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Structures of Scutellones D and E Determined from X-Ray Diffraction, Spectral and Chemical Evidence. Neoclerodane-Type Diterpenoids from *Scutellaria rivularis* WALL.

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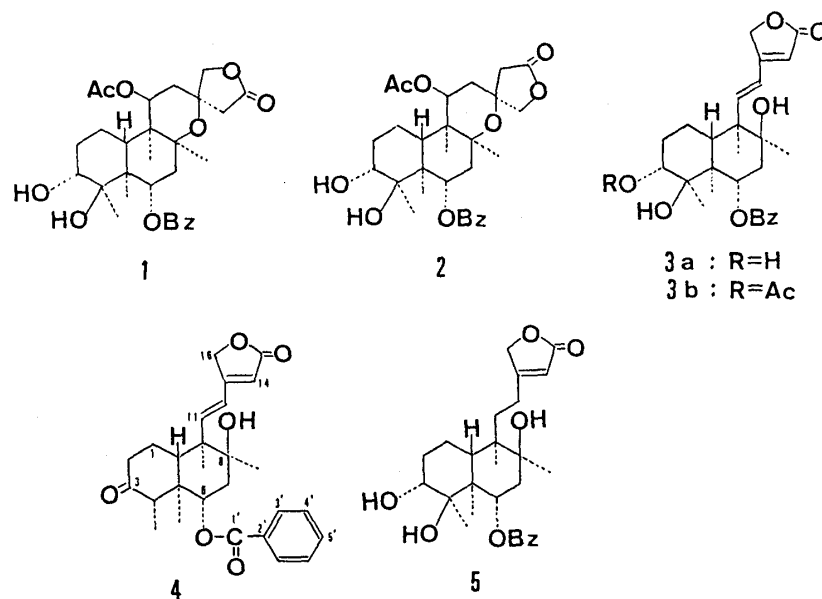
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A new neoclerodane-type diterpenoid lactone, scutellone E, together with a known compound, scutellone D (scuterivulactone D), has been isolated from aerial parts of *Scutellaria rivularis* WALL. Their structures have been determined from X-ray diffraction, spectral and chemical evidence.

Keywords—*Scutellaria rivularis*; Labiatae; neoclerodane diterpenoid; scutellone D (scuterivulactone D); scutellone E; X-ray analysis

The whole herb of *Scutellaria rivularis* WALL. (Labiatae) has been used for the treatment of tumors, hepatitis, liver cirrhosis and other diseases in China and Taiwan.¹⁾ The ethanol extract from aerial parts of this plant was separated by silica gel column chromatography followed by Sephadex LH-20 column chromatography to give six neoclerodane-type diterpenoid lactones (scutellones A, B, C, D, E and F), one oleanane-type triterpenoid (scutellaric acid) and eighteen flavonoid constituents.^{2,3)} The structure of scutellone A (**1**) was determined⁴⁾ by X-ray analysis. Recently, Tomimori *et al.*^{5,6)} reported the isolation of five clerodane-type diterpenoids, scuterivulactones A, B, C₁, C₂ and D, from the same source. The structures of scuterivulactones C₁, C₂,⁵⁾ and D⁶⁾ were determined as **1**, **2**, and **3a**, respectively, by means of two dimensional nuclear magnetic resonance (2-D NMR) spectroscopy including INADEQUATE and ¹H-¹³C long-range COSY. This report deals with structural



elucidation of scutellone D (scuterivulactone D) (**3a**) and scutellone E (**4**) on the basis of X-ray diffraction spectral and chemical evidence.

Compound (**3a**) is identical with scuterivulactone D, and therefore the physical data of **3a** are exactly the same as in the literature.⁶⁾ Partial hydrogenation of **3a** (Pd-C as the catalyst and ethyl acetate as the solvent) afforded dihydroscutellone D (**5**) (mp 199–200 °C), which exhibits NMR (CDCl₃) signals at δ 2.63 (2H, m, H-12) instead of two olefinic protons, and at δ 5.87 (1H, br s, H-14). When **3a** was treated with acetic anhydride in pyridine, it gave the 3-O-acetate (**3b**) [mp 244–246 °C; lit. 242–244 °C⁶⁾]. The relative configuration and the structure of the 3-O-acetate of scutellone D (**3b**) were determined by X-ray diffraction analysis.

