. Zone 1 was about one-fourth of the way down the column, zone 2 was near the middle and zone 3 was near the bottom. The zones were cut out (the indicator streaks removed with a scalpel) and eluted with 100 cc. of acetone and sirups of a light yellow color were obtained on solvent removal; yields: zone 1, 106 mg.; zone 2, 122 mg.; zone 3, 76 mg.; total yield, 304 mg.

The material from zone 1 yielded crystals from ethanol (95%); yield 31 mg. (0.05% of the original cane blackstrap molasses, calculated as D-mannitol) m. p. 116° $[\alpha]^{27}D$ +24° (*c* 2.8, 2-dm. tube, $CHCl_3$). Recrystallization yielded rhombic crystals; m. p. 119–120° unchanged on admixture with an authentic specimen of D-mannitol hexaacetate, inositol test[27] (−). The accepted constants for D-mannitol hexaacetate are: m. p. 120°, $[\alpha]^{20}D$ +26° ($CHCl_3$).

Anal. Calcd. for $C_6H_8O_6(COCH_3)_6$: C, 49.74; H, 6.04; CH_3CO, 13.8 cc. 0.1 *N* NaOH per 100 mg. Found: C, 49.83; H, 5.77; CH_3CO, 13.8 cc.

An amount of 50 mg. of the above material was saponified as described above for inositol hexaacetate; yield 12 mg., m. p. 164–165°, unchanged on admixture with an authentic specimen of D-mannitol (accepted m. p. 166°). Molisch (−), Fehling reduction (−).

The material from zone 2 (122 mg.) yielded crystals from ethanol; yield 50 mg. (several crops), m. p. 128–129°. Pure material was obtained on recrystallization from ethanol, yield 30 mg. (0.07% of the original cane blackstrap molasses calculated as D-glucose), m. p. 131–132° unchanged on admixture with an authentic specimen of β-D-glucose pentaacetate, $[\alpha]^{25}D$ +4.4 ± 0.8° (*c* 1.7, 2-dm. tube, $CHCl_3$), Molisch (+), Fehling reduction (+), Seliwanoff test for ketoses (−). This substance was therefore adequately identified as β-D-glucose pentaacetate (accepted constants: m. p. 132°, $[\alpha]^{20}D$ +3.8° in $CHCl_3$). The mother liquor material from zone 2 gave a negative Scherer[27] inositol test.

(23) A synthetic, hydrated magnesium acid silicate manufactured by the Westvaco Chlorine Products Co., South Charleston, West Virginia.

(24) All benzene employed was free of thiophene.

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

(26) All ethanol employed in the chromatographic work was absolute.

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

The material from zone 3 (76 mg.) was found to contain further amounts (50 mg.) of glycerol triacetate, identified by micro b. p. A negative Scherer[27] inositol test was obtained on the residue left in zone 3 after removal of the glycerol triacetate.

Identification of Inositol in Cane Juice.—The cane juice (1500 g.) was concentrated to *ca.* 1300 g. under reduced pressure in sterilized equipment to remove toluene and brought to its original volume with sterilized distilled water. It was fermented as described above for the cane blackstrap molasses, employing the same amount of yeast. It was deionized, without dilution, in the same manner (soln. ash changed from 0.31% to 0.014%) and the non-fermented residue isolated; yield *ca.* 10 g. of a dark amber colored, hygroscopic sirup with little odor and a bitter taste. The reducing sugar content (modified Scales method) of this residue was found to be 0.1 (calcd. as % of original cane juice). This material was acetylated as described above for cane blackstrap molasses; yield 1.0 g. of Fraction A′ (corresponding to A; an amorphous, dark-colored solid that was partially soluble in acetone but only slightly soluble in chloroform or benzene) and 20.9 g. of chloroform extracted material (golden yellow sirup). The glycerol triacetate (14.6 g.) was removed as described above and a portion (1 g.) of the residual sirup (total amount, 6.0 g., Fraction B′, corresponding to B) was dissolved in 5 cc. of benzene and added at the top of a column (35 mm. in diameter and 230 mm. long) containing 45 g. of 5/1 (by wt.) "Magnesol"[28]/"Celite."[25] The chromatogram was developed with 1350 cc. of 500/1 (by vol.) benzene/ethanol. A well-defined zone about half way down the column was located, isolated and eluted as described above; yield 50 mg. of a golden yellow sirup. This sirup yielded crystals from hot ethanol (abs.); yield 17 mg., m. p. 214–215°, unchanged on admixture with an authentic specimen of inositol hexaacetate, Molisch (−), Fehling (−), Scherer[27] inositol test (+). This crystalline material, in relation to the previously described work with cane blackstrap molasses, was considered to be adequately identified as inositol hexaacetate.

