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SOME ORGANIC ACIDS OF SUGAR CANE MOLASSES

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In continuation of the work in this Laboratory on the non-volatile acids of fruits and plant products, an investigation was made of the acids of sugar cane molasses.[1] While considerable work has been done on this subject, it appeared to be worth while to determine the acids of molasses by the ester distillation method.

The organic acids of cane molasses comprise the acids naturally occurring in cane juice, chiefly aconitic acid, and the acids formed during the process of manufacture, such as formic, acetic and lactic acids. Of the amino acids formed from the hydrolysis of proteins, aspartic and glutamic acids have been found in molasses. Dark colored uncrystallizable acids are also present, but none of these is considered in the present paper.

The presence of aconitic acid in cane juice was first noted by Arno Behr,[2] who separated it in the form of an acid ammonium salt.

Malaguti[3] records that although oxygen is without influence on neutral sugar solutions, formic acid is formed in the presence of weak acids. He concluded that the acid caused partial inversion and that the formic acid resulted from the action of oxygen on invert sugar. As early as 1851, formic and acetic acids were mentioned by Michaelis[4] as being present in beet molasses, and this has been confirmed by later investigations.

It has been shown by numerous investigators, among whom may be mentioned Niedschlag,[5] Isaac[6] and Tollens,[7] that acetic and lactic acids are formed by the action of alkalies on sucrose, and Tollens states that the lime treatment of beet juice probably produces most of the lactic acid found in the products of sugar manufacture, and that this acid may amount to 0.5% of the molasses.

More recently the acids in sugar cane juice have been investigated by Yoder,[8] who found aconitic acid and a small quantity of malic acid. From 6 liters of juice Yoder isolated 3 g. of aconitic acid and 0.046 g. of malic acid. He reported the absence of tartaric, succinic and citric acids.

[1] Acknowledgment is made to Dr. C. A. Browne, Assistant Chief of the Bureau of Chemistry and Soils, for suggesting this problem and for his helpful and sustained interest throughout the progress of the work.

[2] Behr, *Ber.*, **10**, 351 (1877).

[3] Malaguti, *Ann. Chemie*, [2] **59**, 412 (1835).

[4] Michaelis, *Z. Verh. Rubenzucker-ind.*, **1**, 114 (1851).

[5] Niedschlag, *Die Deutsche Zucker-ind.*, **12**, 159 (1887).

[6] Isaac, *Chem. News*, **66**, 39 (1892).

[7] Tollens, *Z. Verh. deutsche Zucker-ind.*, (O. S.) **39**, 322 (1889); *Ann.*, **255**, 228 (1889).

[8] Yoder, *J. Ind. Eng. Chem.*, **3**, 640 (1911).

The material used in the investigation reported in this paper was a sample of molasses from the Central Fajardo, Porto Rico.[9] The analysis accompanying the sample was as follows: Brix, 88.90; dry substance, 83.86; apparent purity, 31.00; true purity, 36.54; glucose, 28.86; sucrose, 30.64; ash, 7.52; dye value,[10] 3373.

The alkalinity of the ash was equivalent to 57.1 cc. of normal hydrochloric acid per 100 g. of sample and the free acid corresponded to 26.0 cc. of normal hydrochloric acid per 100 g.

The volatile acids consisted of a mixture of formic and acetic acids, 0.097 g. of formic and 0.2 g. of acetic acid being recovered per 100 g. of molasses.

The predominating non-volatile acid was aconitic acid (0.8%). Small quantities of malic, citric and lactic acids were found.

Experimental

Volatile Acids.—Volatile acids were distilled from three kilos of molasses to which sufficient hydrochloric acid had been added to liberate the combined acids. The distillate was neutralized with standard barium hydroxide solution and evaporated to dryness. The dried barium salts were weighed and from the weight, taken in conjunction with the quantity of barium necessary to neutralize the distillate, the proportion of barium formate and barium acetate was calculated.

