Notes

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Flavonoids of Various *Prunus* Species. VIII. The Flavonoids in the Wood of *Prunus mume*

MASAO HASEGAWA

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The pattern of the flavonoid compounds obtained from the wood of *Prunus mume* Siebold et Zuccarini, called "mume" in Japanese and belonging to section *Prunophora*, differs from the other species of section *Cerasus* reported before.[1] The specific constituent kaempferid-7-glucoside (3,5-dihydroxy-7-glucosidoxy-4′-methoxyflavone) was obtained instead of genkwanin and chrysin which had been found to be characteristic of the latter section.

Kaempferid-7-glucoside was hydrolyzed by acid into one mole each of kaempferid and glucose. The glycoside produced an olive green coloration with ferric chloride. The absorption maximum at 367 mμ showed the presence of a free hydroxyl group at the 3 position. The maximum absorption of the glycoside was identical with that of its aglycone, kaempferid, and this fact indicated that the hydroxyl group in the 3 position of the glycoside was free from sugar.[2] (The solvent used was redistilled methanol.) The hydrolysis product from the methylated glucoside gave no coloration with ferric chloride. From these facts, it was evident that the hydroxyl group at position 7 was the one involved in glycoside formation. Since this compound has not previously been reported as a natural product, the name "mumenin" is proposed for it.

In addition to mumenin, the compounds naringenin (5,7,4′-trihydroxyflavanone), prunin (the 7-glucoside of naringenin), (+)-catechin, (−)-epicatechin, leucoanthocyanidin, and a new flavanone glycoside have also been obtained. Details on the last two compounds will be reported in a later paper. The yield of the new flavanone glycoside was poor and purification of the leucoanthocyanin has thus far been unsuccessful.

EXPERIMENTAL

Wood chips (500 g.) of *Prunus mume* (cut down in February), prepared from a stem of 8-cm. diameter, were extracted twice with 4 l. methanol for 3 hr. each. A total of 2.1 kg. wood chips was extracted. After filtration and subsequent distillation of the methanol, the residual 200 ml. of sirup was extracted 6 times with 400-ml. portions of ether. The ether-insoluble residue was then extracted 10 times with 400-ml. portions of ethyl acetate.

Ether-soluble portion. After distillation of the ether, the remaining 26 g. of sirup was extracted with 300 ml. hot benzene for 30 min. The residue was extracted with hot water (150 ml.), and finally dissolved in 20 ml. methanol.

After concentration of the benzene solution to 50 ml., white crystals of naringenin were gradually deposited.

The hot water-soluble portion, red brown in color, was extracted by ether with Soxhlet liquid percolator for 1 hr. After evaporation of the ether, the residue was dissolved in 100 ml. water. This water-soluble portion was extracted twice with 100 ml. ether to give a pale yellow solution. After evaporation of the ether, the residue was recrystallized from 20 ml. water. Crystals of (+)-catechin deposited, and, after two recrystallizations, the yield of (+)-catechin was 30 mg.

The mother liquor of the water-soluble portion was repeatedly extracted with ethyl acetate. After evaporation of the ethyl acetate, the residue was dissolved in 50 ml. acetone and an equal volume of benzene was added. The pale yellow supernatant solution was evaporated to dryness. The whitish yellow residue was dissolved in 10 ml. of ethyl acetate and after a week (−)-epicatechin precipitated; yield 1.0 g.

From the methanol-soluble portion, no crystalline substance was obtained, but naringenin and eriodictyol were found in this fraction by paper chromatography.[3]

Ethyl acetate-soluble portion. The combined ethyl acetate solution was evaporated to 200 ml., then 200 ml. of water was added. On standing for 2 days in an ice box, an oily blackish brown mass was precipitated on the bottom of the flask. After decanting, the residue was mixed with 20 ml. of methanol and allowed to stand for a week at room temperature. Yellow crystals of mumenin gradually appeared; yield, 2.4 g. The mother liquor was evaporated on a water bath to one third its volume; yellow crystals of mumenin deposited on standing, yield 3.0 g.

