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Studies on the Nepalese Crude Drugs. VI.[1] On the Flavonoid Constituents of the Root of *Scutellaria discolor* COLEBR. (2)[2]

TSUYOSHI TOMIMORI,*,[a] YUKINORI MIYAICHI,[a] YOSHITAKA IMOTO,[a] HARUHISA KIZU[a] and TSUNEO NAMBA[b]

School of Pharmacy, Hokuriku University,[a] 3 Ho, Kanagawa-machi, Kanazawa 920–11, Japan and Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University,[b] 2630, Sugitani, Toyama 930–01, Japan

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From the root of *Scutellaria discolor* COLEBR., two new flavones (I and II) were isolated together with pinocembrin, 7-hydroxy-5,8-dimethoxyflavone, 5,7,4′-trihydroxy-8-methoxyflavone, 5,7,2′-trihydroxy-8,6′-dimethoxyflavone and norwogonin 7-*O*-β-D-glucuronopyranoside. Compounds I and II were identified as 7-hydroxy-5,8,2′-trimethoxyflavone and 5,7-dihydroxy-8,2′,6′-trimethoxyflavone, respectively, based on spectral and chemical data.

Keywords——*Scutellaria discolor*; Labiatae; flavonoid; 7-hydroxy-5,8,2′-trimethoxyflavone; 5,7-dihydroxy-8,2′,6′-trimethoxyflavone; pinocembrin; 7-hydroxy-5,8-dimethoxyflavone; 5,7,4′-trihydroxy-8-methoxyflavone; 5,7,2′-trihydroxy-8,6′-dimethoxyflavone; norwogonin 7-*O*-β-D-glucuronopyranoside

In the previous paper,[1] we reported the structural identification of ten flavonoids which were isolated from the root of *Scutellaria discolor* COLEBR. collected in Central Nepal. In our further studies on the constituents of this plant, two new flavones (I and II) and five known flavonoids (III-VII) were isolated. The present paper deals with their structural determination.

Compounds I-VII showed positive color reactions to Mg–HCl, and had absorption bands assignable to hydroxyls, conjugated carbonyl groups and aromatic rings in the infrared (IR) spectra.

Compound I was obtained as pale yellow needles, mp 249 °C (dec.), $C_{18}H_{16}O_6$. The ultraviolet (UV) spectrum and diagnostic shift suggested the presence of a hydroxyl at the C-7 position and the absence of a chelated hydroxyl at the C-5 position in the flavone nucleus.[3] The proton nuclear magnetic resonance (H^1-NMR) spectrum of I showed the presence of three methoxyls (3.78, 3.82, 3.93 ppm), one hydroxyl (10.55 ppm) and one C-3 proton (6.66 ppm). In the aromatic region of the spectrum, there were a singlet (1H, 6.48 ppm) and a multiplet (4H, 7.08—7.86 ppm) attributable to the A- and the B-ring protons, respectively. Methylation of I with CH_2N_2 yielded a monomethyl ether (Ia), mp 187 °C, $C_{19}H_{18}O_6$, $FeCl_3$ (−), which was identical with 5,7,8,2′-tetramethoxyflavone[1] prepared from skullcapflavone I (5,2′-dihydroxy-7,8-dimethoxy-flavone)[4] by Kuhn's methylation.[5]

From these results, the sturcture of I was identified as 7-hydroxy-5,8,2′-trimethoxy-flavone. This was further supported by its carbon nuclear magnetic resonance (^{13}C-NMR) spectrum, in which the signal patterns of the A-ring and of the B-ring were almost identical with those of 7-hydroxy-5,8-dimethoxyflavone (V)[6,7d] and 5,7-dihydroxy-8,2′-dimethoxy-flavone,[6] respectively.

