ally, but it was found that different rates could be obtained using different portions of the same data. However, the rate was not second order, since it was independent of the concentrations of the reactants except at very high concentrations of chromic anhydride relative to 2-methylfenchol, when it was somewhat lower (see Table IV). In most of the runs the concentration of the intermediate having $\lambda_{max}$ 393 m$\mu$ was recorded early enough to observe the density curve passing through a maximum. In these runs the rate was determined from data taken beyond the maximum point.

**Rate of Oxidation of 1-Methyl-$\alpha$-fenchene by Chromic Anhydride in Acetic Acid.**—Standard solutions of chromic anhydride and 1-methyl-$\alpha$-fenchene, m.p. 62.5–64°, in acetic acid were prepared and thermostated at 25°. Aliquots were mixed just prior to making a run, transferred to the Cary cell and allowed to stand for 30 seconds before following the reaction at 347 m$\mu$. At no time during these oxidations was there an indication of a maximum at 393 m$\mu$. The second-order rate constants were calculated directly and graphically from the optical density data (Table V). Similar measurements were performed on mixed olefin.

TABLE V

RATE OF OXIDATION OF 1-METHYL-$\alpha$-FENCHENE (IV) AND MIXED OLEFIN BY CHROMIC ANHYDRIDE IN ACETIC ACID AT 25°

| No. of runs | (Olefin) $\times 10^{-2}$ | ($CrO_3$) $\times 10^{-2}$ | $k_2$, mole$^{-1}$ sec.$^{-1}$ |
|---|---|---|---|
| 6 (IV) | 0.960–1.92 | 1.03–2.06 | $5.68 \pm 0.1 \times 10^{-3}$ |
| 14 (III–IV) | 0.963–6.0 | 0.965–1.6 | $1.03 \pm 0.04 \times 10^{-4}$ |

**Chromic Anhydride Oxidation of 1-Methyl-$\alpha$-fenchene.**—1-Methyl-$\alpha$-fenchene (0.50 g.) was dissolved in 6 ml. of acetic acid, and a solution of 0.87 g. of chromic anhydride in 13 ml. of acetic acid and 0.5 ml. of water was added dropwise during 15 min., the temperature never exceeding 25°. This mixture was then heated to *ca.* 90° for 3.5 hr., after which it was poured into 30 g. of ice-water and extracted first with five 10-ml. portions of pentane and then with four 10-ml. portions of ether. Each of these was then extracted with three 10-ml. portions of 5% sodium carbonate.

The pentane solution was dried over magnesium sulfate, the solvent was evaporated on the steam-bath and the residue was distilled at room temperature (1 mm.): 105 mg. The infrared spectrum of this product had peaks at 5.75, 5.85 and 6.00 $\mu$, showing the presence of camphor (fenchone), another carbonyl substance and olefin. Comparison of the absorption peaks at 9.55 and 9.77 $\mu$ with those of authentic mixtures of camphor and fenchone indicated the identity of the mixture to be chiefly camphor containing not more than 6% fenchone. The ether layer, when worked up in the same manner, gave a very small amount of additional product of similar composition.

The combined carbonate extracts were acidified with concentrated hydrochloric acid and extracted with ether. After drying over magnesium sulfate, the solvent was evaporated and the residue was sublimed at 40° (1 mm.), 93 mg. The infrared spectrum, although similar to that of camphane-2-carboxylic acid, indicated a mixture of acids of almost the same composition as that obtained from the chromic acid oxidation of the mixed olefins, 1-methyl-$\alpha$-fenchene and 1-methylcamphene.

In order to show that the pure olefin was not significantly rearranged by acetic acid under the conditions of the oxidation just described, 364 mg. of 1-methyl-$\alpha$-fenchene in 10 ml. of acetic acid was held at 33° for 2 hr. and then at 80–90° for 2.5 hr. The olefin was then recovered by the method of workup described above for the oxidation, from which 146 mg. of starting substance was recovered having an infrared spectrum indistinguishable from that of the pure olefin.

