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Scutellaric Acid, a New Triterpene from *Scutellaria rivularis*

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The structure of scutellaric acid, a new oleanane type triterpene acid isolated from *Scutellaria rivularis*, was determined on the basis of spectral and chemical correlations.

Keywords——*Scutellaria rivularis*; Ban Zhi Lian; Labiatae; oleanane-type triterpene acid; scutellaric acid; chemical evidence; physical evidence

Ban Zhi Lian (dried whole plants of *Scutellaria rivularis* WALL., Labiatae) has been used to treat hepatitis and other diseases in China and Taiwan. The chemical constituents of this plant have been investigated in detail.[1] In our previous reports[1f,h] we have described the isolation of six new neoclerodane-type diterpenoid lactones,[2-5] scutellones A, B, C, D, E, and F, one new oleanane-type triterpenoid acid, scutellaric acid, and eighteen flavonoid constituents. Recently, Tomimori *et al.* isolated five clerodane-type diterpenoids, of which scuterivulactones C_1[6] and D[7] were identical with scutellones A, and D, respectively. In this paper we describe our study of the remaining triterpene acid, scutellaric acid.

Scutellaric acid (**1a**), needles from acetone, has the molecular formula $C_{30}H_{48}O_4$ on the basis of elementary analysis. The infrared (IR) spectra showed three functional groups, hydroxyl (3400 cm^{-1}), carboxylic acid (3200—2500, 1690, and 910 cm^{-1}), and olefin (1630 cm^{-1}). The nuclear magnetic resonance (NMR) spectra revealed that scutellaric acid (**1a**) contains six methyl groups, one hydroxymethyl group, one secondary hydroxyl group, one trisubstituted olefin, and one carboxylic acid. From the above data in addition to the signal at δ 2.74 (1H, dd, J=9.9, 1.8 Hz, H-18)[8] scutellaric acid is considered to be an oleanane-type triterpene acid with one primary alcohol and one secondary alcohol. The electron impact mass spectrum (EIMS) of scutellaric acid exhibited the M^+ peak at m/z 472 and peaks at 454 ($M^+ - H_2O$), 248, 233, 203, 133, and 119 (Chart 1)[9] further supported the structure of **1a**. The functional groups in **1a** were identified from the following chemical evidence. Treatment of **1a** with CH_2N_2 in CH_3OH gave a methyl ester (**1b**) [δ $CDCl_3$ 3.60 (3H, s)]. The reaction of Ac_2O and **1a** in pyridine at room temperature gave a diacetate (**1c**) [δ $CDCl_3$ 2.01 and 2.06 (each 3H, s), 3.93 and 4.16 (each 1H, d, J=11.4 Hz), and 4.94 (1H, t, J=1.8 Hz)] which was subsequently treated with CH_2N_2 in CH_3OH to afford **1d** [δ $CDCl_3$ 3.60 (3H, s), 3.93 and 4.19 (each 1H, d, J=11.4 Hz)]. The hydroxyl group located at C-3 must be in α-axial orientation, since H-3 shows a small coupling constant. By comparison of the chemical shift of the methylene protons of $-CH_2OAc$ in **1c** and **1d** with those of known compounds,[10] the primary hydroxyl group of **1a** was concluded to be linked to C-24. No reaction product was observed with 2,2-dimethoxypropane and **1b** under acidic conditions. This also suggests that the glycol is *trans* diaxial. Based on the above evidence, the structure of scutellaric acid (**1a**) can be assigned as 3α,24-dihydroxyolean-12-en-28-oic acid, a new triterpene acid.

