

COMPOSITION OF CANE JUICE AND CANE FINAL MOLASSES

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BIOGRAPHICAL NOTE

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Dr. Melville L. Wolfrom received his B.A. degree from The Ohio State University in 1924. During and preceding his undergraduate training, he worked as a control and analytical chemist in various industrial laboratories in Cleveland, Ohio, and served as a developmental chemist with the National Carbon Company in Fremont, Ohio, and the United States Gypsum Company at Gypsum, Ohio, and Chicago, Illinois. He received his Ph.D. degree in organic chemistry from Northwestern University in 1927 under W. Lee Lewis.

Dr. Wolfrom then served for two years as a National Research Council Fellow in Chemistry and studied at The Ohio State University with W. L. Evans, at the Rockefeller Institute for Medical Research with P. A. Levene, and at the National Bureau of Standards with C. S. Hudson. During 1939 he held a Guggenheim Memorial Foundation Fellowship and studied with P. Karrer at the University of Zurich in Switzerland.

He was appointed instructor in chemistry at The Ohio State University in 1929 and has been professor there since 1940. His principal research interests have been in the chemistry of carbohydrates and other natural products.

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Foreword

The title of this monograph is very broad, yet the report does not undertake to mention all recorded literature on molasses or to compile all the claims that have been made and conclusions that have been drawn by previous workers. The material here has been selected by special tests. The purpose of the authors has been to record all the components of cane juice and molasses *that have been positively and unequivocally identified according to the exact criteria of modern organic chemistry*. Many inferential conclusions from inadequate and indirect data have therefore been omitted.

The authors have endeavored to screen the entire literature of chemistry for information meeting these standards. In addition, they include conclusions from their own work under Project No. 4 of Sugar Research Foundation which was carried out between 1944 and 1951 at The Ohio State University. The present report, therefore, will serve as a final account of the project as well as a critical survey of the literature.

The U. S. Department of Agriculture contributed to the work in 1950-1951 and has been the principal sponsor since July 1, 1951, when Foundation support ceased.

The main purposes in studying molasses composition have been two in number. First, a knowledge of molasses components should help explain why we have molasses, that is, just what kinds of substance forming or accumulating in the liquors are responsible for eventually inhibiting sugar crystallization. Second, a knowledge of non-sugar molasses constituents, including those beneficial or helpful to microorganisms, should assist the efficient use of this product for making alcohol yeast, citric acid, or other products of microbiology as well as chemical transformations. A third purpose, that is, to discover ingredients worth recovering for immediate sale, is a relatively minor one.

About 53 organic compounds so far have been identified in cane juice and about 44 in molasses. The greatest remaining problem is to discover

just how the high molecular weight ingredients of molasses ("sugar polymers") are formed from cane juice constituents, to elucidate their structures and to learn how to minimize their formation or eliminate them. The work already carried out should form a basis for discovering the differences between juices that are readily clarified and those that are "refractory," or those which differ otherwise in practical handling in the mill. Such information should eventually guide the breeder in producing more satisfactory new varieties.

Both cane juice and molasses are very complex mixtures and difficult to resolve. The newly-developed methods of chromatographic separation have contributed a great deal to current progress. The authors of this report did extensive original work in the early stages to adapt chromatography to sugar separations.

The goal of complete knowledge of cane juice and molasses composition is more nearly in sight now than ever before, though much still remains to be done.

HENRY B. HASS
President

ROBERT C. HOCKETT
Former Scientific Director

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I. INTRODUCTION

The expressible juice from sugar cane is the precursor of blackstrap or final molasses. Components of the juice constitute the major part of this by-product. However, a significant portion of the final molasses consists of the altered reaction products formed under factory conditions from the juice constituents. A summary of the composition of cane juice is presented as a necessary background for any discussion of cane final molasses. Cane juice is an aqueous solution circulating in the living plant and carrying materials required for growth and metabolism. It is therefore extremely complex. It distinguishes itself from other plant juices or saps by its characteristically high content of sucrose. The

cane juices referred to in this writing are "screened" crusher juices; they closely approximate the normal plant juice. The range in variability of these juices is demonstrated by their empirical analyses¹ (basis whole juice): water, 78–86%; sucrose, 10–20%; reducing sugars, 0.5–2.5%; other organic compounds, 0.5–1.0%; ash, 0.3–0.7%; pH 5.2–6.2. In the early part of the 1951 season a typical Florida crusher juice showed a pH of 5.8 and contained (basis whole juice): 18.55° Brix solids at 20°C.; sucrose, 16.22%; reducing sugars, 0.5%.² The pressure on the crusher rolls which express this juice from the shredded cane may be as much as 75 tons per foot of roll width.¹ Leaving the crushers the cane (bagasse) is extracted during passage through a series of 3-roll mills; considerable water is added and these liquids are then much less representative of the actual juice in the cane.

II. COMPOSITION OF CANE JUICE

1. Carbohydrates

a. Normal.—The production of sucrose from sugar cane is revealed in the earliest records of modern civilization.^{3,4} The application of the scientific method to this process began slightly more than a century ago.⁵ Three species of the genus *Saccharum* are grown for the industrial production of sugar; they are *spontaneum*, *robustum* and *officinarium*.³ The sugar cane is a reed or grass and is propagated by joint cuttings ("plant cane"). Several cuttings ("stubble cane") are made from each planting.⁶ Seedlings are required for new varieties and hybrids. As the seeds are very difficult to germinate, this work has been carried out in the experiment stations and has led to such variety designations as POJ 234 (variety 234 of Proefstation Oost Java), BH (Barbados Hybrid) or D (Demarara, British Guiana).³ The cane seldom matures before harvest in Louisiana, the danger of frost there confining the growing period to about nine months. This period is about fifteen months in Cuba and is eighteen to twenty-four months in Hawaii.

(1) E. R. Riegel, "Industrial Chemistry," 5th ed., Reinhold Publishing Corp., New York, 1949.

(2) Private communication from Dr. B. A. Bourne of the United States Sugar Corp., Clewiston, Florida.

(3) Noel Deerr, "History of Sugar," Chapman and Hall, London, 1949, Vol. I, p. 12.

(4) E. O. von Lippmann, "Geschichte des Zuckers," 2nd ed., J. Springer, Berlin, 1929.

(5) J. B. Avenquin, *J. chim. méd. pharmacie toxicologie*, [2] 1, 132 (1836).

(6) A. Van Hook, "Sugar," The Ronald Press Co., New York, 1949, p. 23.

Reducing sugars, 4–8%,⁷ are known to be present in cane juice; they are D-glucose and D-fructose. The acetylation of lyophilized (freeze-dried) normal cane juice solids followed by chromatography on a magnesium acid silicate of these acetates led to the isolation and proper identification of the sugars as crystalline derivatives.⁸

Methods of approximate analysis in solution for sucrose, D-glucose and D-fructose have been extensively developed and refined.⁹ They depend upon the fact that sucrose is a non-reducing disaccharide of rather high optical rotation, $[\alpha]_D +66^\circ$, and that on acid hydrolysis to its reducing sugar components, the mixture is levorotatory because of the high equilibrium levorotation, $[\alpha]_D -92^\circ$, displayed by D-fructose over that of D-glucose, $[\alpha]_D +52.5^\circ$. The fact that D-fructose is highly tautomeric and changes its equilibrium rotation markedly with temperature is also employed in analysis.

The other carbohydrates in cane juice are the soluble polysaccharides vaguely classified under the terms "hemi-celluloses, soluble gums and pectins." It is possible that some of these polysaccharides may enter the juice during the milling of the cane as the plant cell structure is destroyed. A gummy product has been isolated from cane fiber by alkali extraction followed by alcohol precipitation. Acid hydrolysis of this substance yielded crystalline D-xylose and L-arabinose.¹⁰ Such gums in Trinidad cane juices were isolated by alcohol precipitation at suitable hydrogen ion concentration and assayed for pentose content by the Tollens 2-furaldehyde assay; the results showed an "apparent pentosan" content of 0.04–0.07%¹¹ of the Brix solids.

