Flavonoids of Various Prunus Species. I. The Flavonoids in the Wood of *Prunus yedoensis*

BY MASAO HASEGAWA AND TERUO SHIRATO

RECEIVED MAY 5, 1952

Introduction

Prunus yedoensis is the most commonly cultivated species of cherry tree in Japan. From the bark of this tree, Asahina[1] isolated sakuranin and proved that it is the 5-glucoside of sakuranetin, 4′,5-dihydroxy-7-methoxyflavanone.[2] Using the procedure of Asahina, *et al.*,[2] sakuranin has been easily obtained in the present study from the sample bark, of *Prunus yedoensis*. No sakuranin or sakuranetin, however, have been found in the heartwood or sapwood of this tree. Instead, detailed investigation has revealed the presence of genkwanin, naringenin and *d*-catechin in the heartwood and *d*-catechin and a new glucoside of naringenin in the sapwood.

Genkwanin was isolated for the first time from a Chinese drug "Yuen Hua" (flower buds of *Daphne Genkwa*) and characterized as 4′,5-dihydroxy-7-methoxyflavone by Nakao and Tseng.[3] It has also previously been isolated from the bark of *Prunus puddum*[4] and from the bark of *P. serrulata* var. *spontanea*[5] as a glycoside called glucogenkwanin.[6] The well-known naringenin and *d*-catechin are 4′,5,7-trihydroxyflavanone and 3,3′,4′,5,7-pentahydroxyflavane, respectively.

Since the new glucoside of naringenin gives a brownish coloration when treated with ferric chloride, it is evident that the hydroxyl group in the position 5 of naringenin is free. When this glucoside was methylated by means of an insufficient quantity of diazomethane and then hydrolyzed, it gave a mixture of the unchanged and a methylated naringenin. This mixture was examined by paper chromatography, using benzene–ligroin (1:1) saturated with water, and added with a small quantity of methanol as the mobile phase.[7] By comparing with the spots given by the authentic specimens in the same chromatogram, we were able to detect with ease naringenin and isosakuranetin (naringenin 4′-methyl ether), but no trace of sakuranetin. Then it must be concluded that this glucoside is naringenin-7-glucoside and is therefore new to the literature. The name "prunin" is now proposed for it.

Prunin must be definitely different from salipurpin (naringenin-5-glucoside), which was isolated from *Salix purpurea* of Europe by Charaux and Rabaté[8] and determined in its constitution by Zemplén, *et al.*,[9] because the latter is said to give no coloration with ferric chloride.

(1) Y. Asahina, *J. Pharm. Soc. Japan*, **28**, 213 (1908).
(2) Y. Asahina, J. Shinoda and M. Inubuse, *ibid.*, **47**, 133 (1927).
(3) M. Nakao and K. Tseng, *ibid.*, **52**, 342, 903 (1932).
(4) D. Chakravarti and R. P. Ghosh, *J. Indian Chem. Soc.*, **21**, 171 (1944).
(5) M. Ohta and S. Nishikawa, *J. Pharm. Soc. Japan*, **67**, 40 (1947).
(6) M. Ohta, personal communication.
(7) G. Lindstedt, *Acta Chem. Scand.*, **4**, 448 (1950); *C. A.*, **45**, 2482b (1951).
(8) G. Charaux and J. Rabaté, *Compt. rend. Acad. Sci. Paris*, **192**, 1478 (1931).
(9) G. Zemplén, R. Bognár and I. Székely, *Ber.*, **76**, 386 (1943).

A sample of floribundin, a naringenin glucoside from the flowers of *Acacia floribunda*,[10] which was sent by Prof. R. Paris, also gave a negative ferric chloride reaction. No direct comparison could be made with the naringenin glycoside isolated from the flowers of *Antirrhinum majus* by Seikel and Geissman.[11] These workers did not obtain this glycoside in crystalline form.