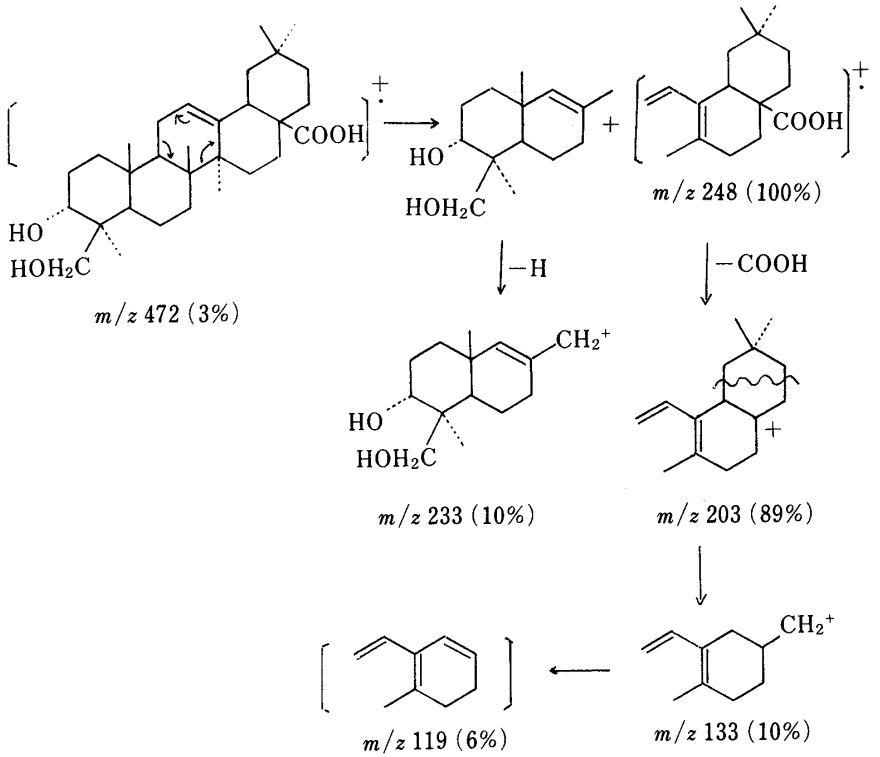

Chart 1

1a : $R_1=R_2=H$, $R_3=\overset{H}{\underset{OH}{<}}$

1b : $R_1=Me$, $R_2=H$, $R_3=\overset{H}{\underset{OH}{<}}$

1c : $R_1=H$, $R_2=Ac$, $R_3=\overset{H}{\underset{OAc}{<}}$

1d : $R_1=Me$, $R_2=Ac$, $R_3=\overset{H}{\underset{OAc}{<}}$

1e : $R_1=Me$, $R_2=Ac$, $R_3=\overset{H}{\underset{OH}{<}}$

1f : $R_1=Me$, $R_2=Ac$, $R_3=O$

1g : $R_1=Me$, $R_2=H$, $R_3=O$

1h : $R_1=Me$, $R_2=Ac$, $R_3=\overset{OH}{\underset{H}{<}}$

Fig. 1

The chemical correlation of scutellaric acid (**1a**) to methyl 24-hydroxy-3-oxoolean-12-en-28-oate (**1g**) was achieved as follows. Compound **1b** was reacted with acetic anhydride in pyridine at 0°C for 1 h to yield a diacetate (**1d**) and monoacetate (**1e**) in 1 : 9 ratio. The latter [δ $CDCl_3$ 2.0 (3H, s)] was subsequently oxidized with Jones reagent to afford a ketone (**1f**) which was different from the monoacetate of methyl hederagonate (**2**) on comparison of their physical data.[11] The product **1g**, obtained from **1f** by acidic methanolysis, was identical with methyl 24-hydroxy-3-oxoolean-12-en-28-oate.[12] This result confirmed the structure of scutallaric acid as shown formula **1a**. Sodium borohydride reduction of **1f** in methanol gave **1h** [δ $CDCl_3$ 3.25 (1H, dd, J=8.5, 1.5 Hz)].

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO A-102 spectrometer. ^{1}H-NMR spectra were run on a Brucker AM 300 at 300 MHz with tetramethylsilane (TMS) as an internal standard. EIMS were taken on a JOEL-JMS-100.

Extraction and Isolation——The aerial part of *Scutellaria rivularis* (6.2 kg) was extracted with ethanol four times. The purification of scutellone A, B, C, D, E, F, and scutellaric acid, in addition to eighteen flavonoid consituents, was described in detail in the previous report.[1f,h]

Scutellaric Acid (**1a**): mp 275—277 °C. $[\alpha]_D^{27}$ +35.5° (c=1.0 in MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 3200—2500, 1690, 1630, 910. ^{1}H-NMR (DMSO-d_6) δ: 0.68, 0.82, 0.87, 0.87, 0.88, 1.10 (each 3H, s), 2.74 (1H, dd, J=9.9, 1.8 Hz, H-18), 3.19, 3.43 (each 1H, d, J=10.8 Hz, $-C\underline{H}_2OH$), 3.55 (1H, t, J=1.8 Hz, H-3), 5.15 (1H, t, J=2.0 Hz, H-12), 11.9 (1H, br s, −COOH, disappeared on D_2O exchange). *Anal.* Calcd for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.05; H, 10.21.

