[CONTRIBUTION FROM THE GOVERNMENT FOREST EXPERIMENT STATION OF JAPAN]

Flavonoids of Various *Prunus* Species. IV. The Flavonoids in the Wood of *Prunus donarium* var. *spontanea*

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Eriodictyol, genkwanin, sakuranin and a flavanone glucoside have been isolated from the wood of *Prunus donarium* var. *spontanea*, a common wild cherry tree in Japan. The glycoside proved to be an isosakuranetin-7-glucoside, the natural form of isosakuranin synthesized by Zemplén in 1942.

This paper[1] deals with the flavonoids of the wood of *Prunus donarium* Siebold var. *spontanea* Makino, a large cherry tree growing wild in the central and southern parts of Japan. The flavonoid pattern of this species differs from any of those of the species already reported and included eriodictyol, genkwanin, sakuranin and a flavanone glycoside. The latter gives on hydrolysis one mole each of isosakuranetin and glucose. Its monomethyl ether contains two methoxyl groups, one of which was originally on the aglycone, the other having been introduced by methylation. It shows no coloration with ferric chloride, and proved to be identical with prunin dimethyl ether.[1] In view of these facts, the glycoside must be without doubt isosakuranetin-7-glucoside.

Zemplén,[2] previously has synthesized an isosakuranetin glucoside and ascribed to it the structure of a 7-glucoside. This synthetic glucoside melted at 214° and showed the specific rotation $[\alpha]^{24}\text{D}$ 73.4°, while ours melted at 190° and showed $[\alpha]^{13}\text{D}$ $-41.4°$.

Our glycoside gave on hydrolysis with emulsin an optically active isosakuranetin, which, on heating with hydrochloric acid, was converted to the optically inactive racemate. The discrepancy seems thus to be most probably attributable to the difference in the optical activity of the aglycone.

Experimental

Isolation of Eriodictyol, Isosakuranin, Sakuranin and Genkwanin from the Wood.—Wood chips (1.2 kg.) from a branch (7.5 cm. diameter) of *Prunus donarium* var. *spontanea*, were extracted in 300-g. lots with two portions of 3 l. boiling methanol, and the methanol extracts were concentrated by distillation under ordinary pressure. The concentrate was then evaporated in a dish on a boiling water-bath again to about 300 ml. After extracting several times with ether, the aqueous layer was repeatedly extracted with ethyl acetate until the mother liquor gave no color with magnesium and hydrochloric acid.

The combined ether was evaporated and the residue was treated with petroleum ether to remove easily-soluble resinous substances. The insoluble portion was dissolved in dilute methanol and, after boiling, allowed to stand for some time; a black sticky mass separated. From the filtrate, a crystalline substance deposited gradually. After 3 recrystallizations, the substance melted at 263° and was identified readily as eriodictyol; yield 0.5 g.

The ethyl acetate extracts were combined, evaporated to about 200 ml., and mixed with an equal volume of water. An oily mass which had separated was filtered, and from the filtrate colorless crystals were obtained. These were filtered and washed with ethyl acetate; yield 4.5 g. When recrystallized from ethanol and then from acetone, the substance (isosakuranin) was obtained in colorless needles, and decomposed at 190°. The mother liquor separated from isosakuranin was extracted again with ethyl acetate, and the extracts were concentrated to 100 ml. and mixed with an equal volume of water. After standing for some time, crystals deposited; yield 0.4 g. When recrystallized from ethyl acetate and then dilute methanol, the substance was obtained as colorless needles, m.p. 212°, identified as sakuranin. The mother liquor from sakuranin was dissolved in a large quantity of water, aqueous lead acetate was added, and the precipitate was filtered. The precipitate and the filtrate were separately treated with hydrogen sulfide. As no crystalline substance was isolated from both parts by the method employed, these portions were combined, and extracted with ether and then with ethyl acetate. The ethereal solution was evaporated and the residue was dissolved in a little methanol. There was deposited a crystalline substance; yield 0.2 g. This crystallized from methanol in minute yellow needles, which melted at 282° (genkwanin). The ethyl acetate solution was treated as above and 0.4 g. of sakuranin was obtained.