Compound II was obtained as pale yellow needles, mp 206 °C (dec.), $C_{18}H_{16}O_7$. Bathochromic shifts in the UV spectrum caused by addition of diagnostic reagents suggested

the presence of a 5,7-dihydroxy system in II.[3] The ^{1}H-NMR spectrum of II showed the presence of three methoxyls [3.72 ppm (3H), 3.80 ppm (6H)], one hydroxyl (10.50 ppm), one chelated hydroxyl (12.51 ppm) and one C-3 proton (6.34 ppm). In the aromatic region of the spectrum, the remaining four protons appeared as a singlet (1H, 6.28 ppm), a doublet (2H, 6.83 ppm, $J=8.3$ Hz) and a triplet (1H, 7.52 ppm, $J=8.3$ Hz). The latter three signals could be assigned to C-3′, 5′ and C-4′ protons, respectively, from their chemical shifts and coupling patterns. These results indicate II to be a 5,7-dihydroxy-2′, 6′-dimethoxyflavone derivative with one methoxyl at the C-6 or C-8 position in the A-ring. Methylation of II with CH_2N_2 gave a monomethyl ether (IIa), mp 199 °C (dec.), $C_{19}H_{18}O_7$, $FeCl_3$ (+), which was identical with 5-hydroxy-7,8,2′, 6-tetramethoxyflavone prepared from rivularin (5,2′-dihydroxy-7,8,6′-trimethoxyflavone)[8] by partial methylation with CH_2N_2.

Compound II was, therefore, identified as 5,7-dihydroxy-8,2′, 6′-trimethoxyflavone. This was further supported by the ^{13}C-NMR spectrum of II, in which the signal patterns of the A-ring and of the B-ring were almost superimposable on those of wogonin (5,7-dihydroxy-8-methoxyflavone)[7] and IIa, respectively.

Compounds III—VII are known flavonoids and were identified as pinocembrin,[9] 7-hydroxy-5,8-dimethoxyflavone,[6,7d] 5,7,4′-trihydroxy-8-methoxyflavone,[10,11a] 5,7,2′-trihydroxy-8,6′-dimethoxyflavone[11b] and norwogonin 7-*O*-β-D-glucuronopyranoside,[12] respectively, by direct comparison with authentic samples.

Experimental[13]

Isolation——"Fractions 3—7" and "*n*-BuOH-soluble fraction," described in the previous paper,[1] were further examined. "Fraction 3" was subjected to chromatography on silica gel [solvent: benzene–$CHCl_3$ (1 : 1)] to give II (30 mg) and III (5 mg). "Fraction 6" was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (100 : 2 : 0.1)] to give V (4 mg) and VI (5 mg). "Fraction 7", containing a mixture of two flavonoids, was chromatographed on silica gel with benzene–AcOEt (100 : 12) to give I (10 mg) and IV (3 mg). The *n*-BuOH-soluble fraction was chromatographed on silica gel [solvent: AcOEt–acetone–H_2O (5 : 4 : 0.5)] to give VII (10 mg).

I (7-Hydroxy-5,8,2′-trimethoxyflavone)——Pale yellow needles (MeOH), mp 249 °C (dec.). *Anal.* Calcd for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found: C, 65.74; H, 4.88. MS *m/z* (%): 328 (M^+, 80), 313 (M^+-CH_3, 100). Mg–HCl (+). *Rf*: 0.18 (TLC-1),[14] 0.05 (TLC-2).[14] UV λ_{max}^{MeOH} nm (log ε): 225 sh (4.35), 270 (4.42), 331 (4.11); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 230 sh (4.37), 280 (4.50), 320 (4.02), 370 (4.02); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 225 sh (4.41), 270 (4.47), 331 (4.19); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 225 sh (4.40), 270 (4.45), 299 sh (4.15), 331 (4.15), 355 sh (4.16); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 279 (4.47), 320 (3.99), 370 (4.00); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 273 (4.42), 327 (4.06). IR ν_{max}^{KBr} cm^{-1}: 3100 (OH) 1630 (conjugated CO), 1600, 1580 (arom. C=C). ^{1}H-NMR: 3.78, 3.82, 3.93 (each 3H, each s, $-OCH_3\times3$), 10.55 (1H, br s, 7-OH), 6.66 (1H, s, 3-H), 6.48 (1H, s, 6-H), 7.86 (1H, dd, $J=7.6$, 1.7 Hz, 6′-H), 7.48—7.65 (1H, m, 4′-H), 7.24 (1H, br d, $J=7.8$ Hz, 3′-H), 7.08—7.24 (1H, m, 5′-H). ^{13}C-NMR: 157.7 (C-2), 112.7 (C-3),176.0 (C-4), 155.1 (C-5), 96.8 (C-6), 155.6 (C-7), 129.0 (C-8), 152.1 (C-9), 107.4 (C-10), 120.0 (C-1′), 157.5 (C-2′), 112.6 (C-3′), 132.6 (C-4′), 121.0 (C-5′), 128.7 (C-6′), 60.9 (C-8-O$\underline{C}H_3$), 55.9 (C-5,2′-O$\underline{C}H_3$).