Crystal Analysis

Compound **3b** crystallized in monoclinic space group *I*2. Lattice parameters $a=17.147(2)$, $b=9.079(3)$, $c=18.099(2)$ Å; $\beta=102.07(1)^\circ$, $Z=4$. Intensity data were collected up to 2θ of 50° with MoK α radiation on a CAD4 diffractometer; 2712 reflections were measured, and among them 1251 ($>2.5\sigma$) were used for the subsequent refinements. The structure was solved by the direct method using the MULTAN program and the atomic parameters were obtained by block diagonal least-squares refinements. The final agreement index *R* is 0.060 with anisotropic temperature factors of all non-hydrogen atoms and isotropic hydrogen atoms with calculated positions. There are water molecules located on the 2-fold axis with only 50% occupancy. The molecular structure and the atomic numbering of **3b** are depicted in Fig. 1. Selected bond lengths and angles are given in Table I. The atomic coordinates are listed in Table II.

Scutellone E (**4**), needles from acetone, has the molecular formula C₂₇H₃₂O₆ on the basis of elementary analysis. Its ultraviolet (UV) absorption appears to be the same as that of **3a**. The infrared (IR) spectrum showed absorption bands at 3440 (OH), 1775 (lactone), 1735 (ester), and 1715 (ketone) cm⁻¹ and the electron impact-mass spectrum (EI-MS) exhibited the

