bined chloroform extracts were evaporated *in vacuo* to give a sirup (0.297 g.) showing $[\alpha]^{27}D$ −13° in methanol (*c* 3.0) and OMe, 36.6%. The sirup crystallized to give 1,3,4-tri-*O*-methyl-D-fructose,[14,28] m.p. 76–77°, undepressed on admixture with an authentic specimen, $[\alpha]^{25}D$ −31° in water (*c* 4.0) after 3 min., changing in 120 min. to −55° (equilibrium value). The mother liquor from the above crystals showed $[\alpha]^{26}D$ −8° in methanol and was treated with acetone containing sulfuric acid as described before[21] but no 1,2-*O*-isopropylidine-3,4,6-tri-*O*-methyl-D-fructose was obtained. The component of the sirup which did not form an isopropylidine derivative (0.16 g.) was oxidized with nitric acid and the resulting acid converted to the amide *via* the methyl ester as described above. In this way there was obtained the diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid,[14,15] m.p. and mixed m.p. 191–192°.

(d) **3,4-Di-*O*-methyl-D-fructose.**—A portion of fraction C, Table III (0.05 g.), OMe 24.2%, was oxidized with nitric acid as described above and the acid converted to the corresponding amide in the usual way. There was isolated the diamide of methyl 3,4-di-*O*-methyl-D-fructofuranoside-1,6-dicarboxylic acid,[14,15] m.p. and mixed m.p. with an authentic specimen 191–192°, $[\alpha]^{22}D$ −72° in water (*c* 0.4).

Quantitative Analysis of the Cleavage Fragments of Methylated Glucofructan.—A portion (1.100 g.) of fraction 2, Table II, was hydrolyzed as described above and the aqueous hydrolyzate concentrated to 50 ml. *in vacuo* at 25–30°. To this solution was added barium carbonate (3 g.) and bromine (1 ml.) and the oxidation continued at room temperature in the dark for 3 days. The excess bromine was removed by aeration, the solution filtered and the residue washed well with water. The combined filtrate and washings were freed from barium ions with sulfuric acid and then the aqueous solution treated with silver carbonate to remove the hydrochloric acid produced. After filtration the aqueous solution was passed through "Amberlite IR 120" cation- and "Duolite A4" anion-exchange resins and the neutral eluate evaporated *in vacuo* to a sirup (0.863 g.) which was separated into the component methyl-fructoses by column chromatography as described above.

The results were as follows: 1,3,4,6-tetra-*O*-methyl-D-fructose (0.379 g.), $[\alpha]^{23}D$ +13° in ethanol (*c* 4); 1,3,4-tri-*O*-methyl-D-fructose (0.230 g.), m.p. 77°, $[\alpha]^{23}D$ −7° → +16° in methanol (*c* 4.5); and 3,4-di-*O*-methyl-D-fructose (0.190 g.), $[\alpha]^{13}D$ −25° → +28° in methanol (*c* 2). The R_f values of the three component sugars were 0.85, 0.66 and 0.33, respectively, using ethyl methyl ketone:water azeotrope and Whatman No. 1 filter paper.

The acidic material absorbed by the "Duolite A4" anion-exchange resin was eluted with 4% sodium hydroxide and the free acids immediately regenerated by passing the alkaline eluate through "Amberlite IR 120" cation-exchange resin. The acidic eluate from the latter column was concentrated to about 10 ml. under reduced pressure and the aqueous solution extracted five times with chloroform. The combined chloroform extracts were evaporated *in vacuo* to give 2,3,4,6-tetra-*O*-methyl-D-gluconolactone as a sirup (0.091 g.), $[\alpha]^{21}D$ +98° in ethanol (*c* 0.3), equiv. wt., 214 (calcd. for $C_{10}H_{18}O_6$; equiv. wt., 234), R_f 0.90 with ethyl methyl ketone:water azeotrope (an authentic sample showed R_f 0.90).