Pectins are present in plant juices. These are now considered to be polymers containing a basic chain, designated pectic acid, of 4-linked D-galactopyranuronic acids, the carboxyl groups of which exist in part as methyl esters and in part as salts. Variable amounts of an araban and a galactan are associated with this polysaccharide.¹² A method of approximate assay for pectic substances¹³ depends upon saponification

(7) Unless otherwise noted, constituent concentrations of cane juice and of cane final molasses are expressed on the basis of the solids contents of the source materials. When the solids contents are unavailable, the normal values of 17% and 80% for cane juice solids and cane final molasses solids, respectively, have been employed to closely estimate these constituent concentrations.

(8) W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **68**, 1720 (1946).

(9) C. A. Browne and F. W. Zerban, "Physical and Chemical Methods of Sugar Analysis," John Wiley and Sons, Inc., New York, 3rd ed., 1941.

(10) C. A. Browne, Jr., and R. E. Blouin, *Louisiana Expt. Sta. Bull.* **91** (1907).

(11) R. G. W. Farnell, *Intern. Sugar J.*, **26**, 480 (1924).

(12) E. L. Hirst and J. K. N. Jones, *Advances in Carbohydrate Chem.*, **2**, 235 (1946).

(13) M. H. Carré and D. Haynes, *Biochem. J.*, **16**, 60 (1922).

followed by the precipitation and weighing of the water-insoluble (but impure) calcium pectate. Application of this procedure to Trinidad cane juices¹¹ showed the presence of 0.0–0.1% of pectins (as calcium pectate) in the Brix solids. Uronic acids are readily decarboxylated on heating with hot mineral acids. An assay for their presence depends upon the determination of the evolved carbon dioxide¹⁴ and when applied to Louisiana cane juice indicated a uronic acid content of 0.44% of the ash-free solids.¹⁵ The uronic acids are derived from the cane fiber and pectins. The methoxyl content of the juice was found by a modified hydriodic acid digestion method¹⁶ to be 0.08% of the ash-free solids.¹⁵ It is presumably derived from the pectins present. Starch is readily detected by iodine coloration and has been reported in the Uba cane juice of South Africa.¹⁷ The starch content of Louisiana cane juice has been determined quantitatively¹⁸ by extraction of the nondialyzable juice fraction with perchloric acid, precipitation of the starch with iodine, decomposition of the starch-iodine complex with alcoholic sodium hydroxide, hydrolysis of the starch with aqueous hydrochloric acid and estimation of the starch content from the copper reduction value of the hydrolyzate.¹⁹ The average starch content of this juice was found to be 0.012–0.018%; the maximum value was 0.035%.¹⁸ The starch is present in the cane just above the nodes, where it apparently serves as a reserve carbohydrate for growth. It is probably not a normal constituent of the plant juice but enters the crusher juice through the mechanical disintegration of the nodes.

Cane juice is a rich source of the cyclic alcohol, *myo*-inositol (the definitive prefix *myo* has been suggested to replace *meso* for the common inositol, m. p. 225°²⁰). It was isolated from cane juice by chromatography²¹; bioassay (Beadle method²²) of the juice showed 0.041% *myo*-inositol.²¹ A logical companion of this constituent is phytin (a calcium-

(14) K. U. Lefèvre and B. Tollens, *Ber.*, **40**, 4513 (1907); A. D. Dickson, H. Otterson and K. P. Link, *J. Am. Chem. Soc.*, **52**, 1775 (1930).

(15) C. A. Browne and M. Phillips, *Intern. Sugar J.*, **41**, 430 (1939).

(16) M. Phillips, *J. Assoc. Off. Agr. Chemists*, **15**, 118 (1932).

(17) L. Fevilherade, *Proc. S. African Sugar Tech. Assoc.*, **71** (1929); *Chem. Abstracts*, **24**, 3127 (1930).

(18) R. T. Balch, B. A. Smith and L. F. Martin, *Sugar J.*, **15**, No. 6, 39 (1952); R. T. Balch, *ibid.*, No. 8, 11 (1953).

(19) G. S. Pucher, C. S. Leavenworth and H. B. Vickery, *Anal. Chem.*, **20**, 850 (1948).

(20) H. G. Fletcher, Jr., L. Anderson and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(21) W. W. Binkley, M. Grace Blair and M. L. Wolfrom, *J. Am. Chem. Soc.*, **67**, 1789 (1945).

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

magnesium salt of *myo*-inositol hexaphosphate); it was not found in cane juice.²¹ This juice contained iron which forms a very insoluble salt with *myo*-inositol hexaphosphate and the modified²³ phytin assay,²⁴ depending upon the precipitation of ferric phytate, was thus not applicable. Phytin must certainly be a juice constituent since it is found in blackstrap molasses.²¹ The amount of organic phosphate in cane juice (average *ca.* 18.7 mg. of phosphoric anhydride per 100 g. of Brix solids) has been estimated from the difference between the total and inorganic phosphorus contents; the presence of hexose phosphates is suggested.^{25,25a}

b. Bacterial.—Slime-producing bacteria are associated with the cane plant and these attack cane damaged by frost or by other agents. Some of these bacteria belong to the *Leuconostoc mesenteroides* and *Leuconostoc dextranicum* classifications and form many strains that are difficultly distinguishable. They contain a transglycosidase enzyme system which acts upon sucrose to transfer the *D*-glucose glycosidic linkage from *D*-fructose to the *D*-glucose of another sucrose molecule with the concomitant liberation of *D*-fructose. This process continues and a polysaccharide molecule is built up containing α -*D*-glucopyranose units linked 1 \rightarrow 6 and 1 \rightarrow 4 with the former predominating. In some products other linkages may be present.²⁶ These substances are termed dextrans²⁷⁻²⁹ and have been the subject of considerable investigation since the recently indicated use of acid-modified dextrans as blood extenders. Other bacteria associated with cane and cane products, such as *Bacillus mesentericus*, contain a transglycosidase enzyme system that transfers the *D*-fructose glycosidic linkage in sucrose to the *D*-fructose unit of another sucrose molecule with the liberation of *D*-glucose. The polysaccharides formed are termed levans²⁸⁻³⁰ and consist of *D*-fructofuranose units joined 2 \rightarrow 6. The dextran-producing bacteria are thermolabile and are destroyed in the mill processing whereas the levan-producing bacteria persist.

D-Mannitol is not a normal constituent of cane juice²¹ but is always

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

(24) W. Heubner and H. Stadler, *Biochem. Z.*, **64**, 422 (1941).

(25) P. Honig, "Phosphates in Clarified Cane Juice," *West Indies Sugar Corp. Rept.* **1** (1952); *Abstracts Papers Am. Chem. Soc.*, **121**, 15P (1952).

(25a) L. F. Wiggins, *Intern. Sugar J.*, **54**, 324 (1952).

(26) R. L. Lohmar, *J. Am. Chem. Soc.*, **74**, 4974 (1952).

(27) E. Durin, *Compt. rend.*, **83**, 128 (1876).

(28) T. H. Evans and H. Hibbert, *Advances in Carbohydrate Chem.*, **2**, 203 (1946); *Sugar Research Foundation, Sci. Rept. Series*, **6** (1947).

(29) E. J. Hehre, *Transactions N. Y. Acad. Sci.*, [II] **10**, No. 6, 188 (1948).

(30) R. G. Smith, *Intern. Sugar J.*, **4**, 430 (1902); R. G. Smith and T. Steel, *J. Soc. Chem. Ind.*, **21**, 1381 (1902).

present in final molasses.²¹ It is introduced by bacterial action. There are many types of bacteria that produce D-mannitol from carbohydrates, especially from D-fructose.³¹ The occurrence of gross amounts of D-mannitol in damaged and bacterially infected cane has been described.³²

2. Enzymes

Hydrolyzing and color-producing enzymes or enzyme systems are active in raw cane juice and both contribute to the formation of molasses. The cane plant contains the sucrose-hydrolyzing enzyme invertase³² and thus differs from the sugar beet, where it is absent. This enzyme is present in the juice and produces a simultaneous decrease of sucrose and an increase in reducing sugars. The color-producing enzyme systems are represented by an oxidase (laccase), a peroxidase and tyrosinase.³²⁻³⁶ These are oxidizing enzymes that produce phenols and quinones from aromatic compounds present in the plant juice which then react with ferric ion to produce dark colored complexes.