From 1.2 kg. of the *Prunus* wood, 0.5 g. of eriodictyol, 4.5 g. of isosakuranin, 0.8 g. of sakuranin and 0.2 g. of genkwanin were thus obtained.

Eriodictyol: colorless needles of m.p. 263°. *Anal.* Calcd. for $C_{15}H_{12}O_6$: C, 62.50; H, 4.16. Found: C, 62.11; H, 4.26.

Eriodictyol Acetate.—Eriodictyol (0.2 g.) was treated with 4 ml. each of acetic anhydride and pyridine. After standing 24 hours, cold water was added, the acetate was washed with water and recrystallized from methanol. Colorless needles were obtained which melted at 138°, both alone and on mixing with the sample obtained from the wood of *Prunus campanulata*.[1]

Eriodictyol 7,3′,4′-Trimethyl Ether.—Eriodictyol (0.2 g.) was dissolved in 30 ml. of acetone, then 3 g. of potassium carbonate and 0.5 ml. of dimethyl sulfate were added and the whole was heated for 1 hour. When the reaction was over, the liquid was evaporated after removal of mineral salts, mixed with water, and the solidified mass was washed with water and recrystallized from methanol; colorless needles of m.p. 136°, yield 0.1 g.

Anal. Calcd. for $C_{15}H_9O_3(OCH_3)_3$: OCH_3, 28.18. Found: OCH_3, 28.13.

Sakuranin.—The glycoside melted at 212° and gave no color reaction with ferric chloride.

Anal. Calcd. for $C_{22}H_{24}O_{10}$: C, 58.92; H, 5.35. Found: C, 58.48; H, 5.36.

Hydrolysis of Sakuranin.—A mixture of sakuranin (0.504 g.) and 40 ml. of 3% sulfuric acid was heated on a boiling water-bath. No sooner had the crystals of sakuranin dissolved, than crystals of the aglycone sakuranetin separated in their place. After heating 3 hr., the deposited crystals were filtered and dissolved in ether. The mother liquor was exhaustively extracted with ether and the ethereal solution together with that mentioned above was evaporated. The residue was crystallized from dilute methanol and colorless needles which melted at 154° were obtained in a yield of 0.30 g. No depression of melting point occurred when admixed with sakuranetin obtained from sakuranin of *Prunus speciosa*.[1]

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.15; H, 4.86; OCH_3, 10.83. Found: C, 67.08; H, 4.97; OCH_3, 10.71.

Water was added to the filtrate separated from sakuranetin to make 50 ml. With 10 ml. of this solution, the sugar was determined quantitatively according to the method of Bertrand. It was found that the reducing power of each 10 ml. of the solution was equal to 11.7 and 11.6 ml. of 0.1 *N* $KMnO_4$ (corresponding to 38.7 and 37.9 mg. of glucose,

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[1] For earlier papers in this series, see M. Hasegawa and T. Shirato, THIS JOURNAL, **74**, 6114 (1952); **76**, 5559, 5560 (1954).

[2] G. Zemplén, R. Bognár and L. Mester, *Ber.*, **75**, 1432 (1942).

respectively); Calcd.: glucose, 0.202 g. Found: glucose, 0.1915 g.

The rest of the solution then was neutralized with barium carbonate, filtered, and evaporated. An osazone which melted at 208°, both alone and on admixture with the authentic specimen, was obtained easily.

Sakuranetin Acetate.—Sakuranetin was acetylated as usual and the acetate melted at 132°; yield 0.2 g.

