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The Phytochemical Study of the Yamamomo-Fruit (*Myrica rubra S. and Z.*)

By

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The plant pigments of red, blue or violet colour which occur in flowers, fruits or leaves, were regarded by chemists to have an intimate connection, from the chemical and physiological points of view, with the flavons of the yellow colour found in plant tissues. The extensive research by A. E. Everest[1] on the isolation of the glucoside of myricetin and violanin—a glucoside of delphinidin—in the same petals of purple black violas, was highly esteemed in these respects, with that by R. Willstätter and H. Mallison[2] on the conversion of quercetin into cyanidin chloride by chemical means.

The present phytochemical study of the Yamamomo-fruit was undertaken to afford some strong evidence for the feeling of investigators that the anthocyans are directly derived in plant tissues from the flavons.

The Yamamomo-tree grown in the southern parts of Japan, was favoured in those districts as its bark has afforded a raw material - for a mordant dye[3] due to the presence of myricetin, and also its beautiful reddish or white juicy fruits gave a fresh sour taste. Hitherto nothing was known of the chemical nature of these red fruits, or of the substances to which their beauty and taste were due.

Myricetin in the Barks of Yamamomo-Tree.

800 grm. of the air-dried bark were extracted with boiling water, and crude myricitrin was obtained on cooling the hot extract, which was

1   Proc. Roy. Soc., [B]; **90**, 251 (1918).
2   Sitzber. d. Kgl. Preuss. Akad. d. Wiss., 769 (1914).
3   K. Shirai: The plants for dyestuff (in Japanese), p. 89 (1918).

purified by recrystallization from dilute alcohol. The yield was 80 grm. It melts at 199°, and gave on analysis: $C = 51.02$; $H = 4.92$; theory requires $C = 50.40$; $H = 4.80$ for $C_{21}H_{20}O_{12} \cdot 2H_2O$.

5 grm. of the substance by hydrolysis with 0.25 per cent. sulphuric acid, yielded 3.1 grm. of myricetin and rhamnose M. p. 180-181° (its phenyl osazone M. p. 95-97°), and on analysis the former gave $C = 53.60$; $H = 3.72$; theory requires $C = 53.56$; $H = 3.60$ for $C_{15}H_{10}O_8 \cdot H_2O$. The chemical properties of our myricetin and its rhamnoside agree with those of substances mentioned previously by A. G. Perkin[1] and S. Sato[2].

Anthocyan in the Fruits.

The fruits used in this investigation, were collected in quantity (23 Kilo) at the mature period, 7th of July 1924, from the trees grown at Nakabatake, Hyogoken, from which the raw material of myricetin was obtained, through the courtesy of Prof. T. Tanaka of the College of Agriculture, Kyushu Imp. University. The edible part of the fruit separated by hand from the stones, had the juice pressed out, and 2 Kilo. of the marc, thus obtained, were treated with 4 litres of 2 per cent. methyl alcohol hydrochloric acid for 3 hours to extract the pigment according to the method used by Willstätter[3] in extracting the petals' pigments, the solution was filtered and the marc washed with the acidified alcohol. The filtrate combined together with the washings, and 5 litres of combined bluish-red coloured solution, mixed with 15 litres of ether and the mixture was allowed to stand over night. The dark red aqueous layer was deposited on the bottom of the vessel, separated from the upper one, and concentrated to a thick syrup in a vacuum over sulphuric acid. The syrup thus obtained was dissolved in methyl alcohol and again precipitated with ether. The precipitate was dissolved completely in methyl alcohol and half of its volume of 10 per cent. hydrochloric acid was added to the solution whereby the pigment separated slowly in the form of brownish-red granular particles which separated from the solution and dried in a vacuum over sulphuric acid and potassium hydroxide. It weighed 1.1 grm. The crude pigment thus obtained could not be extracted completely with amylalcohol from a 0.5 per cent. hydrochloric acid solution, indicating the presence of some diglucoside[4]. The purplish-red amylalcohol solution was treated repeatedly with 0.5 per cent.

1   J. Chem. Soc., **81**, 204 (1902).
2   Kogiokagakuzasshi, **17**, 23 (1914).
3   Lieb. Ann., **412**, 178 (1916).
4   R. Willstätter u. E. H. Zöllinger: Lieb. Ann., **412**, 200 (1917).

hydrochloric acid, whereby a large part of the colouring matter was passed into the dilute acid solution, but it was impossible to remove all the pigment from the amylalcohol solution, since, as stated by Willstätter[1], the free anthocyanidins remain in the amylacohol in this treatment. Thus, our pigment was regarded as being constituted mainly of monoglucoside accompanied by a small amount of diglucoside and free anthocyanidin.