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[CONTRIBUTION FROM THE GOVERNMENT FOREST EXPERIMENT STATION, JAPAN]

# Flavonoids of Various *Prunus* Species. VI. The Flavonoids in the Wood of *Prunus aequinoctialis*, *P. nipponica*, *P. Maximowiczii* and *P. avium*

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From the wood of *Prunus aequinoctialis*, naringenin, aromadendrin, sakuranetin, eriodictyol, genistein, prunetin, verecundin, prunin, genistin and chrysin-7-glucoside were obtained. The name aequinoctin is proposed for the chrysin-7-glucoside, new to the natural product literature. The wood of *P. nipponica* contains *d*-catechin, naringenin, sakuranetin, eriodictyol, taxifolin, genistein, prunetin, aequinoctin (a little), chrysin (trace) and genistin (a little); and that of *P. Maximowiczii*, *d*-catechin (a little), naringenin, sakuranetin, eriodictyol, taxifolin (a little), aromadendrin (trace), chrysin (trace), prunetin and genistein. The wood of *P. avium* contains *d*-catechin, naringenin, prunin, aromadendrin (trace), eriodictyol, taxifolin, chrysin, aequinoctin, genistein, prunetin and genistein.

This report deals with the flavonoids obtained from the wood of *Prunus aequinoctialis* Miyoshi, which is distributed in the mountainous region of Japan. The flavonoid compounds which were isolated are naringenin, aromadendrin, sakuranetin, eriodictyol, genistein, prunetin, verecundin,[1] prunin,[2] genistin[3] and chrysin-7-glucoside.

Chrysin-7-glucoside is hydrolyzed by acids into one mole each of chrysin and glucose, and, since it shows a violet-brown coloration with ferric chloride, only one possibility remains for the glycosidic bond in it. Since it is hitherto not described as having been isolated from a natural product, I propose the name "aequinoctin" for it.

In 1944, Zemplén, Bognár and Mechner[4] reported the synthesis of chrysin-7-glucoside and gave 242 and 196° as the melting points of it and its pentaacetate, respectively. These coincide well with those of aequinoctin (m.p. 245°) and its pentaacetate (m.p. 197°). Zemplén, *et al.*, considered that the glycoside toringin has the constitution chrysin-7-glucoside, as revealed in the title of their article "Synthesis of the glucoside toringin." Toringin, however, is chrysin-5-glucoside as was elucidated by Hirose[5] and synthesized by Hattori and Shimokoriyama.[6] It does not give any coloration with ferric chloride.

(1) M. Hasegawa and T. Shirato, THIS JOURNAL, **79**, 450 (1957).
(2) M. Hasegawa and T. Shirato, *ibid.*, **74**, 6114 (1952).
(3) E. D. Walter, *ibid.*, **63**, 3273 (1941).
(4) G. Zemplén, R. Bognár and J. Mechner, *Ber.*, **77B**, 99 (1944).
(5) Y. Hirose, *J. Chem. Soc. Japan*, **41**, 187 (1920); Proceedings of the Society Meeting of Jan. 13, 1920.
(6) S. Hattori and M. Shimokoriyama, *Acta Phytochim. (Japan)*, **13**, 109 (1943).

The flavonoid patterns of *Prunus nipponica*, *P. Maximowiczii* and *P. avium* are similar to that of *P. aequinoctialis:* namely, *P. nipponica* contains *d*-catechin, naringenin, sakuranetin, eriodictyol, taxifolin, prunetin, genistein, prunin, chrysin (trace), aequinoctin, genistin (a little); and *P. Maximowiczii* contains *d*-catechin (a little), naringenin, sakuranetin, eriodictyol, taxifolin (a little), aromadendrin (a little), prunetin, genistein and chrysin (trace). The wood of *P. avium* contains *d*-catechin, naringenin, prunin, aromadendrin, eriodictyol, taxifolin, chrysin, aequinoctin, genistein.

Aromadendrin is 3,4′,5,7-tetrahydroxyflavanone; *d*-catechin is 3,3′,4′,5,7-pentahydroxyflavane; chrysin is 5,7-dihydroxyflavone; eriodictyol is 3′,4′,5,7-tetrahydroxyflavanone; genistein is 4′,5,7-trihydroxyisoflavone; genistin is the 7-glucoside of genistein; naringenin is 4′,5,7-trihydroxyflavanone; prunetin is 4′,5-dihydroxy-7-methoxyisoflavone; prunin is the 7-glucoside of naringenin; pinocembrin is 5,7-dihydroxyflavanone; sakuranetin is 4′,5-dihydroxy-7-methoxyflavanone; taxifolin is 3,3′,4′,5,7-pentahydroxyflavanone; and verecundin is pinocembrin-5-glucoside.

