

Studies on the Constituents of *Scutellaria* Species. XIII.¹⁾ On the Flavonoid Constituents of the Root of *Scutellaria rivularis* WALL.²⁾

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Four new flavonoids (I—IV) were isolated from the root of *Scutellaria rivularis* WALL, together with 7-*O*- β -D-glucuronopyranosides of baicalein, wogonin, carthamidin and isocarthamidin. The structures of I—IV were shown to be 7-hydroxy-5,8-dimethoxyflavone 7-*O*- β -D-glucuronopyranoside, 5,7,8,2'-tetrahydroxyflavone 7-*O*- β -D-glucuronopyranoside, 5,2'-dihydroxy-7,8,6'-trimethoxyflavone 2'-*O*- β -D-glucuronopyranoside and 5,2',6'-trihydroxy-7,8-dimethoxyflavone 2'-*O*- β -D-glucuronopyranoside, respectively, on the basis of the chemical and spectral data.

Keywords *Scutellaria rivularis*; Labiatae; flavonoid; flavone glucuronide; structure elucidation

The Chinese crude drug "Bai zhi lian" (半枝莲) is the dried whole herb of *Scutellaria rivularis* WALL (Labiatae), and has been used for the treatment of tumors, hepatitis, liver cirrhosis and other diseases in China and Taiwan.^{3,4)} Regarding the chemical constituents of this drug, it has been reported that the following compounds were isolated: a mixture of sterol glucosides,⁵⁾ unknown alkaloids,^{6,7)} diterpenoids,⁸⁻¹⁶⁾ a triterpene acid¹⁷⁾ and more than thirty kinds of flavonoids^{5,6,18,19)} including unknown bisflavones.⁵⁾

In previous papers we reported the structural identification of neoclerodane diterpenoids⁸⁻¹⁰⁾ and flavonoid aglycones¹⁹⁾ isolated from the ethanol extract of Ban zhi lian. As the continuation of our work on this crude drug, we have now investigated the constituents of the fresh root of *Scutellaria rivularis* and isolated four new flavone glucuronide (I—IV) besides baicalin, wogonin 7-*O*-glucuronide, carthamidin 7-*O*-glucuronide and isocarthamidin 7-*O*-glucuronide. The present paper deals with their structural determination.

Compound I was obtained as colorless needles, mp 205 °C (dec.), C₂₃H₂₂O₁₁, giving a positive Mg-HCl test. On methanolysis, I yielded 5-*O*-methylwogonin and a sugar fraction, which was identified as methyl glucuronopyranoside methyl ester (S₁) and the methyl glycoside of glucurono-6,3-lactone (S₂), by gas-liquid chromatography (GLC). In the proton (¹H-) and carbon-13 (¹³C-) nuclear magnetic resonance (NMR) spectra of I, an anomeric proton signal at 5.40 ppm (d, *J* = 5.9 Hz) and a set of carbon signals due to the sugar moiety including an anomeric carbon signal at 100.3 ppm (d, *J* = 165.5 Hz) indicated the presence of a β -glucuronopyranosyl unit in I. These facts indicated I to be a 5-*O*-methylwogonin glucuronide. I was esterified with 5% HCl-MeOH to give a monomethyl ester, mp 201—203 °C, C₂₄H₂₄O₁₁, which was identified as 5-*O*-methylwogonin 7-*O*- β -D-glucuronopyranoside methyl ester prepared from wogonin 7-*O*- β -D-glucuronopyranoside methyl ester²⁰⁾ by methylation with CH₂N₂.

I was, therefore, determined to be 7-hydroxy-5,8-dimethoxyflavone 7-*O*- β -D-glucuronopyranoside.

Compound II was obtained as yellow needles, mp 174 °C (dec.), C₂₁H₁₈O₁₂, Mg-HCl(+). On methanolysis, II yielded an aglycone (IIa), mp 320 °C (dec.), C₁₅H₁₀O₆, and the same sugar fraction (S₁ and S₂) as I. The physical properties and the ¹H- and ¹³C-NMR spectra suggested that IIa is identical with 5,7,8,2'-tetrahydroxyflavone which has already been synthesized.²¹⁾ The identification was

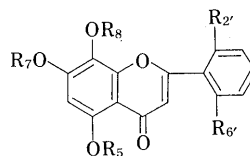
confirmed by comparison of the ¹H- and ¹³C-NMR spectra with those of an authentic sample which was prepared from 5,7-dihydroxy-8,2'-dimethoxyflavone^{19a,22,23a)} by demethylation with pyridine hydrobromide. In the ¹³C-NMR spectrum of II, the A-ring carbon signals coincided well with those of norwogonin 7-*O*- β -D-glucuronopyranoside,^{23b)} suggesting that II is a 7-*O*-glucuronide of IIa, II, on methylation with CH₂N₂, gave a dimethyl ether monomethyl ester (IIb), mp 205—206 °C (dec.), C₂₄H₂₄O₁₂, which was identified as 5,7-dihydroxy-8,2'-dimethoxyflavone 7-*O*- β -D-glucuronopyranoside methyl ester by direct comparison with an authentic specimen prepared from 5,7-dihydroxy-8,2'-dimethoxyflavone 7-*O*- β -D-glucuronopyranoside (IIc)²²⁾ by partial methylation.

