[CONTRIBUTION FROM THE BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE NON-VOLATILE ACIDS OF THE PEAR, QUINCE, APPLE, LOGANBERRY, BLUEBERRY, CRANBERRY, LEMON AND POMEGRANATE

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RECEIVED JANUARY 21, 1927 PUBLISHED MAY 10, 1927

As the present contribution is a continuation of work on the non-volatile acids of fruits recorded in THIS JOURNAL,[1] a detailed description of the method employed will not be given here.

The acids were separated from the concentrated juice by means of lead acetate, liberated from their lead salts with sulfuric acid, and converted into ethyl esters. The esters were fractionated at 10 mm. pressure. Final identification was made by means of the hydrazides prepared from the various fractions.

Experimental Part

Pears (Bartlett).—Nineteen kg. of fruit afforded 25 g. of crude esters. Fractionated at 10 mm., the following fractions were obtained: (1) 86–90°, 1.3 g., α_D—0.7°; (2) 90–120°, 0.8 g., α_D—2.1°; (3) 120–130°, 3.5 g., α_D—10.25°; (4) 130–150°, 1.8 g., α_D—9.75°; (5) 150–165°, 1.9 g., α_D—6.2°; (6) 165–170°, 10.9 g., α_D—0.7°. From fractions boiling under 120° a hydrazide was obtained which is easily soluble in alcohol and melts at 80–82°. These fractions contain the ester of levulinic acid, a contamination resulting from levulinic acid formed by the action of hydrochloric acid on carbohydrates carried down by the lead acetate. This could have been avoided by passing carbon dioxide through a suspension of the lead precipitate in water to break up lead-sugar compounds.[2]

From Fractions 2, 3 and 4, *l*-malic dihydrazide, m. p. 177–179°, was obtained. Fraction 6 yielded citric trihydrazide; m. p. (anhydrous) 145–147°.

Quinces.—Nineteen and one-half kg. of fruit yielded 59 g. of crude esters. Fractionated at 10 mm., it gave: (1) 100–115°, 0.7 g.; (2) 124–126°, 48.7 g., α_D—12.0°; (3) residue in still, 0.8 g. Fraction 1 consisted of *l*-malic ester, with a small amount of levulinic ester, while Fraction 2 is the ester of *l*-malic acid, affording the characteristic hydrazide melting at 177–179°. Examination of the residue showed the absence of triethyl citrate.

Apples (Winesap).—Nineteen kg. of winesap apples yielded 61 g. of crude esters, practically all of which, at 10 mm., distils at 125–130°,

[1] Nelson, THIS JOURNAL, **46**, 2337, 2506 (1924); **47**, 568, 1177 (1925).

[2] Franzen and Schumacher, *Z. physiol. Chem.*, **115**, 22 (1921). Franzen and Helwert, *ibid.*, **122**, 48 (1922).

the distillate having an optical rotation of —12.15° and yielding the hydrazide of *l*-malic acid. The final drops distilling at about 150° afforded a hydrazide which appeared to be a mixture of *l*-malic hydrazide with citric hydrazide. Optical crystallographic examination[3] confirmed the presence of a small amount of crystals of citric trihydrazide in the mixture.

Apples (York Imperial).—Eighteen kg. of York Imperial apples yielded 54 g. of crude esters, in which only *l*-malic ester could be identified. No citric acid hydrazide was detected in the hydrazide obtained from the final runnings.

Loganberries.—Nine kg. of canned loganberries yielded 140 g. of crude esters. Fractionated at 10 mm., it gave: (1) 125–130°, 2.2 g., α_D—11.5°; (2) 130–145°, 1.15 g., α_D—10.75°; (3) 145–155°, 2.1 g., α_D—7.25°; (4) 155–168°, 0.5 g.; (5) 168–170°, 119 g., α_D—0.25°; (6) 170–175°, 2.7 g., α_D—1.2°. Fractions 1, 2 and 3 yielded *l*-malic hydrazide, m. p. 178–179°; Fractions 5 and 6 afforded the characteristic citric trihydrazide melting at 147–149°. Fraction 6 was carefully examined for triethyl isocitrate, with negative results. It was thought that isocitiic acid, the characteristic acid of the blackberry, might also be present in the loganberry.

Blueberries.—Nine kg. of blueberries gave 18 g. of crude esters. Distilled at 10 mm., it gave: (1) 125–140°, a few drops; (2) 140–165°, 0.6 g.; (3) 165–170°, 11.5 g.; (4) 170–180°, 1.4 g.; (5) condenser washings.