## Experimental

**Isolation of Flavonoids from the Wood of *Prunus aequinoctialis*.**—Wood chips (500 g.) of *Prunus aequinoctialis* prepared from a stem of 10-cm. diameter were twice extracted with 4-l. portions of methanol for 3 hr. each. After filtration and distillation of the methanol, the residual 600 ml. of sirup was extracted fifteen times with 400-ml. portions of ordinary ether. The residue, insoluble in ether, was extracted ten times with 400-ml. portions of ordinary ethyl acetate.

**Ether-soluble Portion.**—Distillation of the ether gave 90 g. of sirup which was triturated with 500 ml. of petroleum ether (b.p. 30–60°) to remove certain oils. The residue (47 g.) was dissolved in 300 ml. of hot methanol and cooled to room temperature. After standing seven days (room temp.), 1.5 g. of crystals of prunetin were removed by filtration and identified by the following methods. The filtrate was concentrated to half-volume and allowed to stand two days at room temperature. Genistein (0.9 g.) was removed by filtration.

The mother liquor was then evaporated, and the residue was extracted with ordinary ether. After evaporation of the ether, the residue (22 g.) was recrystallized from dilute methanol to give naringenin as colorless needles of m.p. 248° (yield 3.7 g.).

**Ethyl Acetate Soluble Portion.**—The combined ethyl acetate solution was concentrated to 500 ml., followed by the addition of 100 ml. of water. After standing for a week, a crystalline mass (a mixture of genistin and verecundin) gradually appeared on the interface of the two liquids and was collected by filtration. The filtered mass was extracted with hot ordinary ethyl acetate, from which verecundin gradually appeared after cooling to room temp. Recrystallized from ordinary ethyl acetate, verecundin was obtained as colorless needles of m.p. 135°; the yield was 0.2 g.

The portion insoluble in hot ethyl acetate was recrystallized from 80% methanol to give genistin as almost colorless prisms of m.p. 259° (yield 3.5 g.).

The mother liquor freed from genistin and verecundin was then shaken with ordinary ethyl acetate. After evaporation of ethyl acetate, the residue was dissolved in hot methanol and allowed to stand; thereupon aequinoctin deposited as yellow needles of m.p. 245° (yield 1.4 g.).

After evaporation of methanol, the sirupy residue was allowed to stand, and crystals of prunin appeared slowly. Further recrystallization from a small quantity of methanol gave prunin as colorless needles of m.p. 224°. The yield was 5.0 g.

**The Second Method of Evaporation.**—One lot of wood chips (500 g.) was extracted with 4 l. of methanol and was divided into ordinary ether and ordinary ethyl acetate soluble portions as described above.

**The Ether-soluble Portion.**—After evaporation of the ether, the residue was extracted with 300 ml. of hot benzene and then with hot water (500 ml.) and finally the residue was dissolved in 20 ml. of hot methanol.

After evaporation of benzene from the solution, the residue was dissolved in 5 ml. of cold methanol. After removing thick oily substances by filtration, colorless crystalline substances appeared. This precipitate was recrystallized from methanol. The first crop was sakuranetin and the second, naringenin. The yield of sakuranetin was very scanty and that of naringenin was 0.1 g.

The fraction soluble in hot water was extracted with ordinary ether. After evaporation of the ether, the residue was dissolved in 10 ml. of water. After standing several days, aromadendrin appeared as colorless crystals. Aromadendrin was recrystallized from water and obtained as colorless prisms of m.p. 227°, yield 20 mg.

From the portion soluble in methanol prunetin, genistein, eriodictyol and naringenin were stepwise obtained by crystallization. The yields were 0.05, 0.3, 0.07 and 0.8 g., respectively.

**The Ethyl Acetate Soluble Portion.**—No flavonoid was obtained in workable amount from the portion soluble in ethyl acetate.

**Naringenin.** *Anal.* Calcd. for $C_{15}H_{12}O_5$: C, 66.17; H, 4.41. Found: C, 66.27; H, 4.52. Naringenin acetate, m.p. 127° (from methanol), m.p. 97° (from ether).