This crude pigment gave a dull red solution with water which readily decolourised. It dissolved in very dilute hydrochloric acid, and 7 per cent. sulphuric acid, and on adding water or an alkali solution to the acid solution the colour changes from clear brownish-red to blue. It also was easily soluble in methyl and ethyl alcohols giving a beautiful bluish-red solution. When ferric chloride was added to the alcoholic solution, a fine blue colour was produced, which on the addition of water changed to violet. Without further purification of this pigment, it was hydrolysed. It was dissolved in 0·05 per cent. hot hydrochloric acid, the insoluble matter formed filtered off, and concentrated hydrochloric acid was added, and then the mixture was boiled for $2\frac{1}{2}$ minutes, whereby the anthocyanidin chloride crystallized. After standing the acid solution in an ice box over night, the crystals were filtered, washed with cold 20 per cent. hydrochloric acid and dried in vacuo over sulphuric acid and potassium hydroxide. The yield was 0·1 grm. from 0·5 grm. of the crude sample. It was dissolved in a small volume of alcohol and half of its volume of 10 per cent. hydrochloric acid was added. On allowing the alcohol to evaporate, the anthocyanidin chloride separated out as crystalline aggregates, filtered and washed with 20 per cent. hydrochloric acid and dried on a porcelain tile. The anthocyanidin chloride thus obtained was dark red in colour, having a green reflex. When it was treated with a little water, it gave a dull brownish-red solution. It was fairly soluble in dilute hydrochloric acid (0·5–1 per cent.) and cold 7 per cent. sulphuric acid, giving a brownish-red solution which turned to a blue colour by adding water, sodium hydroxide or sodium carbonate, and with lead or copper acetate it gave a·fine blue or a reddish violet precipitate. In methyl and ethyl alcohols it dissolved easily with a bright bluish-red colour. On addition of ferric chloride to the alcoholic solution, it gave a fine intense blue colouration which, diluted with water, changed to a violet.

It was confirmed by the Zeisel method that the methoxyl group was present in the pigment, accordingly it was demethylated by means of

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1 R. Willstätter u. E. H. Zöllinger: Lieb. Ann., **412**, 200 (1917).

hydroiodic acid, and anthocyanidin iodide was obtained in dark red crystals, which were converted into the chloride. The demethylated anthocyanidin chloride thus obtained was soluble in methyl and ethyl alcohols, water and dilute hydrochloric acid, and sparingly in 7 per cent. sulphuric acid. The alcoholic solution with ferric chloride and the acidic solution with sodium carbonate gave equally a blue colouration.

The solubilities and colour reactions above-cited are similar to those of delphinidin chloride described by Willstätter and Mieg[1].

For the identification, the absorption spectrum of 0·0800 grm. substance in 1 litre ethyl alcohol (1 mol. in 4,687 litres as with delphinidin chloride : $C_{15}H_{11}Cl·2H_2O$.) was examined with the following results :

Column 2·5 mm. 532
„ 5·0 „ 526
„ 10·0 „ 500
„ 15·0 „ 495

The results, showing the characteristic band in the region of the green spectrum, agree fairly well with those of delphinidin chloride.[2] Although the analytical data of the pure sample were lacking, due to the small quantity of the material, it must be admitted that the demethylated anthocyanidin chloride is no doubt delphinidin chloride, and accordingly that a methyl ether of delphinidin occurs in the pigment isolated from the fruit.

The sugar solution obtained as one of the cleavage products from the anthocyan by hydrolysis was concentrated to 25 c.c., after clarified with lead carbonate, and its optical rotatory and reducing powers were determined with the following results : $\alpha = 0·80°$ in a 10 cm. tube, whence 0·38 grm. d-glucose in the solution which fairly agree with the results calculated from the reducing power of the Fehling's solution (0·39 grm. of the reducing sugar calculated as d-glucose). Phenylglucosazone M. p. 204–205° was isolated in a pure state from the above solution. It was concluded from these experimental results that the sugar combined with the anthocyanidin was d-glucose.

When the rhamnoside of myricetin which occurs in the bark of the Yamamomo-tree, transported to the sap of the fruit, so far as we have studied, where the conversion of methyl pentose into d-glucose—hexose—on the one side, and on the other, the methylation of delphinidin which was regarded as resulting from the reduction of myricetin, took place.

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1 Lieb. Ann., **408**, 125 (1915).
2 Ibid., **75**.

The Sugars in the Fruit.

The chemical study of the sugars in the fruit, therefore, attracted attention and 13·5 litres of the pressed juice separated from the marc were used for this purpose and also for the isolation of some acids.