3. Vitamins

Raw cane juice is not a rich source of vitamins; it does contain a little vitamin A, some of the B vitamins and probably a trace of vitamin D.³⁶ A more complete assay of the B-group vitamins revealed the presence of thiamine, riboflavin, pantothenic acid, niacin, and biotin.³⁷ Cane juice is a good source for the reported fat-soluble "antistiffness factor," a yield of 0.1 g. (50,000,000 units) being obtained from 55 gal. of raw juice.³⁸ Application of counter-current distribution with a methanol-isoöctane system to a cane juice concentrate containing this "antistiffness factor" and sublimation of one of the three resulting fractions produced a sublimate with an infrared spectrum like stigmasterol.³⁹ This sterol has the same chemical and biological properties as the "antistiffness

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

(32) C. A. Browne, Jr., *J. Am. Chem. Soc.*, **28**, 453 (1906); C. F. Walton, Jr., and C. A. Fort, *Ind. Eng. Chem.*, **23**, 1295 (1931).

(33) M. Raciborski, *Jaarverslag 1898 van het proefsta. voor Suikerriet West Java*, 15 (1899).

(34) F. W. Zerban and E. C. Freeland, *Louisiana Agr. Expt. Sta. Bull.* **165** (1919).

(35) F. W. Zerban, *Ind. Eng. Chem.*, **12**, 744 (1920).

(36) E. M. Nelson and D. B. Jones, *J. Agr. Research*, **41**, 749 (1930).

(37) W. R. Jackson and T. J. Macek, *Ind. Eng. Chem.*, **36**, 261 (1944).

(38) J. Van Wagendonk and Rosalind Wulzen, *J. Biol. Chem.*, **164**, 597 (1946).

(39) H. Rosenkrantz, A. T. Milhorat, M. Farber and A. E. Milman, *Proc. Soc. Exptl. Biol. Med.*, **76**, 408 (1951).

factor."⁴⁰ *myo*-Inositol, a vitamin complement and growth factor, is present in relatively large quantities. It is generally included as a member of the B-group of vitamins although its position in human nutrition is still not adequately defined.⁴¹

4. Nitrogen Compounds

Both simple and complex organic nitrogen compounds occur in cane juice. Loosely defined albumins and simpler proteins are the complex substances and they may represent as much as 25% of the organic nitrogen compounds of the juice.⁴² None of these proteins has been isolated or characterized. Analysis of a high-nitrogen juice (Florida) with ion-exchange resins showed that at least 75% of the nitrogen substance behaved like amino acids.⁴³ *L*(*levo*)-Asparagine is the most abundant amino acid of cane juice, from which it has been isolated and adequately identified.⁴⁴ *L*(*dextro*)-Glutamine and tyrosine were likewise isolated and identified⁴⁴; the cane juice employed was Puerto Rican.

Application of amino acid paper chromatography to the cations removed from cane juice by ion-exchange resins indicated the presence of leucine (or isoleucine), valine, γ -aminobutyric acid, alanine, glycine, serine, asparagine, glutamic and aspartic acids, lysine and glutamine.^{45,46} A preliminary chromatographic fractionation on clay enhanced the clarity of the spots, which were further identified by spot enhancement with authentic specimens of these substances.⁴³ Tyrosine was detected only after a preliminary concentration on a column of powdered cellulose.⁴³ Microbiological amino acid assays⁴⁶ of the organic cationic fraction of a Florida cane juice showed *L*-leucine,^{47,48} 0.0025%; *L*-isoleucine,^{47,48} 0.0010%; *L*-valine,^{47,48} 0.0018%; *L*-tryptophan,⁴⁹ 0.0034%;

(40) E. Kaiser and Rosalind Wulzen, *Arch. Biochem. Biophys.*, **31**, 327 (1951).

(41) D. W. Woolley, *J. Nutrition*, **28**, 305 (1944).

(42) F. A. F. C. Went, *Jahrb. wiss. Botan.*, **31**, 289 (1898); *Chem. Zentr.*, II, 367 (1898).

(43) G. N. Kowkabany, W. W. Binkley and M. L. Wolfrom, *Abstracts Papers 12th Intern. Congr. Pure Applied Chem.*, 166 (1951); *Agr. Food Chem.*, **1**, 84 (1953).

(44) F. W. Zerban, *8th Intern. Congr. Pure Applied Chem.*, 103 (1912); *Chem. Abstracts*, **6**, 3337 (1912).

(45) O. E. Pratt and L. F. Wiggins, *Proc. Brit. West Indies Sugar Technol.*, **29** (1949); L. F. Wiggins and J. H. Williams, *ibid.*, **40** (1951).

(46) Performed by the Food Research Laboratories, Inc., Long Island City, N. Y., for the Sugar Research Foundation, Inc., New York, N. Y.

(47) Method of S. Shankman, *J. Biol. Chem.*, **150**, 305 (1943).

(48) Method of J. R. McMahan and E. E. Snell, *J. Biol. Chem.*, **152**, 83 (1944).

(49) Method of R. D. Greene and A. Black, *J. Biol. Chem.*, **155**, 1 (1944).

L-methionine,⁵⁰ 0.0009%. L-Lysine, L-arginine, L-threonine, L-histidine and L-phenylalanine were sought but not detected.^{46,50} Although qualitatively the general amino acid picture was always the same, the total amounts of amino acids in the top portions of Barbados cane varied with the variety from 1 to 12.5 millimoles per liter of cane juice; it was highest in the top and lower portions of the cane and was at a minimum near the middle. It was at a maximum in the early stages of growth.⁴⁵

5. Non-nitrogenous Acids

Juice expressed from normal cane does not contain volatile acids; soured juice or juice from damaged cane possesses volatile acids, largely acetic.⁵¹ The principal organic acid of cane juice is aconitic. Its presence was based, as early as 1877,⁵² on the isolation of a nearly pure specimen of the acid but proper identification was not recorded until 1919.⁵³ The concentration of this acid in Louisiana juice solids varies from 0.3 to 1.6% and it may represent as much as 90% of the non-volatile acids.⁵⁴ The occurrence in cane juice of such acids as malic,⁵⁵⁻⁵⁸ succinic,^{56,57,59} glycolic,⁶⁰ formic,⁶¹ and oxalic,^{55,58,62,63} has been based only on qualitative tests and analytical methods. The recent application of a chromatographic procedure,⁶⁴ employing silicic acid, to an anionic fraction of cane juice has led to the isolation and identification of fumaric and succinic acids in addition to aconitic acid.⁶⁵ Aconitic acid exhibits geometric isomerism and exists in *cis* and *trans* forms.⁶⁶ These are interconvertible in solution, temperature and pH being factors.⁶⁷ It is

(50) Method of J. L. Stokes, M. Gunness, Irla M. Dwyer and Muriel C. Caswell, *J. Biol. Chem.*, **160**, 35 (1945).

(51) C. A. Fort and J. I. Lauritzen, *Ind. Eng. Chem., Anal. Ed.*, **10**, 251 (1938).

(52) A. Behr, *Ber.*, **10**, 351 (1877).

(53) C. S. Taylor, *J. Chem. Soc.*, 115, 886 (1919).

(54) R. T. Balch, C. B. Broeg and J. A. Ambler, *Intern. Sugar J.*, **48**, 186 (1946).

(55) A. Payen, *Compt. rend.*, **28**, 613 (1849).

(56) H. Winter, *Z. Ver. deut. Zuckerind.*, **38**, 780 (1888).

(57) J. van Breda de Haan, *Jaarverslag 1891 van het proefsta. voor Suikerriet West Java*, 9 (1892).

(58) P. A. Yoder, *J. Ind. Eng. Chem.*, **3**, 640 (1911).

(59) T. Tanabe, *Repts. Tiansan Formosa Expt. Sta.*, **4**, 33 (1937).

(60) E. C. Shorey, *J. Am. Chem. Soc.*, **21**, 45 (1899).

(61) F. W. Zerban, *J. Assoc. Off. Agr. Chemists*, **15**, 355 (1932).

(62) J. E. Quintus Bosz, *Arch. Suikerind.*, **28**, 969 (1920).

(63) H. C. Prinsen Geerligs, *Arch. Suikerind. Nederland en Ned.-Indie*, **1**, 230 (1940).

(64) C. S. Marvel and R. D. Rands, *J. Am. Chem. Soc.*, **72**, 2642 (1950).