The melting points of naringenin and its derivatives did not alter on mixing with authentic specimens obtained from *Prunus yedoensis*.[2]

**Aromadendrin.**—The paper-chromatographic data agreed with the authentic specimens obtained from *Cercidiphyllum japonicum*.[7] The developing solvents were: *m*-cresol: acetic acid:water, 24:1:25; $R_f$ value 0.76; isopropyl alcohol:water, 22:78; $R_f$ value 0.65; acetic acid:water, 60:40; $R_f$ 0.85. The melting point did not show any depression when mixed with authentic aromadendrin.

**Sakuranetin.**—The paper-chromatographic data completely agreed with those of authentic sakuranetin. The color reaction given by diazotized benzidine was more faint than that of isosakuranetin.

**Eriodictyol.**—Methanolic solution gave a brown coloration with ferric chloride, and when one drop of water was added, the coloration turned to green. Eriodictyol gave colorless needles of m.p. 262° from methanol. The paper-chromatographic data and mixed melting point agreed with eriodictyol obtained from *Prunus campanulata*.[8]

**Prunetin.**—Methanolic solution gave a violet-brown coloration with ferric chloride and slight yellow coloration with magnesium powder and concentrated hydrochloric acid in alcoholic solution.

*Anal.* Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.22; $OCH_3$, 10.91. Found: C, 67.72; H, 4.44; $OCH_3$, 10.55.

Prunetin acetate, m.p. 224°. Prunetin-4′-methyl ether, m.p. 145°. *Anal.* Calcd. for $C_{15}H_8O_3(CH_3O)_2$: $OCH_3$, 20.80. Found: $OCH_3$, 20.79.

The melting points of prunetin and its derivatives did not alter on mixing with the corresponding compounds prepared from an authentic specimen obtained from *Prunus verecunda*.

**Verecundin.**—Verecundin gave no coloration with ferric chloride and an orange one with magnesium powder and concentrated hydrochloric acid in methanol solution. No depression of melting point was observed when mixed with verecundin of authenticated structure obtained previously.

*Anal.* Calcd. for $C_{21}H_{22}O_9 \cdot 2H_2O$: C, 55.60; H, 5.72. Found: C, 55.49; H, 5.95.

**Genistin.**—Genistin gave a violet-brown coloration with ferric chloride in methanol.

*Anal.* Calcd. for $C_{21}H_{20}O_{10}$: C, 58.38; H, 4.63. Found: C, 58.41; H, 4.56.

A deep depression was observed when this preparation was mixed with sophoricoside (genistein-4′-glucoside)[9] obtained from the fruit pods of *Sophora japonica* by Mr. M. Matsuda.

**Hydrolysis of Genistin.**—Genistin (0.8836 g.) was heated with 100 ml. of 6% methanolic hydrochloric acid for 3 hr. After addition of 50 ml. of water, the mixture was heated

(7) M. Hasegawa, Miscellaneous Reports of the Research Institute for National Resources, Nos. 17–18, 57 (1950).

(8) M. Hasegawa and T. Shirato, THIS JOURNAL, **76**, 5560 (1954).

(9) G. Zemplén, R. Bognár and L. Farkas, *Ber.*, **76**, 267 (1953).

further for 3 hr. over a direct flame. After evaporation of methanol, the residual solution was allowed to stand overnight. After genistein was filtered, the filtrate was concentrated in a vacuum desiccator over potassium hydroxide granules and examined chromatographically. Glucose was the only sugar found. The melting point of the phenylosazone (m.p. 208°) prepared from the above filtrate did not alter when mixed with an authentic specimen of glucosazone.

The filtered aglycone was washed and dried; yield 0.5485 g. After repeated recrystallization from methanol, the aglycone melted at 297°. The aglycone was identical with the genistein obtained from *Prunus verecunda*. Genistein acetate, m.p. 201°; genistein-7,4'-dimethyl ether, m.p. 145°.

**Genistin Acetate.**—A mixture of 0.2 g. of genistin, 4 ml. of pyridine and 4 ml. of acetic anhydride in a small test-tube was set aside for 48 hr. Cold water was then added and the precipitate of acetylgenistin filtered, washed and recrystallized from methanol, giving colorless needles of m.p. 188°, yield 0.2 g.

**Aequinoctin (Chrysin-7-glucoside).**—This glycoside showed a brown coloration with ferric chloride and an orange one with magnesium and concentrated hydrochloric acid in ethanol solution. It was recrystallized from methanol and gave yellow needles of m.p. 245°.

