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The Constituents of the Leaves of *Comanthosphace japonica* S. Moore (Labiatae)[1]: Isolation of Two New Flavone Glycosides, Comanthosides A and B

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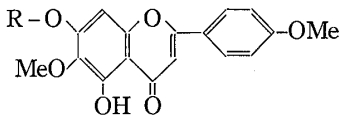
Two new flavone glycosides, comanthoside A (I), $C_{24}H_{24}O_{12}$, mp 252—255°, and comanthoside B (II), $C_{23}H_{22}O_{12}$, mp 209—210°, have been isolated from fresh leaves of *Comanthosphace japonica* S. Moore (Labiatae). The structures of I and II have been determined as 5,7-dihydroxy-6,4′-dimethoxyflavone-7-O-β-D-glucuronic acid methyl ester and -7-O-β-D-glucuronide, respectively.

Compounds I and II were also detected in *C. stellipila* S.M. by PPC and TLC.

Keywords——*Comanthosphace japonica* S. Moore; *Comanthosphace stellipila* S. Moore; Labiatae; pectolinarigenin; comanthoside A; pectolinarigenin-7-O-β-D-glucuronic acid methyl ester; comanthoside B; pectolinarigenin-7-O-β-D-glucuronide

As a part of our program to investigate unutilized natural resources, the constituents of the leaves of *Comanthosphace japonica* S. Moore (Labiatae) were examined. We now wish to report the structural elucidation of two new flavone glycosides, to which we gave the names comanthoside A (I) and comanthoside B (II), isolated from the ethyl acetate extract, and the identification of pectolinarigenin (III) obtained from the ethereal extract of the fresh leaves.



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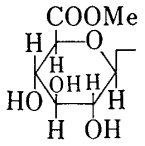
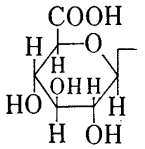
	comanthoside A (I)
	comanthoside B (II)
H	pectolinarigenin (III)

Chart 1

The crude solid from the ethyl acetate extract was chromatographed on a silica gel column to give comanthosides A (I) and B (II), eluting with a chloroform–methanol mixture.

Comanthoside A (I), 252—255°, was recrystallized from methanol as pale yellow needles and determined to be $C_{24}H_{24}O_{12}$ by elemental analysis. I gave an orange-yellow color in the flavone reaction (Mg-HCl and Zn–HCl), reacted greenish-brown to ferric chloride solution

1) This paper is part VIII of Studies on Unutilized Resources. Part VII: M. Arisawa, M. Shimizu, and N. Morita, *Yakugaku Zasshi*, **92**, 747 (1972).

2) Location: *a*) *Shugitani 2630, Toyama 930-01, Japan*; *b*) *Kashima, Namerikawa 936, Japan.*

and gave a positive reaction to Fehling's solution. The ultraviolet (UV) absorption spectrum of I showed maxima at 276, 328 nm (log ε 4.15, 4.27). The UV maxima changed to 259 (s.), 286 (s.), 298, 344 nm on addition of aluminum chloride, but were unchanged on addition of sodium acetate. The infrared (IR) spectrum of I exhibited absorption bands at 3300—3400 (hydroxyl), 1750 (ester), 1670 (carbonyl), and 1615 cm^{-1} (aromatic). The above data clearly indicated that I was a 5-hydroxy flavone glycoside. The proton nuclear magnetic resonance (PMR) spectrum of the trimethylsilyl (TMS) ether of I taken in carbon tetrachloride showed a pair of 2H doublets (J=9.0 Hz) at 7.67 and 6.82 ppm, assignable to the protons located at the 2′, 6′ and 3′, 5′ positions, and two 1H singlets at 6.46 and 6.36 ppm assignable to the protons located at the 8 and 3 positions, respectively. A 1H doublet (J=6.5 Hz), centered at 5.12 ppm, was attributed to the anomeric proton of a β-linked sugar.

Acetylation of I afforded colorless needles, mp 189—191° (IV), with the composition $C_{32}H_{32}O_{16}$ as determined by elemental analysis. In the PMR spectrum of IV, three 3H singlets at 3.86, 3.79, and 3.74 ppm, indicated three methoxyl groups, and the 3H and 9H, singlets at 2.45 and 2.07 ppm were attributed to one aromatic acetyl and three aliphatic acetyl groups, respectively. Ammonolysis of I with ammonium hydroxide afforded an amide as fine pale yellow needles with mp 275—277°. Saponification of I with 5% potassium hydroxide afforded yellow needles with mp 207—209°, which were identical with those of comanthoside B (II) described below.

Comanthoside B (II), mp 209—210°, gave an empirical formula of $C_{23}H_{22}O_{12}$ on elemental analysis. Hydrolysis of II with conc. hydrochloric acid afforded glucuronic acid and an aglycone which was identical with authentic pectolinarigenin. Methylation of II with dimethyl sulfate and anhydrous potassiun carbonate in acetone afforded colorless needles with mp 197°, which were identical with clerodendroside (5,6,7-trihydroxy-4′-methoxyflavone-7-O-β-D-glucuronide) methylate.[3]

Based on these findings, the structures of comanthosides A and B can be represented as 5,7-dihydroxy-6,4′-dimethoxyflavone-7-O-β-D-glucuronic acid methyl ester and 5,7-dihydroxy-6,4′-dimethoxyflavone-7-O-β-D-glucuronide, respectively. Comanthosides A and B were also found in the leaves of *C. stellipila* S. Moore by paper and thin-layer chromatography.