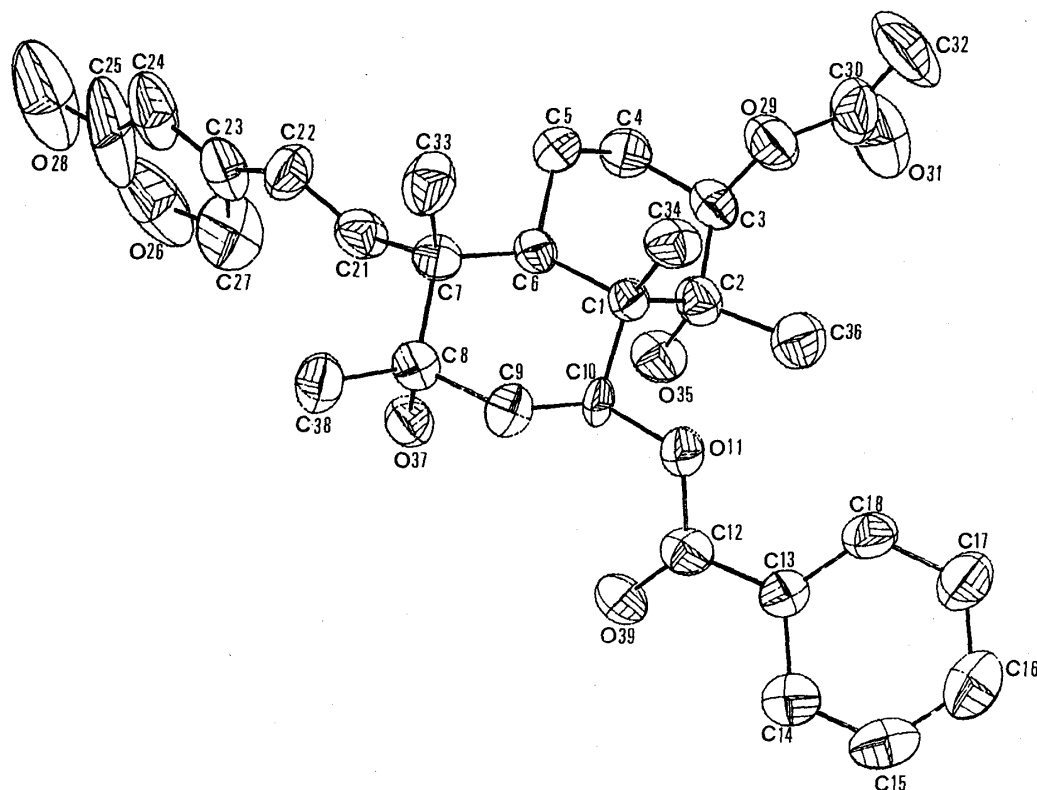


Fig. 1. Molecular Structure and Atomic Numbering of **3b**

TABLE I. Selected Bond Lengths and Angles of 3b

C(1)–C(2)	1.55 (3)	O(11)–C(12)	1.340 (22)
C(1)–C(6)	1.57 (3)	O(12)–C(13)	1.48 (3)
C(1)–C(10)	1.527 (24)	C(12)–O(39)	1.193 (21)
C(1)–C(34)	1.552 (23)	C(13)–C(11)	1.40 (3)
C(2)–C(3)	1.54 (3)	C(13)–C(18)	1.373 (25)
C(2)–O(35)	1.430 (22)	C(14)–C(15)	1.36 (3)
C(2)–C(36)	1.54 (3)	C(15)–C(16)	1.37 (3)
C(3)–C(4)	1.53 (3)	C(16)–C(17)	1.34 (3)
C(3)–O(29)	1.458 (21)	C(17)–C(18)	1.38 (3)
C(4)–C(5)	1.50 (3)	C(21)–C(22)	1.33 (3)
C(5)–C(6)	1.53 (3)	C(22)–C(23)	1.44 (3)
C(6)–C(7)	1.55 (3)	C(23)–C(24)	1.32 (4)
C(7)–C(8)	1.56 (3)	C(23)–C(27)	1.45 (4)
C(7)–C(21)	1.51 (3)	C(24)–C(25)	1.36 (4)
C(7)–C(33)	1.54 (3)	C(25)–O(26)	1.33 (5)
C(8)–C(9)	1.54 (3)	C(25)–O(28)	1.25 (3)
C(8)–O(37)	1.450 (23)	O(26)–C(27)	1.45 (3)
C(8)–C(38)	1.54 (3)	O(29)–C(30)	1.337 (21)
C(9)–C(10)	1.51 (3)	C(30)–O(31)	1.24 (3)
C(10)–O(11)	1.471 (20)	C(30)–C(32)	1.41 (3)
C(2)–C(1)–C(6)	105.3 (13)	C(8)–C(9)–C(10)	111.3 (14)
C(2)–C(1)–C(10)	111.2 (15)	C(1)–C(10)–C(9)	114.7 (16)
C(2)–C(1)–C(34)	111.5 (14)	C(1)–C(10)–O(11)	108.8 (12)
C(6)–C(1)–C(10)	105.7 (13)	C(9)–C(10)–O(11)	106.4 (13)
C(6)–C(1)–C(34)	112.9 (15)	C(10)–O(11)–C(12)	119.2 (13)
C(10)–C(1)–C(34)	110.1 (14)	O(11)–C(12)–C(13)	113.0 (14)
C(1)–C(2)–C(3)	113.5 (17)	O(11)–C(12)–O(39)	122.1 (18)
C(1)–C(2)–O(35)	107.9 (13)	C(13)–C(12)–O(39)	124.9 (18)
C(1)–C(2)–C(36)	115.7 (15)	C(12)–C(13)–C(14)	116.9 (15)
C(3)–C(2)–O(35)	106.6 (14)	C(12)–C(13)–C(18)	123.5 (16)
C(3)–C(2)–C(36)	106.1 (15)	C(14)–C(13)–C(18)	119.4 (17)
O(35)–C(2)–C(36)	106.5 (17)	C(13)–C(14)–C(15)	118.1 (17)
C(2)–C(3)–C(4)	114.4 (15)	C(14)–C(15)–C(16)	122.1 (19)
C(2)–C(3)–O(29)	110.2 (14)	C(15)–C(16)–C(17)	119.8 (21)
C(4)–C(3)–O(29)	107.5 (17)	C(16)–C(17)–C(18)	120.1 (20)
C(3)–C(4)–C(5)	111.1 (15)	C(13)–C(18)–C(17)	120.5 (18)
C(4)–C(5)–C(6)	111.2 (17)	C(7)–C(21)–C(22)	131.5 (19)
C(1)–C(6)–C(5)	110.8 (13)	C(21)–C(22)–C(23)	122.2 (22)
C(1)–C(6)–C(7)	118.8 (14)	C(22)–C(23)–C(24)	126 (3)
C(5)–C(6)–C(7)	112.3 (16)	C(22)–C(23)–C(27)	124.3 (23)
C(6)–C(7)–C(8)	110.4 (16)	C(24)–C(23)–C(27)	108.9 (21)
C(6)–C(7)–C(21)	106.7 (14)	C(23)–C(24)–C(25)	110 (3)
C(6)–C(7)–C(33)	112.8 (14)	C(24)–C(25)–O(26)	110.2 (25)
C(8)–C(7)–C(21)	105.7 (14)	C(24)–C(25)–O(28)	130 (4)
C(8)–C(7)–C(33)	111.1 (15)	O(26)–C(25)–O(28)	119 (3)
C(21)–C(7)–C(33)	109.7 (17)	C(25)–O(26)–C(27)	108.0 (21)
C(7)–C(8)–C(9)	110.0 (14)	C(23)–C(27)–O(26)	102.9 (25)
C(7)–C(8)–O(37)	108.8 (14)	C(3)–O(29)–C(30)	121.1 (17)
C(7)–C(8)–C(38)	115.2 (17)	O(29)–C(30)–O(31)	120.0 (21)
C(9)–C(8)–O(37)	104.0 (16)	O(29)–C(30)–C(32)	115.1 (23)
C(9)–C(8)–C(38)	109.8 (15)	O(31)–C(30)–C(32)	124.8 (22)
O(37)–C(8)–C(38)	108.1 (14)		

