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Studies on the Nepalese Crude Drugs.[1] IX. On the Flavonoid Constituents of the Root of *Scutellaria scandens* BUCH.-HAM. *ex* D. DON[2]

YUKINORI MIYAICHI,[a] YOSHITAKA IMOTO,[a] TSUYOSHI TOMIMORI,*[,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 scandens* BUCH.-HAM. *ex* D. DON, five new flavanones (I—V) were isolated, together with oroxylin A, dihydrooroxylin A, wogonin, chrysin, baicalein, dihydrobaicalein, norwogonin, wogonin 7-*O*-glucuronide, chrysin 7-*O*-glucuronide, baicalin and dihydrobaicalin. Compounds I—V were identified as (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone, (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-glucopyranoside, (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-feruloyl)glucopyranoside, (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-sinapoyl)glucopyranoside and (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-vanilloyl)glucopyranoside, respectively, based on spectral and chemical data.

Keywords——*Scutellaria scandens*; Labiatae; root; flavonoid; flavone; flavanone; structure elucidation

Scutellaria scandens BUCH.-HAM. *ex* D. DON is a perennial herb of the family Labiatae, which is distributed in Nepal and India.[3] In Nepal, the plant is called "charpate," and its leaves are used as a folk remedy for cuts and insect stings.

As regards the constituents of this plant, no work has been reported. As part of our studies on Nepalese crude drugs and on the flavonoid constituents of Scutellaria species, we have now examined this plant. As described in the experimental section, five new flavanones (I—V) were isolated together with eleven known flavonoids (VI—XVI) from the ethanol extract of the root of this plant which was collected in Central Nepal. This paper deals with their structural identification.

Compound I was obtained as colorless needles, mp 122 °C (dec.), $C_{16}H_{14}O_7$, Mg–HCl test (+). It gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the infrared (IR) spectrum. The ultraviolet (UV) spectrum of I was characteristic of the 5,7-dihydroxyflavanone series.[4] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I showed the signals of one methoxyl (3.68 ppm), three hydroxyls (8.84, 9.09 and 10.73 ppm), one chelated hydroxyl (12.16 ppm) and an ABX-type grouping due to the C-2 (5.61 ppm) and C-3 protons (2.70 and 3.12 ppm). The mass spectrum of I exhibited a fragment ion peak originating from the B-ring at *m/z* 136 ($C_8H_8O_2$) and one from the A-ring at *m/z* 183 ($C_8H_7O_5$). These findings indicated I to be a flavanone possessing two hydroxyls (on C-5 and 7) and one methoxyl in the A-ring and two hydroxyls in the B-ring. In the aromatic region of the ^{1}H-NMR spectrum, the remaining four protons were observed as a singlet (6.02 ppm, 1H), a double doublet (6.58 ppm, 1H, J=2.5 and 8.3 Hz) and two doublets (6.70 ppm, 1H, J=8.3 Hz; 6.84 ppm, 1H, J=2.5 Hz). The former singlet could be assigned to

the C-8 proton by long-range selective proton decoupling (LSPD)[5] in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum as follows. In the ^{1}H non-decoupling ^{13}C-NMR spectrum of I, the signal of the carbon attached to an isolated aromatic hydrogen was observed at 95.1 ppm in the form of a doublet ($J=164.7$), which did not change when the chelated hydroxyl proton at the C-5 position was selectively irradiated, indicating that the isolated aromatic proton was not at the position *ortho* (C-6) to the chelated hydroxyl (C-5). These data indicated that the substitution pattern of the A-ring was 5,7-dihydroxy-6-methoxy. The latter three signals were assigned to the C-3′, C-4′ and C-6′ protons, respectively, from their chemical shifts and coupling patterns. These assignments were further supported by the ^{13}C-NMR spectrum of I, in which the signal patterns of the A-ring and of the B-ring were almost identical with those of 5,7-dihydroxy-6-methoxyflavanone (compound VII)[6] and (2*S*)-5,2′,5′-trihydroxy-7,8-dimethoxyflavanone,[7] respectively.

