[Contribution from the Bureau of Chemistry, United States Department of Agriculture]

THE NON-VOLATILE ACIDS OF THE BLACKBERRY

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Received November 21, 1924 Published February 5, 1925

Very little information can be found in the literature on the acids of the blackberry. Bigelow and Dunbar,[1] in their study of the acid content of fruits, state that "some samples of blackberries appeared to contain only citric acid, some contained malic acid in traces with no citric, while others gave no reactions for either malic or citric."

Franzen and Keyssner,[2] who examined the acids from blackberry leaves (*Rubus fruticosus*) found, besides considerable lactic acid and a little malic, succinic and oxalic acids, three unidentified acids, the hydrazides of which melted at 181–182°, 201–202° and 201–202°, respectively, the third being derived from the filtrate from the lead precipitate and not identical with the second.

An investigation was undertaken in the Bureau of Chemistry to ascertain the identity of the characteristic acid of the blackberry. The methods employed by Franzen and his co-workers[3] were employed. The fruit used in this work was bought on the Washington market toward the close of the blackberry season. An analysis of the first lot of fruit (hereafter designated as Lot 1) by C. P. Lathrop, using the methods of the A. O. A. C. for the ordinary determinations, gave the following results: total solids, 16.42%; water-insoluble solids, 9.06%; total sugars as invert, 4.69%; alcohol precipitate, 0.503%; pectic acid, 0.311%; ash, 0.424%; alkalinity number of ash, 13.6; acidity, 88.0 cc. of 0.1 *N* acid per 100 g.; P_2O_5 in ash, 37.8 mg. per 100 g. of sample; citric acid (pentabromo-acetone method), 0.002%; tartaric acid (Kling method),[4] none; *l*-malic acid, none detected, but uranyl acetate gives a positive increase in rotation of +4.0° Ventzke per 100 g.

The predominating acid in the blackberry was found to be an optically active isocitric acid. This acid has not previously been reported as occurring in nature. It was prepared by Fittig,[5] who obtained it on treating trichloromethyl-paraconic acid with barium hydroxide and presumably in its optically inactive modification. Fittig describes isocitric acid, $HOOC.CH_2.CH(COOH)CH(OH)COOH$, as a very hygroscopic, light yellow substance, becoming solid only on standing for several weeks in a vacuum

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[1] Bigelow and Dunbar, *J. Ind. Eng. Chem.*, **9**, 762 (1917).

[2] Franzen and Keyssner, *Z. physiol. Chem.*, **129**, 309 (1923).

[3] Franzen and others, *ibid.*, (a) **115**, 9, 37 (1921); (b) **122**, 46 (1922); (c) **124**, 65 (1922); (d) **127**, 14 (1923); ref. 2.

[4] A. Kling, *Bull. soc. chim.*, [4] **7**, 567, 886 (1910).

[5] Fittig, *Ann.*, **255**, 47 (1889).

desiccator. Thus prepared, he found that it was not the pure acid, but a mixture of the acid with its lactone, and that the acid is converted into its lactone at a temperature of 100°.

When the predominating acid from blackberries was recovered from its barium salt and dried, it was obtained in the form of lacto-isocitric acid, $C_6H_6O_6$, which reacts like both a lactone and an acid.

A comparison of the boiling point of the ethyl ester and the melting point of the hydrazide of this acid with the results obtained on fraction 11 by Franzen and Keyssner,[6] indicates that the unknown acid found by them in blackberry leaves may be the acid here described.

	Acid from blackberries	Acid from blackberry leaves
Boiling point of ethyl ester	175–180° (10 mm.)	180–190° (12 mm.)
Melting point of hydrazide	178–179°	181–182°

Experimental Part

Three crates of blackberries, weighing from 17.4 to 18 kilograms each, were used in this work. One (Lot 1) was bought in the latter part of the blackberry season in 1923, and the other two (Lots 2 and 3) in 1924. Each crate was treated separately.

The juice was concentrated to a sirup and treated with several volumes of alcohol in order to precipitate the pectin. After filtering off the pectin, the alcoholic extract wsa diluted to 4 liters with alcohol and the acidity was determined by titration. A slight excess of lead acetate, in water solution, was added and the mixture was thoroughly stirred. The precipitate of lead salts was filtered off and washed and then decomposed with a slight excess of sulfuric acid. After the removal of the precipitated lead sulfate, the excess of sulfuric acid was determined in the filtrate, its equivalent of barium hydroxide was added, and the solution was filtered and evaporated. The final traces of water were removed by repeated evaporation with absolute alcohol. The acid mixture was then dissolved in 500 cc. of absolute alcohol containing 2.5% of hydrogen chloride and refluxed for five hours, the alcohol being distilled as completely as possible on a salt-bath. This operation was repeated, using 500 cc. of absolute alcohol containing 2.5% of hydrogen chloride and, after the removal of the alcohol by distillation, the esters were dissolved in ether. The ethereal solution was shaken with potassium hydroxide solution, which was drawn off, and the ethereal solution, after being dried with sodium sulfate, was evaporated, yielding the ethyl esters of the blackberry acids.

