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

Tsuyoshi TOMIMORI,* Yoshitaka IMOTO and Yukinori MIYAICHI

School of Pharmacy, Hokuriku University, 3 Ho, Kanagawa-machi, Kanazawa 920–11, Japan. *Received July 2, 1990*

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" (半枝蓮) 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 acid[17] 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 diterpenoids[8–10] and flavonoid aglycones[19] 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.), $C_{23}H_{22}O_{11}$, 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 (S_1) and the methyl glycoside of glucurono-6,3-lactone (S_2), by gas-liquid chromatography (GLC). In the proton (^{1}H-) and carbon-13 (^{13}C-) 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, $C_{24}H_{24}O_{11}$, which was identified as 5-*O*-methylwogonin 7-*O*-β-D-glucuronopyranoside methyl ester prepared from wogonin 7-*O*-β-D-glucuronopyranoside methyl ester[20] by methylation with CH_2N_2.

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

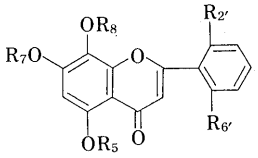
Compound II was obtained as yellow needles, mp 174 °C (dec.), $C_{21}H_{18}O_{12}$, Mg–HCl(+). On methanolysis, II yielded an aglycone (IIa), mp 320 °C (dec.), $C_{15}H_{10}O_6$, and the same sugar fraction (S_1 and S_2) as I. The physical properties and the ^{1}H- and ^{13}C-NMR spectra suggested that IIa is identical with 5,7,8,2′-tetrahydroxyflavone which has already been synthesized.[21] The identification was confirmed by comparison of the ^{1}H- and ^{13}C-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 ^{13}C-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 CH_2N_2, gave a dimethyl ether monomethyl ester (IIb), mp 205—206 °C (dec.), $C_{24}H_{24}O_{12}$, 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.), $C_{24}H_{24}O_{13}$, Mg–HCl(+). On methanolysis, III yielded rivularin (5,2′-dihydroxy-7,8,6′-trimethoxyflavone)[22] and the same sugar fraction (S_1 and S_2) as I. The ultraviolet (UV) spectrum of III showing a bathochromic shift by the addition of $AlCl_3$/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 ^{1}H-NMR spectrum of III (12.69 ppm). III is, therefore, rivularin 2′-*O*-glucuronide. On methylation with CH_2N_2, III gave a monomethyl ester (IIIa), mp 129—131 °C (dec.), $C_{25}H_{26}O_{13}$, $FeCl_3$(+). Subsequently, IIIa was reduced with $NaBH_4$ to give a corresponding glucoside, mp 157 °C (dec.), $C_{24}H_{26}O_{12}$, 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.), $C_{23}H_{22}O_{13}$, Mg–HCl(+). The ^{1}H- and ^{13}C-NMR spectra suggested IV to be a trihydroxydimethoxyflavone glucuronide. On methanolysis, IV gave 5,2′,6′-trihydroxy-7,8-dimethoxyflavone[25] and a sugar fraction (S_1 and S_2). IV was methylated with CH_2N_2 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-gluA	OMe
IV	H	Me	Me	O-gluA	OH