Experimental

All melting points are uncorrected and were taken on a Yanaco micro melting point apparatus MP-3. IR spectra were recorded as KBr tablets on a JASCO IR-S spectrophotometer. UV spectra were measured using a Hitachi spectrophotometer, model 124. PMR spectra were obtained on a JNM-PMX 60 machine, and the signals are given as chemical shifts in δ values (ppm) with TMS as an internal standard, using the following abbreviations: s.=singlet, d.=doublet, m.=multiplet, b.=broad. Column chromatography was performed on Wakogel C-200 and TLC was carried out with Kieselgel nach Stahl. PPC was performed on Toyo filter paper No. 51 using the descending technique. Solvent systems used were: A, *n*-BuOH–AcOH–H_2O (4:1:2); B, 60% AcOH; C, 30% AcOH; D, 15% AcOH; E, $CHCl_3$–MeOH–H_2O (33:22:3); F, MeCOEt–AcOH–MeOH (3:1:1).

Extraction and Separation——Fresh leaves of the materials (*C. japonica* S. Moore was collected at Ooiwa, Kamiichi, Toyama, and *C. stellipila* S. Moore was collected at Okinoshima, Shimane) were cut and extracted with hot MeOH. Removal of MeOH by evaporation yielded the extract. The extract of *C. japonica* S.M. was reextracted with ether and then ethyl acetate. Crude crystals were obtained from the ethereal extract and recrystallized from MeOH to give yellow needles (III). The ethyl acetate extract afforded a crude solid which was chromatographed on a silica gel column. The column was eluted with a mixture of $CHCl_3$–MeOH (20:1) to afford pale yellow needles (I), and subsequently with a (5:1) mixture to afford fine yellow needles (II). The extract of *C. stellipila* S. M. was subjected to examination by PPC and TLC.

Pectolinarigenin (III)——mp 209—210°. UV λ_{max}^{MeOH} nm: 275, 330. Acetate mp 151°. PMR (acetate in $CDCl_3$) δ (ppm): 2.38 (3H, s., OAc), 2.49 (3H, s., OAc), 3.89 (6H, s., OMe×2), 6.52 (1H, s., C_3-H), 6.96 (2H, d., J=9.0 Hz, $C_{3',5'}$-H), 7.23 (1H, s., C_8-H), 7.77 (2H, d., J=9.0 Hz, $C_{2',6'}$-H).

Comanthoside A (I)——Recrystallization from MeOH gave pale yellow needles, mp 252—255°, with a greenish-brown color reaction to $FeCl_3$, orange-yellow to Mg–HCl and Zn–HCl, and giving a positive reaction

3) N. Morita, M. Arisawa, H. Ozawa, C.S. Chen, and W.S. Kan, *Yakugaku Zasshi*, **97**, 976 (1977).

to Fehling's solution; the needles appeared dark brown under UV light. PPC *Rf*: A, 0.75; B, 0.89; C, 0.73; D, 0.41. TLC t_R: E, 0.85. *Anal.* Calcd. for $C_{24}H_{24}O_{12}$: C, 57.12; H, 4.80. Found: C, 57.21; H, 5.03. UV λ_{max}^{MeOH} nm (log ε): 276 (4.15), 328 (4.27). UV $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 259 (s.) (3.87), 286 (s.) (3.90), 298 (4.19), 344 (4.24). UV $\lambda_{max}^{MeOH-AcONa}$ nm (log ε): 276 (4.15), 328 (4.28). IR ν_{max}^{KBr} cm^{-1}: 3300—3400, 1750, 1670, 1615. PMR (TMS ether of I, in CCl_4) δ (ppm): 3.5—4.0 (totally 13H, m., (COOMe (3.64), OMe×2 (3.85), and aliphatic H×4)), 5.12 (1H, d., J=6.5 Hz, anomeric H), 6.36 (1H, s., C_3-H), 6.46 (1H, s., C_8-H), 6.82 (2H, d., J=9.0 Hz, $C_{3'5'}$-H), 7.67 (2H, d., J=9.0 Hz, $C_{2'6'}$-H).

Comanthoside A Acetate (IV)——To a solution of I in pyridine, acetic anhydride was added. After heating on a water bath for 3 hr, the reaction mixture was worked up in the usual manner. Recrystallization from MeOH gave colorless needles, mp 189—191,° showing no color with $FeCl_3$. *Anal.* Calcd. for $C_{32}H_{32}O_{16}$: C, 57.12; H, 4.80. Found: C, 57.30; H, 4.98. PMR ($CDCl_3$) δ (ppm): 2.07 (9H, s., OAc×3), 2.45 (3H, s., OAc), 3.74 (3H, s., OMe), 3.79 (3H, s., OMe), 3.86 (3H, s., OMe), 4.28 (1H, b., aliphatic H), 5.30 (4H, b., aliphatic H×4), 6.46 (1H, s., C_3-H), 6.93 (2H, d., J=9.0 Hz, $C_{3'5'}$-H), 7.08 (1H, s., C_8-H), 7.74 (2H, d., J=9.0 Hz, $C_{2'6'}$-H).