It is known that flavanones having 2*S*-configuration exhibit a positive Cotton effect due to n–π^* transition ($\sim$330 nm) and a negative Cotton effect due to π–π^* transition (270—290 nm) in the circular dichroism (CD) spectra.[8] The CD curve of I exhibited positive and negative maxima at 330 and 285 nm, respectively, which established the 2*S*-configuration. Based on the above findings, compound I was determined to be (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone.

Compound II was obtained as colorless needles, mp 255 °C (dec.), $C_{22}H_{24}O_{12}$, Mg–HCl test (+). The IR spectrum gave absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings. The UV spectrum and diagnostic shifts suggested II to be a 5,7-dihydroxyflavanone derivative.[4]

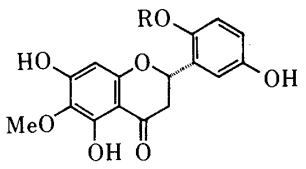
On enzymatic hydrolysis with β-glucosidase, II afforded compound I and D-glucose. These findings indicated II to be 5,7,2′,5′-tetrahydroxy-6-methoxyflavanone carrying a D-glucose moiety at the C-2′ or C-5′ position.

The position of the D-glucose was determined by comparison of the ^{1}H-NMR spectrum of II with that of I. On going from I to II, the proton signal due to H-3′ was displaced downfield by 0.35 ppm, while other signals were almost unaffected. This indicated that the D-glucosyl group of II is located at the 2′-hydroxy group. This was further substantiated by the ^{13}C-NMR spectrum. It is known that glycosylation of a phenolic hydroxyl group results in a downfield shift of the carbon resonances due to the *ortho* and *para* carbons.[9] On going from I to II, the signals due to C-1′, C-3′ and C-5′ were deshielded by 1.4—4.2 ppm.

The β-configuration of glycosidic linkage was indicated by the ^{1}H- and ^{13}C-NMR spectra of II, based on the observation of the anomeric proton signal at 4.58 ppm ($J=6.0$ Hz) and the anomeric carbon signal at 103.2 ppm ($J=160.3$ Hz). The 2*S*-configuration was confirmed in the same way as in the case of I. From these results, the structure of II was determined to be (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-glucopyranoside.

Compound III was obtained as pale yellow needles, mp 176 °C (dec.), $C_{32}H_{32}O_{15}$, and gave a positive Mg–HCl test. The UV spectrum and diagnostic shifts suggested III to be a 5,7-dihydroxyflavanone derivative.[4] The IR spectrum indicated the presence of an α,β-unsaturated carbonyl group at 1700 cm^{-1}, other than the 4-positional carbonyl group of flavanone.

On alkaline hydrolysis III afforded compound II and *trans*-ferulic acid, which suggested III to be a mono-ferulate of II. In the ^{13}C-NMR spectrum of III, the carbon signals due to the flavanone moiety were observed to be almost superimposable on those of II, suggesting that the acyl group in III was attached to the glucose moiety. In order to clarify the site of binding of the acyl group, we used the acylation shifts[9a,10] in ^{13}C-NMR spectroscopy. In the ^{13}C-NMR spectrum of III, the signals due to C-1″ and C-3″ were shifted upfield in comparison with those of II, while other signals remained almost unaffected. These data indicated that the feruloyl group was linked to C-2″.



I: R=H
II: R=glc
III: R=glc^2—feruloyl
IV: R=glc^2—sinapoyl
V: R=glc^2—vanilloyl
glc: β-D-glucopyranosyl

Fig. 1

Compound IV, mp 172 °C (dec.), $C_{33}H_{34}O_{16}$, Mg–HCl test (+), and compound V, mp 202 °C (dec.), $C_{30}H_{30}O_{15}$, Mg–HCl test (+), were obtained as pale yellow needles, respectively.

On alkaline hydrolysis IV and V afforded *trans*-sinapic acid and vanillic acid, respectively, together with compound II as a common deacyl compound, which suggested IV and V to be a mono-sinapate and a mono-vanillate of II, respectively. The positions of the acyl groups in both compounds were determined to be C-2″ from their ^{13}C-NMR spectral data as in the case of III.

