point was raised to 70–71° by recrystallization from dilute ethanol with the removal of small amounts of the least soluble material by filtration before dilution and cooling.

Anal. Calcd. for $C_{12}H_{18}N_2O_4S$: C, 50.33; H, 6.34. Found: C, 50.43; H, 6.25.

Experiment B.—When 0.58 g. of I, 0.33 g. of nitroethane and 0.34 g. of potassium hydroxide in a small amount of methanol were employed according to the procedure of the preceding experiment, 0.12 g. (18%) of the adduct containing two sulfonanilide units (m.p. 175–176°) and 0.35 g. (44%) of the 1:1 adduct were obtained.

With 1-Nitropropane. Experiment A.—Following the procedure described under experiment A of nitroethane, 0.500 g. of I was caused to react with 0.695 g. of 1-nitropropane in the presence of one drop of saturated potassium hydroxide–methanol solution. The less soluble product was N,N',3-triethyl-3-nitropentane-1,5-disulfonanilide obtained in 0.187 g. yield (31%, m.p. 124–126°, raised to 130–131° by recrystallization from absolute ethanol).

Anal. Calcd. for $C_{21}H_{33}N_3O_6S_2$: C, 53.98; H, 6.50. Found: C, 53.50; H, 6.26.

When the filtrate from the initial crystallization was concentrated to approximately 5 ml. and cooled to 0°, 0.250 g. (35%) of N-ethyl-3-nitropentanesulfonanilide (m.p. 50–54°, raised to 59–60° by recrystallization from small portions of absolute ethanol) was obtained.

Anal. Calcd. for $C_{13}H_{20}N_2O_4S$: C, 51.98; H, 6.71. Found: C, 52.46; H, 6.68.

Experiment B.—Using 0.37 g. of 1-nitropropane, 0.57 g. of I and 0.45 g. of potassium hydroxide in approximately 4 ml. of methanol according to the procedure of the previous experiment, only a trace of the 2:1 adduct was obtained. A 0.32-g. quantity (40%, m.p. 47–50°) of the 1:1 adduct was obtained.

With 2-Nitropropane.—To 0.100 g. of I was added 0.045 g. of 2-nitropropane and one drop of saturated potassium hydroxide–methanol solution. The resulting solution was allowed to remain at room temperature for 12 hours and then acidified with 3 *N* acetic acid. The solid which precipitated was separated, washed with small portions of water and dried. The yield of N-ethyl-3-methyl-3-nitrobutanesulfonanilide obtained was 0.120 g. (83%, m.p. 89–90°). The analytical sample was recrystallized from absolute ethanol, but the melting point was not changed.

Anal. Calcd. for $C_{13}H_{20}N_2O_4S$: C, 51.98; H, 6.71. Found: C, 52.04; H, 6.68.

Acknowledgment.—Some of the analyses reported herein were carried out by Miss Peggie Garrison.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF GEORGIA
ATHENS, GEORGIA

Flavonoids of Various Prunus Species. II. The Flavonoids in the Wood of *Prunus speciosa*

BY MASAO HASEGAWA AND TERUO SHIRATO

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In a previous paper,[1] we reported the presence in the wood of *Prunus yedoensis* of four flavonoid constituents (prunin, genkwanin, naringenin and *d*-catechin). We have now obtained two flavonoid compounds from the wood of *Prunus speciosa* (Ohshimazakura in Japanese), cultivated in the Akanuma Experimental Nursery in Saitama Prefecture. No crystalline substance was obtained from the ethereal fraction, but two glycosides of a flavanone and a flavone, respectively, were isolated from the ethyl acetate fraction.

On the basis of its melting point, elementary analysis and hydrolysis products (one mole each of sakuranetin and glucose), the flavanone glycoside was identified as sakuranin, which was first isolated by Asahina[2] from the bark of *Prunus yedoensis* and *P. donarium*.

The flavone glycoside consisted of one mole each of glucose and of aglycone which showed the properties of genkwanin. Ohta and Nishikawa[3] isolated a genkwanin glucoside from the bark of *Prunus serrulata*. Ohta[4] showed it to be a 5-glucoside, which he named glucogenkwanin. The flavone glucoside and glucogenkwanin have the same melting point which was not depressed by admixture of the two.

The same results were obtained with a sample of *P. speciosa* from Ohshima Island, where this tree grows wild.

Experimental

Isolation of Flavonoids.—Wood chips (400 g.) from living stems (7 cm. diam.) were extracted with 3 l. of boiling methanol for 3 hr., and the extraction repeated. The methanolic filtrate was concentrated to a sirup, mixed with 200 ml. of water, heated on a water-bath and filtered. The filtrate was then repeatedly extracted with ether. After evaporation of the ether, the residue was mixed with an equal volume of ethyl acetate, and allowed to stand overnight. More ethyl acetate was added to dissolve the oily mass which had separated. After a while, white crystals gradually separated. These were filtered off; then yellow crystals appeared. The white crystals were sakuranin (yield of crude substance, 1.0 g.) which were recrystallized from dilute methanol to give white needles, m.p. 212°. The yellow crystals were glucogenkwanin (yield of crude substance, 0.4 g.) which were recrystallized from methanol to give microscopic needles, m.p. 273°.