The molecular ratios of the four cleavage fragments deduced from the above results are as follows: 2,3,4,6-tetra-*O*-methyl-D-glucose (1); 1,3,4,6-tetra-*O*-methyl-D-fructose (3), 1,3,4,-tri-*O*-methyl-D-fructose (2) and 3,4-di-*O*-methyl-D-fructose (2).

ST. PAUL, MINNESOTA

(28) S. W. Challinor, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, 676 (1934).

[CONTRIBUTION FROM THE GOVERNMENT FOREST EXPERIMENT STATION, JAPAN]

Flavonoids of Various *Prunus* Species. V. The Flavonoids in the Wood of *Prunus verecunda*

BY MASAO HASEGAWA AND TERUO SHIRATO

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From the wood of *Prunus verecunda* Koehne, pinocembrin, pinocembrin-5-glucoside, genistein, prunetin, isosakuranetin, isosakuranin, naringenin, genkwanin, eriodictyol and taxifolin were isolated. The name "verecundin" was proposed for the second-named new glycoside.

This report deals with the flavonoids of the wood of *Prunus verecunda* Koehne, which is distributed in the mountainous region of middle Japan. The flavonoid pattern of this tree differs somewhat from any of those described before[1–4] for other *Prunus* species.

The flavonoid compounds which were isolated are pinocembrin-5-glucoside (5,7-dihydroxyflavanone 5-glucoside), genistein (5,7,4′-trihydroxyisoflavone), prunetin (5,4′-dihydroxy-7-methoxyisoflavone), pinocembrin (5,7-dihydroxyflavanone), isosakuranetin (5,7-dihydroxy-4′-methoxyflavanone), isosakuranin (5,7-dihydroxy-4′-methoxyflavanone 7-glucoside), naringenin (5,7,4′-trihydroxyflavanone), genkwanin (5,4′-dihydroxy-7-methoxyflavone), eriodictyol (5,7,3′,4′-tetrahydroxyflavanone), taxifolin (3,5,7,3′,4′-pentahydroxyflavanone), and among these substances, the first four are the new additions to the *Prunus* constituents.

(1) M. Hasegawa and T. Shirato, THIS JOURNAL, **74**, 6114 (1952).
(2) M. Hasegawa and T. Shirato, *ibid.*, **76**, 5559 (1954).
(3) M. Hasegawa and T. Shirato, *ibid.*, **76**, 5560 (1954).
(4) M. Hasegawa and T. Shirato, *ibid.*, **77**, 3557 (1955).

Of these, the first-named glycoside is new. It is hydrolyzed by dilute mineral acids to give one mole each of pinocembrin[5] and glucose, and the analysis agreed with the formula $C_{21}H_{22}O_9$. It gives no coloration with ferric chloride showing that the 5-hydroxyl group of pinocembrin is not present owing to glycoside formation. It gives merely an orange coloration when reduced in methanol with magnesium powder and concd. hydrochloric acid. By methylation with diazomethane, one hydroxyl group is methylated. We wish to propose the name "verecundin" for this glycoside.

Experimental

Isolation of Flavonoids.—Wood chips (500 g.) of *Prunus verecunda* prepared from a stem of 7.5 cm. diameter were twice extracted with 3-l. portions of methanol for 3 hours. The filtered methanol extracts were concentrated to a sirup. A total of 2.4 kg. of wood chips were thus treated. The combined sirup was extracted repeatedly with ether and then with ethyl acetate.

Ether-soluble Portion.—After evaporation of the ether, the residue was twice extracted with 300 ml. of hot benzene

(5) H. Erdtman, *Svensk Papperstidn.*, **46**, 226 (1943); *C. A.*, **37**, 5862 (1943).

followed by filtering. An amorphous substance gradually deposited from the hot benzene, and some additional amount was obtained from the mother liquor. The yield was 20 g. This amorphous substance was recrystallized from benzene and then from dilute methanol. The first precipitate was pinocembrin (yield 0.3 g.) and the second was isosakuranetin (yield 1.5 g.). Pinocembrin was then recrystallized from methanol to give colorless needles of m.p. 197°. Isosakuranetin was obtained from methanol as colorless prisms of m.p. 177°.[4] Beside these two substances naringenin was also detected by paper chromatography in the fraction soluble in benzene.