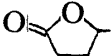
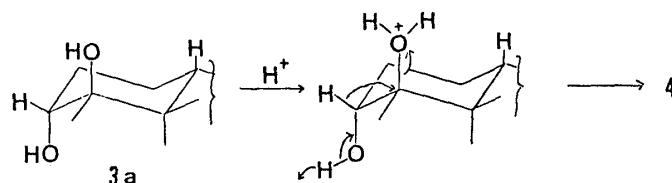
M^+ peak at m/z 452 and fragment ion peaks at m/z 330 ($M^+ - C_6H_5COOH$), 312 ($M^+ - H_2O - C_6H_5COOH$), 204 ($M^+ - H_2O - C_6H_5COOH -$  $C \equiv C$, 100%), 122

TABLE II. Atomic Coordinates of **3b**

Name	x	y	z
C(1)	0.18749	0.62416	0.47121
C(2)	0.23012	0.50577	0.52730
C(3)	0.26476	0.56697	0.60705
C(4)	0.31599	0.70495	0.60794
C(5)	0.27497	0.81678	0.55209
C(6)	0.25060	0.75020	0.47275
C(7)	0.23477	0.86977	0.41011
C(8)	0.21050	0.79655	0.33046
C(9)	0.14371	0.68235	0.33028
C(10)	0.16931	0.56600	0.39026
O(11)	0.10318	0.45990	0.38195
C(12)	0.09407	0.35949	0.32650
C(13)	0.03072	0.25047	0.33033
C(14)	0.00880	0.15526	0.26877
C(15)	-0.04571	0.01872	0.27281
C(16)	-0.07779	0.03020	0.33568
C(17)	-0.05670	0.12132	0.39468
C(18)	-0.00259	0.23259	0.39248
C(21)	0.31342	0.91519	0.41133
C(22)	0.33334	1.08647	0.41413
C(23)	0.41129	1.13532	0.40784
C(24)	0.43740	1.27226	0.41176
C(25)	0.51356	1.27618	0.10105
O(26)	0.53910	1.14006	0.39150
C(27)	0.47440	1.03833	0.39491
O(28)	0.55993	1.38326	0.40147
O(29)	0.20054	0.60293	0.64557
C(30)	0.19428	0.53507	0.70966
O(31)	0.24296	0.43899	0.73685
C(32)	0.12902	0.57994	0.74051
C(33)	0.17247	0.98403	0.42230
C(34)	0.10969	0.67881	0.49324
O(35)	0.29613	0.45037	0.49874
C(36)	0.17928	0.37165	0.53870
O(37)	0.27688	0.70937	0.31677
C(38)	0.18537	0.90418	0.26421
O(39)	0.13457	0.35939	0.28035
O(40)	0.00000	0.17717	0.00000

(C₆H₅COOH) and 105 (C₆H₅CO). The fragment ion peak at m/z 204 was also present in **3a** with 67% relative intensity. The ¹H- and ¹³C-NMR spectra were as follows: ¹H-NMR (CDCl₃) δ: 0.91 (3H, d, $J=6.6$ Hz), 1.09, 1.13 and 1.15 (each 3H, s), 2.65 (1H, q, $J=6.6$ Hz, H-4), 5.02 and 5.04 (each 1H, d, $J=16.0$ Hz, H-16), 5.47 (1H, dd, $J=8.2, 4.9$ Hz, H-6), 5.93 (1H, s, H-14), 6.44 [2H, s, H-11, H-12; (DMSO-*d*₆) δ: 6.39 and 6.47 (each 1H, d, $J=16.8$ Hz)], 7.60 (3H, m), 7.93 (2H, dd, $J=7.3, 2.4$ Hz); ¹³C-NMR (by DEPT method) (CDCl₃) δ: 9.7, 10.3, 15.6 and 26.5 (1 °C, four methyl groups), 24.1, 28.5, 41.3 and 70.7 (2 °C: C-1, C-7, C-2, C-16, respectively), 46.5, 57.6, 75.5, 114.9, 122.0, 128.4, 129.4, 133.0, 147.2 (3 °C: C-10, C-4, C-6, C-14, C-12, C-4', C-3', C-5', C-11, respectively), and 45.1, 48.1, 76.5, 130.5, 161.7, 165.3, 173.4, 210.9 (4 °C: C-5, C-9, C-8, C-2', C-13, C-1', C-15, C-3, respectively). A comparison of the physical data of scutellone D (**3a**) and scutellone E (**4**) suggested that **4** possesses the same structural skeleton as **3a** with the exception of one ketone group instead of an α-glycol function. When **3a** was heated in CHCl₃ with *p*-toluenesulfonic acid for 2 h, **4** was isolated