Hence, II was determined to be 5,7,8,2'-tetrahydroxyflavone 7-*O*- β -D-glucuronopyranoside.

Compound III was obtained as pale yellow needles, mp 186—188 °C (dec.), C₂₄H₂₄O₁₃, Mg-HCl(+). On methanolysis, III yielded rivularin (5,2'-dihydroxy-7,8,6'-trimethoxyflavone)²²⁾ and the same sugar fraction (S₁ and S₂) as I. The ultraviolet (UV) spectrum of III showing a bathochromic shift by the addition of AlCl₃/HCl indicated the presence of a free hydroxyl at C-5.²⁴⁾ The presence of a chelated hydroxyl at C-5 was also shown by the ¹H-NMR spectrum of III (12.69 ppm). III is, therefore, rivularin 2'-*O*-glucuronide. On methylation with CH₂N₂, III gave a monomethyl ester (IIIa), mp 129—131 °C (dec.), C₂₅H₂₆O₁₃, FeCl₃(+). Subsequently, IIIa was reduced with NaBH₄ to give a corresponding glucoside, mp 157 °C (dec.), C₂₄H₂₆O₁₂, which was identified as rivularin 2'-*O*- β -D-glucopyranoside.^{23a)}

III was, therefore, determined to be 5,2'-dihydroxy-7,8,6'-trimethoxyflavone 2'-*O*- β -D-glucuronopyranoside.

Compound IV was obtained as pale yellow needles, mp 276—277 °C (dec.), C₂₃H₂₂O₁₃, Mg-HCl(+). The ¹H- and ¹³C-NMR spectra suggested IV to be a trihydroxydimethoxyflavone glucuronide. On methanolysis, IV gave 5,2',6'-trihydroxy-7,8-dimethoxyflavone²⁵⁾ and a sugar fraction (S₁ and S₂). IV was methylated with CH₂N₂ to give IIIa. Hence IV was determined to be 5,2',6'-trihydroxy-



	R ₅	R ₇	R ₈	R _{2'}	R _{6'}
I	Me	gluA	Me	H	H
II	H	gluA	H	OH	H
III	H	Me	Me	O-glucA	OMe
IV	H	Me	Me	O-glucA	OH

glucA = β -D-glucuronopyranosyl

Fig. 1

7,8-dimethoxyflavone 2'-O- β -D-glucuronopyranoside.

Compounds V—VIII are known flavone glucuronides and were identified as baicalin,²⁶⁾ wogonin 7-O-glucuronide,²⁶⁾ carthamidin 7-O-glucuronide²⁷⁾ and isocarthamidin 7-O-glucuronide,²⁷⁾ respectively, by direct comparison with authentic samples.

Experimental

General Procedures The instruments used to obtain the physical data were the same as those described in part XI²⁸⁾ except for the following. Electron impact-mass spectra (EI-MS) were taken on a JEOL JMS-DX-300 mass spectrometer. Fast atom bombardment-mass spectra (FAB-MS) were taken on a MSFAB-06B equipped with a FAB accessory. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. GLC was run on a Shimadzu GC-6AM unit with a flame ionization detector: column, a glass column (2 m $\times$ 4 mm i.d.) packed with 5% SE-30 on Chromosorb W (60–80 mesh); column temperature, programed from 150 °C (20 min hold) to 240 °C at 5 °C/min.

Material *Scutellaria rivularis* was cultivated in the botanical garden of Hokuriku University for two years, and harvested in August, 1987.

Extraction and Isolation The fresh root (1 kg) was extracted with boiling EtOH. The EtOH extract was partitioned between ether and H₂O. The H₂O layer was passed through a column of Toyopearl HW-40, and successively eluted with 5%, 10%, 15% and 20% acetone. The 20% acetone eluate containing flavone glucuronides (as the salts) was concentrated to remove acetone, then passed through an ODS column and eluted with H₂O, 5% (fractions 1 to 3), 10% (fractions 4 to 6), 15% and 20% CH₃CN. Fractions 1 to 6 were acidified with 1 N H₂SO₄, liberating free flavone glucuronides to form the precipitates 1 to 6, respectively. The precipitates 1, 2, 3 and 5 were purified by recrystallization to give compounds I, II, V (baicalin) and IV, respectively. Precipitate 4 was chromatographed on silica gel [solvent: AcOEt–MeCOEt–HCOOH–H₂O (7:3:0.5:0.5)] to give VII (carthamidin glucuronide) and VIII (isocarthamidin glucuronide). Precipitate 6 was chromatographed on silica gel [solvent: CHCl₃–MeOH–HCOOH–H₂O (100:15:1.5:1.5)] to give III and VI (wogonin glucuronide). Yields: I (80 mg), II (80 mg), III (60 mg), IV (50 mg), V (400 mg), VI (40 mg), VII (25 mg), VIII (25 mg).