The absolute configurations at C-2 in III—V were confirmed as *S* from the CD spectra. From these data, compounds III—V were determined to be (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-feruloyl)glucopyranoside, (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-sinapoyl)glucopyranoside and (2*S*)-5,7,2′,5′-tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-vanilloyl)glucopyranoside, respectively.

Compounds VI—XVI are known flavonoids and were identified as oroxylin A,[1] dihydrooroxylin A,[6] wogonin,[7] chrysin,[11] baicalein,[12] dihydrobaicalein,[13] norwogonin,[13] wogonin 7-*O*-glucuronide,[7] chrysin 7-*O*-glucuronide,[1,14] baicalin[1] and dihydrobaicalin,[13] respectively, by direct comparison with authentic samples.

Experimental

General Procedures——All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were determined with addition of diagnostic reagents by standard procedures[4] on a Hitachi recording spectrophotometer, type 323. IR spectra in KBr disk were run on a JASCO IR-A-2 spectrometer. NMR spectra were taken in dimethylsulfoxide-d_6 (DMSO-d_6) on a JEOL JNM-FX-100 spectrometer (^{1}H-NMR at 100 MHz and ^{13}C-NMR at 25 MHz), and the chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; m, multiplet; br, broad). Electron impact-mass spectra (EI-MS) were taken on a JEOL JMS-DX-300 mass spectrometer. Fast atom bombardment-mass spectra (FAB-MS) were obtained on an MSFAB-06B equipped with a FAB accessory. CD spectra were run on a JASCO J-20A automatic recording spectropolarimeter. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Sugar was converted to the TMS–ether of 1-(L-α-methylbenzylamino)-1-deoxyalditol (TMS-MBA-alditol) and assayed by gas-liquid chromatography (GLC) according to Oshima and Kumanotani.[15] Apparatus, Shimadzu GC-6AM unit with a flame ionization detector; column, a fused-silica WCOT column with Carbowax 20M (Shinwa Kako Co., 25 m × 0.2 mm); carrier gas, He; column temperature, programmed from 110 °C (1 min hold) to 170 °C (10 min hold) at 2 °C/min (lit.,[15] 158 °C). 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 : 10 : 1 : 0.3) (TLC-1), benzene–AcOEt–AcOH (50 : 50 : 2) (TLC-2), $CHCl_3$–MeOH–H_2O–HCOOH (25 : 8 : 1 : 0.5) (TLC-3), AcOEt–methyl ethyl ketone–H_2O–HCOOH (70 : 30 : 10 : 1) (TLC-4). Spots were detected by spraying dilute H_2SO_4 followed by heating. TLC for sugar was conducted on Avicel SF (Funakoshi) with *n*-BuOH–AcOH–H_2O (4 : 1 : 5) (TLC-5) and detection was done by spraying aniline hydrogen phthalate reagent.

Extraction and Separation——The dried root (700 g) of *Scutellaria scandens* BUCH.-HAM. *ex* D. DON, collected in Nepal in 1985, was extracted with boiling EtOH. The EtOH extract was concentrated to dryness to give a residue (29.3 g), which was suspended in H_2O and successively extracted with Et_2O and *n*-BuOH. The Et_2O layer was concentrated and the residue (3.2 g) was chromatographed on Sephadex LH-20 (100 g) with a gradient of $CHCl_3$–MeOH (1 : 0→0 : 1) to give four fractions, fr. 1—4, in the order of elution. Fraction 1 was subjected to rechromatography on silica gel (solvent: benzene) to give VI, VII and a mixture of two flavonoids (VIII and IX). The mixture was passed through a silica gel column (solvent: $CHCl_3$) to give VIII and IX. Fraction 2 gave XI. Fraction 3 was subjected to repeated chromatography on silica gel [solvent: benzene–AcOEt (10 : 1)] to give X and XII. Fraction