The esters were fractionated at 10 mm. and refractionated at the same pressure.

The hydrazides of the acids were prepared from all the fractions by dissolving 0.7–0.8 g. in 2.5–3.0 cc. of absolute alcohol, adding 0.5 cc. of hydrazine hydrate and, after thorough mixing, allowing to stand overnight.

The results obtained on Lot 1 are given in Table I.

The hydrazide of Fraction 1 melts at the temperature of oxalic dihydrazide. The hydrazide of Fraction 2 gives no depression in melting point when mixed with oxalic dihydrazide, and is that substance. The hydrazide of Fraction 3 gives a sharp depression in melting point when

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[6] Ref. 3 e, p. 312.

mixed with succinic dihydrazide. It is not succinic dihydrazide and was not identified, and the preparation of the benzylidene compound yielded no definite result.

TABLE I

RESULTS WITH LOT 1

Fraction No.	1	2	3	4	5	6	7	8
B. p. at 10 mm.	50–55	65–85	105–115	124–135	135–145	145–155	155–165	175–180
Weight (g.)	0.9	0.7	2.0	3.0	3.0	1.5	2.0	40.0
Rotation	...	...	−1.1°	−5.0°	−6.5°	−6.0°	...	−7.75°
Hydrazide, m. p.	237	235–236	160–168	175–177	167–173	170–175	172–176	178–179

Fraction 4 afforded a hydrazide which gave no depression in melting point when mixed with *l*-malic dihydrazide, and is that substance. The hydrazides of Fractions 5, 6 and 7 are impure *l*-malic hydrazides.

The hydrazide of Fraction 8 gave a sharp depression in melting point when mixed with *l*-malic dihydrazide. It is the hydrazide of an unidentified acid.

The main part of Fraction 8 was saponified with barium hydroxide solution, and the acid was recovered by careful precipitation of the barium with sulfuric acid. The acid is very soluble in water and was obtained in crystalline form only by drying in a vacuum oven with a high vacuum at 100°. It softens at 130° and melts clear at 146°.

Anal.[7] Subs., 0.1434, 0.1223: CO_2, 0.2192, 0.1867; H_2O, 0.0461, 0.0377. Calcd. for $C_6H_6O_6$: C, 41.37; H, 3.47. Found: C, 41.68, 41.53; H, 3.59, 3.44.

Subs., 0.1652: 18.8 cc. of 0.1 *N* NaOH required to neutralize and a further 9.2 cc. of 0.1 *N* NaOH when boiled with an excess for 15 minutes. From the total quantity of sodium hydroxide consumed, assuming 3 atoms of Na to be required, the molecular weight of the acid is found to be 177. Calcd. for $C_6H_6O_6$: 174.

The esters from Lot 2 (64 g.) and Lot 3 (68.6 g.) were also twice fractionated at 10 mm.

TABLE II

RESULTS WITH LOTS 2 AND 3

Fraction No.	1	2	3	4	5
B. p., °C.	90–100	112–125	125–135	135–175	175–178
Wt., g.	(2) 0.52 (3) 0.6	(2) 0.44 (3) 0.7	(2) 4.72 (3) 3.8	(2) run into 3 (3) 2.5	(2) 50.2 (3) 50.6
Rotation, α_D			(2) −8.3° (3) −9.0°		(2) +14.75° (3) +13.1°
Hydrazide, m. p.	(2) 174–177	(2) 173–176	(2) 174–176	(2) 157–161	(2) 170–172
Benzylidene hydrazide, m. p.	(2) 210–213	(2) 215–217			

The hydrazides of Fractions 1, 2 and 3, when mixed with *l*-malic dihydrazide, gave no depression in melting point. The benzylidene hydrazide of Fractions 1 and 2, obtained from the filtrates from the *l*-malic di-

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[7] Acknowledgment is due to Mr. R. M. Hann of this Bureau for the organic analyses.

hydrazide, when mixed with succinic benzylidene dihydrazide gave no depression in melting point. The hydrazide of Fraction 4 is a mixture. Fraction 5 gave a hydrazide which showed a sharp depression in melting point when mixed with *l*-malic dihydrazide. It is the hydrazide of the characteristic blackberry acid.

No fractions corresponding with Fractions 1 and 2 of Lot 1 (in which oxalic acid was found) were obtained.

Examination of Fraction 5, boiling at 175–178°, at 10 mm., showed the following results.

Anal. Calcd. for triethyl isocitrate, $C_{12}H_{20}O_7$: C, 52.17; H, 7.24; sap. equiv. 609.7. Found, Lot 2: C, 52.50, 52.77; H, 7.06, 6.99; sap. equiv., 614; Lot 3, sap. equiv., 619.7.

