Studies on the Constituents of Scutellaria Species. IX.1) On the Flavonoid Constituents of the Root of Scutellaria indica L.2)

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Four new flavanones (I—IV) and a new flavone (V) were isolated from the root of Scutellaria indica L., together with 2(S)-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone, (±)-5,2'-dihydroxy-6,7,6'-trimethoxyflavanone, 5,7-dihydroxy-8,2'-dimethoxy flavone, rivularin, 5,2',6'-trihydroxy-7,8-dimethoxyflavone, scutevurin, 5,7,4'-trihydroxy-6-methoxyflavone, wogonin, alpinetin, caldamomin and wogonin 7-O-glucuronide. Compounds I—V (Fig. 1) were identified based on spectral data and simple chemical modifications.

Keywords—Scutellaria indica; Labiatae; flavonoid; flavone; flavanone; chalcone; structure elucidation

Scutellaria indica L. is a perennial herb of the family Labiatae, which is widely distributed in Japan, Taiwan, China, Korea and Indo-China. The dried whole herb of this plant is a crude drug which is known as “han xin cao” (鴨血草) in China and has been used as an antidote, analgesic and hemostatic for the treatment of hemoptysis, hematemesis and other diseases.3) So far, only a flavone glycoside, scutellarin, has been isolated as a constituent of this plant.4) As a part of our studies on the flavonoid constituents of Scutellaria species, we have now examined this plant.

As described in the experimental part, four new flavanones (I—IV) and a new flavone (V) were isolated together with eleven known flavonoids (VI—XVI) from the ethanol extract of the root of this plant which was collected in Taiwan. This paper deals with their structural identification.

Compound I was obtained as colorless needles, mp 197 °C (dec.), C16H14O6, 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-dihydroxy flavanone series.5) The proton nuclear magnetic resonance (1H-NMR) spectrum of I showed the signals of one methoxyl (3.67 ppm), two hydroxyls (9.6 and 10.8 ppm), one chelated hydroxyl (11.93 ppm) and an ABX type grouping due to the C-2 (5.76 ppm) and C-3 protons (2.76 and 3.23 ppm). In the aromatic region of the spectrum, the signals of the remaining five protons occurred as a singlet (6.01 ppm, 1H) due to the A-ring proton, and a double doublet (7.23 ppm, 1H, J = 7.9 and 7.5 Hz), a broad triplet (6.89 ppm, 1H, J = 7.5 Hz) and two doublets (7.48 ppm, 1H, J = 7.5 Hz; 6.92 ppm, 1H, J = 7.9 Hz) due to the B-ring protons.

On methylation by Kuhn’s method,6) I gave a trimethyl ether, mp 174 °C (dec.), C19H20O6, which was identical with 5,7,8,2'-tetramethoxyflavanone, prepared from 2(S)-5,7-dihydroxy-8,2'-dimethoxyflavanone.7)
Compound I was, therefore, considered to be a monomethyl ether of 5,7,8,2'-tetrahydroxyflavanone. In the carbon-13 nuclear magnetic resonance (13C-NMR) spectrum of I, the methoxyl carbon signal appeared downfield at 60.4 ppm, which indicated the methoxyl to be on the C-8 carbon, being di-ortho-substituted by two oxygen functions.8)

It is known that flavanones having 2(S)-configuration exhibit a positive Cotton effect due to \( \pi-\pi^* \) transition \((\sim 330 \text{ nm})\) and a negative Cotton effect due to \( \pi-\pi^* \) transition \((270\text{—}290 \text{ nm})\) in the circular dichroism (CD) spectra.9) The CD curve of I exhibited positive and negative maxima at 309 and 288 nm, respectively, which established the 2(S)-configuration. Based on the above findings, compound I was determined to be 2(S)-5,7,2'-trihydroxy-8-methoxyflavanone.

Compound II was obtained as colorless needles, mp 202 °C, \( \text{C}_{15}\text{H}_{12}\text{O}_5 \), Mg-HCl test (+). The IR spectrum gave absorption bands corresponding to hydroxyl and conjugated carbonyl groups and aromatic rings. The UV spectrum and diagnostic shifts suggested II to be a 5,7-dihydroxyflavanone derivative.5) The \(^1\text{H}-\text{NMR} \) spectrum showed the presence of one chelated hydroxyl \((12.15 \text{ ppm})\) and C-2 \((5.72 \text{ ppm})\) and C-3 \((2.71, 3.23 \text{ ppm})\) protons. In the aromatic region of the spectrum, a pair of doublets \((J=2.0 \text{ Hz})\) \((5.94 \text{ ppm}, 1\text{H}; 5.91 \text{ ppm}, 1\text{H})\) for the A-ring protons and two double doublets \((7.21 \text{ ppm}, 1\text{H}, J=7.6 \text{ and } 7.8 \text{ Hz}; 6.87 \text{ ppm}, 1\text{H}, J=7.6 \text{ and } 7.8 \text{ Hz})\) and two doublets \((6.89 \text{ ppm}, 1\text{H}, J=7.8 \text{ Hz}; 7.44 \text{ ppm}, 1\text{H}, J=7.8 \text{ Hz})\) due to the B-ring protons were seen. These chemical shifts and splitting patterns of the B-ring protons suggest that II possesses the same B-ring \((2'\text{-OH})\) as I. This was also supported by the 13C-NMR spectrum, in which the carbon signals due to the B-ring of II were observed to be almost superimposable on those of I. 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'-trihydroxy-8-methoxyflavanone.

