glasses are attained much too slowly to be measured by the Nernst method, and that conclusions relative to the applicability of the third law of thermodynamics to glasses cannot be based upon measurements by the Nernst method.

LAFAYETTE, INDIANA RECEIVED SEPTEMBER 1, 1937

[CONTRIBUTION FROM THE FOOD RESEARCH DIVISION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPT. OF AGRICULTURE]

Some Constituents of the Cannonball Fruit (Couroupita Guianensis, Aubl.)[1]

BY E. K. NELSON AND D. H. WHEELER

The cannonball tree, *Couroupita guianensis*, was first described and named by Fusée Aublet.[2] Descriptions of the tree and fruit have also been given by W. M. Buswell,[3] and by B. E. Dahlgren.[4]

The cannonball tree is a strictly tropical tree of the dense, humid forests of South America, but according to Buswell there is one specimen in the United States at Fort Myers, Florida, growing and fruiting apparently much as it would in its native forests.

The flowers are four to five inches in diameter, and the fruits, six to eight inches in diameter, are round and encased in a hard shell. The pulp fills the entire shell and apparently varies in color according to the degree of maturity but the odor is peculiar and disagreeable to most people. According to Dahlgren the fresh pulp is said to be used by the natives in the preparation of a cooling, medicinal drink.

The present investigation was made on two fruits sent to Washington by Atherton Lee, director of the Federal Experiment Station at Mayaguez, Puerto Rico.

The pulp from one of these weighed 1739 g.; it was mashed and distilled with steam at a slightly reduced pressure. The distillate was extracted with ether and on carefully evaporating the ether a very small quantity of volatile oil was left which had the peculiar odor of the fruit. Shaking with dilute sodium hydroxide dissolved this oil with the exception of a slight turbidity which was removed by filtration.

Carbon dioxide passed into the clear solution precipitated a phenolic substance which gave a dirty violet color in alcoholic solution with ferric chloride. The solution left after extraction of the phenol with ether was acidified and again extracted with ether, and a partly crystalline acid with an odor similar to capric acid was separated. Dried on a porous plate, the crystalline acid melted at *ca.* 130°. The amount of the volatile oil precluded further study.

The pulp magma, remaining in the still, was pressed out and the juice extracted with ether, which removed a red coloring matter which, according to Dr. C. E. Sando of the Food Research Division, gives every indication of being a carotinoid pigment, probably lycopin.

The juice was then made to a volume of 2000 cc., 10 cc. required 27.7 cc. 0.1 *N* sodium hydroxide to neutralize it, corresponding to 2.03% acid in the pulp, calculated as citric.

The solution was neutralized with sodium hydroxide and the acids precipitated with lead acetate. The acids were recovered from the lead precipitate and esterified by refluxing with absolute alcohol containing 2.5% of hydrochloric acid.

The esters were dissolved in ether, and the ether solution filtered and washed with dilute solution of sodium hydroxide until neutral. Fifty-seven grams of crude ethyl esters was obtained from the pulp of two fruits. Distilled at 9 mm. without a column, a small part distilled under 170° and most of it between 172 and 174°.

The hydrazide prepared from the higher boiling fraction came down at once and had the appearance of isocitric hydrazide. (Citric hydrazide crystallizes slowly and on the sides of the tube in well-formed crystals.) On filtering the hydrazide it went to a paste on the filter, showing that it was quite impure and that further fractionation was necessary.

Accordingly, the fraction boiling at 172–174° was distilled through an eight-inch (20-cm.) Widmer column at 3.5 mm. and a main fraction boiling constantly at 151° was obtained. This fraction gave the characteristic crystals of citric trihydrazide, melting at 103–105° and in the anhydrous form at 146–147°.

Optical crystallographic examination[5] showed its identity with citric trihydrazide.

The fraction boiling under 170° at 9 mm. and the residue from the redistillation of the 172–174° fraction were fractionated from a 10-cc. bulb through a Vigreux column, at 3.5 mm. From the lower boiling fraction a small amount of distillate was obtained which gave a hydrazide melting at 178–179°. Admixture with *l*-malic hydrazide gave no depression in melting point.

The residue in the flask from the main fraction was redistilled similarly, and an ester boiling at 158–160° was separated. This gave an immediate precipitate of hy-

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[1] Food Research Division Contribution No. 344.

[2] Fusée Aublet, "Histoire des plantes de la Guiane Françoise," Vol. II, 708 (1775).

[3] W. M. Buswell, *The Florida Naturalist*, n. s. II, 105 (1929); *The American Botanist*, XXXIII, 97 (1927).