Methylation of I: An MeOH–Et_2O (3 : 2) solution (3 ml) of I (5 mg) was treated with ethereal CH_2N_2 (1 ml) for a short time. After removal of the solvent, the residue was chromatographed on silica gel (10 g) using $CHCl_3$ as an eluent and recrystallized from MeOH to give Ia (yield 3 mg) as colorless needles, mp 187 °C. *Anal.* Calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.78; H, 5.31. MS *m/z* (%): 342 (M^+, 90), 327 (M^+-CH_3, 100). Mg–HCl (+), $FeCl_3$ (−). *Rf*: 0.40 (TLC-1),[14] 0.08 (TLC-2).[14] UV λ_{max}^{MeOH} nm (log ε): 238 sh (4.21), 269 (4.50), 336 (4.19). No change was observed when the spectrum was determined in the presence of NaOMe, $AlCl_3$, $AlCl_3$–HCl, or NaOAc. IR ν_{max}^{KBr} cm^{-1}: no OH, 1630 (conjugated CO), 1590, 1570 (arom. C=C). ^{1}H-NMR: 3.81, 3.88, 3.93, 3.99 (each 3H, each s, $-OCH_3\times4$), 6.69 (2H, s, 3,6-H), 7.87 (1H, dd, $J=7.7$, 1.6 Hz, 6′-H), 7.48—7.66 (1H, m, 4′-H), 7.24 (1H, br d, $J=8.6$ Hz, 3′-H), 7.08—7.24 (1H, m, 5′-H). ^{13}C-NMR: 157.7 (C-2), 112.6 (C-3), 176.1 (C-4), 155.8 (C-5), 93.7 (C-6), 156.5 (C-7), 130.0 (C-8), 151.4 (C-9), 107.9 (C-10), 119.8 (C-1′), 157.7 (C-2′), 112.6 (C-3′), 132.7 (C-4′), 120.9 (C-5′), 128.7 (C-6′), 60.9 (C-8-O$\underline{C}H_3$), 55.9 (C-2′-O$\underline{C}H_3$), 56.3 (C-5,7-O$\underline{C}H_3$). Ia was identical (TLC, UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion) with 5,7,8,2′-tetramethoxyflavone[1] prepared from 5,2′-dihydroxy-7,8-dimethoxyflavone (skullcapflavone I)[4] by Kuhn's methylation.[5]

II (5,7-Dihydroxy-8,2′,6′-trimethoxyflavone)——Pale yellow needles (MeOH), mp 206 °C (dec.). *Anal.* Calcd for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68, Found: C, 62.96; H, 4.66, MS *m/z* (%): 344 (M^+, 48), 329 (M^+-CH_3, 100). Mg–HCl (+). *Rf*: 0.45 (TLC-1),[14] 0.37 (TLC-2).[14] UV λ_{max}^{MeOH} nm (log ε): 267 (4.42), 310 sh (3.92), 350 sh (3.72); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 277 (4.48), 335 sh (3.86), 360 (3.90); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 277 (4.40), 300 sh (4.10), 326 (3.93), 395 (3.72);

$\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 278 (4.41), 300 sh (4.11), 324 (3.91), 395 (3.72); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 277 (4.45), 330 sh (3.84), 360 (3.88); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 270 (4.37), 341 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3300, 3100 (OH), 1650 (conjugated CO), 1610, 1580 (arom. C=C). ^{1}H-NMR: 3.72 (3H, s, 8-OCH_3), 3.80 (6H, s, 2′,6′-OCH_3), 12.51 (1H, s, 5-OH), 10.50 (1H, br s, 7-OH), 6.28 (1H, s, 6-H), 6.34 (1H, s, 3-H), 7.52 (1H, t, J=8.3 Hz, 4′-H), 6.83 (2H, d, J=8.3 Hz, 3′,5,′-H).