7-Hydroxy-5,8-dimethoxyflavone 7-O- β -D-Glucuronopyranoside (I) Colorless needles (from MeOH), mp 205 °C. *Anal.* Calcd for C₂₃H₂₂O₁₁: C, 58.23; H, 4.67. Found: C, 58.01; H, 4.79. Mg–HCl(+). $[\alpha]_D^{20}$ –77.7° (c=0.04, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 272 (4.37), 332 (3.79); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOMe}}$ nm (log ϵ): 272 (4.37), 332 (3.79); $\lambda_{\text{max}}^{\text{MeOH}-\text{AlCl}_3}$ nm (log ϵ): 271 (4.35), 330 (3.82); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOAc}}$ nm (log ϵ): 271 (4.43), 332 (3.88). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3424 (OH), 1720 (COOH), 1643 (conjugated CO), 1601, 1580 (arom. C=C). ¹H-NMR (100 MHz, DMSO-*d*₆): 3.84, 3.91 (each 3H, each s, –OCH₃ $\times$ 2), 3.40–4.00 (m, sugar moiety), 5.40 (1H, d, J=5.9 Hz, anomeric H of sugar), 6.81 (2H, s, 3, 6-H), 7.5–7.7 (3H, m, 3', 4', 5'-H), 7.9–8.1 (2H, m, 2', 6'-H). ¹³C-NMR (25 MHz, DMSO-*d*₆): 159.9 (C-2), 108.2 (C-3), 176.1 (C-4), 155.4 (C-5), 96.9 (C-6), 154.1 (C-7), 131.2 (C-8), 151.6 (C-9), 109.7 (C-10), 131.2 (C-1'), 126.1 (C-2', 6'), 129.4 (C-3', 5'), 131.7 (C-4'), 100.3 (C-1'', J=165.5 Hz), 73.1 (C-2''), 71.3 (C-4''), 76.2 (C-5''), 170.2 (C-6''), 56.3 (C-5-OCH₃), 61.4 (C-8-OCH₃). FAB-MS *m/z* (%): 475 (M⁺ + 1, 30), 299 [C₁₇H₁₄O₅ (aglycone) + 1, 100].

Methanolysis of I: A solution of I (10 mg) in 10% HCl–MeOH (2 ml) was heated under reflux on a water bath for 3 h. The reaction mixture was neutralized with Ag₂CO₃. The precipitates were filtered off and the filtrate was concentrated to give the residue, which was chromatographed on silica gel (10 g) using CHCl₃ as an eluent to give colorless needles (MeOH), mp 290 °C (dec.). This product was identified as 5-O-methyl wogonin^{19a)} by direct comparisons (thin layer chromatography (TLC), UV, infrared (IR), ¹H- and ¹³C-NMR, mixed fusion) with an authentic specimen. The mother liquor of crystallization was shown to contain methyl glucuronopyranoside methyl ester [*t*_R 13 min 24 s (both α and β)] and the methyl glycoside of glucurono-6,3-lactone [*t*_R 6 min 05 s (α , trace), 6 min 48 s (β)] by GLC (as the trimethylsilyl (TMS) ether derivatives).

Methylation of I: I (12 mg) was dissolved in hot 5% HCl–MeOH (5 ml) to which was added ice water (50 ml). The pale yellow powder deposited was crystallized from MeOH to obtain colorless needles, mp 201–203 °C. *Anal.* Calcd for C₂₄H₂₄O₁₁: C, 59.01; H, 4.95. Found: C, 58.92; H, 4.99. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3432 (OH), 1748 (ester), 1644 (conjugated CO), 1604, 1582 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.67 (3H, s, –COOCH₃), 3.83, 3.89 (each 3H, each s, –OCH₃ $\times$ 2), 3.14–3.50 (3H, m, 2'', 3'', 4''-H), 4.21 (1H, d, J=9.5 Hz, 5''-H), 5.44 (1H, d, J=6.9 Hz, anomeric H of