Experimental

Isolation of Naringenin, Genkwanin and *d*-Catechin from the Heartwood.—Three hundred grams of heartwood chips of *Prunus yedoensis* prepared from living stems (those from previously cut dead stems gave almost the same results) was twice extracted with 3-l. portions of methanol for 3 hours. The methanolic filtrates were concentrated to about 150 ml. on a water-bath and then filtered. Yellow crystals of crude genkwanin separated out gradually in the course of a week or two. On recrystallization from methanol, yellow needles, m.p. 267°, uncor., were obtained with a yield of 0.4 g. After 3 recrystallizations from methanol the m.p. was 282°.

The mother liquor was repeatedly extracted with ethyl acetate, and the combined extract was evaporated to dryness and the residue dissolved in hot water. The aqueous solution was extracted with ether until the extract gave no color with magnesium powder and concentrated hydrochloric acid. After the combined ethereal extract was evaporated, the residue was dissolved in methanol and allowed to stand. Naringenin gradually deposited in white needles of m.p. 236° (yield 5.1 g.). After 3 recrystallizations from methanol, m.p. was 248°.

The mother liquor treated with ether was evaporated to dryness, the residue was extracted with hot water, and the solution was filtered. From the filtrate, *d*-catechin was precipitated, yield 1.1 g. After 5 recrystallizations from water, colorless needles of *d*-catechin (m.p. 97°) were obtained. From the water-insoluble portion, a further small amount of naringenin was eventually obtained.

I. **Genkwanin.**—The crystals contained no water of crystallization.

Anal. Calcd. for $C_{15}H_9O_4(OCH_3)$: C, 67.13; H, 4.89; OCH_3, 10.91. Found: C, 66.59; H, 4.43; OCH_3, 10.41.

Absorption: λ_{max} 355 mμ, log ε 4.38; λ_{max} 256 mμ, log ε 4.28; λ_{min} 290 mμ, log ε 4.13.

The diacetate of genkwanin was obtained in white needles of m.p. 202°.

Anal. Calcd. for $C_{15}H_7O_4(OCH_3)(CH_3CO)_2$: OCH_3, 8.42. Found: OCH_3, 8.60.

4′-Monomethyl ether of genkwanin was prepared by gently boiling a mixture of genkwanin (0.2 g.), acetone (30 ml.), dimethyl sulfate (1 ml.) and potassium carbonate (3 g.) on a water-bath; yellow prisms of m.p. 171°. This melting point was not altered by admixing with authentic specimens of acacetin 7-methyl ether (5-hydroxy-7,4′-dimethoxyflavone) of m.p. 171°.

Anal. Calcd. for $C_{15}H_8O_3(OCH_3)_2$: OCH_3, 20.80. Found: OCH_3, 20.66.

The 5-acetate of this methyl ether was colorless needles of m.p. 195°.

II. **Naringenin.**—The crystals of naringenin contained no water of crystallization.

Anal. Calcd. for $C_{15}H_{10}O_5$: C, 66.17; H, 4.41. Found: C, 66.75; H, 4.66.

Absorption: λ_{max} 312 mμ (inflection); λ_{max} 288 mμ, log ε 4.23; λ_{min} 252 mμ, log ε 3.17.

Naringenin triacetate was obtained in colorless long needles of m.p. 126°, as described by Seikel and Geissman[11]; 3.45 mg. subst., 2.41 ml. 0.01 *N* NaOH; calcd. CH_3CO, 32.41; found CH_3CO, 32.34.

The 7,4′-dimethyl ether was prepared by heating an acetone solution of naringenin with dimethyl sulfate and potassium carbonate; colorless crystals of m.p. 120°; 4.870 mg. subst., 7.441 mg. AgI; calcd. OCH_3, 20.66; found: OCH_3, 21.06.

The acetate of this methyl ether was colorless needles of m.p. 161°.

(10) R. Paris, *Compt. rend. acad. sci., Paris*, **231**, 72 (1950).
(11) M. Seikel and T. A. Geissman, THIS JOURNAL, **72**, 5725 (1950).

III. *d*-Catechin.—Water of crystallization: 12.64 mg. subst. (dried 1 hour over P_2O_5 in a vacuum) H_2O, 2.56 mg.

Anal. Calcd. for $C_{15}H_{14}O_6 \cdot 4H_2O$: H_2O, 20.25. Found: H_2O, 19.89.

