SCUTELLONE C AND F, TWO NEW NEOCLERODANE TYPE DITERPENOIDS FROM SCUTELLARIA RIVULARIS

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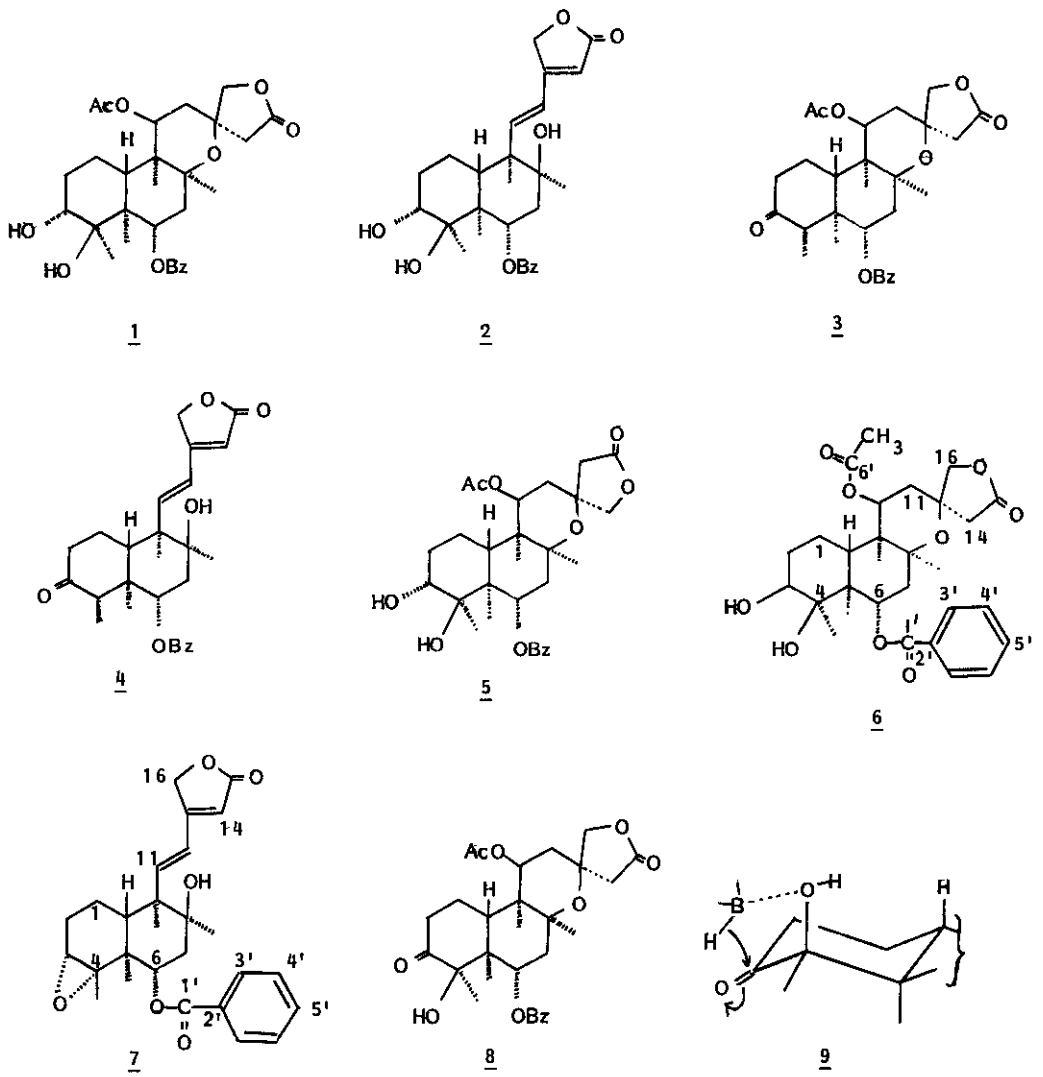
Abstract — Two new neoclerodane type diterpenoids, scutellone C and F, had been isolated from aerial parts of Scutellaria rivularis Wall.. Their structures were elucidated by spectral and chemical evidence.