4 gave crude I, which was purified by chromatography on silica gel (solvent: Et_2O) to give purified I. The *n*-BuOH-soluble portion was concentrated and the residue (18.2 g) was chromatographed on Sephadex LH-20 (100 g) with a gradient of MeOH–H_2O (1 : 10→1 : 0) as an eluent to give three fractions (frs. 5—7). Fraction 5 was re-chromatographed on silica gel [solvent: $CHCl_3$–MeOH–H_2O (100 : 5 : 0.3)] to give III and a mixture of two flavonoids, which was chromatographed on silica gel with a gradient of AcOEt–acetone (1 : 0→0 : 1) to give IV and V. Fraction 6 was subjected to repeated chromatography on Sephadex LH-20 [solvent: AcOEt–MeOH (1 : 0→0 : 1)] to give II and XVI. Fraction 7 was chromatographed on RP-8 with a gradient of MeOH–H_2O (0 : 1→1 : 0) to give XIII, XIV and XV. Yields: I (50 mg), II (60 mg), III (40 mg), IV (30 mg), V (30 mg), VI (10 mg), VII (15 mg), VIII (30 mg), IX (20 mg), X (50 mg), XI (30 mg), XII (10 mg), XIII (20 mg), XIV (15 mg), XV (70 mg), XVI (30 mg).

I ((2*S*)-5,7,2′,5′-Tetrahydroxy-6-methoxyflavanone)——Colorless needles (MeOH/H_2O), mp 122 °C (dec.). *Anal.* Calcd for $C_{16}H_{14}O_7$: C, 60.38; H, 4.43. Found: C, 60.05; H, 4.46. Mg–HCl (+). *Rf*: 0.52 (TLC-1), 0.46 (TLC-2). UV λ_{max}^{MeOH} nm (log ε): 295 (4.07), 340 sh (3.26); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 248 (3.84), 323 (4.26), 334 sh (4.21), 410 sh (3.13); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 297 sh (4.05), 305 (4.07), 316 sh (4.04), 392 (2.91); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 312 (4.18), 392 (3.06); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 254 sh (3.59), 330 (4.27); $\lambda_{max}^{MeOH-NaOAc-H_3BO_3}$ nm (log ε): 298 (4.02), 330 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (conjugated CO), 1600 (arom. C=C). ^{1}H-NMR: 3.68 (3H, s, 6-OCH_3), 2.70 (1H, dd, *J* = 17.2, 3.3 Hz, *cis* 3-H), 3.12 (1H, dd, *J* = 17.2, 12.5 Hz, *trans* 3-H), 5.61 (1H, dd, *J* = 12.5, 3.3 Hz, 2-H), 6.02 (1H, s, 8-H), 6.58 (1H, dd, *J* = 8.3, 2.5 Hz, 4′-H), 6.70 (1H, d, *J* = 8.3 Hz, 3′-H), 6.84 (1H, d, *J* = 2.5 Hz, 6′-H), 8.84, 9.09 (each 1H, each s, 5′ and 2′-OH), 10.73 (1H, br s, 7-OH), 12.16 (1H, s, 5-OH). ^{13}C-NMR: 74.2 (C-2), 41.3 (C-3), 197.2 (C-4), 155.4 (C-5), 129.2 (C-6), 159.7 (C-7), 95.1 (C-8, $J_{(C-6)-(6-H)}$ = 164.7 Hz), 158.4 (C-9), 101.9 (C-10), 125.6 (C-1′), 146.6 (C-2′), 116.4 (C-3′), 115.9 (C-4′), 150.1 (C-5′), 113.2 (C-6′), 60.1 (C-6-O<u>C</u>H_3). EI-MS *m/z* (%): 318 (M^+, 85), 300 ($C_{16}H_{12}O_6$, 50), 285 ($C_{15}H_9O_6$, 100), 183 ($C_8H_7O_5$, 68), 136 ($C_8H_8O_2$, 20). CD (*c* = 0.0001, MeOH) $[\theta]^{15}$ (nm): +7337 (330) (positive maximum), −47165 (285) (negative maximum).