The fraction insoluble in hot benzene was extracted with 500 ml. of hot water and the water-soluble portion was repeatedly extracted with ether. After evaporation of the ether, the residue was recrystallized from dilute methanol to give taxifolin as colorless prisms of m.p. 235° (yield 0.5 g.).

The fraction insoluble in hot water was dissolved in dilute methanol and allowed to stand. Yellow crystals gradually deposited. The yellow crystals were recrystallized from a large volume of methanol. Genkwanin, genistein and prunetin were stepwise obtained. The yields were 0.5, 1.0 and 0.3 g., respectively.

The mother liquor was decolorized with charcoal. A small amount of water was added to give a slight turbidity. Naringenin was then gradually deposited (yield 2.5 g.). After repeated recrystallizations from methanol the melting point rose to 248°. After evaporating the methanol, the mother liquor was extracted with ether. The ether was evaporated and the residue was dissolved in methanol using charcoal. Eriodictyol crystallized out as colorless needles of m.p. 264° (yield 0.3 g.).

Ethyl Acetate-soluble Portion.—After evaporation of ethyl acetate, the residue was extracted with hot ethyl acetate. To the concentrated ethyl acetate solution (300 ml.) 200 ml. of water was added. After standing, crystalline substances gradually separated on the interface of the two liquids. Afterwards further amount was obtained from the mother liquor (total yield of the crude crystalline substance, 7.0 g.). These colorless crystals were recrystallized from a mixture of 300 ml. of ethyl acetate and 100 ml. of water. At first, verecundin (5 g.), and then isosakuranin (0.3 g.) were deposited. Verecundin was further recrystallized from methanol, acetone and then ethanol and was obtained as colorless needles of m.p. 135°. After recrystallization from 70% methanol, the isosakuranin melted at 190°.

Pinocembrin.—An ethanolic solution gave a violet-red coloration with ferric chloride and an orange one with magnesium powder and hydrochloric acid; ultraviolet absorption: λ_{max} 288 mμ, log ε 4.35; λ_{max} 314 mμ, log ε 3.78; λ_{min} 249 mμ, log ε 3.20.

Anal. Calcd. for $C_{15}H_{12}O_4$: C, 70.03; H, 4.66. Found: C, 70.20, H, 4.65.

The mixed melting point with authentic pinocembrin supplied by Prof. H. Erdtman was undepressed.

The diacetate of pinocembrin was obtained as colorless needles of m.p. 122°.

Pinocembrin 5,7-Dimethyl Ether.—One-tenth gram of pinocembrin, 0.5 ml. of dimethyl sulfate, 2 g. of potassium carbonate and 30 ml. of acetone were heated for one hour on a water-bath. After filtering, the solvent was evaporated and to the residue was added 50 ml. of water. These were allowed to stand. The oily mass which had deposited gradually crystallized. The crystalline mass was recrystallized from methanol giving colorless needles of m.p. 169°, yield 50 mg.

Anal. Calcd. for $C_{15}H_{10}O_2(CH_3O)_2$: OCH_3, 21.83. Found: OCH_3, 21.80.

Isosakuranetin: specific rotation, 0.4185 g. of subst., 25 ml. of acetone–pyridine (21.5:3.5, v./v.), 2.2 dm. tube, α −1°, $[\alpha]^{14}D$ −27.1°.

Isosakuranetin Acetate.—This was obtained as colorless needles of m.p. 121° by treating isosakuranetin with acetic anhydride with the addition of a drop of pyridine. The melting points of isosakuranetin and its acetate were undepressed by admixture with the authentic specimens.