(65) See Addendum, p. 314.

(66) R. E. Miller and S. M. Cantor, *Advances in Carbohydrate Chem.*, **6**, 231 (1951).

(67) J. A. Ambler and E. J. Roberts, *J. Org. Chem.*, **13**, 399 (1948).

generally assumed that the natural form of aconitic acid is mainly *cis* because the enzyme system aconitase, widely distributed in plants and animals, converts citric acid to isocitric acid through the intermediate *cis*-aconitic acid. The pH of cane juice lies in the range 5.2–6.2 where the equilibrium would favor the *cis*-form.⁶⁷

6. Pigments

Normal cane juice, as it circulates in the intact plant, is colorless³⁴; however, the tissue breakdown produced in grinding the cane for sucrose extraction permits the colloidal suspension and solution of pigmented substances not normally present. The tannins⁶⁸ and water-soluble anthocyanins³⁴ are the major color contributors together with the color-producing enzyme systems described previously (p. 6). One of the anthocyanins has been isolated from the rind of Purple Mauritius cane and converted with methanolic hydrogen chloride to a red-brown crystalline solid, $C_{28}H_{33}O_{17}Cl \cdot 4H_2O$ ⁶⁹; it is a diglucoside and it possesses one methoxyl group. Qualitative tests show that the aglycon is probably a mono-*O*-methyl-delphinidine. Chlorophyll is largely insoluble in the cane juice and is removed in the mill scums.³⁴ That a small amount is soluble, however, is established by the chromatographic isolation of a green-colored fraction from final molasses⁷⁰ giving the ultraviolet absorption spectrum of chlorophyll a.

An amorphous, incrustating solid, resembling lignin and designated "saccharetin" is mechanically dispersed in the juice during the grinding of the cane. It is a water-insoluble, weakly acidic substance with the reported formula $(C_6H_7O_2)_n$.⁷¹ It dissolved readily in alkali to yield an intense yellow solution, gave a red color with phloroglucinol and hydrochloric acid, and an orange-yellow color with aniline sulfate and sulfuric acid. Dry distillation of "saccharetin" yielded pyrogallol; alkali fusion gave protocatechuic acid and pyrocatechol; acid hydrolysis produced vanillic acid and vanillin. The lignin of bagasse has recently been isolated by an enzymic method and characterized.⁷²

Anthocyanins, tannins and "saccharetin" contain phenolic groups; their color contribution is increased by the iron dissolved from the

(68) F. W. Zerban, *J. Ind. Eng. Chem.*, **10**, 814 (1918).

(69) C. J. Dasa Rao, D. G. Walawalkar and B. S. Srikantan, *J. Indian Chem. Soc.*, **15**, 27 (1938).

(70) W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **70**, 290 (1948).

(71) L. G. Langguth Steuerwald, *Arch. Suikerind.*, **19**, 1543 (1911); *Chem. Abstracts*, **6**, 691 (1912).

(72) G. de Stevens and F. F. Nord, *J. Am. Chem. Soc.*, **73**, 4622 (1951); **74**, 3326 (1952); **75**, 305 (1953); *Proc. Natl. Acad. Sci. U.S.*, **39**, 80 (1953).

sugar mill and by the contact of air with the warm alkaline defecation solutions.⁷³

7. Waxes, Sterols and Lipids

A small amount of the wax coating of sugar cane is dispersed in the juice during grinding. Extraction with petroleum ether will remove it from the raw juice.⁷⁴ The wax and other similar substances are almost completely removed by the normal juice defecation and are found in the settlings (muds, filter-press cake); a minute quantity is carried through the entire process and is found in the final molasses (p. 21). The crude wax content of Louisiana whole cane is 0.2% and that of press cake may run to 22% (dry basis).⁷⁵ Dried filter-press muds from the northern British West Indies islands and from Jamaica, Trinidad and British Guiana have an average wax content of 14.7, 11.3, 8.8 and 7.0%, respectively.⁷⁶

Chromatography, on alumina, fractionated Louisiana cuticle cane wax (scraped from the stalk surface) into three groups: free acids, free alcohols and other substances (esters, ketones, hydrocarbons).⁷⁷ Slight to almost complete hydrolysis occurred during the formation of these chromatograms. Identification of the individual components of the fractions was not completed. An empirical analysis of Louisiana cuticle cane wax is presented in Table I.

TABLE I
Chemical Data on Louisiana Sugarcane Cuticle Waxes⁷⁵

Source	Saponification Value	Acid Value	Iodine Value	Acetyl Value
Co* 290	40.5	16.0	8.0	91.8
Co* 281	56.7	23.8	15.6	—

* Seedling variety from Coimbatore Experiment Station (India).

The nature of the non-acid fraction of the wax is of interest.⁷⁵ Some glycerol is present but the main alcohols are the higher-carbon monohydric alcohols, ceryl ($n\text{-C}_{25}\text{H}_{51}\text{—CH}_2\text{OH}$, isolated from Louisiana wax) and melissyl (isolated from Cuban molasses⁷⁰). The latter is probably a

(73) F. W. Zerban, *Sugar Research Foundation, Tech. Rept. Series*, 2 (1947).

(74) N. G. Chatterjee, *J. Indian Chem. Soc., Ind. and News Ed.*, 3, 183 (1940).

(75) R. T. Balch, *Sugar Research Foundation, Tech. Rept. Series*, 3 (1947).

(76) L. F. Wiggins, *Proc. Brit. West Indies Sugar Technol.*, 16 (1950).

(77) T. W. Findley (with J. B. Brown), Ph.D. Dissertation, The Ohio State University, 1950.

mixture of $n\text{-C}_{29}\text{H}_{59}\text{-CH}_2\text{OH}$ and $n\text{-C}_{31}\text{H}_{63}\text{-CH}_2\text{OH}$. The sterol content (free and combined) of Louisiana wax is in the range of 5–10%.⁷⁵ The sterol fraction consists⁷⁵ of 3 parts of stigmasterol (m. p. 171°, $[\alpha]_D -51^\circ$ in chloroform; acetate: m. p. 144°, $[\alpha]_D -55^\circ$ in chloroform) and 7 parts of a mixture of β -sitosterol and γ -sitosterol (m. p. 137–143°, $[\alpha]_D -38^\circ$ in chloroform).

8. Inorganic Components

Crusher juice and whole raw cane juice contain representative inorganic constituents; clarified juice is limed. The inorganic substances

TABLE II
Inorganic Components of Juice from Louisiana-grown Cane Varieties Coimbatore 281 and 290⁷⁶

Component	Co. 281 ^a	Co. 290 ^a
K ₂ O	1.603%	1.792%
Na ₂ O	0.060	0.056
CaO	0.216	0.189
MgO	0.248	0.263
Al ₂ O ₃	0.088	0.067
Fe ₂ O ₃	0.0104	0.01
MnO	0.0034	0.0031
SO ₂	0.543	0.455
P ₂ O ₅	0.415	0.400
Cl	0.191	0.246
SiO ₂	0.094	0.096

^a Expressed as per cent on solids.

or ash contents vary greatly among the different varieties of cane within a confined area and in a single variety grown in the different cane producing areas. The principal cations of cane juice are potassium, magnesium, calcium, aluminum, sodium, iron and manganese; anions are sulfate, phosphate, chloride and silicate (Table II). Raw juice contains more inorganic cations than inorganic anions even though it is acid, pH 5.2–6.2; this acidity is due to the presence of organic acids.

9. Summary

Table III summarizes the data on cane juice constituents.

(78) C. A. Fort and N. McKaig, *U. S. Dept. Agr., Tech. Bull.* 688 (1939).