The distillate required 657 cc. of 0.25 *N* barium hydroxide to neutralize it, and the barium salts weighed 20.089 g. From these data the weight of the barium formate was calculated to be 7.195 g. and that of the barium acetate, 12.894 g.

Non-Volatile Acids.—The non-volatile acids were precipitated as lead salts from two kilos of molasses. The acids recovered from the lead salts were dissolved in water and extracted four times with ether. The ether removed 4.06 g. of a crystalline acid melting at 185 to 186° and giving no depression in melting point on admixture with aconitic acid. The aqueous solution was then evaporated to dryness, esterified in the usual manner and the esters, 7.8 g., were fractionated at 10 mm. Less than 1 cc. distilled under 150°. This distillate afforded a hydrazide crystallizing like malic hydrazide, melting at 178 to 179° and showing no depression in melting point when mixed with malic hydrazide.

Fraction 2, when redistilled, boiled at 160° and weighed 4.5 g. As the hydrazide of aconitic acid is very hygroscopic and not easily identified, this fraction was saponified, acidified and extracted with ether, yielding pure aconitic acid. Four and one-half grams of ethyl aconitate correspond to three grams of aconitic acid.

The third fraction, boiling above 160°, measured 0.5 cc. When this fraction was treated with hydrazine hydrate, a hydrazide separated which had the appearance of citric hydrazide. It melted at 100 to 103°. Optical crystallographic examination[11] proved its identity as citric hydrazide.

In order to avoid losses by esterification, a direct, continuous ether extraction was made on the acids recovered by precipitation of three kilos of molasses with lead acetate.

[9] The Carbohydrate Division of this Bureau furnished material for this investigation with the analysis of the sample.

[10] Badollet and Paine, *Int. Sugar Journal*, **28**, 23, 97, 137 (1926).

[11] This examination was made by G. L. Keenan of the Food, Drug and Insecticide Administration.

The concentrated solution of the acids was extracted in a Bacon-Dunbar "perforation" outfit until only negligible quantities of extractive were removed by the ether. In this manner 23.9 g. of aconitic acid was obtained, or 7.97 g. per kilo.

The acid solution remaining after the extraction by ether was neutralized with calcium carbonate, the excess being removed by filtration. The solution, on boiling, deposited a considerable precipitate. Filtered and dried, this weighed 1.76 g. The acid isolated from this calcium salt melted at 142 to 144° and gave no depression when mixed with citric acid. Optical crystallographic examination confirmed its identity as citric acid.

A special examination of Dominican molasses was made for lactic acid. Four hundred grams of molasses was acidified, diluted with 200 cc. of water and extracted for twenty-four hours with a rapid stream of ether in a perforation outfit. The ether-soluble acids were neutralized with barium hydroxide, diluted to 100 cc. and then 200 cc. of alcohol was added. After this solution had stood overnight, the undissolved barium salts were filtered off. The filtrate was freed from barium with the required quantity of sulfuric acid, the filtrate from barium sulfate was boiled with an excess of zinc carbonate and filtered. The filtrate was concentrated to 5 cc., 15 cc. of alcohol was added and the crystalline precipitate was filtered, dried and weighed. It weighed 0.315 g., corresponding to 0.19 g. of lactic acid, or 0.05% of the molasses. Optical crystallographic examination, oxidation to acetaldehyde and the hydroxy acid test with ferric chloride established its identity as zinc lactate.

The acids recovered from the insoluble barium salt were subjected to fractional crystallization. All fractions were aconitic acid. No succinic acid or tricarballylic acid could be separated from any of the fractions or identified by optical crystallographic examinations.

Summary

The acids of molasses were found to be formic acid, about 0.1%; acetic acid, 0.2%; aconitic acid, 0.8%; lactic acid, 0.05% and small quantities of malic and citric acids. By establishing the presence of formic, acetic, aconitic, malic and lactic acids in sugar cane molasses, the results of previous investigators were confirmed. Citric acid has not previously been reported as a constituent of sugar cane molasses.

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