II ((2*S*)-5,7,2′,5′-Tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-Glucopyranoside)——Colorless needles (MeOH/H_2O) mp 255 °C (dec.). $[\alpha]_D^{15}$ −146.0° (*c* = 0.04, MeOH). *Anal.* Calcd for $C_{22}H_{24}O_{12}$: C, 55.00; H, 5.04. Found: C, 54.64; H, 5.06. Mg–HCl (+). *Rf*: 0.37 (TLC-3), 0.49 (TLC-4). UV λ_{max}^{MeOH} nm (log ε): 294 (4.29), 334 (3.53); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 242 sh (4.17), 330 (4.46); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 299 (4.16), 315 (4.15), 396 (3.15); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 314 (4.26), 396 (3.23); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 253 sh (3.75), 290 sh (3.77), 330 (4.39); $\lambda_{max}^{MeOH-NaOAc-H_3BO_3}$ nm (log ε): 295 (4.17), 332 (3.99). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (conjugated CO), 1605, 1580 (arom. C=C). ^{1}H-NMR: 3.68 (3H, s, 6-OCH_3), 2.71 (1H, dd, *J* = 17.0, 3.3 Hz, *cis* 3-H), 3.03 (1H, dd, *J* = 17.0, 12.0 Hz, *trans* 3-H), 3.0—3.8 (m, sugar moiety), 4.58 (1H, d, *J* = 6.0 Hz, anomeric H of glucose unit), 5.90 (1H, dd, *J* = 12.0, 3.3 Hz, 2-H), 6.03 (1H, s, 8-H), 6.70 (1H, dd, *J* = 8.8, 2.9 Hz, 4′-H), 6.95 (1H, d, *J* = 2.9 Hz, 6′-H), 7.05 (1H, d, *J* = 8.8 Hz, 3′-H), 9.25 (1H, s, 5′-OH), 10.48 (1H, br s, 7-OH), 12.20 (1H, s, 5-OH). ^{13}C-NMR: 73.8 (C-2), 42.1 (C-3), 197.3 (C-4), 155.4 (C-5), 129.3 (C-6), 159.7 (C-7), 95.2 (C-8), 158.4 (C-9), 102.2 (C-10), 129.8 (C-1′), 147.0 (C-2′), 117.8 (C-3′), 115.7 (C-4′), 152.8 (C-5′), 112.8 (C-6′), 103.2 (C-1″, *J* = 160.3 Hz), 73.5 (C-2″), 76.4 (C-3″), 70.0 (C-4″), 77.2 (C-5″), 61.1 (C-6″), 60.2 (C-6-O<u>C</u>H_3). EI-MS *m/z* (%): 318 ($C_{16}H_{14}O_7$, 70), 285 ($C_{15}H_9O_6$, 100). FAB-MS *m/z* (%): 481 (M^++1, 75), 319 ($C_{16}H_{14}O_7$+1, 100). CD (*c* = 0.0001, MeOH) $[\theta]^{15}$ (nm): +6287 (330) (positive maximum), −52096 (285) (negative maximum).

Enzymatic Hydrolysis of II: A solution of II (20 mg) and β-glucosidase (20 mg, from almond, Sigma) was adjusted to pH 5.0 (dilute HCOOH, 20 ml) and incubated at 37 °C for 15 h. After cooling, the reaction mixture was extracted with AcOEt. The organic layer was washed with water and concentrated to give the residue (9 mg), which was chromatographed on silica gel using Et_2O as an eluent to give colorless needles (MeOH/H_2O), mp 122 °C (dec.). This product was identical with compound I by direct comparison (TLC, UV, IR, ^{1}H- and ^{13}C-NMR). The H_2O layer was concentrated to dryness and extracted with MeOH. The MeOH-soluble portion was concentrated and the residue was passed through an RP-8 column with H_2O to give a syrup, which was shown to contain D-glucose by TLC-5 (*Rf* 0.15) and by GLC [as the TMS-MBA-alditol, t_R 25 min 00 s (TMS-MBA-D-glucitol, t_R 25 min 00 s; TMS-NBA-L-glucitol, t_R 24 min 52 s)].

