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. RECEIVED SEPTEMBER 24, 1937

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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In an unpublished investigation carried out in this Laboratory, unsuccessful attempts were made to prepare a suitable derivative of butane sulfonic acid-1 using as reagents: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.

The salts may be identified by their melting points and also by titrating a weighed portion of the salt with 0.01 *N* sodium hydroxide, using phenolphthalein as an indicator. The corrected melting points and molecular weights of the salts are found in Table I. An analysis for sulfur on the salt of methane sulfonic acid gave 15.81%; calculated 15.72%. Since the observed molecular weights checked well with the calculated values, it was deemed unnecessary to run sulfur analyses on the entire list. Mixed melting points were run on the salts from butane sulfonic acid-1 through octane sulfonic acid-1. Where the two acids differed by one methylene group the depression was found to be about three degrees. Where the acids differed by two methylene groups the depression was about fifteen degrees.

TABLE I

MELTING POINTS OF THE PHENYLHYDRAZINE SALTS OF CERTAIN ALIPHATIC SULFONIC ACIDS

Phenylhydrazonium	M. p., °C. (corr.)	Mol. wt. Obsd.	Mol. wt. Calcd.
Methane sulfonate	193.5–194 (dec.)	206.5	204.0
Ethane sulfonate	182.8	218.0	218.2
Propane sulfonate-1	204.5 (dec.)	233.0	232.1
Butane sulfonate-1	114–115	244.5	246.2
Pentane sulfonate-1	108–108.2	258.0	260.2
Hexane sulfonate-1	101–101.6	275.0	274.2
Heptane sulfonate-1	100–100.5	292.5	288.2
Octane sulfonate-1	90–90.5	303.3	302.2

The salts may be prepared on a much smaller scale by using 0.001 mole of the barium salt and corresponding quantities of the other reagents. This will easily give enough material for melting points. If the molecular weight is to be determined, it is suggested that the larger quantities be used.

Summary

1. Phenylhydrazine forms crystalline salts with normal aliphatic sulfonic acids.

2. The salts may be identified by melting point and also by titrating with 0.01 *N* sodium hydroxide to obtain the molecular weight, thus serving as a check on the compound.

3. The constants for the salts of the sulfonic acids from methyl through *n*-octyl are given.

4. The reagent is easily available, the method is simple and the melting points are well defined.

CHAPEL HILL, N. C. RECEIVED SEPTEMBER 23, 1937

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

Natural and Synthetic Rubber. XVIII. The Protein from Natural Rubber and its Amino Acid Constituents

BY THOMAS MIDGLEY, JR., ALBERT L. HENNE AND MARY W. RENOLL

The material remaining after the extraction of natural rubber with organic solvents has been known to contain nitrogen, and to exhibit some protein characteristics.[1,2] The numerous communications on the subject have been compiled and sifted adequately.[3] Only one investigation has been concerned with the amino acids resulting from the hydrolysis of the protein material.[4]

The purpose of the present work was to isolate a product which would be as nearly pure natural rubber protein as possible, to analyze it and to separate and identify the individual amino acids resulting from its hydrolysis.

The starting material was the nitrogen containing residue obtained in the preparation of pure rubber hydrocarbon by fractional precipitation from a mixture of benzene and alcohol.[5] This was a tough brown mass, entirely free of resins, but still containing a large amount of rubber, all the mineral impurities, some sugars and the protein. Depending upon the extent of its treatment, it titrated from 3.5 to 4.2% nitrogen. This material was allowed to swell in a large quantity of xylene kept at 100° in a water-bath and never stirred. Every twenty-four hours, the liquid was decanted with the least possible stirring, and fresh or recovered xylene poured on the rubber to take its place. After a few days, the material became quite crumby and progressively lost its tackiness. After a week, xylene did not remove any more rubber. The material was then boiled with xylene to extract the last traces of rubber. The crude protein separated easily from the xylene. When boiling or stirring was resorted to too early, the protein dispersed in the rubber solution, and considerable loss occurred during decantation; moreover, separation was rendered quite incomplete and difficult to handle.

After removal of the xylene by means of boiling benzene, the protein was placed in an evaporating dish to dry in a current of air; the dry material was a friable, fluffy, tan powder, whose nitrogen content varied from 5 to 8%, and ash content from 15 to 35%. The next step consisted in electrodialyzing at 120 volts through cellophane; this removed the inorganic impurities and carbohydrates.

The purified protein, dried in a vacuum oven at 70° for six hours was a brown, crumby mass; it amounted to about 45% of the material undergoing electrodialysis. It was analyzed for carbon and hydrogen by combustion, and for nitrogen by Kjeldahl, the final result being on an ash-free, dry basis: 57.68% C, 7.54% H, 12.52% N and 22.28% O (computed by difference), corresponding to an

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(1) Weber, *J. Soc. Chem. Ind.*, **19**, 215 (1900).

(2) Spence, *India Rubber J.*, **2**, 766 (1907).

(3) Dinsmore, *Ind. Eng. Chem.*, **18**, 1140 (1926).

(4) Belgrave, *Malayan Agr. J.*, **13**, 154 (1925).

(5) Midgley, Henne and Renoll, THIS JOURNAL, **53**, 2733 (1931).