TABLE III
 Cane Juice Constituents

Component	Juice Origin ^a	Concentration, % ¹	Reference
Carbohydrates			
Sucrose	La.	78-84	78
D-Glucose	La. }	4.3-7.8	78
D-Fructose	La. }		
myo-Inositol	La.	0.041	21
Phytin	Cuba	—	21
Pentosans	Trin.	0.04-0.07	11
Methoxyl	La.	0.08	15
Pectins	Trin.	0.0-0.1	11
Uronic acids	La.	0.44	15
Starch	La.	0.012-0.018	18
Organic phosphate	Cuba	0.0187	25
Enzymes			
Invertase	La.	—	32, 34
Oxidase	La.	—	34
Peroxidase	La.	—	34
Tyrosinase	La.	—	34
Vitamins			
Vitamin A	La.	—	36
Biotin	La.	2.2×10^{-5}	37
	Cuba	17.6×10^{-5}	37
Vitamin D (?)	La.	—	36
Niacin	La.	4.9×10^{-5}	37
	Cuba	4.5×10^{-5}	37
Pantothenic acid	La.	218×10^{-5}	37
	Cuba	99.4×10^{-5}	37
Riboflavin	La.	3.1×10^{-5}	37
	Cuba	4.9×10^{-5}	37
Thiamine	La.	5.3×10^{-5}	37
	Cuba	10.5×10^{-5}	37
Nitrogen compounds			
Amino acids			
Alanine	Fla., Jam.	—	43, 45
γ -Aminobutyric acid	Fla., Jam.	—	43, 45
L(<i>levo</i>)-Asparagine	Fla., P. R., Jam.	—	43-45
Aspartic acid	Fla., Jam.	—	43, 45
Glutamic acid	Fla., Jam.	—	43, 45
L(<i>dextro</i>)-Glutamine	Fla., P. R., Jam.	—	43-45
Glycine	Fla., Jam.	—	43, 45
L-Isoleucine	Fla.	0.0010	46
L-Leucine	Fla.	0.0025	46
Lysine	Jam.	—	45
L-Methionine	Fla.	0.009	46
Serine	Fla., Jam.	—	43, 45
L-Tryptophan	Fla.	0.0034	46
Tyrosine	Fla., P. R.	—	43, 44
L-Valine	Fla.	0.0018	46

TABLE III (Continued)

Component	Juice Origin ^a	Concentration, % ¹	Reference
Proteins	Java	—	42
	La.	0.33-0.49	78
Non-nitrogenous acids ⁶⁵	La.	1.79-3.48	78
	Trin.	0.6-1.2	79
Aconitic	La.	0.3-2.1	54, 65
Fumaric	La.	—	65
Glycolic	Hawaii	—	61
Malic	Java	—	55-58
Oxalic	La.	—	55, 62, 63
Succinic	La.	—	65
Pigments	Trin.	0.73-1.58	79
Anthocyanins	La.	—	34
Chlorophyll	La., Cuba	—	34, 70
"Saccharetin"	La.	—	34
Tannins	La.	—	68
Waxes, sterols and lipids	La., Trin.	0.73-1.58	78, 79
Nonsaponifiable fraction	La.	38.9 ^b	75
Palmitic acid	La.	16.9 ^b	75
Oleic acid	La.	9.1 ^b	75
Linoleic acid	La.	31.4 ^b	75
Linolenic acid	La.	1.0 ^b	75
Inorganic components	La.	2.6-3.6	78
	Trin.	1.2-3.0	79

^a Barb. = Barbados; Fla. = Florida; Jam. = Jamaica; La. = Louisiana; P. R. = Puerto Rico; Trin. = Trinidad.

^b Percentage of crude cane wax from press cake.

III. COMPOSITION OF CANE FINAL MOLASSES

During the production of sucrose from cane juice, crystallization inhibitors collect in the residual sirups or mother liquors; these sirups are called molasses. When the accumulation of these inhibitors is so great that the recovery of sucrose is no longer economically feasible, this molasses is known as the final or blackstrap molasses. The term "blackstrap" originated in the Dutch sugar industry from black "stroop" meaning black sirup.⁸⁰ The molasses obtained in the early stages of sucrose production has a pleasant, palatable flavor and is used in the preparation of edible molasses. While some molasses intermediate between edible and blackstrap are best suited for the production of calcium magnesium aconitate,⁸¹ far more aconitate is recovered from blackstrap (diluted to 55° Brix) which is available all year. In the recrystalli-

(79) F. Hardy, *Planter Sugar Mfr.*, 79, 445 (1927).

(80) Noel Deerr, *Intern. Sugar J.*, 47, 123 (1945).

(81) L. Godchaux, II, *Sugar J.*, 10, 2 (1947).

zation or refining of the crude sucrose, the final mother liquor concentrate is known as refinery blackstrap molasses. It can be considered as consisting of the accumulated blackstrap molasses originally adherent to the crude sucrose crystals.

In the crystallization of sucrose from cane juice, the pH of the normal juice entering the process lies in the range 5–6. This juice contains a little water added during the crushing process. Calcium hydroxide is added to bring the pH to 8 ± 0.5 and the mixture is heated to $220^{\circ}F.$ and maintained around $200^{\circ}F.$ for several hours. This is the defecation process and results in a clarification of the liquid with the precipitation of suspended materials, proteins, waxes and fats.^{81a} It is closely controlled and varies slightly with the processor. After passing through the settlers the pH is now approximately 7. The muds from the settlers are removed with continuous rotary filters, resulting in filter cake. The clarified juice is heated to $225^{\circ}F.$ in the first of a bank (usually four) of multiple-effect vacuum evaporators for a period of ten minutes or less and for longer periods at lower temperatures in subsequent evaporators as the juice is concentrated to a sirup. The crystallization of sucrose from this sirup is a batch operation and is accomplished with single vacuum evaporators ("pans"). During these processes the mother liquors are recirculated and fresh defecated juice is added. The final residual sirup is blackstrap molasses; its pH is 5.8.

1. Carbohydrates

As in cane juice, sucrose is the principal sugar of cane blackstrap molasses. However, the ratio of sucrose to apparent reducing sugars has dropped from 10–15:1 in the juice to 1.5–2.5:1 in the molasses. Patents have been issued for the recovery of sucrose from molasses but general acceptance of these processes by the industry is still lacking. Some of the methods depend on the removal from molasses of the reducing sugars and other impurities by lime,⁸² invertase-free yeast,⁸³ barium hydroxide,⁸⁴ or fuller's earth clay.⁸⁵ Other methods are based upon the use of solvents⁸⁶ and of ion-exchange resins.⁸⁷ The significant simple sugars are

(81a) P. Honig, *Sugar*, 47, No. 6, 31 (1952).

(82) E. E. Battelle, U. S. Pats. 1,044,003 (1913), 1,044,004 (1913).

(83) H. De F. Olivarius, U. S. Pats. 1,730,473 (1929), 1,788,628 (1931).

(84) A. L. Holven, U. S. Pats. 1,878,144 (1933), 1,878,145 (1933).

(85) M. L. Wolfrom and W. W. Binkley, U. S. Pat. 2,504,169 (1950); *J. Am. Chem. Soc.*, 69, 664 (1947).

(86) J. H. Payne, U. S. Pat. 2,501,914 (1950).

(87) N. V. Octrooien Maatschappij, Dutch Pat. 58,496 (1946); *Chem. Abstracts*, 41, 4667 (1947).

D-glucose and D-fructose. The actual isolation of crystalline D-glucose was accomplished by the chromatography of Cuban blackstrap molasses⁸⁸ on fuller's earth clay⁸⁹; D-fructose was obtained as a sirup which yielded crystalline acetates of the β -D-pyranose and *keto* forms of this sugar after acetylation and chromatography⁹⁰ on magnesium acid silicate.

The precipitation from molasses of D-mannose as its phenylhydrazone was reported over 50 years ago.⁹¹ Recent investigation of the phenylhydrazone from Cuban molasses did not reveal any D-mannose phenylhydrazone⁹²; the presence of this sugar in cane molasses cannot be considered as demonstrated.

Early investigators⁹³ considered an unestablished sugar designated "glutose" to be an unfermentable, molasses component and to be isolable from this unfermentable residue as its phenylosazone. Later work⁹⁴ showed that "glutose phenylosazone" was an impure D-glucose phenylosazone.

The presence of D-psicose (D-ribo-hexulose, D-allulose) in distillery slop (from the fermentation of cane molasses) is claimed,⁹⁵ but sharp experimental support is lacking.

Diheterolevulosans (difructose dianhydrides) are obtained by refluxing concentrated aqueous solutions of D-fructose.^{96,97} Chromatography of Cuban blackstrap molasses in a pilot-plant-scale chromatogram on fuller's earth clay did not reveal the presence of these substances.⁹⁸

Traces of the complex carbohydrates contained in the juice survive the processing and appear in the molasses; analytical data indicate that they probably consist largely of pectins and pentosans.¹⁰ Quantitative estimations based on the ash-free solids of Louisiana molasses revealed the presence of 2% uronic acids and 0.5% methoxyl.¹⁵ Heat-modified

(88) W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **72**, 4778 (1950).