Sakuranetin 4'-Monomethyl Ether.—Three-tenths gram of sakuranetin was methylated in acetone with dimethyl sulfate and potassium carbonate. Sakuranetin 4'-monomethyl ether consists of colorless needles of m.p. 122°, yield 0.2 g.

Anal. Calcd. for $C_{15}H_{10}O_5(OCH_3)_3$: OCH_3, 20.66. Found: OCH_3, 20.10.

Isosakuranin.—The glycoside isosakuranin melted and at the same time decomposed at 190°. Methanolic solution showed a purplish-brown coloration with ferric chloride, and with magnesium and concd. hydrochloric acid it gave a reddish-purple coloration. The glycoside is difficultly soluble in hot water, ethanol, methanol, acetone and ethyl acetate, and insoluble in ether, benzene, chloroform and petroleum ether. R_f values: 0.75 (*m*-cresol–acetic acid–water 24:1:15), 0.95 (butanol–acetic acid–water 4:1:5). Absorption: λ_{max} 304 mμ (inflection), 283 mμ, λ_{min}. 250 mμ. Specific rotatory power: 0.2030 g. of subst. in 25 ml. of 60% acetone, 2-dm. tube, α_D −0.75°, $[\alpha]^{13}_D$ −41.4°.

Anal. Calcd. for $C_{22}H_{24}O_{10}\cdot 1^1/_2H_2O$: C, 58.92; H, 5.35; OCH_3, 6.91; H_2O of crystn., 5.68. Found: C, 59.10; H, 5.38; OCH_3, 6.99; H_2O of crystn., 5.65.

Isosakuranin Acetate.—The glycoside (0.2 g.) was dissolved in a mixture of 2 ml. each of acetic anhydride and pyridine and the solution was allowed to stand for 24 hr. After adding cold water, the solidified mass was washed with water and recrystallized from methanol to give colorless prisms of m.p. 147°. The acetate showed no color reaction with ferric chloride and gave a purplish-red coloration with magnesium and hydrochloric acid.

Anal. Calcd. for $C_{32}H_{34}O_{15}$: C, 58.35; H, 5.16. Found: C, 58.53; H, 5.12.

Isosakuranin Monomethyl Ether.—Isosakuranin (0.2 g.) was dissolved in 50 ml. of acetone, and then 0.5 ml. of dimethyl sulfate and 2 g. of anhydrous potassium carbonate were added. After heating on a water-bath for 1 hr., the solution from which mineral salts had been removed was evaporated. After cooling, ether was added to the resulting solution, and the whole was stirred thoroughly. Crystals were filtered, washed with water and recrystallized from methanol. Colorless needles were obtained, which melted at 228°, showed no color reaction with ferric chloride, but a red one with magnesium and hydrochloric acid; yield 0.1 g. On admixture with prunin dimethyl ether (231°), isosakuranin monomethyl ether melted at 228°, indicating their identity.

Anal. Calcd. for $C_{23}H_{26}O_{10}$: OCH_3, 13.44. Found: OCH_3, 13.40.

Hydrolysis of Isosakuranin with Acid.—Isosakuranin (0.623 g.) was hydrolyzed by heating on a boiling water-bath with 60 ml. of 3% hydrochloric acid, a small quantity of acetone being added in order to solubilize the sparingly soluble glycoside. The acetone was evaporated gradually, crystals which separated out were filtered and the mother liquor was heated 3 hr. further for completion of hydrolysis. After cooling, the hydrolyzate was extracted with ether, and the crystals were dissolved in ether. The ethereal solutions were combined, evaporated and the residue was recrystallized from dilute methanol. Colorless prisms which melted at 193° were obtained; yield 0.40 g. Neither depression of melting point when admixed with isosakuranetin from poncirin,[3] nor rotatory power was observed.

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.13; H, 4.86. Found: C, 66.78; H, 5.04.