III ((2*S*)-5,7,2′,5′-Tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-Feruloyl)glucopyranoside)——Pale yellow needles (MeOH/H_2O) mp 176 °C (dec.). $[\alpha]_D^{15}$ −116.9° (*c* = 0.03, MeOH). *Anal.* Calcd for $C_{32}H_{32}O_{15}$: C, 58.53; H, 4.91. Found: C, 58.32; H, 4.90. Mg–HCl (+). *Rf*: 0.50 (TLC-3), 0.59 (TLC-4). UV λ_{max}^{MeOH} nm (log ε): 295 (4.38), 335 (4.19); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 245 sh (4.27), 332 (4.44), 382 (4.35); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 296 sh (4.32), 318 (4.41), 345 sh (4.10); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 313 (4.44), 343 sh (4.11); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 253 sh (4.11), 293 sh (4.11), 328 (4.51), 396 sh (3.75); $\lambda_{max}^{MeOH-NaOAc-H_3BO_3}$ nm (log ε): 297 (4.37), 332 (4.33). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (ester), 1630 (conjugated CO), 1590 (arom. C=C). ^{1}H-NMR (f; feruloyl moiety): 3.66 (3H, s, 6-OCH_3), 3.79 (3H, s, f3-OCH_3), 2.69 (1H, br d, *J* = 17.2 Hz, *cis* 3-H), 2.93 (1H, dd, *J* = 17.2, 11 2 Hz, *trans* 3-H), 3.1—3.8 (m, sugar moiety), 4.90 (1H, d, *J* = 5.9 Hz, anomeric H of glucose unit), 5.33 (1H, br d, *J* = 11.2 Hz, 2-H), 5.84 (1H, s, 8-H), 6.31 (1H, d, fα-H, *J* = 15.6 Hz), 6.69 (1H, dd, *J* = 9.1, 2.5 Hz, 4′-H), 6.74 (2H, s, f5, f6-H), 6.89 (1H, d, *J* = 2.5 Hz, 6′-H), 7.06 (1H, d, *J* = 9.1 Hz, 3′-H), 7.13 (1H, s, f2′-H), 7.25 (1H, d, *J* = 15.6 Hz, fβ-H), 9.27 (1H, s, 5′-OH), 9.57 (1H, s, f4-OH), 10.57 (1H, s, 7-OH), 12.22 (1H, s, 5-OH). ^{13}C-NMR (f, feruloyl moiety; *, may be reversed): 73.8 (C-2), 41.5 (C-3), 196.4 (C-4), 155.3 (C-5), 129.3 (C-6), 159.6 (C-7), 94.9 (C-8), 157.8 (C-9), 101.7 (C-10), 129.3 (C-1′), 146.2 (C-2′), 117.3 (C-3′), 115.5 (C-4′), 153.0 (C-5′), 112.6 (C-6′), 100.3 (C-1″), 73.8 (C-2″*), 73.5 (C-3″*), 70.1 (C-4″), 77.4 (C-5″), 60.7

(C-6″), 166.2 (fC=O), 114.1 (fC-α), 145.7 (fC-β), 125.4 (fC-1), 110.8 (fC-2), 148.0 (fC-3), 149.5 (fC-4), 115.5 (fC-5), 123.3 (fC-6), 55.9 (fC-3-O$\underline{C}$H$_3$), 59.9 (C-6-O$\underline{C}$H$_3$). EI-MS *m/z* (%): 318 ($C_{16}H_{14}O_7$, 100), 300 ($C_{16}H_{12}O_6$, 80), 177 ($C_{10}H_9O_3$, 60). FAB-MS *m/z* (%): 657 ($M^+ + 1$, 35), 319 ($C_{16}H_{14}O_7 + 1$, 70), CD ($c = 0.0001$, MeOH) $[\theta]^{15}$ (nm): +19172 (328) (positive maximum), −89469 (285) (negative maximum).

Alkaline Hydrolysis of III: A solution of III (20 mg) in 1% KOH was allowed to stand overnight. After acidification with HCOOH, the reaction mixture was extracted with Et_2O. The organic layer was washed with H_2O and concentrated to dryness, then the residue was recrystallized from MeOH/H_2O to give colorless needles (6 mg), mp 168 °C; this product was identical with ferulic acid by direct comparison (TLC, UV, IR and mixed fusion). The aqueous layer was passed through an RP-8 column, which was washed with H_2O, then eluted with 30% MeOH. The eluate was concentrated to dryness and the residue was recrystallized from MeOH/H_2O to give colorless needles (11 mg), mp 143 °C (dec.); this product was identified as compound II by comparison with an authentic sample (TLC, UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