The acid was isolated from 36.3 g. of Fraction 5 by saponifying it with potassium hydroxide, adding an excess of phosphoric acid and percolating the solution with ether in a continuous extraction outfit. Some phosphoric acid being removed with the organic acid, the operation was repeated until only a trace of phosphoric acid was left; this was removed by a little calcium hydroxide. The acid appears as a thick, viscous, yellowish sirup, which becomes partially crystalline on drying in a vacuum oven at 100° and standing for a long time in a desiccator.

A solution of 0.9344 g. in 50 cc. of water, with the calculated amount of sodium hydroxide required to neutralize it, was used for polarimetric examinations. This solution in a 100mm. tube gave $\alpha_D = -0.26°$; $[\alpha]_D = -13.9°$.

With uranyl acetate, $\alpha_D = +3.43°$ and $[\alpha]_D = +183.5°$, or an increase in specific rotation of $+197.4°$.

The BARIUM SALT was prepared by saponifying the ester with the calculated amount of barium hydroxide.

It is crystalline and much less soluble in boiling water than in cold water.

Anal. Calcd. for the isocitrate, $Ba_3(C_6H_5O_7)_2 + H_2O$: Ba, 50.92. Found: 50.75. (Ref. 5.)

The SILVER SALT was prepared by precipitating a neutral solution of the sodium salt with silver nitrate.

Anal. Calcd. for $Ag_3C_6H_5O_7$: Ag, 63.13; C, 14.04; H, 0.97. Found: Ag, 62.87; C, 14.35; H, 1.22.

Isocitric acid should not yield pentabromo-acetone by the tentative method of the A. O. A. C. for citric acid. On the other hand, it should be possible to convert it into aconitic acid if lactone formation can be avoided. Actually, it was found that little or no aconitic acid was formed by heating with dil. sulfuric acid, according to the method of Hentschel[8] for making aconitic acid from citric acid. On the other hand, by heating the barium salt of the acid from blackberries to 300°, lactone formation was avoided, and a considerable amount of aconitic acid was separated and identified. The acid isolated from the heated barium salt melted at 182–184°. When mixed with aconitic acid of melting point 184–186°, a melting point of 183–185° was obtained.

Crystallographic examination by Dr. E. T. Wherry of this Bureau also showed its identity with aconitic acid.[9]

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[8] Hentschel, *J. prakt. Chem.*, [2] **35**, 206 (1887).

[9] Dr. Wherry and Mr. Keenan of the Bureau of Chemistry have determined the refractive indices of a known sample of aconitic acid as $\alpha = 1.490$, $\gamma = 1.610$; grains too minute for further data. On examining the acid described in this paper, they find

As aconitic acid could be formed as well by the contamination of Fraction 5 by triethyl citrate, a test was made for citric acid by the pentabromoacetone method on 0.8 g. of the ester and on 0.4 g. of the free acid. Only a trace of citric acid was found. Therefore, the aconitic acid obtained must come from isocitric acid. Further proof that the acid is isocitric acid was obtained on fusing it with potassium hydroxide, the products being acetic and oxalic acids. Citric acid, on fusion with potassium hydroxide yields the same products.

The ethyl isocitrate obtained from the first lot of blackberries was levorotatory, whereas that obtained from the second and third lots was dextrorotatory. The indications are, therefore, that isocitric acid may occur in blackberries in both optically active modifications.

As its optical rotation is increased in the positive direction by uranyl salts, instead of in the negative direction as in the case of *l*-malic acid, determinations of the latter acid in its presence would be disturbed. The solubility of barium isocitrate in cold water would make its separation from barium malate difficult, and it would be better to attempt such a separation from boiling water.

Summary

Investigation of the acids of the blackberry shows that the predominating and characteristic acid is optically active isocitric acid, constituting about five-sixths of the total acids of the fruit. *l*-Malic acid was present in moderate amount, together with traces of oxalic, succinic and citric acids.

Further study is required in order to devise methods for the estimation of isocitric acid in fruits.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

THE DISSOCIATION INTO FREE RADICALS OF SUBSTITUTED DIXANTHYLS. I. DIBENZYL- AND DIBUTYLDIXANTHYL

BY JAMES B. CONANT AND ARTHUR W. SLOAN

RECEIVED DECEMBER 5, 1924 PUBLISHED FEBRUARY 5, 1925

It was shown in a preliminary paper from this Laboratory[1] that the salts of certain organic bases and pseudo bases were reduced by vanadous chloride with the formation of substituted ethanes, the general reaction being represented by the following equations: $AX + VX_2 \longrightarrow A— + VX_3$; $2A— \longrightarrow A—A$. The discovery of this convenient method of preparing ethanes directly from the corresponding carbinols makes possible the synthesis of many interesting types of substituted ethanes hitherto

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that it is identical with respect to these properties, thus confirming its identity with aconitic acid.

[1] THIS JOURNAL, **45,** 2466 (1923).