gluA = 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*-glucuronide[27] 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 XI[28] 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 × 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 H_2O. The H_2O 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 H_2O, 5% (fractions 1 to 3), 10% (fractions 4 to 6), 15% and 20% CH_3CN. Fractions 1 to 6 were acidified with 1 N H_2SO_4, 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–H_2O (7:3:0.5:0.5)] to give VII (carthamidin glucuronide) and VIII (isocarthamidin glucuronide). Precipitate 6 was chromatographed on silica gel [solvent: $CHCl_3$–MeOH–HCOOH–H_2O (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 $C_{23}H_{22}O_{11}$: 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 λ_{max}^{MeOH} nm (log *ε*): 272 (4.37), 332 (3.79); $\lambda_{max}^{MeOH-NaOMe}$ nm (log *ε*): 272 (4.37), 332 (3.79); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log *ε*): 271 (4.35), 330 (3.82); $\lambda_{max}^{MeOH-NaOAc}$ nm (log *ε*): 271 (4.43), 332 (3.88). IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 1720 (COOH), 1643 (conjugated CO), 1601, 1580 (arom. C=C). ^{1}H-NMR (100 MHz, DMSO-d_6): 3.84, 3.91 (each 3H, each s, –OCH_3 × 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). ^{13}C-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″), 75.5 (C-3″), 71.3 (C-4″), 76.2 (C-5″), 170.2 (C-6″), 56.3 (C-5-O$\underline{C}H_3$), 61.4 (C-8-O$\underline{C}H_3$). FAB-MS *m/z* (%): 475 (M^+ + 1, 30), 299 [$C_{17}H_{14}O_5$ (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 Ag_2CO_3. The precipitates were filtered off and the filtrate was concentrated to give the residue, which was chromatographed on silica gel (10 g) using $CHCl_3$ 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), ^{1}H- and ^{13}C-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 $C_{24}H_{24}O_{11}$: C, 59.01; H, 4.95. Found: C, 58.92; H, 4.99. IR ν_{max}^{KBr} cm^{-1}: 3432 (OH), 1748 (ester), 1644 (conjugated CO), 1604, 1582 (arom. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.67 (3H, s, –$COOCH_3$), 3.83, 3.89 (each 3H, each s, –OCH_3 × 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). ^{13}C-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 (–$COO\underline{C}H_3$), 56.2 (C-5-O$\underline{C}H_3$), 61.4 (C-8-O$\underline{C}H_3$). EI-MS *m/z* (%): 488 (M^+, 7), 298 [$C_{17}H_{14}O_5$ (aglycone), 82], 283 (aglycone – CH_3, 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, ^{1}H- and ^{13}C-NMR, mixed fusion) with an authentic specimen, which was prepared from wogonin 7-*O*-*β*-D-glucuronopyranoside methyl ester[20] by methylation with CH_2N_2 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 $C_{21}H_{18}O_{12}$: 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 λ_{max}^{MeOH} nm (log *ε*): 279 (4.20), 336 (3.80); $\lambda_{max}^{MeOH-NaOMe}$ nm (log *ε*): 258 sh (3.92), 286 (4.03), 333 (3.50), 400 (3.83); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log *ε*): 287 (4.19), 300 (4.18), 355 (3.95), 426 (3.54); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log *ε*): 286 (4.20), 298 sh (4.18), 351 (3.94), 426 (3.54); $\lambda_{max}^{MeOH-NaOAc}$ nm (log *ε*): 281 (4.34), 330 (4.07), 400 (3.64); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log *ε*): 279 (4.35), 336 (3.97). IR ν_{max}^{KBr} cm^{-1}: 3426 (OH), 1735 (COOH), 1663 (conjugated CO), 1620, 1585 (arom. C=C). ^{1}H-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 br s, –OH × 2), 12.31 (1H, s, 5-OH). ^{13}C-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 [$C_{15}H_{10}O_6$ (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 S_1 and S_2 by GLC as in the case of I.

IIa: Yellow needles (from MeOH), mp 323 °C. *Anal.* Calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.77; H, 3.59. Mg–HCl(+). UV λ_{max}^{MeOH} nm (log *ε*): 228 sh (4.32), 279 (4.51), 337 (4.15); $\lambda_{max}^{MeOH-NaOMe}$ nm (log *ε*): 238 (4.34), 295 (4.20), 404 (4.08); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log *ε*): 277 sh (4.18), 288 (4.26), 312 (4.24), 335 sh (4.14), 366 (4.04); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log *ε*): 252 sh (4.07), 287 (4.36), 300 sh (4.29), 354 (4.13), 425 (3.66); $\lambda_{max}^{MeOH-NaOAc}$ nm (log *ε*): 285 (4.15), 325 sh (3.95), 390 (3.31); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log *ε*): 286 (4.38), 335 sh (4.01). IR ν_{max}^{KBr} cm^{-1}: 3424 (OH), 1662 (conjugated CO), 1628, 1586 (arom. C=C). ^{1}H-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, br t, *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 br s, 8, 7, 2′-OH), 12.33 (1H, s, 5-OH). ^{13}C-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 ($C_7H_4O_5$, 80). The aglycone (IIa) was identified as 5,7,8,2′-tetrahydroxyflavone by direct comparisons (TLC, UV, IR, ^{1}H- and ^{13}C-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 Na_2SO_4. 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 CH_2N_2 to give a dimethyl ether monomethyl ester (IIb), pale yellow needles (from MeOH), mp 205—206 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1748 (ester), 1660 (conjugated CO), 1616, 1582 (arom. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.67 (3H, s, –$COOCH_3$), 3.84, 3.94 (each 3H, each s, –OCH_3 × 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). ^{13}C-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 (–$COO\underline{C}H_3$), 56.8

(C-2′-OCH$_3$), 62.1 (C-8-OCH$_3$).