The ethanol extract from aerial parts of "Ban Zhi Lian" (dried *Scutellaria rivularis* Wall., *Labiatae*) was separated by silica gel column chromatography followed by Sephadex LH-20 column chromatography to give six new neoclerodane type diterpenoid lactones (scutellone A, B, C, D, E, and F), one oleanane type triterpenoid (scutellaric acid) in addition to eighteen flavonoid constituents.[1,2] The structures of scutellone A (1)[3] and D (2)[4] were determined by X-ray diffraction, spectral and chemical evidence, and the structures of scutellone B (3)[5] and E (4)[4] were elucidated by spectral evidence and chemical correlation. Recently, Tomimori reported the isolation of five clerodane type diterpenoids, scuterivulactone A, B, C_1, C_2 and D, from the same source. The structures of scuterivulactone C_1, C_2[6], and D[7] were identified as scutellone A (1), (5), and scutellone D (2), respectively, by means of 2-D nmr spectroscopy including INADEQUATE and ^1H-^{13}C long-range COSY. In this paper we describe our study of the other remained two structures of scutellone C (6) and F (7).

Scutellone C (6), needles from acetone, has the molecular formula $C_{29}H_{38}O_9$ on the basis of elementary analysis and mass spectra [$(M + H)^+$ at m/z 531[6] and other peaks at m/z 513, 494, 390, 122 and 105]. The ir absorption bands also express similarity to that of scutellone A (1). The ^1H and ^{13}C-nmr spectra were as follows (the assigment was based on ^1H-^1H COSY spectra, ^1H-^{13}C COSY spectra, and DEPT method): ^1H nmr ($CDCl_3$) δ 0.99, 1.20, 1.25, 1.34, and 2.04 (each 3H, s), 2.73 and 2.81 (each 1H, d, J = 17.0 Hz, H-14), 4.15 and 4.35 (each 1H, d, J = 9 Hz, H-16), 4.22 (1H, dd, J = 12.3, 4.5 Hz, H-3), 5.32 (1H, dd, J = 11.3, 4.0 Hz, H-11), 5.78 (1H, t, J = 8.0 Hz, H-6), 7.44 (3H, m), and 7.95 (2H, dd, J = 7.3, 1.2 Hz); ^{13}C nmr ($CDCl_3$) δ 10.3, 16.5, 17.2, 21.1, and 23.8 (1°C; five methyl groups), 22.1, 33.0, 35.2, 37.9, 42.7 and 79.0 (2°C; C-1, C-12, C-2, C-7, C-14, and C-16, respectively), 37.5, 67.4, 71.5, 73.1, 128. 3, 129.4 and 132.9 (3°C; C-10, C-3, C-6, C-11,