*Anal.* Calcd. for $C_{21}H_{20}O_9 \cdot H_2O$: C, 56.88; H, 5.19. Found: C, 56.92; H, 5.13.

**Hydrolysis of Aequinoctin.**—The glycoside (57.6 mg.) was dissolved in 30 ml. of concentrated sulfuric acid; after a while this solution was added to 50 ml. of hot water, and the solution was heated 5 minutes more on a water-bath. After cooling, the precipitated aglycone was collected, washed and dried; yield 33.0 mg.

For the estimation of the sugar produced by hydrolysis of aequinoctin, which hydrolysis was effected by heating the glycoside with 100 ml. of 6% methanolic hydrochloric acid for 6 hr. on a water-bath, 50 ml. of water was added and the methanol was evaporated by heating. The solution was further heated for 1 hr. over a direct flame and then allowed to stand overnight. Of the filtrate, glucose was estimated by the method described previously under genistin.

The aglycone was recrystallized from methanol twice and obtained as yellow needles of m.p. 276°. It gave a violet-brown coloration in a methanolic solution with ferric chloride and an orange one with magnesium powder and concentrated hydrochloric acid in methanol.

*Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 70.86; H, 3.93. Found: C, 70.95; H, 4.12.

Chrysin acetate, m.p. 196°. Chrysin-7-methyl ether, m.p. 163°. *Anal.* Calcd. for $C_{15}H_9O_3(OCH_3)$: $OCH_3$, 11.56. Found: $OCH_3$, 11.85.

The melting point of this methyl ether did not alter on mixture with tectochrysin.[9]

**Aequinoctin Pentaacetate.**—A mixture of the glycoside (0.1 g.), pyridine (3 ml.) and acetic anhydride (3 ml.) in a small test-tube was allowed to stand for 48 hr. Cold water was then added, and the precipitate was filtered, washed and recrystallized from methanol to give colorless needles of m.p. 197°.

*Anal.* Calcd. for $C_{31}H_{30}O_{14} \cdot H_2O$: C, 57.76; H, 4.81. Found: C, 57.82; H, 5.12.

**Aequinoctin-5-methyl Ether.**—This was prepared by heating an acetone solution of aequinoctin with dimethyl sulfate and potassium carbonate; faint yellow needles, m.p. 214°.

*Anal.* Calcd. for $C_{22}H_{22}O_9$: $OCH_3$, 7.21. Found: $OCH_3$, 7.34.

**Prunin.**—After repeated recrystallization from methanol, prunin melted at 224°. This glycoside proved to be identical with authentic prunin on chromatographic comparison and by mixed melting point test. Prunin acetate, m.p. 190°. Prunin 5,4'-dimethyl ether, m.p. 232°.

**Flavonoids of *Prunus nipponica* and *P. Maximowiczii*.**—Five hundred grams of wood chips each of these species was extracted and the extract worked up as above mentioned. There were obtained *d*-catechin (0.1 g.), naringenin (0.6 g.), sakuranetin (0.2 g.), eriodictyol (0.1 g.), taxifolin (0.2 g.), aequinoctin (a small amount), chrysin (trace), prunetin (0.5 g.), genistein (0.1 g.), prunin (0.1 g.), genistin (a small amount) from the former and *d*-catechin (a small amount), naringenin (0.8 g.), sakuranetin (0.5 g.), eriodictyol (0.1 g.), taxifolin (a small amount), aromadendrin (trace), prunetin (0.2 g.), genistein (0.4 g.) and chrysin (trace) from the latter.

It is very curious that no glycoside was obtained in the wood of the latter plant, even when 3 kg. of material was used. Whether this is to be ascribed to the special behavior of this tree or all of the glycosides which existed have disappeared by hydrolysis, is not known at present.

**Flavonoids of *Prunus avium*.**—Three hundred grams of wood of this species was treated by the same method as was applied to the preceding two species. There were obtained *d*-catechin (2 g.), naringenin (0.2 g.), eriodictyol (0.1 g.), taxifolin (0.1 g.), aromadendrin (trace), chrysin (0.6 g.), prunetin (0.1 g.) and genistein (0.2 g.). From 1 of the same wood aequinoctin (1.1 g.), genistin (0.2 g.) and prunin (0.1 g.) were obtained by another procedure.

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