IV ((2*S*)-5,7,2′,5′-Tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-Sinapoyl)glucopyranoside)——Pale yellow needles (MeOH/H_2O) mp 172 °C (dec.). $[\alpha]_D^{15}$ −136.1° ($c = 0.05$, MeOH). *Anal.* Calcd for $C_{33}H_{34}O_{16}$: C, 57.72; H, 4.99. Found: C, 58.01; H, 4.95. Mg–HCl (+). *Rf*: 0.52 (TLC-3), 0.54 (TLC-4). UV λ_{max}^{MeOH} nm (log ε): 295 (4.23), 337 (4.08); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 242 (4.19), 265 sh (4.01), 320 sh (4.01), 370 (4.33), 400 sh (4.24); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 297 sh (4.33), 317 (4.45), 345 sh (4.21); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 314 (4.48), 343 sh (4.20); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 247 sh (4.27), 328 (4.55), 400 sh (3.70); $\lambda_{max}^{MeOH-NaOAc-H_3BO_3}$ nm (log ε): 298 (4.36), 332 (4.33). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (ester), 1630 (conjugated CO), 1600 (arom. C=C). ^{1}H-NMR (s; sinapoyl moiety): 3.62 (3H, s, 6-OCH$_3$), 3.79 (6H, s, s3,s4-OCH$_3$), 2.70 (1H, br d, $J = 17.3$ Hz, *cis* 3-H), 2.94 (1H, dd, $J = 17.3$, 11.2 Hz, *trans* 3-H), 3.1—3.8 (m, sugar moiety), 4.90 (1H, d, $J = 6.1$ Hz, anomeric H of glucose unit), 5.35 (1H, br d, $J = 11.2$ Hz, 2-H), 5.87 (1H, s, 8-H), 6.38 (1H, d, sα-H, $J = 15.6$ Hz), 6.69 (1H, dd, $J = 8.8$, 2.9 Hz, 4′-H), 6.80 (2H, s, s2,s6-H), 6.90 (1H, d, $J = 2.9$ Hz, 6′-H), 7.06 (1H, d, $J = 8.8$ Hz, 3′-H), 7.30 (1H, d, $J = 15.6$ Hz, sβ-H), 8.91 (1H, s, s4-OH), 9.27 (1H, s, 5′-OH), 10.55 (1H, s, 7-OH), 12.21 (1H, s, 5-OH). ^{13}C-NMR (s, sinapoyl moiety; *, may be reversed): 73.8 (C-2), 41.6 (C-3), 196.4 (C-4), 155.2 (C-5), 129.2 (C-6), 159.5 (C-7), 94.9 (C-8), 157.8 (C-9), 101.7 (C-10), 129.4 (C-1′), 146.2 (C-2′), 117.4 (C-3′), 115.5 (C-4′), 153.0 (C-5′), 112.7 (C-6′), 100.3 (C-1″), 73.7 (C-2″*), 73.5 (C-3″*), 70.1 (C-4″), 77.4 (C-5″), 60.8 (C-6″), 166.1 (sC=O), 114.6 (sC-α), 146.0 (sC-β), 124.3 (sC-1), 106.2 (sC-2,6), 148.1 (sC-3,5), 138.5 (sC-4), 56.0 (sC-3,5-O$\underline{C}$H$_3$), 59.9 (C-6-O$\underline{C}$H$_3$). FAB-MS *m/z* (%): 657 ($M^+ + 1$, 10), 319 ($C_{16}H_{14}O_7 + 1$, 30). CD ($c = 0.0001$, MeOH) $[\theta]^{15}$ (nm): +20079 (327) (positive maximum), −100395 (290) (negative maximum).

Alkaline hydrolysis of IV: A solution of IV was hydrolyzed with 1% KOH and worked up in the same way as III to identify sinapic acid and II.