After evaporation of the remainder of solvent from the ethyl acetate fraction, the residue was dissolved in 150 ml. of benzene. A red resinous substance precipitated. The supernatant yellow solution was then evaporated to dryness, leaving a yellow substance. The red resinous precipitate was repeatedly treated with this acetone-benzene procedure to give a total of 32 g. of yellow substance. This yellow substance was extracted in a Soxhlet with ether for 20 hr. The residual substance (23 g.) was dissolved in 130 ml. warm water, and after cooling, 100 ml. ethyl acetate was added. After a month in an ice box, a crystalline mass (a mixture of a new flavanone glycoside and prunin) gradually appeared on the interface of the two liquids and was collected by filtration with a yield of 1.8 g. on recrystallization from 30 ml. ethyl acetate. Prunin was obtained as colorless needles. After three recrystallizations from methanol, the melting point of prunin rose to 224°[4]; yield 0.3 g. After evaporation of ethyl acetate, the residue was recrystallized twice from dilute methanol to produce colorless long needles of a new flavanone glycoside, m.p. 141°; yield 0.2 g.

The ethyl acetate-soluble portion was evaporated, and the residue was treated by the acetone-benzene method described above. From this portion, a substance containing

(1) M. Hasegawa and T. Shirato, *J. Am. Chem. Soc.*, **74**, 6114 (1952); **76**, 5559, 5560 (1954); **77**, 3557 (1955); **79**, 450 (1957); and M. Hasegawa, *J. Am. Chem. Soc.*, **79**, 1738 (1957).

(2) S. Hattori, *Acta Phytochim. (Japan)*, **6**, 131 (1932).

(3) M. Hasegawa, *J. Japan. Forestry Soc.*, **38**, 107 (1956).

(4) All melting points are uncorrected.

leucoanthocyanin was obtained as a yellowish powder; yield, 7.8 g.

The reddish brown precipitate obtained by the acetone-benzene method was dissolved in 20 ml. methanol, and, after 2 weeks, 1.7 g. of mumenin was obtained as yellow crystals.

Thus, from 2.1 kg. wood chips of *Prunus mume*, 30 mg. (+)-catechin, 1.0 g. (−)-epicatechin, 32 mg. naringenin, 0.3 g. prunin, 0.2 g. of a new flavanone glycoside, 7.1 g. mumenin, and 7.8 g. leucoanthocyanin were obtained.

(+)-Catechin. The melting point of (+)-catechin (97°) as well as of its anhydrous substance did not alter when mixed with authentic specimens obtained from *Prunus yedoensis*.[1]

(−)-Epicatechin. After recrystallization from water, it was obtained as plates of m.p. 236°. It gave a green coloration with ferric chloride. The chromatographic data (R_f 0.18 in *m*-cresol:acetic acid:water 25:1:24, R_f 0.52 in isopropyl alcohol:water 22.78) agreed with that of an authentic specimen which was kindly supplied by Prof. Tsujimura.[5] The melting point was not depressed by admixture with the authentic specimen (m.p. 236°). Acetate: m.p. 150°.

Naringenin. After 3 recrystallizations from dilute methanol, naringenin melted at 246°, yield: 32 mg. The mixed melting point with an authentic specimen (m.p. 248°) obtained from *Prunus yedoensis*[1] was the same.

Prunin. This glycoside proved to be identical with authentic prunin (m.p. 224°) on chromatographic comparison and by mixed melting point determination. On hydrolysis with 2% hydrochloric acid, it produced naringenin of m.p. 246° and glucose (ascertained by paper chromatography).

Mumenin. This glycoside showed a greenish brown coloration with ferric chloride and an orange one with magnesium powder and hydrochloric acid in methanol solution. Mumenin crystals are difficultly soluble in most organic solvents except pyridine and dioxane. It was recrystallized from pyridine-water and gave microscopic yellow prisms of m.p. 278°. R_f: 0.95 (*m*-cresol:acetic acid:water 25:1:24), and 0.10 (isopropyl alcohol:water 22:78). Absorption: λ_{max} 260 mμ, 320 mμ, 367 mμ; λ_{min} 239 mμ, 285 mμ, 329 mμ.