Hydrolysis of Isosakuranin with Emulsin.—One gram of isosakuranin was suspended in 100 ml. of 0.5% emulsin solution and allowed to stand 8 days at 27° with occasional stirring. The mixture was shaken with ether, and the ethereal extract gave after evaporation a crystalline mass (0.4 g.). When recrystallized from dilute methanol, colorless prisms of isosakuranetin of m.p. 177° were obtained. Specific rotatory power: 0.367 g. of subst. in 25 ml. of pyridine–acetone (3.5 ml. + 21.5 ml.), 2.2-dm. tube, α_D −0.90°, $[\alpha]^{16}_D$ −27.8°.

Anal. Calcd. for $C_{16}H_{14}O_5$: C, 67.15; H, 4.86. Found: C, 67.10; H, 4.92.

Racemization of Isosakuranetin by Heating with Acid.—A half gram of isosakuranetin (m.p. 177°), obtained by the hydrolysis of isosakuranin with emulsin, 50 ml. of 5% hydrochloric acid and 20 ml. methanol were heated on a water-bath for 3 hr. After evaporation of methanol the residue (0.4 g.) was recrystallized repeatedly from methanol, and colorless prisms of m.p. 193° were obtained. No depression of melting point was observed when admixed with isosakuranetin, obtained by hydrolysis with mineral acid. It possessed no rotatory power.

Isosakuranetin Acetate.—Isosakuranetin (0.1 g.) was dissolved in 1 ml. each of acetic anhydride and pyridine. After standing 3 hr., cold water was added to the mixture. The solidified mass was recrystallized from methanol, giving colorless prisms which melted at 115°, yield 0.15 g.

Isosakuranetin Monomethyl Ether.—Isosakuranetin (0.1 g.) was methylated in 20 ml. of anhydrous acetone with 0.5 ml. of dimethyl sulfate and 3 g. of potassium carbonate. After heating for 1 hr., the solution was filtered, evaporated, and the residue was treated with water. The solidified mass was washed with water and dissolved in methanol. Colorless prisms of m.p. 118° separated from the solution; yield 0.05 g.

Anal. Calcd. for $C_{17}H_{16}O_5$: OCH_3, 20.66. Found: OCH_3, 20.95.

Determination of the Sugar in Isosakuranin.—The hydrolysis solution from which the aglycone had been removed was made up with water to 100 ml. in a volumetric flask, and, with 10 ml. of this solution, the sugar was determined according to the method of Bertrand. The reducing power of each 10 ml. was found to be equal to 7.78 and 7.86 ml. of 0.1 *M* $KMnO_4$ (corresponding to 24.89 and 25.15 mg. glucose, respectively). Calcd.: glucose, 0.1503 g. Found: glucose, 0.2515, 0.1489 g.

The solution was evaporated on a boiling water-bath and dried in a desiccator over sodium hydroxide under reduced pressure. After removing hydrochloric acid, chromatograms were prepared, using butanol–acetic acid–water (4:1:1) as solvent and benzidine as a developing agent. It gave only a single spot, and this corresponded to glucose. An osazone was obtained, which decomposed at 208°, both alone and on admixture with an authentic specimen of glucosazone.

Genkwanin: microscopic yellow needles of m.p. 282°.

Anal. Calcd. for $C_{15}H_9O_4(OCH_3)$: C, 67.60; H, 4.22. Found: C, 67.41; H, 3.99.

Genkwanin Monomethyl Ether.—Genkwanin (0.1 g.) was methylated by heating in 50 ml. of anhydrous acetone with 1 ml. of dimethyl sulfate and 4 g. of anhydrous potassium carbonate for 1 hr. on a water-bath. The filtered solution was evaporated, the residue was treated with water, and the precipitate was recrystallized from methanol. Monomethyl genkwanin consists of yellow needles which melted at 173°, both alone and on admixture with acacetin 7-methyl ether.

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(3) S. Hattroi, M. Hasegawa and M. Shimokoriyama, *Acta Phytochimica (Japan)*, **14**, 1 (1944).