Compound III was obtained as colorless needles, mp 192 °C, \( \text{C}_{17}\text{H}_{16}\text{O}_7 \), Mg-HCl test (+), and gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum and diagnostic shifts suggested the presence of a hydroxyl group at the C-5 position and the absence of a free hydroxyl at the C-7 position.5) The H-NMR spectrum of III showed the signals of two methoxys \((3.66 \text{ and } 3.87 \text{ ppm})\), two hydroxys \((8.87 \text{ and } 9.11 \text{ ppm})\), one chelated hydroxyl \((12.03 \text{ ppm})\) and an ABX type grouping due to the C-2 \((5.70 \text{ ppm})\) and C-3 protons \((2.80 \text{ and } 3.18 \text{ ppm})\). The mass spectrum of III exhibited a fragment ion peak originating from the B-ring at \(m/z \text{ 136} \ (\text{C}_{18}\text{H}_{8}\text{O}_2^+)\). These findings indicated III to be a flavanone possessing one hydroxyl (on C-5) and two methoxyls in the A-ring and two hydroxyls in the B-ring. In the aromatic region of the \(^1\text{H}-\text{NMR} \) spectrum, the remaining four protons were observed as a singlet \((6.25 \text{ ppm}, 1\text{H})\) and a double doublet \((6.62 \text{ ppm}, 1\text{H}, J=2.0 \text{ and } 9.3 \text{ Hz})\) and two doublets \((6.75 \text{ ppm}, 1\text{H}, J=9.3 \text{ Hz}; 6.92 \text{ ppm}, 1\text{H}, J=2.0 \text{ Hz})\). The former singlet could be assigned to the C-6 proton by long-range selective proton decoupling (LSPD)10) in the \(^{13}\text{C}-\text{NMR} \) spectrum as follows. In the H non-decoupling \(^{13}\text{C}-\text{NMR} \) spectrum of III, the signal of the carbon attached to an isolated aromatic hydrogen was observed at 93.0 ppm in the form of a double doublet \((J=163.2 \text{ and } 7.4 \text{ Hz})\), which changed to a doublet when the chelated hydroxyl proton at the C-5 position was selectively irradiated, indicating that the isolated aromatic proton was present at the position ortho \((\text{C}-6)\) to the chelated hydroxyl \((\text{C}-5)\). These data indicated that the substitution pattern of the A-ring was 5-hydroxy-7,8-dimethoxy. This was further confirmed by the \(^{13}\text{C}-\text{NMR} \) spectrum of III, in which the signal pattern of the A-ring was almost identical with that of 5,2'-dihydroxy-7,8,6'-trimethoxyflavanone (VI).7) The latter three signals were assigned to the C-3', C-4' and C-6' protons, respectively, from their chemical shifts and coupling patterns. This was further confirmed by the \(^{13}\text{C}-^1\text{H} \) shift correlation spectrum (COSY) and the \(^{13}\text{C}-^1\text{H} \) long-range COSY.11)

The 2(S)-configuration of III was confirmed in the same way as in the case of I.
Compound III was, therefore, determined to be $2(S)$-5,2',5'-trihydroxy-7,8-dimethoxyflavanone.

Compound IV was obtained as pale yellow needles, mp 143°C (dec.), C$_{24}$H$_{26}$O$_{13}$, Mg-HCl test positive. It gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum and diagnostic shifts suggested the presence of a hydroxyl at the C-5 position and the absence of a free hydroxyl at the C-7 position.5)

On methanolysis, IV yielded 5,2'-dihydroxy-7,8,6'-trimethoxyflavanone (VI),7) 5,2'-dihydroxy-6,7,6'-trimethoxyflavanone (VII),7) methyl glucuronopyranoside methylester and the methyl glycoside of glucurono-6,3-lactone.

The absence of a substituent at the C-6 position in IV was confirmed by the LSPD method.10) The aglycone of IV is, therefore, VI, and VII was considered to be a secondary product formed from VI by ring isomerization (similar to the interconvertibility of hemiphloin and isohephloin by acid).12)

In the $^1$H- and $^{13}$C-NMR spectra of IV, the anomic proton signal at 5.00 ppm (d, $J$= 6.3 Hz) and a set of carbon signals between 71.4 ppm and 170.3 ppm including an anomic carbon signal at 101.1 ppm (d, $J$= 164.7 Hz) indicated the presence of a $\beta$-glucuronopyranosyl unit.