Taxifolin.—Paper chromatographic data agreed with those of the authentic specimen of taxifolin.[3] The acetate of taxifolin was obtained as colorless prisms of m.p. 132°. Taxifolin tetramethyl ether was prepared by the ordinary method using dimethyl sulfate, potassium carbonate and acetone; colorless long needles of m.p. 171°.

The melting points of taxifolin and its derivatives did not alter an admixture with the authentic specimen of taxifolin[3] and the derivatives, respectively.

Genkwanin.—The melting points of genkwanin and its derivative did not alter by mixing with authentic genkwanin and its derivatives obtained from *Prunus yedoensis*,[1] respectively; genkwanin-4′-monomethyl ether, m.p. 173°.

Genistein.—This gave colorless needles of m.p. 296°[6] from methanol. Its methanolic solution gave a violet-brown coloration with ferric chloride. The melting point was not depressed by admixing with authentic genistein, obtained by Mr. H. Matsuda of the Botanical Institute of the University of Tokyo from the fruit pods of *Stiphnolobium japonicum* (*Sophora japonica*) by the method of Charaux and Rabaté[7]; genistein-7,4′-dimethyl ether, colorless needles of m.p. 145°; genistein trimethyl ester, colorless needles of m.p. 163°; genistein-7,4′-dimethyl ether 5-acetate, colorless needles, m.p. 204°; genistein acetate, colorless needles of m.p. 201°.

Prunetin.—Prunetin gave colorless needles of m.p. 240° from methanol. It gave a violet-brown coloration with ferric chloride in methanolic solution.

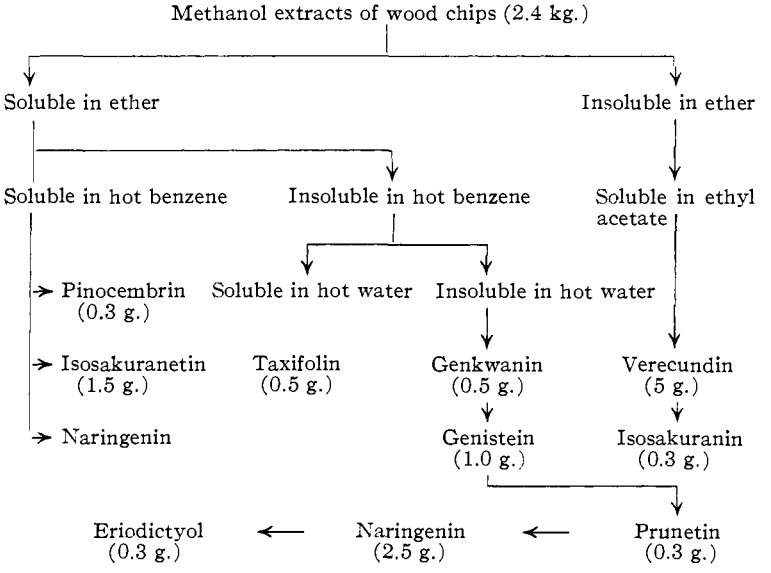
Anal. Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.22; OCH_3, 10.91. Found: C, 67.54; H, 4.04; OCH_3, 11.14

Prunetin-4′-monomethyl ether was prepared by heating an acetone solution of prunetin with dimethyl sulfate and potassium carbonate. Colorless needles of m.p. 145° were obtained. This derivative did not show any depression of melting point when mixed with genistein-7,4′-dimethyl ether.

Naringenin.—After repeated recrystallisation from methanol, the compound melted at 248°. Various properties of this compound agreed well with naringenin described before.[1,3] A mixed melting point with authentic naringenin remained the same; naringenin-7,4′-dimethyl ether, m.p. 122°.

Eriodictyol was recrystallized from methanol, and obtained as colorless needles of m.p. 264°. Its methanolic solution gave a greenish-brown coloration with ferric chloride, this color changing to green when one drop of water was added. A mixed melting test also proved the identity.