sugar), 6.77 (1H, s, 3 or 6-H), 6.80 (1H, s, 3 or 6-H), 7.58 (3H, m, 3', 4', 5'-H), 8.01 (2H, m, 2', 6'-H). ¹³C-NMR (100 MHz, DMSO-*d*₆): 159.7 (C-2), 108.0 (C-3), 175.9 (C-4), 153.9 (C-5), 96.5 (C-6), 155.2 (C-7), 131.0 (C-8), 151.4 (C-9), 109.5 (C-10), 131.0 (C-1'), 125.9 (C-2', 6'), 129.2 (C-3', 5'), 131.6 (C-4'), 100.0 (C-1''), 72.9 (C-2''), 75.8 (C-3''), 71.2 (C-4''), 75.1 (C-5''), 169.0 (C-6''), 52.1 (–COOCH₃), 56.2 (C-5-OCH₃), 61.4 (C-8-OCH₃). EI-MS *m/z* (%): 488 (M⁺, 7), 298 [C₁₇H₁₄O₅ (aglycone), 82], 283 (aglycone–CH₃, 100), 269 (aglycone–COH, 13). This product was identified as 5-O-methylwogonin 7-O- β -D-glucuronopyranoside methyl ester by direct comparisons (UV, IR, ¹H- and ¹³C-NMR, mixed fusion) with an authentic specimen, which was prepared from wogonin 7-O- β -D-glucuronopyranoside methyl ester²⁰⁾ by methylation with CH₂N₂ in the usual way.

5,7,8,2'-Tetrahydroxyflavone 7-O- β -D-Glucuronopyranoside (II) Yellow needles (from MeOH), mp 174 °C (dec.). *Anal.* Calcd for C₂₁H₁₈O₁₂: C, 54.55; H, 3.92. Found: C, 54.75; H, 4.01. $[\alpha]_D^{20}$ –75.9° (c=0.03, MeOH). Mg–HCl(+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 279 (4.20), 336 (3.80); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOMe}}$ nm (log ϵ): 258 sh (3.92), 286 (4.03), 333 (3.50), 400 (3.83); $\lambda_{\text{max}}^{\text{MeOH}-\text{AlCl}_3}$ nm (log ϵ): 287 (4.19), 300 (4.18), 355 (3.95), 426 (3.54); $\lambda_{\text{max}}^{\text{MeOH}-\text{AlCl}_3-\text{HCl}}$ nm (log ϵ): 286 (4.20), 298 sh (4.18), 351 (3.94), 426 (3.54); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOAc}}$ nm (log ϵ): 281 (4.34), 330 (4.07), 400 (3.64); $\lambda_{\text{max}}^{\text{MeOH}-\text{H}_3\text{BO}_3-\text{NaOAc}}$ nm (log ϵ): 279 (4.35), 336 (3.97). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3426 (OH), 1735 (COOH), 1663 (conjugated CO), 1620, 1585 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.4–4.0 (m, sugar moiety), 5.18 (1H, d, J=6.4 Hz, anomeric H of sugar), 6.64 (1H, s, 6-H), 7.14 (1H, s, 3-H), 6.96–7.12 (2H, m, 3', 5'-H), 7.30 (1H, ddd, J=7.8, 7.6, 1.5 Hz, 4'-H), 7.99 (1H, dd, J=7.8, 1.5 Hz, 6'-H), 8.74, 10.88 (each 1H, each brs, –OH $\times$ 2), 12.31 (1H, s, 5-OH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 161.9 (C-2), 109.2 (C-3), 183.0 (C-4), 151.2 (C-5), 98.4 (C-6), 152.5 (C-7), 127.2 (C-8), 145.2 (C-9), 105.5 (C-10), 117.5 (C-1'), 157.2 (C-2'), 117.4 (C-3'), 133.2 (C-4'), 119.8 (C-5'), 128.9 (C-6'), 100.8 (C-1'', J=164.7 Hz), 73.1 (C-2''), 75.3 (C-3''), 71.5 (C-4''), 75.6 (C-5''), 170.3 (C-6''). FAB-MS *m/z* (%): 463 (M⁺ + 1, 33), 287 [C₁₅H₁₀O₆ (aglycone) + 1, 100].

Methanolysis of II: A solution of II (10 mg) in 10% HCl–MeOH (2 ml) was heated under reflux for 3 h and worked up in the same way as I to give an aglycone (IIa) and a sugar fraction, the latter of which was identified as S₁ and S₂ by GLC as in the case of I.