[4] B. E. Dahlgren, Field Museum of Natural History, Botany Leaflet No. 6 (1924).

[5] Optical crystallographic examinations were made by G. L. Keenan, of the Food and Drug Administration, U. S. Department of Agriculture.

drazide crystallizing in small needles melting at 177–179°. Optical crystallographic examination shows it to be isocitric trihydrazide. The optical rotation of the ester taken in a 100 mm. micropolariscope tube is −12.25°. Ethyl isocitrate, obtained from the acid of the blackberry,[6] was found to be optically active.

If the fraction obtained from the ethyl esters of acids isolated from fruits, boiling in the neighborhood of triethyl citrate gives an immediate precipitate in alcoholic solution with hydrazine hydrate, and this comes down in a solid mass, the presence of isocitric acid should be suspected, and a careful separation made with an efficient fractionating column.

The filtrate from the lead precipitate, after removal of lead by potassium oxalate, was concentrated. A test with Fehling's solution indicated the presence of reducing sugar.

Summary

The fruit of the cannonball tree contains a small amount of volatile oil, composed of a phenolic substance and acids, to which the characteristic odor of the fruit is due.

A red color is present, which is evidently a carotinoid pigment.

The acids consist chiefly of citric acid with small amounts of malic and isocitric acids.

This is the second fruit examined in which isocitric acid has been identified, although it is not the predominating acid as is the case with the blackberry.

WASHINGTON, D. C.

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[6] E. K. Nelson, THIS JOURNAL, **47**, 568 (1925); **52**, 2928 (1930).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

Sulfur Studies. XIII. The Identification of Some Aliphatic Sulfonic Acids

BY P. H. LATIMER AND R. W. BOST

In connection with studies on the preparation of aliphatic sulfonic acids being carried on in this Laboratory, it was found desirable to develop a simple method of identifying these acids.

Practically all of the work that has been recorded has dealt with aromatic sulfonic acids. Aniline has been used to detect sulfonic acids in petroleum,[1] and also for the removal of sulfonic acids from acid sludge.[2] Acid salts of sulfopropionic acid with aniline and α-naphthylamine also have been prepared.[3]

In an unpublished investigation carried out in this Laboratory, unsuccessful attempts were made to prepare a suitable derivative of butane sulfonic acid-1 using as reagents: 2,4-dinitrochlorobenzene, aniline, *p*-toluidine, *p*-nitrobenzyl bromide and *p*-phenylphenacyl bromide.[4]

The authors have found that phenylhydrazine makes an ideal reagent for the identification of aliphatic sulfonic acids. Its basic dissociation constant is of such a magnitude that stable salts are formed which may be purified easily. The melting points of the salts are sharp and well defined and the derivative is of such a nature that a molecular weight may be run by titration with standard alkali, thus serving as a double check on the identity of the acid under investigation. Since aliphatic sulfonic acids are usually isolated as, and purified through, their barium salts, the authors started at this point with the method of identification. The general procedure for preparing these derivatives is described below.

The sulfonic acids were prepared by the action of ammonium sulfite on the corresponding alkyl bromide and isolated as the barium salt. A detailed method of their preparation and purification will be given in a later paper.

Experimental

One hundredth mole of the barium salt of the sulfonic acid was dissolved in as little hot water as possible and the free acid was released by treating with slightly less than an equivalent quantity of one normal sulfuric acid. The barium sulfate was removed by centrifuging. To the solution of the free sulfonic acids was added 0.02 mole of phenylhydrazine, dissolved in a few drops of alcohol. The solution was concentrated, under a hood, to 20–30 ml. On cooling, the salt separated and was usually colored a light tan by a trace of unreacted phenylhydrazine. The crude phenylhydrazine salts of the acids, up through normal amyl, were best purified by dissolving in the smallest possible quantity of hot absolute alcohol, cooling and precipitating the salt by the addition of several volumes of anhydrous ether. The salt, which separates here as very thin, white plates, was filtered and washed with anhydrous ether. Two recrystallizations were sufficient. The crude phenylhydrazine salts of the higher acids were purified by taking the finely powdered, dry, crude salt and heating with 30–40 ml. of anhydrous ether.

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[1] J. Scott, *Petroleum World*, **18**, 20 (1921).

[2] H. Limburg, U. S. Patent, 1,937,521.

[3] A. P. N. Franchimont and H. J. Backer, *Rec. trav. chim.*, **39**, 689 (1920).

[4] W. W. Williams, Doctoral dissertation, University of North Carolina, June, 1936.