^{13}C-NMR: 161.2 (C-2), 112.2 (C-3), 182.0 (C-4), 156.5 (C-5), 99.2 (C-6), 157.4 (C-7), 127.8 (C-8), 150.6 (C-9), 103.8 (C-10), 110.3 (C-1′), 158.3 (C-2′,6′), 104.5 (C-3′,5′), 133.0 (C-4′), 60.9 (C-8-O$\underline{C}H_3$), 56.1 (C-2′,6′-O$\underline{C}H_3$).

Methylation of II: II (7 mg) was methylated with CH_2N_2 in the same manner as in the case of methylation of I to give IIa (5 mg) as pale yellow needles (MeOH), mp 199 °C (dec.). *Anal.* Calcd for $C_{19}H_{18}O_7$: C, 63.68; H, 5.06. Found: C, 63.42; H, 5.07. MS *m/z* (%): 358 (M^+, 46), 343 (M^+-CH_3, 100). Mg–HCl (+), $FeCl_3$ (+). *Rf*: 0.77 (TLC-1),[14] 0.31 (TLC-2).[14] UV λ_{max}^{MeOH} nm (log ε): 266 (4.48), 338 (3.84); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 267 (4.47), 365 (3.72); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 276 (4.48), 300 sh (4.16), 323 (3.98), 400 (3.83); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 276 (4.48), 300 sh (4.17), 320 (3.97), 400 (3.84); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 266 (4.47), 338 (3.83); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 266 (4.52), 338 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1650 (conjugated CO), 1600, 1580 (arom. C=C). ^{1}H-NMR: 3.70, 3.92 (each 3H, each s, $-OCH_3\times2$), 3.80 (6H, s, 2′,6′-OCH_3), 12.63 (1H, s, 5-OH), 6.32 (1H, s, 3-H), 6.62 (1H, s, 6-H), 7.52 (1H, t, J=8.3 Hz, 4′-H), 6.83 (2H, d, J=8.3 Hz, 3′,5′-H). ^{13}C-NMR: 161.7 (C-2), 112.2 (C-3), 182.2 (C-4), 157.0 (C-5), 96.2 (C-6), 158.7 (C-7), 128.6 (C-8), 149.9 (C-9), 104.1 (C-10), 110.2 (C-1′), 158.3 (C-2′,6′), 104.6 (C-3′,5′), 133.1 (C-4′), 61.0 (C-8-OCH), 56.5 (C-7-O$\underline{C}H_3$), 56.2 (C-2′,6′-O$\underline{C}H_3$). IIa was identical (TLC, UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion) with 5-hydroxy-7,8,2′,6′-tetramethoxyflavone prepared from rivularin (5,2′-dihydroxy-7,8,6′-trimethoxyflavone[8]) by methylation with CH_2N_2.

Identification of III—VII——III (mp 195 °C (dec.)), IV (mp 290 °C), V (mp 302 °C), VI (mp 266 °C) and VII (mp 245 °C (dec.)) were identified as pinocembrin, 7-hydroxy-5,8-dimethoxyflavone, 5,7,4′-trihydroxy-8-methoxyflavone, 5,7,2′-trihydroxy-8,6′-dimethoxyflavone and norwogonin 7-*O*-β-D-glucuronopyranoside, respectively, by direct comparisons with authentic specimens (TLC, UV, IR, ^{1}H-and ^{13}C-NMR, mixed fusion).

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References and Notes

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13) The instruments used to obtain the physical data were the same as described in the previous paper.[1]
14) Thin layer chromatography (TLC) was carried out on Kieselgel 60 F_{254} (Merck) with the following solvent systems: $CHCl_3$–MeOH–H_2O–AcOH (100:4:0.2:0.1) (TLC-1), *n*-hexane–acetone–AcOH (60:40:0.1) (TLC-2).