(89) B. W. Lew, M. L. Wolfrom and R. M. Goepp, Jr., *J. Am. Chem. Soc.*, **68**, 1449 (1946).

(90) W. H. McNeely, W. W. Binkley and M. L. Wolfrom, *J. Am. Chem. Soc.*, **67**, 527 (1945).

(91) C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **16**, 260, 280 (1897).

(92) M. Grace Blair (with M. L. Wolfrom), Ph. D. Dissertation, The Ohio State University, 1947.

(93) H. C. Prinsen Geerligs, *Intern. Sugar J.*, **40**, 345 (1938). Review article.

(94) L. Sattler, *Advances in Carbohydrate Chem.*, **3**, 113 (1948).

(95) F. W. Zerban and L. Sattler, *Ind. Eng. Chem.*, **34**, 1180 (1942).

(96) L. Sattler and F. W. Zerban, *Ind. Eng. Chem.*, **37**, 1133 (1945).

(97) M. L. Wolfrom and M. Grace Blair, *J. Am. Chem. Soc.*, **70**, 2406 (1948).

(98) W. W. Binkley and M. L. Wolfrom, *Sugar Research Foundation, Member Rept.* **25**, 21 (1950).

starches are probably contained in the molasses from the starch-bearing cane juices.^{17,18}

Crystalline *myo*-inositol (see p. 4) was isolated from Cuban final molasses by chromatography on fuller's earth clay.⁸⁸ The *myo*-inositol content (0.261%) of this molasses was determined by fermentation with yeast, acetylation of the unfermented residue, chromatography on magnesium acid silicate of the acetylated residue and the isolation of crystalline *myo*-inositol hexaacetate. The value obtained by bioassay was 0.238%.²¹ The phytin content of Cuban molasses was estimated to be 0.22–0.23% by bioassay.²¹ A small amount of *D*-mannitol as the crystalline hexaacetate was obtained from this molasses by these chromatographic procedures.⁸⁸ In addition to the *D*-mannitol produced by the bacteria in the sugar mill, this hexitol may be introduced bacterially in the cane juice, thus leading to its accumulation in the final molasses. Extension of these chromatographic techniques to the unfermented residue of Cuban molasses led to the isolation of erythritol and *D*-arabitol (as their respective acetates) as trace constituents⁸⁸; the yeast may be the source of these substances.

Another important carbohydrate group of cane final molasses, and one which has been little studied, consists of the products formed by the action of heat, alkali and amino acids upon the reducing sugars. This is sometimes termed the non-fermentable fraction as it remains after yeast fermentation although it is thereby badly contaminated with metabolic products from the yeast.

The reducing sugars *D*-glucose and *D*-fructose are first formed by the action of invertase on sucrose. These are then subjected to the action of hot alkali (*pH ca.* 8) during the defecation and to considerable heat treatment at *pH* 6–7 thereafter (see p. 14). Monosaccharide decomposition involving dehydration, disproportionation, and fragmentation, is involved. Dehydration leads necessarily to dicarbonyl compounds^{99,100} which are probably significant reaction intermediates and may form furan derivatives (Fig. 1 and Sections III-4 and III-5). Chain fragmentation by reverse aldolization¹⁰¹ may play a part (Fig. 1 and Section III-4). In addition, the amino acids present undoubtedly react with the reducing sugars, or their dehydration products, to yield dark colored polymeric substances containing nitrogen. This is known as the Mail-

(99) M. L. Wolfrom, E. G. Wallace and E. A. Metcalf, *J. Am. Chem. Soc.*, **64**, 265 (1942).

(100) M. L. Wolfrom, R. D. Schuetz and L. F. Cavalieri, *J. Am. Chem. Soc.*, **71**, 3518 (1949).

(101) J. F. Haskins and M. J. Hogsed, *J. Org. Chem.*, **15**, 1275 (1950).

lard¹⁰² or "browning" reaction.¹⁰³⁻¹⁰⁵ It leads to the production of color and finally to the separation of brown to black solids known as "melanoidins."

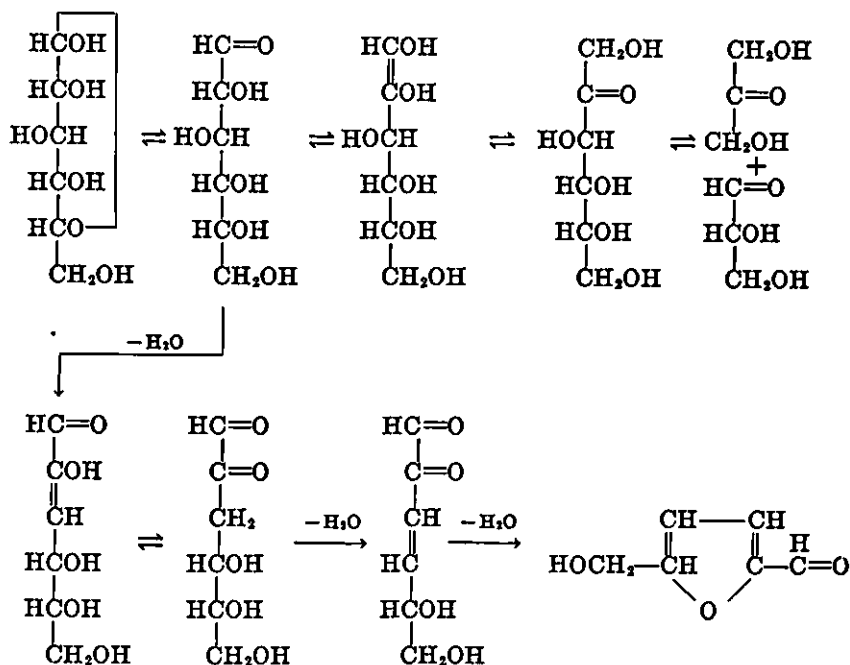


FIG. 1.—Possible Routes of Sugar Decomposition.

These various products of sugar decomposition are reducing^{88,106} so that the consideration of this analytically measurable property as being due solely to D-glucose and D-fructose is in error. Their total content has been estimated¹⁰⁷ to be 10.1 and 10.7% of the solids in final molasses samples from Louisiana and Cuba, respectively. This fraction was separated by clay chromatography⁸⁸ and its more complex components were segregated by dialysis and freeze-drying. The product so obtained was a brown, bitter, non-hygroscopic solid that contained nitrogen (1.7%) and exhibited a slight Fehling reduction. The major fraction of the

(102) L.-C. Maillard, *Compt. rend.*, **154**, 66 (1912); *Ann. chim.*, [9] **5**, 258 (1916).

(103) J. P. Danehy and W. W. Pigman, *Advances in Food Research*, **3**, 241 (1951).

(104) M. L. Wolfrom, R. C. Schlicht, A. W. Langer, Jr., and C. S. Rooney, *J. Am. Chem. Soc.*, **75**, 1013 (1953).

(105) L. Sattler and F. W. Zerban, *Ind. Eng. Chem.*, **41**, 1401 (1949).

(106) C. Erb and F. W. Zerban, *Ind. Eng. Chem.*, **39**, 1597 (1947).

(107) C. A. Fort, *Sugar*, **41**, No. 11, 36 (1946).

material when subjected to dialysis passed through the membrane in the manner of relatively low molecular weight substances.¹⁰⁸

2. Vitamins

The desugaring of cane juice concentrates the heat- and alkali-stable vitamins in the final molasses. Even after this accumulation, only *myo*-inositol may have reached the level of minimum dietary requirements.¹⁰⁹ Niacin, pantothenic acid and riboflavin are also present in significant quantities¹⁰⁹; the thiamine, pyridoxin, pantothenic acid, biotin and folic acid contents of molasses have been estimated by bioassay.^{110,111} The biotin content of Hawaiian and Cuban molasses was 2.1 and 1.7 gammas per gram, respectively.¹¹² The "antistiffness factor" (closely related to stigmasterol) has been found in cane molasses.^{38,39} The distillery slop from the yeast fermentation of molasses is marketed as a vitamin concentrate; this product also contains vitamins originating in the yeast.