1 2 3 4 5 6 7 8 9

Fig. 1

C-4', C-3', and C-5', respectively), 42.4, 48.0, 77.4, 78.0, 82.0, 130.5, 165.3, 170.1, and 174.1 (4°C; C-9, C-5, C-4, C-13, C-8, C-2', C-1', C-6', and C-15, respectively). In ^{1}H-^{13}C COSY spectra, δ 2.73 and 2.81 and 42.7, 4.15 and 4.35 and 79.0, 4.22 and 67.4, 5.31 and 73.1, and 5.78 and 71.5 exhibited strong correlation. These evidence also confirmed the structure. By comparison with the physical data of scutellone A (1) and scutellone C (6), the structure of scutellone C (6) can be assigned as C-3 epimer of (1). When (1) was oxidized with pyridinium chlorochromate (PCC) in CH_2Cl_2, ketone (8) [mp 225-226°C] was obtained. Attempt to prepare (6) from (8) by reducing with sodium borohydride in methanol gave (1) only. The reason of this result is that boron atom was chelated with C_{β}-4 hydroxyl group [intermediate (9)] and then hydride attacked carbonyl from β-face to give α-oriented hydroxyl. The structure of scutellone C (6) was elucidated as C-3 epimer of scutellone A (1) by fact that scutellone C (6) also yielded ketone (8) by CrO_3 oridation. Scutellone F (7), $C_{27}H_{32}O_6$, was obtained as colorless needles (from acetone). The ir spectrum showed absorption bands at 3460 (-OH), 1775 and 1740 (lactone), 1710 (ester), 1640 (olefin), 1600 and 1590 (benzenoid), and 1270 and 845 cm^{-1} (epoxide). The EIMS exhibited the M^+ peak at m/z 452 (100 %), and fragment ion peaks at m/z 330 ($M^+ - C_6H_5COOH$), 312 ($M^+ - H_2O - C_6H_5COOH$), 204 ($M^+ - H_2O - C_6H_5COOH -$ ), 122 (C_6H_5COOH), and 105 (C_6H_5CO). The fragment ion peak at m/z 204 was also present in scutellone D (2) and E (4) with 67 % and 100 % relative intensity, respectively. The uv absorption of (7) was similar to scutellone D (2) and E (4) that suggested the presence of the same partial structure. Our assignment of the structure of scutellone F (7) was based on ^{1}H-^{13}C nmr, ^{1}H-^{1}H COSY spectra, ^{1}H-^{13}C COSY spectra and DEPT method. The ^{1}H- and ^{13}C spectra were shown as follows: ^{1}H nmr ($CDCl_3$) 1.03, 1.06, 1.16 and 1.35 (each 3H, s), 2.76 (1H, br s, H-3), 4.93 and 5.03 (each 1H, d, J = 16.5 Hz, H-16), 5.55 (1H, t, J = 7.8 Hz, H-6), 5.87 (1H, s, H-14), 6.27 and 6.37 (each 1H, d, J = 16.5 Hz, H-12 and H-11, respectively), 7.50 (3H, m), 8.00 (2H, dd, J = 7.2, 1.5 Hz); ^{13}C nmr ($CDCl_3$) δ 13.3, 15.5, 21.1 and 27.6 (1°C; four methyl groups), 16.7, 29.6, 38.8, and 70.6 (2°C; C-1, C-2, C-7, and C-16, respectively), 44.0, 63.0, 75.3, 114.4, 121.7, 128.4, 129.4, 133.2, and 147.6 (3°C; C-10, C-3, C-6, C-14, C-12, C-4', C-3', C-5' and C-11, respectively), 41.7, 48.1, 65.6, 74.5, 130.3, 162.3, 165.5, and 174.1 (4°C; C-5, C-9, C-4, C-8, C-2', C-13, C-1' and C-15, respectively). The presence of trisubstituted epoxide function was confirmed by ^{1}H-^{13}C COSY spectra in which δ 2.76 (H-3) and 63.0 (C-3) showed strong correlation. Based on the above evidence, the structure of scutellone F (7) must possess the same structural skeleton as scutellone D (2) with the exception of a trisubstituted epoxide group in replacement of a α-glycol function. The epoxide function may be assigned as α-face configuration because the H-3 protons (being β-equatorial orientation) appeared as a slight broad singlet in nmr spectra. The chemical correlation of scutellone F (7) to (2) and (4) was achieved as follows. When scutellone F (7) was treated with p-toluenesulfonic acid in acetone at room temperature for 3hr, scutellone

D (2) (minor) and E (4) (major) were isolated after purification. The result confirmed the structure of scutellone F (7) as another new neocledorane type diterpenoid. The mechanism of this transformation is proposed as in Fig. 1.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. Ir spectra were recorded on a JASCO A-102 spectrometer. ^{1}H - and ^{13}C-Nmr spectra were run on a Bruker AM 300 at 300 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ-values and coupling constants (J) are given in Hz. Electron impact mass spectrometry (EI-MS) and uv spectra were taken on a JOEL-JMS-100 and Hitachi RMS-4 spectrometer, respectively.