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 $C_{24}H_{24}O_{13}$: C, 55.38; H, 4.65. Found: C, 55.19; H, 4.77. Mg–HCl(+). $[\alpha]_D^{30}$ +32.7° (c=0.2, pyridine). UV λ_{max}^{MeOH} nm (log ε): 266 (4.28), 310 sh (3.69), 340 (3.61); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 270 (4.25), 376 (3.79); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 277 (4.39), 320 sh (3.84), 400 (3.67); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 277 (4.38), 320 sh (3.83), 400 (3.68); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 265 (4.39), 310 sh (3.83), 340 (3.71); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 265 (4.38), 310 sh (3.81), 340 (3.71). IR ν_{max}^{KBr} cm^{-1}: 3456 (OH), 1735 (COOH), 1660 (conjugated CO), 1618, 1588 (arom. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.70, 3.78, 3.91 (each 3H, each s, –OCH$_3$×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). ^{13}C-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 (–OCH$_3$), 56.5 (–OCH$_3$), 61.1 (C-8-OCH$_3$). FAB-MS m/z (%): 521 (M^++1, 22). EI-MS m/z (%): 344 [$C_{18}H_{16}O_7$ (aglycone), 54], 329 (aglycone−CH$_3$, 100), 181 ($C_9H_8O_5-CH_3$, 26), 153 ($C_9H_8O_5-CH_3-CO$, 31).

Methanolysis of III: III was methanolysed as in the case of I to give an aglycone mp 259 °C, and S_1 and S_2 (GLC). The aglycone was identified as rivularin[22] by direct comparisons (TLC, UV, IR, ^{1}H- and ^{13}C-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.). $FeCl_3$(+). *Anal.* Calcd for $C_{25}H_{26}O_{13}$: C, 56.18; H, 4.90. Found: C, 56.31; H, 5.02. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1750 (ester), 1662 (conjugated CO), 1618, 1580 (arom. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.64 (3H, s, –COOCH$_3$), 3.70, 3.78, 3.91 (each 3H, each s, –OCH$_3$×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). ^{13}C-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 (–COOCH$_3$), 56.2, 56.5 (–OCH$_3$×2), 61.1 (8-OCH$_3$). EI-MS m/z (%): 534 (M^+, 35), 516 (M^+-H_2O, 22), 501 ($M^+-H_2O-CH_3$, 6), 344 [$C_{18}H_{16}O_7$ (aglycone), 75], 329 (aglycone−CH$_3$, 100).

Reduction of IIIa: $NaBH_4$ (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 H_2O, passed through a silica gel column and evaporated to dryness. The residue was recrystallized from MeOH–H_2O to give pale yellow needles (7 mg), mp 157 °C (dec.). *Anal.* Calcd for $C_{24}H_{26}O_{12}$: 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, ^{1}H- and ^{13}C-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 $C_{23}H_{22}O_{13}$: C, 54.55; H, 4.38. Found: C, 54.71; H, 4.49. Mg–HCl(+). $[\alpha]_D^{25}$ +42.6° (c=0.5, pyridine). UV λ_{max}^{MeOH} nm (log ε): 266 (4.34), 310 sh (3.77), 340 (3.71); $\lambda_{max}^{MeOH-NaOMe}$ nm (log ε): 265 (4.35), 370 (3.90); $\lambda_{max}^{MeOH-AlCl_3}$ nm (log ε): 277 (4.34), 297 sh (4.08), 323 (3.81); 404 (3.66); $\lambda_{max}^{MeOH-AlCl_3-HCl}$ nm (log ε): 277 (4.33), 297 sh (4.06), 322 sh (3.80); 404 (3.66); $\lambda_{max}^{MeOH-NaOAc}$ nm (log ε): 266 (4.43), 310 sh (3.86), 345 (3.84); $\lambda_{max}^{MeOH-H_3BO_3-NaOAc}$ nm (log ε): 264 (4.45), 347 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3454 (OH), 1736 (–COOH), 1662 (conjugated CO), 1616, 1575 (arom. C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): 3.73, 3.92 (each 3H, each s, –OCH$_3$×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). ^{13}C-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-OCH$_3$), 61.0 (8-OCH$_3$). FAB-MS m/z (%): 507 (M^++1, 3), 154 ($C_9H_8O_5-CO-CH_3$+1, 100). EI-MS m/z (%): 330 [$C_{17}H_{14}O_7$ (aglycone), 51], 315 (aglycone−CH$_3$, 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 (S_1 and S_2), the former of which was identified as 5,2′,6′-trihydroxy-7,8-dimethoxyflavone[25] by direct comparisons (TLC, UV, IR, ^{1}H- and ^{13}C-NMR, mixed fusion).

Methylation of IV: IV was methylated with CH_2N_2 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, ^{1}H- and ^{13}C-NMR, mixed fusion).

Acknowledgements We are grateful to Mrs. R. Igarashi and Miss H. Shimomura of this university for elemental analysis and MS measurement.

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 (中葯大辞典)," Shanghai Scientific Technological Publishers, Shanghai, 1977, p. 783.
4) W. S. Kan, "Manual Vegetable Drugs in Taiwan (台湾植物薬材誌)," 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* (中草葯), **12**, 19 (1981).
7) R. D. Xiang, J. F. Zheng and Z. C. Yao, *Zhongcaoyao* (中草葯), **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).