Anal. Calcd. for $C_{22}H_{22}O_{11}.{}^1/_2H_2O$: C, 56.05; H, 4.88; OCH_3, 6.58. Found: C, 56.00; H, 4.85; OCH_3, 6.52.

Mumenin pentaacetate. This acetate was prepared by the usual method using acetic anhydride and one drop of conc. sulfuric acid. It was obtained in colorless long prisms of m.p. 210–212°.

Anal. Calcd. for $C_{34}H_{34}O_{17}$: C, 57.14; H, 4.76. Found: C, 56.76; H, 4.92.

Hydrolysis of mumenin. The glycoside (0.1095 g.), 55 ml. water, and 16 ml. conc. sulfuric acid were heated for 8 hr. under refluxing. After cooling, the precipitated aglycone was collected, washed, and dried: yield, 0.0719 g. After the aglycone was filtered, the filtrate was neutralized with barium hydroxide and then barium carbonate. The neutralized solution was dried over potassium hydroxide granules in a vacuum desiccator, and examined chromatographically. Glucose was the only sugar found.

The aglycone (kaempferid) was recrystallized from dilute methanol 3 times and obtained as yellow prisms of m.p. 228°.[6] Absorption: λ_{max} 266 mμ, 367 mμ; λ_{min} 240 mμ, 280 mμ.

Anal. Calcd. for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.88; H, 4.02.

Kaempferid triacetate, m.p. 197°.

Kaempferid trimethyl ether (kaempferol tetramethyl ether). This derivative was obtained as faint yellow needles of m.p. 158° by heating an acetone solution of kaempferid with dimethyl sulfate and potassium carbonate. The mixed melting point of kaempferid trimethyl ether with the authentic specimen of kaempferol tetramethyl ether was not depressed.

Anal. Calcd. for $C_{19}H_{18}O_6$: OCH_3, 36.25. Found: OCH_3, 36.51.

Mumenin dimethyl ether. Mumenin (0.4 g.) was suspended in 200 ml. acetone. Then 10 g. potassium carbonate and 2 ml. dimethyl sulfate were added and the whole was heated for 16 hr. When the reaction was over, the liquid was evaporated after removal of mineral salts; the residue was mixed with water; and the solidified mass was washed with water and recrystallized from methanol to produce faint yellow prisms of m.p. 248–250°; yield, 0.2 g. Absorption: λ_{max} 260 mμ, 310 mμ (inflection), 344 mμ; λ_{min} 247 mμ, 283 mμ.

Anal. Calcd. for $C_{22}H_{20}O_9(OCH_3)_2$: OCH_3, 18.97. Found: OCH_3, 19.05.

Hydrolysis of mumenin dimethyl ether. A mixture of mumenin dimethyl ether (125 mg.) and 50 ml. of 4% sulfuric acid was heated over a flame for 40 min. After cooling, the precipitate was filtered and recrystallized from methanol to give yellow needles of m.p. 282°.[7] The yield was 64.5 mg. It gave no coloration with ferric chloride.

Anal. Calcd. for $C_{18}H_{16}O_6$: OCH_3, 28.38. Found: OCH_3, 28.40.

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GOVERNMENT FOREST EXPERIMENT STATION
MEGURO, TOKYO, JAPAN

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An Improved Procedure for Preparing Glycerol Ethers[1]

S. C. GUPTA[2] AND F. A. KUMMEROW

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Batyl alcohol, a natural occurring glycerol ether found in the liver of various Elasmobranchii (shark, rays, etc.) was found to be identical with 1-stearyl glycerol ether.[3–5] The 1- and 2-stearyl

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(2) Portion of a thesis presented by S. C. Gupta as partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology.

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