Sakuranin.—It gave no color with ferric chloride. *Anal.* Calcd. for $C_{22}H_{24}O_{10}\cdot 4H_2O$: H_2O, 13.84. Found: H_2O, 12.93.

Anal. Calcd. for $C_{22}H_{24}O_{10}$: C, 58.92; H, 5.35: OCH_3, 6.91. Found: C, 59.18; H, 5.23; OCH_3, 7.19.

Hydrolysis.—Sakuranin (0.3679 g.) was added to 25 ml. of 2% sulfuric acid and heated for 30 minutes over a direct flame under reflux. The cooled liquor was repeatedly extracted with ether. After evaporation of the ether, the residue was recrystallized from dilute methanol to give white needles of sakuranetin, m.p. 152°.

Anal. Calcd. for $C_{16}H_{11}O_5\cdot H_2O$: H_2O, 5.90. Found: H_2O, 6.24. Calcd. for $C_{15}H_{11}O_4(OCH_3)$: OCH_3, 10.83. Found: OCH_3, 11.23.

The acetate of this aglycone was obtained as colorless plates, m.p. 146°.[5]

The mother liquor freed from aglycone was diluted to 50 ml. with water; the rotation in a 2.2 dm. tube at 18° was 0.25°, from which the amount of glucose was calculated to be 0.12 g.; theoretical amount 0.14 g.

This solution was then carefully neutralized with barium carbonate, evaporated on a boiling water-bath to a small volume and filtered. When the filtrate was heated with phenylhydrazine hydrochloride and sodium acetate, glucosazone was formed, which was filtered and recrystallized from dilute methanol; m.p. 208°, alone and on admixture with an authentic specimen.

Glucogenkwanin, m.p. 273°, was insoluble in benzene, chloroform, petroleum ether and ethyl acetate; sparingly soluble in methanol, ethanol and acetone. It gave an orange color with magnesium powder and concentrated HCl, but no color with ferric chloride.

Anal. Calcd. for $C_{22}H_{22}O_{10}\cdot 2H_2O$: C, 53.77: H, 5.40; OCH_3, 6.95; H_2O, 7.47. Found: C, 53.67; H, 6.17; OCH_3, 7.07; H_2O, 7.25.

Hydrolysis.—Glucogenkwanin (53.3 mg.) was dissolved in 1 ml. of concentrated sulfuric acid; after a while it was added to 15 ml. of cold water, and the solution allowed to

(1) M. Hasegawa and T. Shirato, THIS JOURNAL, **74**, 6114 (1952).

(2) Y. Asahina, *J. Pharm. Soc. Japan*, **28**, 213 (1908).

(3) T. Ohta and S. Nishikawa, *ibid.*, **62**, 40 (1942).

(4) T. Ohta, *ibid.*, **72**, 456 (1952).

(5) D. Chakravarti, N. Kundo and R. P. Ghosh, *J. Indian Chem. Soc.*, **25**, 329 (1948).

cool. The precipitated aglycone was rendered easier to filter by warming, and then was collected and dried; yield 34.3 mg.

After repeated recrystallization from methanol, the aglycone melted at 283°; it gave a purplish brown color with ferric chloride and an orange color with magnesium and concentrated hydrochloric acid. The aglycone was identical with the genkwanin obtained from *Prunus yedoensis* as shown by mixed melting point determination.

Glucogenkwanin (0.25 g.) was hydrolyzed by the same procedure. The filtrate was neutralized with barium carbonate and the osazone prepared by the usual method. The melting point 208° did not change when it was mixed with an authentic specimen of glucosazone.

Water (30 ml.) and 8 ml. of concentrated HCl were added to 50 mg. of glucogenkwanin in 50 ml. of methanol, and the mixture heated for 8 hours on a water-bath. After evaporation of the methanol, the aglycone was filtered. The filtrate was concentrated in a vacuum desiccator over KOH granules and examined chromatographically. Glucose was the only sugar found.

Genkwanin Diacetate.—A mixture of 0.2 g. of genkwanin, 4 ml. of acetic anhydride and 4 drops concentrated sulfuric acid in a small test-tube was set aside for an hour. Cold water was then added and the precipitate of acetylgenkwanin filtered, washed and recrystallized from methanol. The melting point 200° was not depressed by admixture with an authentic specimen of genkwanin diacetate, m.p. 204°.

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GOVERNMENT FOREST EXPT. STATION OF JAPAN
MEGURO, TOKYO, JAPAN

Flavonoids of Various *Prunus* Species. III. The Flavonoids in the Wood of *Prunus campanulata*

BY MASAO HASEGAWA AND TERUO SHIRATO

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This paper deals with the flavonoids of the wood of *Prunus campanulata* Maxim[1] which is cultivated in the Arboretum of the Government Forest Experiment Station in Tokyo. The method of extraction has been described previously.[2] Three flavanones were isolated from the ether-soluble fraction of the methanol extract as follows: the first product which separated from hot benzene proved to be naringenin,[2] the second which separated from hot water to be taxifolin[3] (distylin),[4] and the third which separated from a hot methanol–benzene mixture to be eriodictyol.[5] These products were identified by mixed melting point determinations of the compounds and their derivatives. The chromatographic data are given in Table I.