IIa: Yellow needles (from MeOH), mp 323 °C. *Anal.* Calcd for C₁₅H₁₀O₆: C, 62.94; H, 3.52. Found: C, 62.77; H, 3.59. Mg–HCl(+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 228 sh (4.32), 279 (4.51), 337 (4.15); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOMe}}$ nm (log ϵ): 238 (4.34), 295 (4.20), 404 (4.08); $\lambda_{\text{max}}^{\text{MeOH}-\text{AlCl}_3}$ nm (log ϵ): 277 sh (4.18), 288 (4.26), 312 (4.24), 335 sh (4.14), 366 (4.04); $\lambda_{\text{max}}^{\text{MeOH}-\text{AlCl}_3-\text{HCl}}$ nm (log ϵ): 252 sh (4.07), 287 (4.36), 300 sh (4.29), 354 (4.13), 425 (3.66); $\lambda_{\text{max}}^{\text{MeOH}-\text{NaOAc}}$ nm (log ϵ): 285 (4.15), 325 sh (3.95), 390 (3.31); $\lambda_{\text{max}}^{\text{MeOH}-\text{H}_3\text{BO}_3-\text{NaOAc}}$ nm (log ϵ): 286 (4.38), 335 sh (4.01). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3424 (OH), 1662 (conjugated CO), 1628, 1586 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 6.28 (1H, s, 6-H), 7.08 (1H, s, 3-H), 7.04 (2H, m, 3', 5'-H), 7.40 (1H, brt, J=7.1 Hz, 4'-H), 8.05 (1H, dd, J=1.1, 8.1, 6'-H), 8.76, 10.51, 10.81 (each 1H, each brs, 8, 7, 2'-OH), 12.33 (1H, s, 5-OH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 160.8 (C-2), 108.6 (C-3), 182.3 (C-4), 153.0 (C-5), 98.5 (C-6), 153.5 (C-7), 124.9 (C-8), 145.8 (C-9), 103.3 (C-10), 117.3 (C-1'), 156.8 (C-2'), 117.0 (C-3'), 132.8 (C-4'), 119.4 (C-5'), 128.7 (C-6'). EI-MS *m/z* (%): 286 (M⁺, 100), 168 (C₇H₄O₅, 80). The aglycone (IIa) was identified as 5,7,8,2'-tetrahydroxyflavone by direct comparisons (TLC, UV, IR, ¹H- and ¹³C-NMR, mixed fusion) with an authentic sample, which was prepared as follows. To freshly fused pyridine hydrobromide (150 mg) was added 5,7-dihydroxy-8,2'-dimethoxyflavone^{19a,22,23a)} (30 mg) and the mixture was heated at 210–215 °C for 6 min. The reaction mixture was cooled and poured into ice water. After extraction with AcOEt, the extract was washed with water and dried over anhydrous Na₂SO₄. The solution was evaporated to dryness and the residue crystallized from MeOH to give yellow needles, mp 323 °C (lit.,²¹⁾ 323–324 °C).

Methylation of II: II was methylated with CH₂N₂ to give a dimethyl ether monomethyl ester (IIb), pale yellow needles (from MeOH), mp 205–206 °C (dec.). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3428 (OH), 1748 (ester), 1660 (conjugated CO), 1616, 1582 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.67 (3H, s, –COOCH₃), 3.84, 3.94 (each 3H, each s, –OCH₃ $\times$ 2), 6.71 (1H, s, 6-H), 6.92 (1H, s, 3-H), 7.20 (1H, t, 7.6 Hz, 5'-H), 7.28 (1H, d, J=8.8 Hz, 3'-H), 7.61 (1H, dt, J=1.5, 7.9 Hz, 4'-H), 7.88 (1H, dd, J=1.6, 7.8 Hz, 6'-H), 3.31–3.51 (3H, m, 2'', 3'', 4''-H), 4.20 (1H, d, J=9.2 Hz, 5''-H), 5.34 (1H, d, J=7.3 Hz, anomeric H of sugar). ¹³C-NMR (100 MHz, DMSO-*d*₆): 162.5 (C-2), 110.4 (C-3), 183.0 (C-4), 156.6 (C-5), 99.1 (C-6), 156.7 (C-7), 129.9 (C-8), 150.1 (C-9), 105.9 (C-10), 120.1 (C-1'), 158.6 (C-2'), 113.4 (C-3'), 134.2 (C-4'), 121.7 (C-5'), 129.8 (C-6'), 100.2 (C-1''), 73.6 (C-2''), 75.8 (C-3''), 72.0 (C-4''), 76.3 (C-5''), 169.9 (C-6''), 52.7 (–COOCH₃), 56.8

(C-2'-OCH₃), 62.1 (C-8-OCH₃).