Paper chromatographically it also agreed with eriodictyol obtained from *Prunus campanulata*[3]; eriodictyol acetate, m.p. 138°; eriodictyol-7,3′,4′-trimethyl ether, m.p. 136°.



Verecundin.—Verecundin, m.p. 135°, is insoluble in benzene, chloroform and petroleum ether, and sparingly soluble in cold methanol, ethanol and acetone. It gave an orange color when reduced with magnesium powder and hydrochlo-

(6) W. Walz, *Ann.*, **498**, 118 (1931).

(7) C. Charaux and J. Rabaté, *Bull. soc. chim. biol.*, **20**, 454 (1938).

ric acid, but no color with ferric chloride; R_f values: 0.97 (*m*-cresol–acetic acid–water 24:1:25), 0.95 (60% acetic acid); specific rotation, 0.3196 g. of subst., 50 ml. of 60% acetone, 2.2-dm. tube: α_D −1°, $[\alpha]^{14}D$ −71.1°.

Anal. Calcd. for $C_{21}H_{22}O_9 \cdot 2H_2O$: C, 55.50; H, 5.72; H_2O, 7.93. Found: C, 55.30; H, 5.57; water of crystallisation (sample dried for 10 hours *in vacuo* at 80°), 7.99.

Verecundin Acetate.—Two-tenths gram of verecundin was treated with 3 ml. of pyridine and 3 ml. of acetic anhydride in the cold for 24 hours. Cold water was then added and the solidified mass was filtered, washed and recrystallized from methanol. The acetate was obtained as colorless needles of m.p. 191°, yield 0.2 g.

Anal. Calcd. for $C_{31}H_{32}O_{14}$: C, 59.23; H, 5.09. Found: C, 58.88; H, 5.07.

Verecundin Monomethyl Ether.—Two-tenths gram of verecundin in 30 ml. of acetone was added with an ethereal solution of diazomethane prepared from 2 ml. of nitrosomethylurethan. After 24 hours standing, to the ether solution was added 250 ml. of petroleum ether. The precipitate thus obtained was extracted with cold water and then with warm ether. The residue was dissolved in hot water, and then allowed to stand overnight. To the turbid solution, ether was added to clarify the solution, when crystals gradually appeared. The crystals were recrystallized by the above method and colorless needles of m.p. 98° (decomposed at 123°) were obtained. The yield was very scanty. In methanol it gave no coloration with ferric chloride.

Anal. Calcd. for $C_{21}H_{21}O_8(OCH_3)$: OCH_3, 7.17. Found: OCH_3, 7.12.

Hydrolysis.—Verecundin (1.8397 g.), 100 ml. of 5% hydrochloric acid and 70 ml. of acetone were heated on a water-bath for 5 hours. The acetone was then evaporated, and the solution was heated on a flame for 30 minutes. After cooling, the precipitate was filtered; yield 1.0 g.

The mother liquor was extracted with ether. After evaporation of ether, the residue and the aglycone obtained above were recrystallized from dilute methanol to give white needles of pinocembrin of m.p. 198°. The acetate of this aglycone was obtained as colorless needles of m.p. 122°.

The melting point of this aglycone did not show any depression when mixed with authentic pinocembrin.

The mother liquor freed from the aglycone was diluted up to 200 ml. with water. In this solution 0.6335 g. of glucose was found, according to Bertrand's method. If the ratio of pinocembrin to glucose is postulated as 1:1, the theoretical yield of glucose would be 0.7293 g. The residual solution was concentrated in a vacuum desiccator over KOH granules and then examined chromatographically. Glucose was the only sugar found.

Isosakuranin.—After repeated recrystallisation from 70% methanol, isosakuranin melted at 190°. This glycoside proved to be identical with isosakuranin obtained previously[4] as compared chromatographically and by mixed melting test.

Anal. Calcd. for $C_{21}H_{21}O_9(OCH_3)$: OCH_3, 6.92. Found: OCH_3, 6.87.