Methylation of Scutellaric Acid (1a)——Diazomethane in ether solution was added dropwise to a solution of scutellaric acid (**1a**) (20 mg) in MeOH (3 ml). The yellow reaction mixture was evaporated to gave the methyl ester (**1b**) (20 mg) [mp 235—237 °C. IR ν_{max}^{KBr} cm^{-1}: 3490, 3440, 1710. ^{1}H-NMR ($CDCl_3$) δ: 0.67, 0.86, 0.87, 0.90, 1.06, 1.15, 3.60 (each 3H, s), 2.82 (1H, dd, J=10.6, 2.0 Hz), 3.50, 3.70 (each 1H, d, J=10.3 Hz), 3.84 (1H, t, J=1.2 Hz), 5.25 (1H, t, J=1.8 Hz)].

Acetylation of Scutellaric Acid (1a)——A solution of scutellaric acid (**1a**) (20 mg) in pyridine (0.5 ml) and acetic anhydride (0.5 ml) was left overnight at room temperature. The reaction mixture was treated by the usual method and gave a diacetate (**1c**) (21 mg) [mp 274—276 °C. IR ν_{max}^{KBr} cm^{-1}: 3200—2500, 1735, 1690. ^{1}H-NMR ($CDCl_3$) δ: 0.72, 0.89, 0.91, 0.92, 0.92, 1.05, 2.01, 2.06 (each 3H, s), 2.81 (1H, dd, J=9.7, 2.1 Hz), 3.93, 4.16 (each 1H, d, J=11.4 Hz), 4.94 (1H, t, J=1.2 Hz), 5.27 (1H, t, J=1.8 Hz)].

Acetylation of the Methyl Ester (Ib)——The methyl ester (**Ib**) (18 mg) was acetylated under condition similar to those mentioned above and gave **1d** (19 mg) [mp 220—221 °C. IR ν_{max}^{KBr} cm^{-1}: 1735. ^{1}H-NMR ($CDCl_3$) δ: 0.69, 0.87, 0.90, 0.90, 0.92, 1.15, 2.02, 2.06, 3.60 (each 3H, s), 2.85 (1H, dd, J=9.7, 2.1 Hz), 3.93, 4.19 (each 1H, d, J=11.4 Hz), 4.92 (1H, t, J=1.2 Hz), 5.27 (1H, t, J=1.8 Hz)].

Partial Acetylation of the Methyl Ester (1b)——The methyl ester (**1b**) (30 mg) was reacted with Ac_2O (1 ml) ion pyridine (1 ml) at 0 °C for 1 h. The reaction mixture was treated as usual to give a monoacetate (**1e**) (27 mg) [mp 234—236 °C. IR ν_{max}^{KBr} cm^{-1}: 3520, 1730, 1715. ^{1}H-NMR ($CDCl_3$) δ: 0.66, 0.87, 0.89, 0.90, 1.03, 1.12, 2.02, 3.60 (each 3H, s), 2.84 (1h, dd, J=9.8, 2.2 Hz), 3.72 (1H, t, J=1.2 Hz), 3.93, 4.15 (each 1H, d, J=11.1 Hz), 5.26 (1H, t, J=1.8 Hz)] and a diacetate (**1d**) (3 mg).

Oxidation of 1e by Jones Reagent——Jones reagent in acetone was added dropwise to a solution of **1e** (30 mg) in 3 ml of acetone at 0—5 °C. By using the usual treatment, the ketone (**1f**) (20 mg) [mp 164—166 °C. IR ν_{max}^{KBr} cm^{-1}: 1735, 1710. ^{1}H-NMR ($CDCl_3$) δ: 0.74, 0.87, 0.90, 1.09, 1.13, 1.15, 1.97, 3.61 (each 3H, s), 3.93, 4.61 (each 1H, d, J=11.3 Hz), 5.27 (1H, t, J=1.6 Hz)] was isolated.

Conversion of 1f to 1g under Acidic Condition——The ketone (**1f**) (12 mg) and *p*-toluenesulfonic acid (5 mg) in 1 ml of methanol was allowed to stand overnight. After purification, the reaction mixture yielded 1 g (mp 212—213.5 °C), with was identical with methyl 24-hydroxy-3-oxoolean-12-en-28-oate.[12]

Sodium Borohydride Reduce of 1f——Excess of sodium borohydride was added in small portions to a solution of the ketone (**1f**) (7 mg) in 1 ml of methanol, then the solution was poured into water (20 ml) after 4 h. The product (**1h**) had mp 222—224 °C (6 mg). IR ν_{max}^{KBr} cm^{-1}: 3510, 1730, 1715. ^{1}H-NMR ($CDCl_3$) δ: 0.66, 0.87, 0.87, 0.90, 1.10, 1.12, 2.03, 3.60 (each 3H, s), 3.25 (1H, dd, J=8.5, 5.1 Hz), 4.11, 4.33 (each 1H, d, J=11.3 Hz), 5.28 (1H, t, J=1.7 Hz).

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