Fraction 1 yielded *l*-malic hydrazide, m. p. 174–177°, Fraction 2 a mixture of malic and citric hydrazides, and Fractions 3 and 4, citric trihydrazide, melting in its anhydrous form at 146–148°. Examination of the washings from the condenser showed the absence of isocitric acid.

Cranberries.—The volatile acids were separated by distilling 600 g. (acidified with sulfuric acid) with steam. The distillate was filtered, neutralized with sodium hydroxide, and concentrated to 200 cc. Cold, saturated potassium permanganate solution was added until the color remained for five minutes; sodium bisulfite and sulfuric acid were added to dissolve manganese dioxide, and the solution was shaken out several times with ether-petroleum ether mixture. The ether solution was dried with tragacanth and carefully evaporated in a light air current. The crystalline residue weighed 0.3418 g. and lost 0.3258 g. on sublimation. The cranberries, therefore, contain 0.054% of benzoic acid. A second experiment, in which the berries were heated for some time with a solution of sodium hydroxide to saponify esters, yielded 0.069% of benzoic acid.

The non-volatile acids were separated from 4.5 kg. of berries, yielding 49 g. of crude esters. Fractionated at 10 mm., it gave: (1) 125–135°, 8.0 g., α_D—11.4°; (2) 135–145°, 1.1 g.; (3) 145–155°, 1.6 g.; (4) 155–165°, 0.4 g.; (5) 165–170°, 31.6 g., α_D—0.86°. The hydrazides from

[3] Optical crystallographic examinations were kindly made by Mr. G. L. Keenan of the Bureau of Chemistry.

Fractions 1, 2 and 3, m. p. 176–178°, were *l*-malic hydrazide. Fractions 4 and 5 yielded citric hydrazide; m. p. (anhydrous), 146–148°.

Lemon.—Three liters of juice from California lemons yielded 97.8 g. of crude esters. On distillation at 10 mm., two fractions were separated, 155–169°, 1.0 g.; 169–170°, 90.0 g. Fraction 1 was redistilled at atmospheric pressure and about 0.5 g. came over under 290°. This was treated with absolute alcohol and hydrazine hydrate and the crystals which separated after standing overnight, resembling a mixture of the hydrazides of citric and malic acids, were boiled twice with 95% alcohol, dissolved in 10 cc. of warm water and diluted with several volumes of alcohol. The solution, when cool, was seeded with a particle of *l*-malic dihydrazide, when crystals separated. These had the appearance of *l*-malic acid dihydrazide, melted at 178–179°, and showed no depression in melting point when mixed with the known compound. Fraction 2 afforded pure citric trihydrazide of correct melting point. No isocitric acid was found in the final runnings.

Pomegranate.—A quantity of concd. pomegranate juice yielded 78 g. of crude esters. Fractionated at 10 mm. into four fractions it gave: (1) 120–140°, 0.53 g.; (2) 140–160°, 1.3 g.; (3) 160–168°, 1.3 g.; (4) 168–170°, 68.8 g. Fractions 1 and 2 were found to contain ethyl levulinate, due to the imcomplete separation of sugars from the lead precipitate. Fractions 3 and 4 consisted of triethyl citrate, as shown by the melting point of the hydrazide (149° for the anhydrous form and 103–106° for the hydrated form).

Summary

The acids of several fruits were examined by the ester-distillation method. The acids of Bartlett pears were found to be a mixture of two parts of citric acid with one part of malic acid. Winesap apples contain *l*-malic acid with a trace of citric acid, whereas no citric acid was found in the York Imperial. These results are contrary to those obtained by Franzen and Helwert,[4] who found a very considerable amount of citric acid in apples. The variety of apple they used is not stated, but it is assumed that it was a German apple. A mixture of citric acid (about 96%) with *l*-malic acid (about 4%) was found in the loganberry. Isocitric acid, the acid of the blackberry, was not detected. The predominating acid of the blueberry is citric, with a little *l*-malic acid. Cranberries were found to contain a mixture of citric acid (about 80%) with *l*-malic acid (about 20%), in addition to 0.069% of benzoic acid. California lemons contain citric acid with a very small amount of *l*-malic acid. No acid other than citric was found in the pomegranate.

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[4] Franzen and Helwert, *Z. physiol. Chem.*, **127**, 14–38 (1923).