Acknowledgment.—We wish to thank Dr. Masataka Ohmasa of the Government Forest Experiment Station and Prof. Shizuo Hattori of the University of Tokyo for their advice given during this investigation. We are also indebted to Mr. Teizo Maeda of the Government Forest Experiment Station for supplying the wood material, to Prof. H. Erdtman of the Royal Institute of Technology, Stockholm, for generously sending us a specimen of pinocembrin, and to Mr. H. Matsuda for giving us a specimen of genistein. We are also grateful to Prof. Simon H. Wender of the University of Oklahoma for his kindness in revising our manuscript.

MEGURO, TOKYO

[CONTRIBUTION FROM THE MERCK SHARP AND DOHME RESEARCH LABORATORIES]

Cortical Steroids Substituted at C-12[1]

BY D. TAUB, R. D. HOFFSOMMER AND N. L. WENDLER

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The preparation of a number of corticosterone and 11-dehydrocorticosterone derivatives substituted at C-12 is described.

Striking effects on physiological activity have been produced in the adrenal cortical steroids by introduction of substituents at C-9α.[2] In this paper we wish to report the preparation of analogs of corticosterone and 11-dehydrocorticosterone substituted at the alternate α-position, C-12.[3]

The key intermediate in our synthetic route, 11β,12β-oxido-Δ^4-pregnene-21-ol-3,20-dione acetate (Vb) was obtained in 35–40% yield by a five-step sequence from the known 12α-bromo-11-dehydrocorticosterone acetate (I).[4,5] Conversion of the latter into the corresponding 3,20-disemicarbazone II followed by lithium borohydride reduction gave the 3,20-disemicarbazone of 12α-bromocorticosterone (III).[6] Reduction of the hindered 11-carbonyl group of II without concomitant reductive loss of bromine was the crucial step of the sequence and was best accomplished with excess lithium borohydride in tetrahydrofuran at 0° for 6.5 hr.[7] Removal of the semicarbazide residues

(1) A preliminary account of this work was communicated earlier: D. Taub, R. D. Hoffsommer and N. L. Wendler, THIS JOURNAL, **78**, 2912 (1956).

(2) (a) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953); (b) **76**, 1455 (1954); (c) J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1068 (1955); (d) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler, *ibid.*, **77**, 3166 (1955); (e) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *ibid.*, **77**, 4181 (1955); (f) A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *ibid.*, **77**, 4184 (1955); (g) J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, W. P. Schneider, P. F. Beal and J. Korman, *ibid.*, **77**, 4438 (1955); (h) E. Vischer, C. Meystre and A. Wettstein, *Helv. Chim. Acta*, **38**, 1502 (1955).

(3) J. E. Herz, J. Fried and E. F. Sabo [THIS JOURNAL, **78**, 2017 (1956)] have communicated the preparation and properties of the 12α-halo derivatives of 11β-hydroxyprogesterone.

(4) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).

(5) Other starting materials that have been utilized for the preparation of 11β,12β-oxides include the corresponding Δ^{11}-olefins ((a) G. H. Ott and T. Reichstein, *Helv. Chim. Acta*, **26**, 1799 (1943); see also reference 3) and 11α-bromo-12-ketones [(b) J. Schmidlin and A. Wettstein, *ibid.*, **36**, 1241 (1953); (c) J. W. Cornforth, J. M. Osbond and G. H. Phillipps, *J. Chem. Soc.*, 907 (1954)].

(6) The 21-acetate function is cleaved during the reaction.

(7) This procedure is a modification of that utilized by N. L. Wendler, Huang-Minlon and M. Tishler [THIS JOURNAL, **73**, 3818 (1951)] for reduction of the 11-carbonyl group. Under the stated conditions, 15% loss of the 12α-bromo group occurred. Shortening the reaction time resulted in incomplete reduction of the 11-carbonyl function, while lengthening the time or raising the temperature led to increased loss of bromine (see Experimental). By contrast, the 11α-bromo-12-carbonyl system (11α,23ϵ-dibromohecogenin acetate) studied by