3. Nitrogen Compounds

The nitrogen content of the cane blackstraps of North America varies between 0.4 and 1.4%. A surprisingly large fraction, as much as 60-70%, of this nitrogen is present in a relatively simple form.^{43,113} Isolations based on identified crystalline products, obtained from Hawaiian molasses with the aid of ion-exchange resins, are recorded¹¹³ for aspartic acid (as the free acid), glutamic acid (as the hydrochloride), lysine (as the picrate) and the purines guanine (as the hydrochloride) and xanthine (as the free base); qualitative tests were obtained for hypoxanthine and the pyrimidine 5-methyl-cytosine. Aspartic and glutamic acids occur in the plant mainly as their amides asparagine and glutamine but in the mill processing these are hydrolyzed with the evolution of ammonia. The amino acids from Florida molasses were concentrated on fuller's earth clay and were resolved by paper chromatography employing spot enhancement with known specimens⁴³; the presence of asparagine, aspartic acid, glutamic acid, γ -aminobutyric acid, alanine, glycine, leucine or isoleucine, and valine were so established.

Albumins and other protein-like substances of cane juice are precipi-

(108) W. W. Binkley and M. L. Wolfrom, unpublished results.

(109) R. C. Hockett, *J. California State Dental Assn.*, 26, No. 3 Suppl., 72 (1950).

(110) D. Rogers and M. N. Mickelson, *Ind. Eng. Chem.*, 40, 527 (1948).

(111) W. A. Krehl and G. R. Cowgill, unpublished results.

(112) E. E. Snell, R. E. Eaken and R. J. Williams, *J. Am. Chem. Soc.*, 62, 175 (1940).

(113) J. H. Payne with R. F. Gill, Jr., *Hawaiian Planters' Record*, 50, No. 2, 69 (1946).

tated during defecation and only traces of them reach the final molasses. The principal bearers of complex nitrogen are the molasses "browning" products mentioned in the preceding section.

4. *Non-nitrogenous Acids*

Aconitic acid was the first organic acid properly established as a component of cane molasses.⁵³ Louisiana molasses is a rich source and contains more than 6% of this acid in some cases.¹¹⁴ With a potential annual harvest of over 5,000,000 pounds of aconitic acid from Louisiana alone, it is nevertheless only recently that the commercial production of this acid from molasses has been initiated in Louisiana.¹¹⁵ Its isolation depends upon the insolubility and ready crystallizability of dicalcium magnesium *trans*-aconitate hexahydrate.^{114,116-118} Final, or an intermediate or "B", molasses is diluted to 55% solids (55° Brix) with the appropriate wash water from the process (see below) and the pH is adjusted to 7 with lime. The solution is heated to 200°F. and calcium chloride is added. Heating is continued for forty-five minutes whereupon the precipitate is collected. It is resuspended at 195°F. in a volume of water sufficient to adjust the next lot of molasses to 55° Brix. The precipitate is again centrifuged; it contains about 56% aconitic acid.¹¹⁵ Aconitic acid, either as such or after conversion to itaconic acid, is of present-day commercial interest.^{118a}

Malic and citric acids have been adequately identified from molasses as their crystalline hydrazides.¹¹⁹ It is probable that at least the former is a normal juice constituent. Lactic acid was identified as its zinc salt in molasses¹¹⁹; it arises from bacterial action. Formic acid is present¹¹⁹; it probably has an origin, at least in part, in sugar decomposition. Acetic and propionic acids are components and their amounts serve as a rough index of the activity of the microorganisms introduced into the molasses. The microbial count of cane juice, molasses and related products has been determined (Table IV).¹²⁰

Application of modern ion-exchange and silica-gel chromatographic techniques to cane distillery slop (cane molasses yeast fermentation

(114) R. T. Balch, C. B. Broeg and J. A. Ambler, *Sugar*, **40**, No. 10, 32 (1945); **41**, No. 1, 46 (1946); see Ref. 133.

(115) L. Godchaux, II, *Sugar J.*, No. 4, 3 (1949).

(116) E. K. Ventre, J. A. Ambler and S. Byall, U. S. Pat. 2,359,537 (1944).

(117) J. A. Ambler, J. Turer and G. L. Keenan, *J. Am. Chem. Soc.*, **67**, 1 (1945).

(118) E. K. Ventre, U. S. Pat. 2,469,090 (1949).

(118a) R. N. Evans, *Abstracts Papers Am. Chem. Soc.*, **119**, 3Q (1951).

(119) E. K. Nelson, *J. Am. Chem. Soc.*, **51**, 2808 (1929).

(120) C. H. Millstein, L. C. Tobin and C. S. McCleskey, *Sugar J.*, **3**, No. 9, 13 (1941).

residue) led to the isolation of crystalline aconitic acid as the major organic acid component.⁶⁵ The steam distillation of acidified cane distillery slop yielded formic and acetic acids; they were identified by qualitative tests.¹²¹ Esterification and ester distillation followed by the formation of crystalline hydrazides led to the adequate identification of succinic, tricarballylic and perhaps citric as component acids.¹²¹ Ether extraction of diluted acidified slop and fractional precipitation in aqueous ethanol of the barium salts of the extracted acids placed succinic and

TABLE IV
Microbial Count^a of Sugar Mill Products¹²⁰

Product	Mesophilic ^b Bacteria		Thermophilic ^c Bacteria	
	Low	High	Low	High
Raw juice	8,000,000	750,000,000	14	170
Clarifier effluent	0	11	0	8
Press juice	0	51,000	3,700	250,000
Evaporator sirup	200	3,300	300	15,500
Storage tank sirup	1,300	7,100	16,100	38,500
Crystallizer contents	2,000	44,000	350	15,000
Masseccuite	1,200	10,600	1,700	17,100
Raw sugar	340	5,100	100	2,200
Molasses	300	310,000	1,200	16,500

^a Number of bacteria per ml. ^b Optimal growth at 15–40°C. ^c Optimal growth at 40–60°C.

tricarballylic with a trace of aconitic acids in the precipitate and lactic acid (isolated as the crystalline zinc salt) in the supernatant liquor.¹²¹

Other acids (often as esters) have been found in fermented molasses. Usually these substances are products of bacteriological action and they are not normal constituents of unfermented molasses. "Bauer" oil from the yeast fermentation of Cuban blackstrap consists chiefly of the ethyl esters of capric, lauric, myristic and palmitic acids.¹²² The fat from the scums of hot-room Louisiana molasses contained hexanoic (caproic) and octanoic (caprylic) acids.¹⁰ The occurrence of such volatile acids as propionic,¹²³ butyric^{123,124} and valeric acids¹²⁴ requires more adequate establishment.

Qualitative tests showed the probable presence of 5(hydroxymethyl)-2-furaldehyde, acetoin, levulinic and formic acids, and methylglyoxal

(121) E. K. Nelson and C. A. Greenleaf, *Ind. Eng. Chem.*, **21**, 857 (1929).

(122) C. S. Marvel and F. D. Hager, *J. Am. Chem. Soc.*, **46**, 726 (1924).

(123) N. Srinivasan, *Intern. Sugar J.*, **41**, 68 (1939).

(124) E. Humboldt, *Facts About Sugar*, **25**, 18 (1930).

(or acetol) in the volatile decomposition products from the unfermentable residue of a heated D-fructose solution¹⁰⁵; these are sugar decomposition products. The volatile products from a fermented sucrose mixture contained small quantities of acetylmethyl carbinol.¹⁰

5. Pigmented Materials

The principal pigmented substances of final molasses are probably the complex products of the reaction between the reducing sugars and the amino-containing components of the cane juice (see Section III-1). The reducing-sugar self-decomposition in the presence of organic anions, especially aconitate, is probably also a factor and here the anion may serve principally as a buffer and probably does not enter into reaction with the reducing sugar or with its decomposition products. Model experiments¹²⁵ at pH 8 indicate that the main chemical color-producing system in molasses is that of D-fructose and D-glucose with asparagine followed by that of D-fructose and D-glucose in the presence of aconitate ion. Concentrated aqueous solutions of D-fructose heated at sugar mill temperature will produce a dark colored solution in the absence of amino acids.^{96,97}

The polyphenolic colored substances of cane juice, largely tannins and anthocyanins,³⁴ form even more intensely colored iron complexes. Some of these compounds survive defecation and darken further with prolonged exposure to air and ferric ion at elevated temperatures.