Extraction and Isolation

The aerial part of Scutellaria rivularis (6.2 Kg) was extracted with ethanol four times. The combined ethanol solution was evaporated to leave residue which was extracted with ether. The ether extract was chromatographed on silica gel and on Sephadex LH-20 repeatedly. The detailed purification of scutellone A, B, C, D, E, F and scutellaric acid in addition eighteen flavonoid constituents was described in previous report.[2,3]

Scutellone C (6); *mp* 228-230°C; $[\alpha]_D^{20}$ - 20.0° (c 1.0 in $CHCl_3$); ir (KBr) (ν_{cm}-1) 3470, 1780, 1720, 1710, 1600, 1575 and 1480; uv λ_{max} (MeOH) (log ε): 230 (3.98), 273 (3.00) and 282 (2.89) nm. Anal. Calcd for $C_{29}H_{38}O_9$: C, 65.64; H, 7.22. Found C, 65.39; H, 7.25.

Scutellone F (7); mp 213-215°C; $[\alpha]_D^{20}$ + 54.9° (c 1.0 in $CHCl_3$); uv λ_{max} (MeOH) (log ε); 238 (4.16) and 261 (4.38) nm; Anal. Calcd for $C_{27}H_{32}O_6$: C, 71.66; H, 7.13. Found C, 71.89; H, 7.18.

Oxidation of Scutellone A (1) with PCC and Molecular Sieve

Scutellone A (1) (30 mg) was added to a suspension of pyridinium chlorochromate (50 mg) and 4 Å molecular sieve (2 g) in dichloromethane (5 ml)[8]. The reaction mixture was well stirred for four hours then was filtered. The filtrate was subjected to silica gel chromatography and gave ketone (8) (25 mg) (mp 225-226°C); Ir (KBr) (ν_{cm}-1) 3440, 1770, 1710, 1615, 1595, 1270, 1250, 1020, and 720; ^{1}H nmr ($CDCl_3$) δ 0.99, 1.09, 1.15, 1.32 and 2.07 (each 3H, s), 2.74 and 2.84 (each 1H, d, J = 17.0 Hz, H-14), 3.05 (1H, m, H-2 axial), 3.40 (1H, dd, J = 13, 3.5 Hz, H-10), 4.24 and 4.40 (each 1H, d, J = 9.3 Hz, H-16), 5.38 (1H, dd, J = 12.8, 4.0 Hz, H-11), 5.83 (1H, dd, J = 11.5, 4.9 Hz, H-6), 7.42 (1H, t, J = 7.5 Hz), 7.55 (1H, t, J = 7.5 Hz), and 7.95 (2H, d, J = 7.5 Hz).

Reduction of ketone (8) with Sodium Borohydride

Excess sodium borohydride was added in small portions to a solution of ketone (8) (20 mg) in 1 ml of MeOH and let stand for three hours. The reaction mixture was poured into excess water and precipitate appeared. The precipitate was purified by silica gel chromatography to give scutellone A (1) (19 mg) only.

Oxidatin of Scutellone C (6) with CrO_3

A solution of CrO_3 (30 mg) in 0.5 ml of glacial acetic acid containing a few drops of water was added dropwise to a solution of scutellone C (6) (25 mg) in 0.5 ml of glacial acetic acid. The mixture was left standing at room temperature for 1 hr, poured into water and extracted with ether. The extract was purified and yielded ketone (8) (20 mg).

Conversion of Scutellone F (7) to Scutellone D (2) and E (4)

Scutellone F (7) (30 mg) was treated with p-toluenesulfonic acid (10 mg) in acetone (3 ml) at room temperature for 3 hours. The reaction mixture was subjected to silica gel chromatography and gave scutellone D (2) (3 mg) and E (4) (23 mg).

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