The juice after being clarified with basic lead acetate contains 7·7 per cent. of the reducing sugar as d-glucose. Cane sugar, pentose or methyl pentose, could not be proved by the inversion or phloroglucinol test to be present in the sugar solution before and after fermentation with yeast. The reducing power of Fehling's solution and the measurement of the optical rotatory power indicated the glucose-fructose ratio in the juice as 1 : 1·25. d-Glucose was actually isolated from the clarified solution by the usual method, using glacial acetic acid as solvent, and from the mother liquor separated from the crystals of d-glucose, d-fructose was also obtained in a pure state ; both sugars were confirmed by the determination of the melting point and the mutarotation of the aqueous solution :

d-glucose M. p. 145–146.° $[\alpha]_D^{11} = \dfrac{+5\cdot01 \times 100}{2\cdot4888 \times 2} = +100\cdot6°$ after 15 min.

$$[\alpha]_D^{11} = \dfrac{+2\cdot61 \times 100}{2\cdot4888 \times 2} = +52\cdot4° \text{ constant.}$$

d-fructose M. p. 98–104.° $[\alpha]_D^{11} = \dfrac{-8\cdot31 \times 100}{0\cdot09 \times 1} = -92\cdot2°$ constant.

Non-Volatile Acids in the Fruit.

The fruit affords a refreshing and delicious flavour which is owing to the harmony of the organic acids and sugars present in it. What organic acids occur in this fruit which ripens in the hot season in the southern parts of Japan, was a very interesting question from the view point of plant physiology, since organic acids were usually recognized by chemists as formed by the metabolism of the sugars in the plant tissues.

The pressed juice had sp. gr. 1·043, and acidity 0·13 N. titrated with decinormal solution of sodium hydroxide, using the anthocyan in the juice as an indicator, and one part of which was used previously for the isolation of the sugars, and to our interest no volatile free acid could be found in it.

Citric Acid.

5 litres of the juice were treated with an excess of basic lead acetate, the precipitate (A) there formed was separated from the solution and

washed with water. The precipitate (A) suspended in water was then decomposed with hydrogen sulphide gas, and the acid solution, separated from the lead sulphide, was concentrated into a small volume under reduced pressure, this contained 65 grm. of acid calculated to be citric acid, and was optically inactive and did not decolourize bromine water. On standing the concentrated solution, citric acid was deposited in fine crystals which melted at $152.^{\circ}$ On analysis of the anhydrous substance, it gave $C = 37.55$; $H = 4.42$; while theory requires $C = 37.49$; $H = 4.19$ for $C_6H_8O_7$.

Oxalic Acid.

The mother liquor separated from the crystals of citric acid, was converted into calcium salts and concentrated to a small volume whereby precipitate (B) consisting of calcium citrate together with a small quantity of calcium oxalate was separated, in which no tartaric acid[1] could be found. The oxalate was confirmed by analysis of the purified sample; $CaO = 37.85$, while theory requires $CaO = 38.38$ for $C_2O_4Ca \cdot H_2O$.

Calcium citrate was also confirmed by the well-known colour reaction with vaniline and sulphuric acid.

Malic Acid.

The filtrate separated from the precipitate (B) after confirming that no succinic acid occurs in it, was acidified with conc. sulphuric acid, treated with ether to extract malic acid, if present, and the ethereal solution was evaporated to a small volume after neutralizing with potassium hydroxide (indicating 0.124 grm. malic acid present), whereby potassium malate was separated, which was confirmed by the method of L. Rosenthaler.[2]

Lactic Acid.

11 Litres of the juice were neutralized with barium hydroxide, the barium citrate separated, filtrated off and the filtrate was concentrated under reduced pressure to small volume, acidified with sulphuric acid and the lactic acid was extracted with ether. The acid was converted into the zinc salt and analysed : $ZnO = 27.15$; water of crystallization $= 18.34$; theory requires $ZnO = 27.27$; $H_2O = 18.18$ for $(C_3H_5O_3)_2 Zn \cdot 3H_2O$.

The analytical results and the optical measurement of the salt agree well with those of inactive lactic acid.

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1 H. Schmalfuss u. K. Keitel : Zs. physiol. Chem., **138**, 156 (1924).

2 Chem. Centralbl., II, 754 (1912).

Summary.

1. Myricitrin was isolated from the bark of the Yamamomo-tree, and was confirmed to be a rhamnoside of myricetin.

2. The red pigment of the fruit is composed mainly of monoglucoside accompanied by a small quantity of the diglucoside of the anthocyanidin and some free pigment.

3. The demethylated anthocyanidin by Zeisel's method was ascertained to be delphinidin by the colour reactions and spectroscopic method.

4. The sugar group associated with the anthocyanidin was d-glucose.

5. The sugars in the fruit were composed of d-glucose and d-fructose with ratio of 1 : 1.25.

6. The non-volatile free acids in the fruit consist principally of citric acid with a small quantity of malic, oxalic and inactive lactic acids.

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