Ammonolysis of I——I was dissolved in 28% NH_4OH and allowed to stand overnight at room temp. After evaporation to dryness under reduced pressure, recrystallization from MeOH gave fine pale yellow needles, mp 275—277.° *Anal.* Calcd. for $C_{23}H_{23}NO_{11}$: C, 56.42; H, 4.74; N, 2.86. Found: C, 56.42; H, 4.89; N, 3.30. IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 3150, 1690, 1665, 1655, 1610, 1595.

Saponification of I——I was dissolved in 5% KOH and allowed to stand overnight at room temp. After neutralization with dil. HCl, the solution was extracted with ethyl acetate. The ethyl acetate extract afforded crude crystals. Recrystallization from MeOH gave fine yellow needles, mp 207—209°. Mixed mp tests and IR spectral comparison with authentic comanthoside B (II) showed this compound to be identical with II.

Comanthoside B (II)——Recrystallization from MeOH gave fine yellow needles, mp 209—211°, with a greenish-brown color reaction to $FeCl_3$, orange-yellow to Mg–HCl and Zn–HCl, and giving a positive reaction to Fehling's solution; the crystals appeared dark brown under UV light. PPC *Rf*: A, 0.53; B, 0.82; C, 0.67; D, 0.40. TLC *Rt*: E, 0.54. *Anal.* Calcd. for $C_{23}H_{22}O_{12}$: C, 56.31; H, 4.52. Found: C, 56.50; H, 4.74. UV λ_{max}^{MeOH} nm (log ε): 276 (4.16), 329 (4.30). IR ν_{max}^{KBr} cm^{-1}: 3300, 1740, 1660, 1610.

Comanthoside B Acetate——II was acetylated with acetic anhydride and pyridine in the usual manner to afford colorless needles, mp 183—186°, giving no color with $FeCl_3$. *Anal.* Calcd. for $C_{31}H_{30}O_{16}$: C, 56.51; H, 4.56. Found: C, 56.70; H, 4.67. PMR ($CDCl_3$) δ (ppm): 2.12 and 2.21 (totally 9H, OAc×3), 2.45 (3H, s., OAc), 3.82 (3H, s., OMe), 3.85 (3H, s., OMe), 4.30—5.70 (5H, b., aliphatic H×5), 6.46 (1H, s., C_3-H), 6.90 (2H, d., J=9.0 Hz, $C_{3'5'}$-H), 7.12 (1H, s., C_8-H), 7.72 (2H, d., J=9.0 Hz, $C_{2'6'}$-H).

Hydrolysis of II——A solution of II in conc. HCl was heated over an open flame. The reaction mixture was washed with water and the water-insoluble part was recrystallized from MeOH to afford yellow needles, mp 210—212°. Mixed mp test and IR spectral comparison with authentic pectolinarigenin revealed this compound to be identical with the authentic material.

The water-soluble part was evaporated to dryness for examination for sugars. The residue gave a positive reaction to naphthoresorcinol. PPC *Rf*: A, 0.41, 0.20 (reddish-brown, color reaction with 0.1 N aniline hydrogen phthalate, glucuronic acid, 0.40, 0.20). TLC (treated with 0.1 N H_3BO_3) t_R: F, 0.34 (dark blue, color reaction with naphthoresorcinol–H_2SO_4, glucuronic acid, 0.34).

Methylation of II——A mixture of II, K_2CO_3 and dimethyl sulfate in acetone was refluxed for 24 hr, and poured into ice-water. The precipitate was chromatographed over silica gel and crystallized from MeOH to give colorless needles, mp 193—195°. PMR ($CDCl_3$) δ (ppm): 3.60, 3.76, 3.82, 3.88, 3.92, and 4.00 (totally 21H, m., OMe×7), 4.10—4.50 (4H, b., aliphatic H×4), 5.11 (1H, d., J=6.5 Hz, anomeric H), 6.56 (1H, s., C_3-H), 6.95 (2H, d., J=9.0 Hz, $C_{3'5'}$-H), 6.96 (1H, s., C_8-H), 7.78 (2H, d., J=9.0 Hz, $C_{2'6'}$-H). Mixed mp test and IR spectral comparison with authentic clerodendroside methylate revealed this compound to be identical with the authentic material.

Identification of I and II in *C. stellipila* S. M. by PPC and TLC——The aqueous extract was evaporated to dryness and subjected to PPC and TLC. PPC *Rf* : A, 0.85, 0.75, 0.54 (0.86 III, 0.75 I, 0.53 II). TLC t_R: E, 0.96, 0.90, 0.85, 0.54 (0.96 III, 0.90 unknown, 0.85 I, 0.54 II).

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