The melting point of the anhydrous substance rose to 171–173°.

Anal. Calcd. for $C_{15}H_{14}O_6 \cdot 4H_2O$: C, 49.72; H, 6.07. Found: C, 49.39; H, 6.20.

Absorption: λ_{max} 280 mμ, log ε 3.60; λ_{min} 255 mμ, log ε 2.70.

d-Catechin acetate was colorless prisms of m.p. 131°.

Isolation of *d*-Catechin and Prunin from the Sapwood.—Five hundred grams of the sapwood chips were boiled for 3 hours with 3 l. of methanol. The methanolic extract was concentrated to 100 ml., and the solution was extracted with ether. The ethereal layers were evaporated and the residue was recrystallized from water. After 5 recrystallizations, *d*-catechin of m.p. 97° (yield 0.95 g.) was obtained.

The mother liquor was then extracted exhaustively with ethyl acetate. After evaporation of the solvent, the residue was dissolved in 100 ml. of water and 50 ml. of ethyl acetate was added. From the solution an oily mass was gradually separated after standing several days. From the filtered solution ethyl acetate was distilled off, and the residue was dissolved in methanol. The methanolic solution was, after evaporation to a sirup, allowed to stand at room temperature. After about 3 days standing, white crystals of prunin appeared in the solution. The white mass of prunin was collected and crystallized from a small amount of methanol, added with a few drops of water. Prunin was then obtained in colorless needles of m.p. 225°. The yield of the crude substance was 0.7 g.

IV. Prunin.—Prunin is soluble in alcohol, ethyl acetate and acetone, sparingly so in methanol and insoluble in ether benzene and chloroform. A methanolic solution gave a brown-violet coloration with ferric chloride. In alcoholic solution it gave a reddish purple coloration with magnesium powder and concd. hydrochloric acid; specific rotation: 0.311 g. subst., 25 ml. acetone, 1 dm. tube; $\alpha_D = -0.52°$, $[\alpha]_D = -41.8°$.

Absorption: λ_{max} 308 mμ (inflection), log ε 4.12, λ_{max} 283 mμ, log ε 3.44.

The sample was dried over P_2O_5 in a vacuum at 110–115°; 3.084 g. subst., 6.532 g. CO_2, 1.366 g. H_2O.

Anal. Calcd. for $C_{21}H_{22}O_{10}$: C, 58.0; H, 5.0. Found: C, 57.80; H, 4.96.

Hydrolysis of Prunin.—Three-tenths gram of prunin, suspended in 20 ml. of 10% sulfuric acid, was heated 30 minutes on a water-bath. The aglycone which gradually deposited was filtered (yield 0.15 g.), m.p. 246°. This substance was identified with naringenin through a mixed melting point determination. After extracting with ether, the mother liquor was carefully neutralized with barium carbonate, filtered and evaporated on a boiling water-bath to a small volume, and then filtered again. When the filtrate was heated with phenylhydrazine hydrochloride and sodium acetate, glucosazone was formed. After recrystallization from methanol, it melted at 207°, both alone and on admixture with the authentic specimen. By the paper chromatographic method any sugar except glucose could not be detected.

A suspension of 0.31 g. of prunin in 50 ml. of 1% sulfuric acid was boiled for 1 hour. After extraction with ether, the mother liquor was neutralized with 10% sodium hydroxide. In this solution 121.5, 118.5 mg. of glucose was found according to the method of Bertrand. When postulated as naringenin: glucose = 1:1, the theoretical yield of glucose would be 128.9 mg.

Position of the Sugar in Prunin.—Fifty mg. of prunin was methylated by heating in 30 ml. of acetone with 1 mg. of dimethyl sulfate and 5 g. of potassium carbonate for 30 minutes under reflux.