5,2'-Dihydroxy-7,8,6'-trimethoxyflavone 2'-O-β-D-Glucuronopyranoside (III) Pale yellow needles (from MeOH), mp 186–188 °C (dec.). *Anal.* Calcd for C₂₄H₂₄O₁₃: C, 55.38; H, 4.65. Found: C, 55.19; H, 4.77. Mg-HCl(+). $[\alpha]_D^{20} + 32.7^\circ$ ($c=0.2$, pyridine). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 266 (4.28), 310 sh (3.69), 340 (3.61); $\lambda_{\max}^{\text{MeOH}-\text{NaOMe}}$ nm (log ϵ): 270 (4.25), 376 (3.79); $\lambda_{\max}^{\text{MeOH}-\text{AlCl}_3}$ nm (log ϵ): 277 (4.39), 320 sh (3.84), 400 (3.67); $\lambda_{\max}^{\text{MeOH}-\text{AlCl}_3-\text{HCl}}$ nm (log ϵ): 277 (4.38), 320 sh (3.83), 400 (3.68); $\lambda_{\max}^{\text{MeOH}-\text{NaOAc}}$ nm (log ϵ): 265 (4.39), 310 sh (3.83), 340 (3.71); $\lambda_{\max}^{\text{MeOH}-\text{H}_3\text{BO}_3-\text{NaOAc}}$ nm (log ϵ): 265 (4.38), 310 sh (3.81), 340 (3.71). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3456 (OH), 1735 (COOH), 1660 (conjugated CO), 1618, 1588 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.70, 3.78, 3.91 (each 3H, each s, -OCH₃ × 3), 6.32 (1H, s, 6-H), 6.61 (1H, s, 3-H), 6.88 (1H, d, $J=8.4$ Hz, 3' or 5'-H), 6.92 (1H, d, 8.4 Hz, 3' or 5'-H), 7.50 (1H, t, $J=8.4$ Hz, 4'-H), 3.06–3.86 (3H, m, 2'', 3'', 4''-H), 3.84 (1H, d, $J=9.1$ Hz, 5'-H), 5.11 (1H, d, $J=7.7$ Hz, anomeric H of sugar), 12.69 (1H, s, 5-OH). ¹³C-NMR (400 MHz, DMSO-*d*₆): 161.0 (C-2), 112.3 (C-3), 182.1 (C-4), 156.7 (C-5), 95.9 (C-6), 158.3 (C-7), 128.4 (C-8), 149.8 (C-9), 104.2 (C-10), 111.3 (C-1'), 155.6 (C-2'), 107.4 (C-3'), 132.7 (C-4'), 105.5 (C-5'), 158.1 (C-6'), 99.8 (C-1''), 72.8 (C-2''), 75.8 (C-3''), 71.3 (C-4''), 75.2 (C-5''), 170.1 (C-6''), 56.2 (-OCH₃), 56.5 (-OCH₃), 61.1 (C-8-OCH₃). FAB-MS m/z (%): 521 ($M^+ + 1$, 22). EI-MS m/z (%): 344 [C₁₈H₁₆O₇ (aglycone), 54], 329 (aglycone-CH₃, 100), 181 (C₉H₈O₅-CH₃, 26), 153 (C₉H₈O₅-CH₃-CO, 31).

Methanolysis of III: III was methanolysed as in the case of I to give an aglycone mp 259 °C, and S₁ and S₂ (GLC). The aglycone was identified as rivularin²²⁾ by direct comparisons (TLC, UV, IR, ¹H- and ¹³C-NMR) with an authentic specimen.

Methylation of III: III was methylated with 5% HCl/MeOH as I to give a methyl ester (IIIa), pale yellow needles (MeOH), mp 129–131 °C (dec.). FeCl₃(+). *Anal.* Calcd for C₂₅H₂₆O₁₃: C, 56.18; H, 4.90. Found: C, 56.31; H, 5.02. IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3440 (OH), 1750 (ester), 1662 (conjugated CO), 1618, 1580 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.64 (3H, s, -COOCH₃), 3.70, 3.78, 3.91 (each 3H, each s, -OCH₃ × 3), 6.32 (1H, s, 6-H), 6.61 (1H, s, 3-H), 6.88 (1H, d, $J=8.4$ Hz, 3' or 5'-H), 6.92 (1H, d, $J=8.4$ Hz, 3' or 5'-H), 7.50 (1H, t, $J=8.4$ Hz, 4'-H), 3.08–3.49 (3H, m, 2'', 3'', 4''-H), 4.04 (1H, d, $J=9.2$ Hz, 5'-H), 5.17 (1H, d, $J=7.7$ Hz, anomeric H of sugar), 12.68 (1H, s, 5-OH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 160.9 (C-2), 112.3 (C-3), 182.1 (C-4), 156.7 (C-5), 95.9 (C-6), 158.4 (C-7), 128.4 (C-8), 149.8 (C-9), 104.2 (C-10), 111.4 (C-1'), 155.5 (C-2'), 107.5 (C-3'), 132.7 (C-4'), 105.7 (C-5'), 158.1 (C-6'), 99.9 (C-1''), 72.8 (C-2''), 75.5 (C-3''), 71.3 (C-4''), 75.1 (C-5''), 169.1 (C-6''), 51.9 (-COOCH₃), 56.2, 56.5 (-OCH₃ × 2), 61.1 (8-OCH₃). EI-MS m/z (%): 534 (M^+ , 35), 516 ($M^+ - \text{H}_2\text{O}$, 22), 501 ($M^+ - \text{H}_2\text{O} - \text{CH}_3$, 6), 344 [C₁₈H₁₆O₇ (aglycone), 75], 329 (aglycone-CH₃, 100).