The $2(S)$-configuration of IV was confirmed in the same way as in the case of I. Thus, the structure of IV was established as $2(S)$-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone 2'-O-$\beta$-glucuronopyranoside.

Compound V was obtained as yellow needles, mp 247°C (dec.), C$_{23}$H$_{22}$O$_{12}$, Mg-HCl test positive, and gave the absorption bands of hydroxyl and conjugated carbonyl groups and benzene rings in the IR spectrum. The UV spectrum and diagnostic shifts suggested the presence of a hydroxyl at the C-5 position and the absence of a free hydroxyl at the C-7 position.5)

On methanolysis V yielded 5,7-dihydroxy-8,2'-dimethoxyflavone (VIII),7) methyl glucuronopyranoside methylester and the methylglycoside of glucurono-6,3-lactone. In the $^{13}$C-NMR spectrum of V, the signals due to the sugar moiety indicated the presence of a $\beta$-glucuronopyranosyl unit in V.

Hence V was determined to be 5,7-dihydroxy-8,2'-dimethoxyflavone 7-O-$\beta$-glucuronopyranoside. This was confirmed by its $^{13}$C-NMR spectrum, in which the signal patterns of the A-ring and the B-ring were almost identical with those of wogonin 7-O-$\beta$-glucuronopyranoside (XVI)13) and 2'-O-methylskullcapflavone I.7,14) respectively.

Compounds VI—XVI are known flavonoids and were identified as $2(S)$-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone,7) $(\pm)$-5,2'-dihydroxy-6,7,6'-trimethoxyflavanone,7) 5,7-dihydroxy-8,2'-dimethoxyflavone,7) rivularin,15) 5,2',6'-trihydroxy-7,8-dimethoxyflavone,16) scu-

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Fig. 1
tevurin, 16) 5,7,4'-trihydroxy-8-methoxyflavone, 17) wogonin, 7) alpinetin, 12) caldamomin and wogonin 7-O-glucuronide, 7) respectively, by direct comparison with authentic samples.

**Experimental**

**General Procedures** — All melting points were determined on a Yanagimoto micro melting point apparatus and are recorded uncorrected. UV spectra were determined with addition of diagnostic reagents by standard procedures. 1H-NMR and 13C-NMR were recorded on a JEOL JNM-FX-100 spectrometer (1H-NMR at 100 MHz and 13C-NMR at 25 MHz), and 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. CD spectra were run on a JASCO J-20A automatic recording spectropolarimeter. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6AM unit with a flame ionization detector using a glass column (2 m x 4 mm i.d.) packed with 5% SE-30 on Chromosorb W (60-80 mesh); column temperature, programmed from 150 °C (20 min hold) to 240 °C at 5 °C/min. Thin layer chromatography (TLC) was carried out on Kieselgel 60 F254 (Merck) with the following solvent systems: CHCl3-MeOH-h2O-AcOH (100: 4: 0.2: 0.1) (TLC-1), n-hexane-acetone-AcOH (60: 40: 1) (TLC-2), CHCl3-MeOH-h2O-HCOOH (25: 8: 1: 1) (TLC-3), AcOEt-methyl ethyl ketone-h2O-HCOOH (18: 9: 1: 1) (TLC-4). Spots were detected by spraying of dil. H2SO4 followed by heating.

**Extraction and Separation** — The dried root (330 g) of Scutellaria indica L., collected in Taiwan in 1985, was extracted with boiling EtOH. The EtOH extract was concentrated to dryness to give a residue (110.7 g), which was suspended in H2O and successively extracted with Et2O and n-BuOH. The Et2O layer was concentrated and the residue (6.1 g) was chromatographed on silica gel (500 g) using n-hexane-acetone (10: 1 → 1: 0) as an eluent to give five fractions, fr. 1-5, in the order of elution. Fraction 1 gave VI. Fraction 2 was recrystallized from Et2O to give Ia, colorless needles, mp 174 °C (dec.). This product was identical (UV, IR, 1H- and 13C-NMR, recrystallized from MeOH) with 5,7,8,2'-tetramethoxyflavanone.