after purification (50% yield). The result confirmed the relative structure of **4** as a new neocledorane-type diterpenoid. The mechanism is proposed to be as follows:



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. ^1H - 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 hertz (Hz). EI-MS and UV spectra were taken on a JEOL-JMS-100 and Hitachi RMS-4 instruments, 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 a residue which was extracted with ether successively. The ether extract was separated by chromatography on silica gel and then on Sephadex LH-20. The purification of scutellones A, B, C, D, E, F and scutellaric acid, in addition to eighteen flavonoid constituents, was described in previous reports.^{2,3)}

Scutellone D (3a)—Colorless needles, mp 256–258 °C, $[\alpha]_D^{20} +57.4$ ($c=1.00$, CHCl_3). Anal. Calcd for $\text{C}_{27}\text{H}_{34}\text{O}_7$: C, 68.92; H, 7.28. Found: C, 68.67; H, 7.24. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3460, 3370, 1775, 1740, 1665, 1635, 1600, 1585, 1490. EI-MS m/z : 470 (M^+), 452 ($\text{M}^+ - \text{H}_2\text{O}$), 348 ($\text{M}^+ - \text{PhCOOH}$), 330 (100%, $\text{M}^+ - \text{H}_2\text{O} - \text{PhCOOH}$), 287, 204, 180, 122, and 105. ^1H - and ^{13}C NMR data of **3a** are exactly the same as those reported for scuterivulactone D.⁶⁾

Scutellone E (4)—Colorless needles, mp 230–232 °C, $[\alpha]_D^{20} +72.7$ ($c=1.0$, CHCl_3). Anal. Calcd for $\text{C}_{27}\text{H}_{32}\text{O}_6$: C, 71.66; H, 7.13. Found: C, 71.98; H, 7.10. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3440, 1775, 1735, 1715, 1645, 1590, 1480.

Acetylation of Scutellone D (3a)—A solution of **3a** (22 mg) and acetic anhydride (0.5 ml) in pyridine (0.5 ml) was heated at 60 °C for 2 h. The reaction mixture was treated as usual and gave a monoacetate (**3b**) (mp 244–246 °C).⁶⁾

Partial Hydrogenation of Scutellone D (3a)—Compound **3a** (25 mg) was dissolved in 5 ml of ethyl acetate; 10 mg of 10% Pd-C previously suspended in 5 ml of ethyl acetate was added and the mixture was saturated with H_2 . The absorption of H_2 ceased after 4 h, and the catalyst was removed by filtration and washed several times with ethyl acetate. After purification, the combined filtrate and washings yielded dihydroscutellone D (**5**) (22 mg) [mp 199–200 °C. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3500, 3420, 1775, 1745, 1665, 1630, 1600, 1485, 1275, 1125, 715. ^1H -NMR ($\text{DMSO}-d_6$): 0.79, 1.06, 1.09 and 1.31 (each 3H, s), 2.63 (2H, m), 3.98 (1H, br s), 4.84 (2H, br s), 5.83 (1H, dd, $J=8.4, 4.5$ Hz), 5.87 (1H, br s), 7.51 (2H, t, $J=7.8$ Hz), 7.62 (1H, dt, $J=1.1, 7.8$ Hz), 7.93 (2H, dd, $J=7.8, 1.1$ Hz).

Conversion of Scutellone D (3a) to Scutellone E (4) by Acid—Compound **3a** (30 mg) and *p*-toluenesulfonic acid (10 mg) were dissolved in 5 ml of chloroform and the solution was heated under reflux for 2 h. The reaction mixture was subjected to chromatography on silica gel to afford **4** (14 mg), whose identity was confirmed by comparison with an authentic sample.

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