Reduction of IIIa: NaBH₄ (5 mg) was added to a solution of IIIa (10 mg) in MeOH (5 mg) under cooling in an ice-bath, and the mixture was left for 10 min with stirring. After acidification with diluted HCl, the reaction mixture was extracted with AcOEt. The organic layer was washed with H₂O, passed through a silica gel column and evaporated to dryness. The residue was recrystallized from MeOH-H₂O to give pale yellow needles (7 mg), mp 157 °C (dec.). *Anal.* Calcd for C₂₄H₂₆O₁₂: C, 56.91; H, 5.17. Found: C, 56.75; H, 5.20. This product was identical with rivularin 2'-O-β-D-glucopyranoside^{23a)} by direct comparison (TLC, UV, IR, ¹H- and ¹³C-NMR).

5,2',6'-Trihydroxy-7,8-dimethoxyflavone 2'-O-β-D-Glucuronopyranoside (IV) Pale yellow needles (from MeOH), mp 276–277 °C (dec.). *Anal.* Calcd for C₂₃H₂₂O₁₃: C, 54.55; H, 4.38. Found: C, 54.71; H, 4.49. Mg-HCl(+). $[\alpha]_D^{25} + 42.6^\circ$ ($c=0.5$, pyridine). UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 266 (4.34), 310 sh (3.77), 340 (3.71); $\lambda_{\max}^{\text{MeOH}-\text{NaOMe}}$ nm (log ϵ): 265 (4.35), 370 (3.90); $\lambda_{\max}^{\text{MeOH}-\text{AlCl}_3}$ nm (log ϵ): 277 (4.34), 297 sh (4.08), 323 (3.81); 404 (3.66); $\lambda_{\max}^{\text{MeOH}-\text{AlCl}_3-\text{HCl}}$ nm (log ϵ): 277 (4.33), 297 sh (4.06), 322 sh (3.80); 404 (3.66); $\lambda_{\max}^{\text{MeOH}-\text{NaOAc}}$ nm (log ϵ): 266 (4.43), 310 sh (3.86), 345 (3.84); $\lambda_{\max}^{\text{MeOH}-\text{H}_3\text{BO}_3-\text{NaOAc}}$ nm (log ϵ): 264 (4.45), 347 (3.86). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3454 (OH), 1736 (-COOH), 1662 (conjugated CO), 1616, 1575 (arom. C=C). ¹H-NMR (400 MHz, DMSO-*d*₆): 3.73, 3.92 (each 3H, each s, -OCH₃ × 2), 6.32 (1H, s, 6-H), 6.61 (1H, s, 3-H), 6.68 (1H, d, $J=8.4$ Hz, 3' or 5'-H), 6.73 (1H, d, $J=8.4$ Hz, 3' or 5'-H), 7.31 (1H, t, $J=8.4$ Hz, 4'-H), 3.08–3.35 (3H, m, 2'', 3'', 4''-H), 3.90 (1H, d, $J=9.5$ Hz, 5'-H), 5.11 (1H, d, $J=8.0$ Hz, anomeric H of sugar), 10.20 (1H, s, 2'-OH), 12.75 (1H, s, 5-OH). ¹³C-NMR (100 MHz, DMSO-*d*₆): 161.5 (C-2), 112.1 (C-3), 182.1 (C-4), 156.6 (C-5), 95.7 (dd, $J=7.4$, 162.5 Hz, C-6), 158.1 (C-7), 128.3 (C-8), 149.8 (C-9), 104.2 (C-10), 110.1 (C-1'), 156.6 (C-2'), 105.1 (C-3'), 132.1 (C-4'), 109.7 (C-5'), 155.6 (C-6'), 99.6 (d, $J=162.7$ Hz, C-1''), 72.8 (C-2''), 75.7 (C-3''),

71.1 (C-4''), 75.3 (C-5''), 169.9 (C-6''), 56.4 (7-OCH₃), 61.0 (8-OCH₃). FAB-MS m/z (%): 507 ($M^+ + 1$, 3), 154 (C₉H₈O₅-CO-CH₃ + 1, 100). EI-MS m/z (%): 330 [C₁₇H₁₄O₇ (aglycone), 51], 315 (aglycone-CH₃, 100).

Methanolysis of IV: IV was methanolysed as in the case of I to obtain an aglycone, pale yellow needles, mp 286 °C (dec.), and a sugar fraction (S₁ and S₂), the former of which was identified as 5,2',6'-trihydroxy-7,8-dimethoxyflavone²⁵⁾ by direct comparisons (TLC, UV, IR, ¹H- and ¹³C-NMR, mixed fusion).

Methylation of IV: IV was methylated with CH₂N₂ to give IIIa.