Investigation of the Non-fermented Residue from the Fermentation of Pure Sucrose.—A solution of sucrose (225 g.) in 1500 g. of sterile water was fermented in sterilized equipment with 45 g. of yeast for five days at 30° as described above for the fermentation of cane blackstrap molasses. The resultant solution was deionized in the same manner and the non-fermented residue isolated; yield *ca.* 10 g. of a light yellow sirup with a bitter taste and little odor. This sirup was acetylated as described previously; yield: Fraction A″, 0.8 g. of a dark brown solid with a fatty odor; Fraction B″, 25.1 g. Fraction B″ yielded 18.8 g. of glycerol triacetate and 5.9 g. of residual sirup, a portion (400 mg.) of which was chromatographed as described above. Only an additional quantity of glycerol triacetate was found present.

Dephosphorylation of Phytin During Yeast Fermentation.—An amount of 89.5 mg. of calcium phytate[28] of 17% inositol content, determined by the method of Heubner and Stadler[6] as modified by Earley,[7] and which contained no free inositol by the Beadle procedure,[5] was added to 100 cc. of an aqueous solution of D-glucose (15 g.) and fermented with 3 g. of baker's yeast (Fleischmann, starch-free) for three days at 30°. The yeast was removed by centrifugation and the centrifugate was diluted to 1000 cc. and analyzed for its free inositol content by the method of Beadle[5]; found an amount of free inositol corresponding to a phytin hydrolysis of 68%.

Acknowledgment.—Acknowledgment is made to Dr. R. C. Hockett, Scientific Director of the Sugar Research Foundation, for his counsel. Advice was also rendered by Professor G. L. Stahly of the department of bacteriology of The Ohio State University.

Summary

1. Inositol (m. p. 225°) has been isolated from sugar cane juice (as inositol hexaacetate) and from cane blackstrap molasses.

2. The presence of phytin in cane molasses has been detected by biochemical methods.

3. D-Mannitol has been isolated in small amount from a normal sample of cane molasses but was found to be absent in normal cane juice.

4. D-Glucose (as β-D-glucose pentaacetate) was isolated from cane molasses.

5. It was demonstrated that a commercial sample of baker's yeast was able to dephosphorylate phytin.

6. A method of deacetylation employing ion exchange resins has been established.

(28) We are indebted for this material to the Corn Products Refining Company, Argo, Illinois.

COLUMBUS, OHIO RECEIVED JUNE 28, 1945

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

The Action of Diazomethane upon Acyclic Sugar Derivatives. VII.[1] D-Psicose[2]

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In continuation of our studies on the action of diazomethane upon acyclic sugar derivatives, we have synthesized 1-diazo-1-desoxy-*keto*-D-psicose tetraacetate (II) from D-ribonyl chloride tetraacetate (I). D-Ribonic acid tetraacetate has been recorded by Pasternack and Brown[5] and by Ladenburg and co-workers.[6] The latter synthesized it by the direct acetylation of salts of ribonic acid and the former synthesized it through the acetylated amide according to the general procedure of Hurd and Sowden.[7] Chloroform is the solvent of choice for recording the rotations of sugar acetates and we record herein a number of new rotations of D-ribonic acid derivatives in this solvent. Methyl D-ribonate tetraacetate was synthesized in this work and an improved

(1) Previous publication in this series: M. L. Wolfrom, S. M. Olin and E. F. Evans, THIS JOURNAL, **66**, 204 (1944).

(2) Presented in part before the Division of Sugar Chemistry and Technology at the 106th meeting of the American Chemical Society, Pittsburgh, Pennsylvania, September 7, 1943.

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(5) R. Pasternack and E. V. Brown, U. S. Patent 2,237,263 (1941).

(6) K. Ladenburg, M. Tishler, J. W. Wellman and R. D. Babson, THIS JOURNAL, **66**, 1217 (1944).

(7) C. D. Hurd and J. C. Sowden, *ibid.*, **60**, 235 (1938).