**2(S)-5,7,2'-Trihydroxy-8-methoxyflavanone** — Colorless needles (MeOH/H2O), mp 197 °C (decc.). Anal. Calcd for C15H11O5: C, 63.57; H, 4.67. Found: C, 63.33; H, 4.69. Mcg-HCl (+). Rf: 0.19 (TLC-1), 0.28 (TLC-2). UV: Absmax nm (log ε): 340.4 (C-5), 380.6 (C-8), 408.6 (C-4), 420.6 (C-7), 332.4 (C-3), 261.8 (C15H9O5, 100), 167.2 (C7H3O5, 73). CD (c=0.005, MeOH) [α]14 (nm): +16717 (309) (positive maximum), -59554 (288) (negative maximum). Methylation of I by Kuhn’s Method: CH3O+ (0.2 ml) and Ag2O (30 mg) were added to a solution of I (8 mg) in N,N-dimethylformamide (DMF) (0.3 ml), and the reaction mixture was left for 20 h with occasional shaking. Then CHCl3 was added, and after removal of the resulting precipitate by filtration, the filtrate was evaporated to dryness. The residue was chromatographed on silica gel (10 g) using benzene as an eluent to give crude Ia, which was recrystallized from MeOH to give Ia, colorless needles, mp 174 °C (dec.). This product was identical (UV, IR, 1H- and 13C-NMR, mixed fusion) with 5,7,8,2'-tetramethoxyflavanone.
7.6 Hz, 4'-H), 7.44 (1H, d, J = 7.8 Hz, 6'-H), 12.15 (1H, s, 5'-OH), 13C-NMR: 74.5 (C-2), 41.5 (C-3), 197.1 (C-4), 159.0 (C-5), 93.0 (C-6, J(c6) (6H) = 163.3 Hz, J(c6) (5OH) = 7.4 Hz), 156.2 (C-5), 95.9 (C-6), 166.8 (C-7), 95.1 (C-8), 163.4 (C-9), 101.8 (C-10), 124.9 (C-11), 154.4 (C-12), 115.6 (C-13), 129.5 (C-14), 119.2 (C-15), 127.1 (C-16). MS m/z (%): 272 (M+), 60, 254 (C15H10O8, 98), 153 (C13H8O4, 100). 

2.5,8'-Di-O-Methyl-7,8,6'-dimethoxyflavone (III) — Colorless needles (MeOH/H2O), mp 192 °C. Anal. Calcd for C24H20O10: C, 55.18; H, 5.03. Found: C, 55.05; H, 5.06. Mg-HCl (+). Rf: 0.21 (TLC-2). UV A max: 276 nm (log ε): 275 (4.40), 333 (4.03); A max - NaOMe nm (log ε): 243 (4.11), 288 (4.00), 315 (3.76), 390 (3.75); 1H-NMR: 3.66, 3.87 (each 3H, each s, OCH3 x 2), 2.80 (1H, dd, J = 163.2 Hz, J(c6) (5OH) = 7.4 Hz), 4.19 (1H, d, J = 12.9 Hz, 2-H), 6.25 (1H, s, 6-H), 6.62 (1H, d, J = 9.3, 2.0 Hz, 4'-H), 6.75 (1H, d, J = 9.3 Hz, 3'-H), 6.92 (1H, d, J = 2.0 Hz, 6'-H), 8.87, 9.11 (each 1H, each s, 5' and 2'-OH), 12.03 (1H, s, 5'-OH). 13C-NMR: 74.0 (C-2), 41.1 (C-3), 196.5 (C-4), 163.7 (C-5), 95.9 (C-6), 163.7 (C-7), 129.5 (C-8), 153.9 (C-9), 102.4 (C-10), 145.6 (C-11), 116.4 (C-12), 115.9 (C-13), 115.1 (C-14), 150.1 (C-15), 113.2 (C-16), 56.3 (C-7-OCH3), 60.5 (C-8-OCH3). MS m/z (%): 332 (M+, 58), 299 (C15H10O7, 100), 136 (C14H12O4, 25) C (c = 0.005, MeOH) [θ]14 nm: +9501 (308) (positive maximum), —34058 (283) (negative maximum).

Methanolysis of IV: A solution of IV (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. The residue was recrystallized from MeOH/H2O to give a mixture of two types of crystals, which was chromatographed on silica gel (10 mg) using benzene as an eluent to give pale yellow needles (mp 270 °C (dec.)) were identified as 2(S)-5,2'-dihydroxy-7,8,6'-trimethoxyflavanone, ( + )-5,2'-dihydroxy-6,7,6'-methyl glycoside of glucurono-6,3-lactone by GLC.
trimethoxyflavanone, 5,7-dihydroxy-8,2’-dimethoxyflavone, rivularin, 5,2’,6’-trihydroxy-7,8-dimethoxyflavone, scutevurin, 5,7,4’-trihydroxy-8-methoxyflavone, wogonin, alpinetin, caldamomin and wogonin 7-O-glucuronide, respectively, by direct comparisons with authentic specimens (UV, IR, 1H- and 13C-NMR, mixed fusion).

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References and Notes
2) Presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.
11) The spectra were measured in DMSO-d6 on a JEOL GX-400 spectrometer.