Identification of V–VIII V [mp 230 °C (dec.)], VI [mp 270 °C (dec.)], VII [mp 208 °C (dec.)] and VIII [mp 201 °C (dec.)], were identified as baicalin,²⁶⁾ wogonin 7-O-glucuronide,²⁶⁾ carthamidin 7-O-glucuronide,²⁷⁾ and isocarthamidin 7-O-glucuronide,²⁷⁾ respectively, by direct comparisons with authentic specimens (TLC, UV, IR, ¹H- and ¹³C-NMR, mixed fusion).

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References and Notes

- 1) Part XII: Y. Miyaichi, H. Jin, M. Yamamoto, T. Tomimori, M. Mikage and T. Namba, *Shoyakugaku Zasshi*, **44**, 47 (1990).
- 2) Presented at the 34th Annual Meeting of the Japanese Society of Pharmacognosy, Osaka, Oct. 1987.
- 3) Chiang Su New Medical College (ed.), "Dictionary of Chinese Crude Drugs (中藥大辭典)," Shanghai Scientific Technological Publishers, Shanghai, 1977, p. 783.
- 4) W. S. Kan, "Manual Vegetable Drugs in Taiwan (台灣植物藥材誌)," Vol. 2, Chinese Medicine Publishing Inc., Taipei, 1978, p. 57.
- 5) C. J. Chou, K. C. Liu and T. H. Yang, The Annual Reports of the National Research Institute of Chinese Medicine, 1981, p. 91.
- 6) C. C. Wang, *Zhongcaoyao* (中草藥), **12**, 19 (1981).
- 7) R. D. Xiang, J. F. Zheng and Z. C. Yao, *Zhongcaoyao* (中草藥), **13**, 345 (1982).
- 8) T. Kikuchi, K. Tsubono, S. Kadota, H. Kizu, Y. Imoto and T. Tomimori, *Chem. Lett.*, **1987**, 987.
- 9) H. Kizu, Y. Imoto, T. Tomimori, K. Tsubono, S. Kadota and T. Kikuchi, *Chem. Pharm. Bull.*, **35**, 1655 (1987).
- 10) Presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1987, Abstract of Papers, p. 327.
- 11) Y. L. Lin, Y. H. Kuo, G. H. Lee and S. M. Peng, *J. Chem. Res. (S)*, **1987**, 320.
- 12) Y. L. Lin and Y. H. Kuo, *Chem. Express*, **3**, 37 (1988).
- 13) Y. L. Lin and Y. H. Kuo, *Heterocycles*, **27**, 799 (1988).
- 14) Y. L. Lin, Y. H. Kuo, M. C. Chen and Y. Wang, *Chem. Pharm. Bull.*, **36**, 2642 (1988).
- 15) Y. H. Kuo and Y. L. Lin, *Chem. Express*, **6**, 343 (1988).
- 16) Y. L. Lin and Y. H. Kuo, *Chem. Pharm. Bull.*, **37**, 582 (1989).
- 17) Y. H. Kuo, Y. L. Lin and S. M. Lee, *Chem. Pharm. Bull.*, **36**, 3619 (1988).
- 18) C. J. Chou, *J. Taiwan Pharm. Assoc.*, **30**, 36 (1978).
- 19) a) T. Tomimori, Y. Miyaichi, Y. Imoto and H. Kizu, *Shoyakugaku Zasshi*, **38**, 249 (1984); b) *Idem, ibid.*, **40**, 432 (1986).
- 20) T. Tomimori, Y. Miyaichi and H. Kizu, *Yakugaku Zasshi*, **102**, 388 (1982).
- 21) L. Farkas and M. Nogradi, *Chem. Ber.*, **98**, 164 (1965).
- 22) Y. Miyaichi, Y. Imoto, T. Tomimori and C. C. Lin, *Chem. Pharm. Bull.*, **35**, 3720 (1987).
- 23) a) T. Tomimori, Y. Miyaichi, Y. Imoto, H. Kizu and T. Namba, *Chem. Pharm. Bull.*, **36**, 3654 (1988); b) *Idem, ibid.*, **34**, 406 (1986).
- 24) T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1970, Chapter V.
- 25) T. Tomimori, Y. Miyaichi, Y. Imoto, H. Kizu and C. Suzuki, *Yakugaku Zasshi*, **104**, 529 (1984).
- 26) Y. Miyaichi, Y. Imoto, T. Tomimori and T. Namba, *Chem. Pharm. Bull.*, **36**, 2371 (1988).
- 27) Y. Miyaichi, Y. Imoto, H. Saida and T. Tomimori, *Shoyakugaku Zasshi*, **42**, 216 (1988).
- 28) Y. Miyaichi, H. Kizu, T. Tomimori and C. C. Lin, *Chem. Pharm. Bull.*, **37**, 794 (1989).