Virtually pure chlorophyll a was isolated from Cuban molasses by chromatographic and extraction procedures⁷⁰; it was identified by its absorption spectrum; its estimated concentration was 0.00005%.

6. Waxes, Sterols and Lipids

The concentration of fats and related substances in molasses is low; analytical values depend on the extracting solvent.¹²⁶ These tenaciously retained materials can be removed by fractionation of blackstrap on fuller's earth clay.⁷⁰ Chromatography on a calcium silicate of the fat fraction of Cuban molasses led to the isolation of melissyl alcohol, a phytosterol fraction, chlorophyll a and a fat fraction containing a glyceride of linoleic acid.⁷⁰ Stigmasterol and syringic acid are reported as ether-extractable constituents of molasses.¹²⁷

(125) J. N. Schumacher (with M. L. Wolfrom), M. Sc. Thesis, The Ohio State University, 1952.

(126) C. F. Bardorf, *Can. Chem. Met.*, **11**, 231 (1927).

(127) S. Takei and T. Imaki, *Bull. Inst. Phys. Chem. Research (Tokyo)*, **15**, 1055 (1936).

7. Odorants

The ether extract of cane molasses yields an acidic substance with the characteristic odor of raw sugar.¹²⁸ The steam distillation of molasses is stated to yield a "rum oil."¹²⁹ Fractionation of cane final molasses on fuller's earth clay produces a concentrate with a strong molasses odor.⁷⁰ The infrared spectra of the volatile portion of this concentrate indicated the absence of hydroxyl and carbonyl and the presence of a substituted benzene structure, of paraffinic methylene and methyl groups, of an acetate group, and of the $>C=C<$ and $-C\equiv C-$ linkages. The presence of a sulfur function is probable. Further chromatography indicated complexity in this volatile concentrate.¹³⁰

The deionized unfermentable (by yeast) residue from Cuban final molasses has a raisin-like odor.²¹

8. Inorganic Components

The mineral constituents of the raw cane juice persist in the final molasses. The principal difference in relative amounts of these substances in molasses arises from the use of lime in defecation which causes an increase in calcium. Egyptian cane molasses solids contained 0.66% of titanium.¹³¹ The cations are believed to complex with the sugars and to thus inhibit the crystallization of sucrose, which latter is known to form compounds with inorganic salts, such as its well known compound with sodium chloride. Decationization of cane juice with ion exchange resins greatly reduces molasses formation but sucrose inversion is a concomitant problem.¹³²

9. Summary

Table V summarizes the data on the constituents of cane final molasses.

(128) S. Takei and T. Imaki, *Bull. Inst. Phys. Chem. Research (Tokyo)*, **15**, 124 (1936).

(129) E. Arroyo, *Univ. Puerto Rico Agr. Expt. Sta. Research Bull.* **5** (1945); D. Kervégant, "Rhums et eaux-de-vie de canne," Les Editions du Golfe, Vannes, France, 1946.

(130) M. L. Wolfrom, W. W. Binkley and Florinda O. Bobbio, *El Crisol*, in press (1953).

(131) E. O. von Lippmann, *Ber.*, **58**, 426 (1925).

(132) J. H. Payne, H. P. Kortachak and R. F. Gill, Jr., *Ind. Eng. Chem.*, **44**, 1411 (1952).

TABLE V
Cane Final Molasses Constituents

Component	Molasses Origin ^a	Concentration, % ¹	Reference
Carbohydrates	La.	80.52 ^b	107
	Cuba	78.90 ^b	107
Sucrose	La.	37.39	107
	Cuba	47.28	107
"Reducing sugars"	La.	32.72	107
	Cuba	20.98	107
D-Glucose	Cuba	6.9	88
D-Fructose	Cuba	1.6	88
myo-Inositol	Cuba	0.261	21
Phytin	Cuba	0.225	21
D-Mannitol	Cuba	0.6	21
Uronic acids	La.	2.0	15
Methoxyl	La.	0.8	15
Sugar "reaction products"	La.	10.1	107
	Cuba	10.7	107
Vitamins			
Biotin	Cuba	17.0 × 10 ⁻⁵	111
Folic acid	Cuba	0.43 × 10 ⁻⁵	111
Nicotinic acid	Cuba	222 × 10 ⁻⁵	111
Pantothenic acid	Cuba	635 × 10 ⁻⁵	111
Pyridoxine	Cuba	19.1 × 10 ⁻⁵	111
Riboflavin	Cuba	24.4 × 10 ⁻⁵	111
Thiamine	Cuba	8.5 × 10 ⁻⁵	111
Nitrogen Compounds			
Total nitrogen	Cuba	0.89	108
	Fla.	1.40	108
	Hawaii	0.71	108
	La.	0.38	108
	P. R.	1.16	108
Amino Acids			
Alanine	Fla.	—	43
γ-Aminobutyric acid	Fla.	—	43
Asparagine	Fla.	—	43
Aspartic acid	Fla., Hawaii	—	43, 113
Glutamic acid	Fla., Hawaii	—	43, 113
Glycine	Fla.	—	43
Leucine (or isoleucine)	Fla.	—	43
Lysine	Hawaii	—	113
Valine	Fla.	—	43
Nucleic acid bases			
Guanine	Hawaii	—	113
Hypoxanthine	Hawaii	—	113
5-Methylcytosine	Hawaii	—	113
Xanthine	Hawaii	—	113

TABLE V (Continued)

Component	Molasses Origin ^a	Concentration, % ¹	Reference
Non-nitrogenous acids	Cuba	7.59	107
	La.	7.39	107
Aconitic	La.	4.95	133
	P. R.	0.95	119
Malic	P. R.	—	119
Citric	P. R.	—	119
Formic	P. R.	0.12	119
Lactic	P. R.	0.60	119
Acetic	P. R.	0.24	119
Bacteria			
Mesophilic	La.	300–310,000 ^c	120
Thermophilic	La.	1,200–16,500 ^c	120
Pigments			
Chlorophyll a	Cuba	5×10^{-5}	70
“Browning products”	La., Cuba	10.1–10.7	107
Tannins	La.	—	34
Anthocyanins	La.	—	34
Waxes, sterols and lipids	Cuba	0.50	70
Melissyl alcohol	Cuba	—	70
Phytosterol	Cuba	—	70
Stigmasterol	Formosa (?)	—	127
Syringic acid	Formosa (?)	—	127
Odorants			
Molasses odor fraction	Cuba, Hawaii	—	70, 130
Inorganic components	La.	13.46 ^d	107
	Cuba	13.76 ^d	107

^a Fla. = Florida; La. = Louisiana; P. R. = Puerto Rico. ^b Total solids in the molasses. ^c Number of bacteria per ml. of molasses. ^d Carbonate ash.

ADDENDUM

Non-nitrogenous Acids in Cane Juice

Application¹³⁴ to Louisiana cane juice of the procedure of Ramsey and Patterson,¹³⁵ as modified by Marvel and Rands,⁶⁴ leads to the following acid assay: aconitic, 2.07%; malic, 0.28%; citric, 0.22%; mesaconic, 0.058%; succinic, 0.04%; fumaric, 0.023%; glycolic, oxalic and an unknown acid, present. Wiggins^{25a} has tentatively identified, by paper chromatography, succinic, malic, aconitic, citric, glycolic, and possibly glyoxalic acids.

(133) C. A. Fort, B. A. Smith, C. L. Black and L. F. Martin, *Sugar*, **47**, No. 10, 33 (1952).

(134) E. J. Roberts, C. A. Fort and L. F. Martin, *Abstracts Papers Am. Chem. Soc.*, **124**, 10D (1953).

(135) L. L. Ramsey and W. I. Patterson, *J. Assoc. Offic. Agr. Chemists*, **28**, 644 (1945).

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- No. 3. *The Utilization of Sugar Cane Bagasse for Paper, Boards, Plastics, and Chemicals—An Annotated Bibliography*. Compiled by Dr. Clarence J. West, The Institute of Paper Chemistry (March 1946); Supplement (April 1949). Revised edition issued as Technological Report No. 8 (Feb. 1952).
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