

On the Chemistry of Japanese Plants, V. Chemical Composition of *Hydrangena paniculata*, Sieb., I.

By

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(Received September 17, 1924)

The water extract of the bark of Nori-no-ki¹ (*Hydrangena paniculata*, Sieb.) is used in some districts of Japan for the manufacture of paper and is consequently estimated as an important raw material of plant mucilage, and the authors, in the course of the investigation of the mucilage,² were obliged to undertake the present phyto-chemical study of the aqueous extract of the bark by which, if possible, to throw some light on the formation of mucilage and some organic acids occurring in plant tissues.

H. Molish, studying mucilage saps of monocotyledons by micro-chemical methods, has observed that protein, tannin, resinous matter, luteofilin, calcium, magnesium, and phosphoric acid, occur in them.³

The inner bark used in this experiment was obtained by one of the authors from a tree growing wild at Nahari, Tosa, in Sept., 1920; the bark is white in a fresh state, changing to brown on being kept in the open air, especially in a wet state.

4700 gm. of the material mixed twice with its weight of cold water, the viscous liquid, was separated from solid matter by filtration through

¹ J. v. Wiesner: Die Rohstoffe d. Pflanzenreiches, II, 391 (1918); C. Zapeck: Biochemie d. Pflanzen, III, 546 (1921); C. Wehmer: Die Pflanzenstoffe, 267 (1911).

² These Memoirs, 8, 51 (1925).

³ Studien u. d. Milchsaft u. Shleimsaft d. Pflanzen, S. 83 (1901).

cotton cloth and then squeezed, and the operation was repeated 3 or 4 times.

The combined extract, thus obtained, was a non-transparent viscous solution which has a peculiar odour, and is irritating to the skin when fresh. On being exposed to the air, it turns gradually to a greenish black forming a colouring matter, accompanied by fermentation. The technical value of "nori,"¹ which depends on its containing a mucilage, was greatly lessened when fermentation took place, and consequently various measures for protecting it from the action of organisms have been proposed.² The solution was filtered through a silk cloth to clear it and the operation repeated. Although the action of filtration was repeated, some needle crystals suspended in the solution, and observed under the microscope, could not be entirely removed, which were confirmed by chemical means to be composed of calcium oxalate.

The mucilage in the solution was precipitated with alcohol, and separated by decantation from the mother liquor, and washed with alcohol and ether successively, yielding 250 gm. crude mucilage, corresponding to 5.3% of the bark.

The mother liquor separated from the mucilage, was concentrated under reduced pressure to a small volume, and treated with ether to extract some organic substances.

On concentrating the ethereal extract, white crystals were deposited which weighed 5 gm. (0.1% of the bark).

Umbelliferone.³

The substance isolated in a crystalline state from the ethereal solution, melts at 228–229°, soluble in alcohol and ether and gave a blue fluorescence with alkali. It gave on analysis, after drying in vacuo, the following results:

C=66.71; H=4.34; theory requires C=66.67: H=3.7 for $C_9H_6O_3$.

The above-described substance was thus identified as umbelliferone, the occurrence of which in nature appears previously to have been observed, namely, in *Daphne mezereum*⁴ and *Skinmina japonica* Thumb.⁵

¹ Nori means a paste for cementing.

² G. Yoshii: On the Manufacture of Japanese Paper (in Japanese), p. 56 (1897).

³ T. v. Tiemann & C. R. Reimer: Ber. D. Chem. Ges., **12**, 993 (1879); Zwenger & Sommer: Lieb. Ann., **115**, 15 (1860); J. F. Eijkmann: Rec. tra. chim., **3**, 204 (1884), and also refer Bonduraut: Am. J. Pharm., **123** (1887); H. Schroter: Ibid., **19**, 117 (1889); F. B. Power & H. Browning: J. Chem. Soc., **105**, 2280(1914).

⁴ Loc. cit.

⁵ J. F. Eijkmann: Loc. cit.

The authors' substance showing difference in melting-point compared with other's, was then methylated by means of methyl iodide and sodium hydroxide by following the directions of Tiemann and Reimer¹ for its identification.

The methyl ether, thus obtained, purified by recrystallization from dilute alcohol, melting at 115–116°, showed a beautiful blue fluorescence with concentrated sulphuric acid. It was confirmed to be the same substance as umbelliferone methyl ether isolated from the flowers of *Matricaria cham milla* by Power and Browning² and also with herniarin which occurs in *Herniaria hirsta*.³

Resin.

On evaporating off the solvent of the ethereal solution, a resinous substance remained.