No glucosides of these flavanones were detected; only one phlobaphane-like substance was found in the ethyl acetate fraction.

TABLE I[a]

CHROMATOGRAPHIC DATA OF FLAVONOIDS OBTAINED FROM VARIOUS PRUNUS WOODS

Substance	*Rf* Developing agent[b] A	B	C	D	Detecting agent[c]
Eriodictyol		0.72	0.34		E, F, UV
Taxifolin	0.89	.55	.49		E, F, UV
Naringenin		.88	.37	0.05	E, F, UV
Sakuranetin		.95	.26	.71	E, F, UV
Prunin	.70	.75	.63		E, F, UV
Sakuranin	.68		.06		UV
Genkwanin		.98	.00		E, F,
Glucogenkwanin		.91	.20		UV

[a] The chromatograms were run at room temperature, 20–26°. [b] A = butanol:acetic acid:water, 4:1:5; B = *m*-cresol:acetic acid:water, 25:1:24; C = isopropyl alcohol:water, 22:78; D = water-saturated mixture of benzene and ligroin (30:1) containing methanol. [c] E = diazotized benzidine solution; F = 2% methanolic ferric chloride; UV = ultraviolet light. Results with filter paper made in Japan. Fluorescence in ultraviolet light did not occur except in the case of taxifolin, genkwanin and glucogenkwanin, when Whatman No. 1 filter paper was used.

Experimental

Extraction.—Wood chips (500 g.) of *Prunus campanulata* prepared from a dried stem[6] of 15-cm. diameter were twice extracted with 3-l. portions of methanol for 3 hours. The methanol filtrates were concentrated to 500 ml. on a water-bath. The solution was extracted repeatedly with ether, and the combined ether extract evaporated to dryness. The residue was extracted with boiling benzene (about 1500 ml.) and then with hot water (1500 ml.) and finally dissolved in 6 ml. of methanol to which 700 ml. of benzene was added.

Naringenin.—After filtration of the hot benzene extract, naringenin gradually deposited; an additional amount was obtained from the mother liquor. After five recrystallizations from methanol, the compound melted at 248°.

Anal. Calcd. for $C_{15}H_{12}O_5$: C, 66.17; H, 4.41. Found: C, 66.00; H, 4.26.

Naringenin triacetate, colorless needles, m.p. 126°.

The dimethyl ether was prepared by the reaction of naringenin with dimethyl sulfate and potassium carbonate in acetone; colorless long needles, m.p. 121°.

Anal. Calcd. for $C_{15}H_{10}O_3(OCH_3)_2$: OCH_3, 20.66. Found: OCH_3, 20.19.

Taxifolin (distylin).—The fraction soluble in hot water was extracted with ether. After evaporation of the ether, the residue was recrystallized from dilute methanol to give taxifolin as colorless prisms, m.p. 233°.

Anal. Calcd. for $C_{15}H_{12}O_7 \cdot H_2O$[7]: C, 55.90; H, 4.34. Found: C, 55.86; H, 4.46.

Taxifolin pentaacetate was obtained by treating taxifolin with pyridine and acetic anhydride without heating; colorless prisms, m.p. 155°.

Taxifolin.—5,7,3′,4′-Tetramethyl ether was prepared by heating an acetone solution of taxifolin with dimethyl sulfate and potassium carbonate, colorless prisms, m.p. 171°. The acetate of this methyl ether was obtained as colorless prisms, m.p. 169°. The melting points of taxifolin and its derivatives were not altered by admixture with the corresponding compounds prepared from an authentic specimen of taxifolin obtained from the wood of *Larix leptolepis*.

Eriodictyol.—As the methanol–benzene extract was slowly evaporated on a water-bath, eriodictyol deposited; after three recrystallizations from methanol, it was obtained as colorless needles, m.p. 263°.

(1) See M. Hasegawa and T. Shirato, THIS JOURNAL, in press for flavonoids of *Prunus donarium*.

(2) M. Hasegawa and T. Shirato, *ibid.*, **74**, 6114 (1952).

(3) J. C. Pew, *ibid.*, **70**, 3031 (1948); J. Gripenberg, *Acta Chem. Scand.*, **6**, 1152 (1952).

(4) T. Kondo, *J. Fac. Agr. Kyushu Univ.*, **10**, 79 (1951).

(5) J. Shinoda and S. Sato, *J. Pharm. Soc. Japan*, **49**, 7 (1929).

(6) Almost identical results were obtained with chips from a living stem of 4-cm. diameter.

(7) M. Hasegawa and T. Shirato, *J. Chem. Soc. Japan*, **72**, 279 (1951).