After filtering, the solution was evaporated, and the residue was recrystallized from dilute methanol. A small amount of crystals thus obtained was hydrolyzed by boiling with 1% hydrochloric acid for 30 minutes and the resultant solution was shaken several times with ether. The ethereal solution was evaporated to dryness, and the residue was examined by paper chromatography, using a mixture of benzene and ligroin (1:1), saturated with water, and added with a small quantity of methanol as the mobile phase.[7] When developed with 1% methanolic ferric chloride solution, two spots, with R_f values of 0.05 and 0.00, respectively, were obtained. Authentic specimens of naringenin gave an R_f value of 0.05 and that of isosakuranetin 0.00, whereas sakuranetin gave the R_f value 0.73 in the same chromatogram.

Prunin Dimethyl Ether.—Two-tenths gram of prunin was dissolved in 30 ml. of acetone and heated after addition of 5 g. of potassium carbonate and 2 ml. of dimethyl sulfate for 6 hours. At that time, it gave no color reaction with ferric chloride. After filtering, acetone was removed by distillation, and the residue crystallized from dilute methanol in white needles of m.p. 231°; yield poor; 3.21 mg. subst., 3.345 mg. AgI.

Anal. Calcd. for $C_{21}H_{20}O_6(OCH_3)_2$: OCH_3, 13.42. Found: OCH_3, 13.31.

Hydrolysis of Prunin Dimethyl Ether.—Hydrolysis of 50 mg. of prunin dimethyl ether was effected by heating in 20 ml. of 2% hydrochloric acid on a boiling water-bath for an hour. The turbid liquor was extracted 3 times with ether. After the ethereal extract was evaporated, the residue was recrystallized from dilute methanol. White needles of naringenin dimethyl ether (m.p. 187°) were obtained. As only a small quantity was available, no analysis could be made.

Acetate of Prunin. (1).—One-tenth gram of prunin was treated with acetic anhydride (1 ml.) and pyridine (1 ml.) in the cold for one hour. Cold water was then added and the solidified mass was filtered, washed and recrystallized from methanol. Colorless needles of m.p. 187–189° were obtained. The substance gave a purplish color reaction with ferric chloride. Owing to the scarcity of pure substance, no analysis was made.

(2).—One-tenth gram of prunin was mixed with acetic anhydride (1 ml.) and pyridine (3 drops) and the mixture was heated one hour on a water-bath. The reaction mixture was poured into water and the solidified mass was filtered, washed and recrystallized from carbon tetrachloride. Colorless needles of m.p. 138–139° were obtained. This substance was not analyzed because of the lack of a pure specimen.

Acknowledgment.—We wish to thank Dr. Masatake Ohmasa of the Government Forest Experiment Station and Dr. Shizuo Hattori of the University of Tokyo for their advice given during these investigations. We are also indebted to Dr. Rokuya Imazeki and Mr. Masao Kusaka of the Government Forest Experiment Station for supplying the wood used, and to Prof. René Paris of the University of Paris for generously sending a specimen of "floribundin."

We are grateful to Dr. Simon H. Wender of the University of Oklahoma for his kindness in revising our manuscript.

GOVERNMENT FOREST EXPERIMENT STATION OF JAPAN
MEGURO, TOKYO, JAPAN

Hydrolysis and Halide Complexing of Indium(III)

BY L. G. HEPLER AND Z Z. HUGUS, JR.[1]

RECEIVED JULY 28, 1952

In connection with other work in this Laboratory it became desirable to have a knowledge of the hydrolysis constant of In^{+3}. Hattox and DeVries,[2] from *p*H measurements in sulfate solutions at 23°, gave for the hydrolysis constant 2×10^{-4} in their most dilute (0.00631 *M*) solution. Sulfate complexing and bisulfate formation doubtless occurred in their solutions, and correction for these factors is difficult. Moeller[3] has measured the *p*H of aqueous solutions of $InCl_3$, $InBr_3$ and InI_3 over a range of concentrations at 25°. It is evident from

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(2) E. M. Hattox and T. DeVries, THIS JOURNAL, **58**, 2126 (1936).

(3) T. Moeller, *ibid.*, **63**, 1206 (1941); **64**, 953 (1942).