Umbelliferone Glucoside.⁴

The aqueous solution separated from the ethereal layer, was then concentrated under reduced pressure to a small volume, and was left to stand at room temperature, whereby white crystals were deposited. The crystals separated from the mother liquor, purified by recrystallization from a hot aqueous solution, melted at 205°. They were analysed after drying (1) at 105°, (2) in vacuo at 105° for 7 hours, and (3) in air bath heated at 130–135° for 2 hours.

	(1)	(2)	(3)
C	52.34	52.12	52.53
H	5.39	5.32	5.03

The theory requires C=52.64; H=5.26 for C₁₅H₁₈O₉.

It shows $[\alpha]_D^{20} = \frac{-2.66 \times 100}{2 \times 0.9860} = -134.90^\circ$ in an aqueous solution, and

by the action of emulsin and also by heating with 0.5% hydrochloric acid for 3 hours yields umbelliferone and reducing sugars. Umbelliferone split from the glucoside by the aid of emulsin was found to be melted at 228° after being purified from its dilute alcohol solution. The aqueous

¹ Loc. cit.

² Loc. cit.

³ Monat. f. Chem., **10**, 161 (1889).

⁴ J. F. Ejkmann: Loc. cit.; F. Manthner: J. prak. Chem., **91**, 174 (1915).

solution of the glucoside showed a blue fluorescence with alkali only after it had been treated with the acid or emulsin. The above-mentioned characters of the substance rendered the authors to suspect whether it were the same substance as parahydragin¹ or skimmin² which occurs in nature, since the former melts at 178° and the latter at 210°, and, moreover, according to Eijkmann², skimmin loses one molecule of water of crystallization on heating at 135°.

The authors have succeeded in getting d-galactose from the reaction products obtained from the glucoside by hydrolysis, as an osazone which after purification melts at 194-5°. Some other sugar except d-galactose, which yields an osazone soluble in hot aqueous solution, was found present in the hydrolysed liquor together with d-galactose. Confirmation, however, was lacking owing to the small quantity. Accordingly, the authors are induced to believe that their substance, as far as they have studied it, is β -glucoside derived from a biose similar to rutine,³ which, under proper conditions of hydrolysis, would be affected to decompose into the sugar group and umbelliferone, resulting biose in a free state which by further action of the reagent will be hydrolysed into d-galactose and some other sugar.

Magnesium Lactate.

On concentrating further the mother liquor separated from the glucoside, crystals were deposited, separated and recrystallized from the aqueous solution. The air-dried substance gave on analysis the following results:

	Calc. for $(C_3H_5O_2)_2$ Mg $3H_2O$	Found.
Mg	9.46	9.89
H ₂ O	21.07	20.39

Chemical properties, so far studied, agree with those of magnesium lactate.

Calcium Lactate.

Standing in a desiccator, the filtrate from the magnesium salt after

¹ Am. J. Pharm., 551 (1898).

² Loc. cit.

³ C. Charaux: C. R., 178, 1312 (1924).

being concentrated, a calcium salt was obtained which was analysed after it was thrown down as crystals from the hot alcohol solution.

It contains 22.1% of oxide of calcium and 15.96% of water of crystallization, which agree fairly well with the $\text{CaO} = 21.79$; $\text{H}_2\text{O} = 14.29$ required for $(\text{C}_3\text{H}_5\text{O}_3)_2\text{Ca} + \text{H}_2\text{O}$.

The final filtrate still showed an acidic reaction toward a litmus paper and a reducing action of the Fehling solution, which indicates that the same organic acids and sugars occur in it.

The insoluble residue of the bark in water, was then extracted with 95% alcohol. On subjecting the alcoholic extract to distillation to get off the solvent under diminished pressure, there remained in the flask a grey-coloured substance, which was confirmed to be constituted of umbelliferone and resinous matter.

From the aqueous extract of the barks of *Hydrangena paniculata*, Sieb., mucilage, calcium oxalate, resine, fat, umbelliferone, umbelliferone glucoside $\text{C}_{15}\text{H}_{15}\text{O}_9$, magnesium lactate and calcium oxalate were actually isolated.

The occurrence of lactic acid in the living plant tissues, in the present case, has been placed beyond doubt, though the acid is frequently produced from carbohydrates by the action of aerobic microbes when extracts of plant materials are allowed to remain exposed to the air. It is also probable that lactic and oxalic acids have been derived from substances which are formed by breaking up of carbohydrates preserved in the tissues; the exact nature of the chemical changes involved is fascinating in its obscurity.

Moreover, it was supposed that the glucoside and mucilage have an intimate connection in their formation in the plant, and the fact that d-galactose has been isolated from the hydrolytic products of the glucoside should be considered to be an evidence in favour of this view.

May, 1924. Laboratory of Organic- and Bio-Chemistry.