V ((2*S*)-5,7,2′,5′-Tetrahydroxy-6-methoxyflavanone 2′-*O*-β-D-(2-*O*-Vanilloyl)glucopyranoside)——Pale yellow needles (MeOH/H_2O) mp 202 °C (dec.). $[\alpha]_D^{15}$ −97.9° ($c = 0.03$, MeOH). *Anal.* Calcd for $C_{30}H_{30}O_{15}$: C, 57.14; H, 4.80. Found: C, 56.95; H, 4.78. Mg–HCl (+). *Rf*: 0.45 (TLC-3), 0.56 (TLC-4). UV λ_{max}^{MeOH} nm (log ε): 270 sh (4.12), 293 (4.27), 335 (3.40); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 320 (4.54); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 270 (4.08), 297 (4.20), 320 sh (4.02), 380 (3.28); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 270 (4.06), 301 (4.20), 317 sh (4.08), 380 (3.22); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 325 (4.35); $\lambda_{max}^{MeOH-NaOAc-H_3BO_3}$ nm (log ε): 274 (4.07), 294 (4.18), 332 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (ester), 1630 (conjugated CO), 1600 (arom. C=C). ^{1}H-NMR (v; vanilloyl moiety): 3.68 (3H, s, 6-OCH$_3$), 3.71 (3H, s, v3-OCH$_3$), 2.62 (1H, br d, $J = 17.2$ Hz, *cis* 3-H), 2.86 (1H, dd, $J = 17.2$, 11.0 Hz, *trans* 3-H), 3.1—3.8 (m, sugar moiety), 4.97 (1H, d, $J = 6.3$ Hz, anomeric H of glucose unit), 5.25 (1H, dd, $J = 11.0$, 3.9 Hz, 2-H), 5.81 (1H, s, 8-H), 6.68 (2H, br d, 4′,v5-H, $J = 8.8$ Hz), 6.87 (1H, d, $J = 3.0$ Hz, 6′-H), 7.07 (1H, d, $J = 8.8$ Hz, 3′-H), 7.25 (1H, br d, $J = 8.8$ Hz, v6-H), 12.15 (1H, s, 5-OH). ^{13}C-NMR (v, vanilloyl moiety; *, may be reversed): 73.8 (C-2), 41.7 (C-3), 195.9 (C-4), 155.2 (C-5), 129.5 (C-6), 160.7 (C-7), 95.2 (C-8), 157.8 (C-9), 101.4 (C-10), 129.6 (C-1′), 146.2 (C-2′), 117.7 (C-3′), 115.5 (C-4′), 153.1 (C-5′), 112.7 (C-6′), 100.5 (C-1″), 74.0 (C-2″*), 73.7 (C-3″*), 70.2 (C-4″), 77.5 (C-5″), 60.9 (C-6″), 120.4 (vC-1), 112.7 (vC-2), 147.4 (vC-3), 151.8 (vC-4), 115.1 (vC-5), 123.7 (vC-6), 165.2 (v-C=O), 55.5 (vC-3-O$\underline{C}$H$_3$), 60.0 (C-6-O$\underline{C}$H$_3$). FAB-MS *m/z* (%): 631 ($M^+ + 1$, 25), 653 ($M^+ + Na$, 20), 669 ($M^+ + K$, 10). CD: ($c = 0.0001$, MeOH) $[\theta]^{15}$ (nm): +6427 (330) (positive maximum), −47561 (285) (negative maximum).

Alkaline Hydrolysis of V: A solution of V was hydrolyzed with 1% KOH and worked up in the same way as III to identify vanillic acid and II.

Identification of VI—XVI——VI (mp 202 °C (dec.)), VII (mp 166 °C), VIII (mp 203 °C), IX (mp 285 °C), X (mp 255 °C (dec.)), XI (mp 166 °C (dec.)), XII (mp 253 °C (dec.)), XIII (mp 270 °C (dec.)), XIV (mp 226 °C), XV (mp 230 °C (dec.)) and XVI (mp 156 °C (dec.)), were identified as oroxylin A,[1] dihydrooroxylin A,[6] wogonin,[7] chrysin,[11] baicalein,[12] dihydrobaicalein,[13] norwogonin,[13] wogonin 7-*O*-glucuronide,[7] chrysin 7-*O*-glucuronide,[1,14] baicalin[1] and dihydrobaicalin,[13] respectively, by direct comparisons with authentic specimens (UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

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