## Notes

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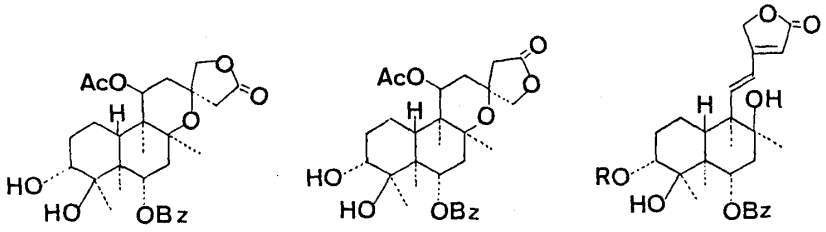
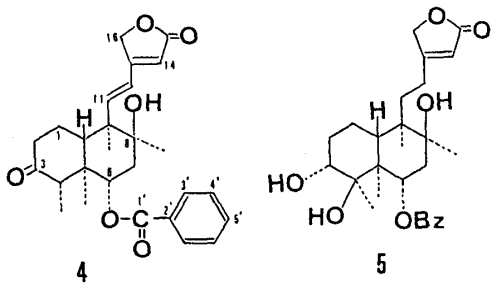
A new neoclerodane-type diterpenoid lactone, scutellone E, together with a known compound, scutellone D (scuterivulactone D), has been isolated from aerial parts of *Scutellaria rivularis* WALL. Their structures have been determined from X-ray diffraction, spectral and chemical evidence.

**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 determined[4] by X-ray analysis. Recently, Tomimori *et al.*[5,6] reported the isolation of five clerodane-type diterpenoids, scuterivulactones A, B, $C_1$, $C_2$ and D, from the same source. The structures of scuterivulactones $C_1$, $C_2$,[5] and D[6] were determined as **1**, **2**, and **3a**, respectively, by means of two dimensional nuclear magnetic resonance (2-D NMR) spectroscopy including INADEQUATE and $^1H$–$^{13}C$ 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 ($CDCl_3$) signals at $\delta$ 2.63 (2H, m, H-12) instead of two olefinic protons, and at $\delta$ 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 °C[6]]. 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 $I2$. Lattice parameters $a=17.147(2)$, $b=9.079(3)$, $c=18.099(2)$ Å; $\beta=102.07(1)°$, $Z=4$. Intensity data were collected up to $2\theta$ of 50° with Mo$K_\alpha$ 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 $C_{27}H_{32}O_6$ 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

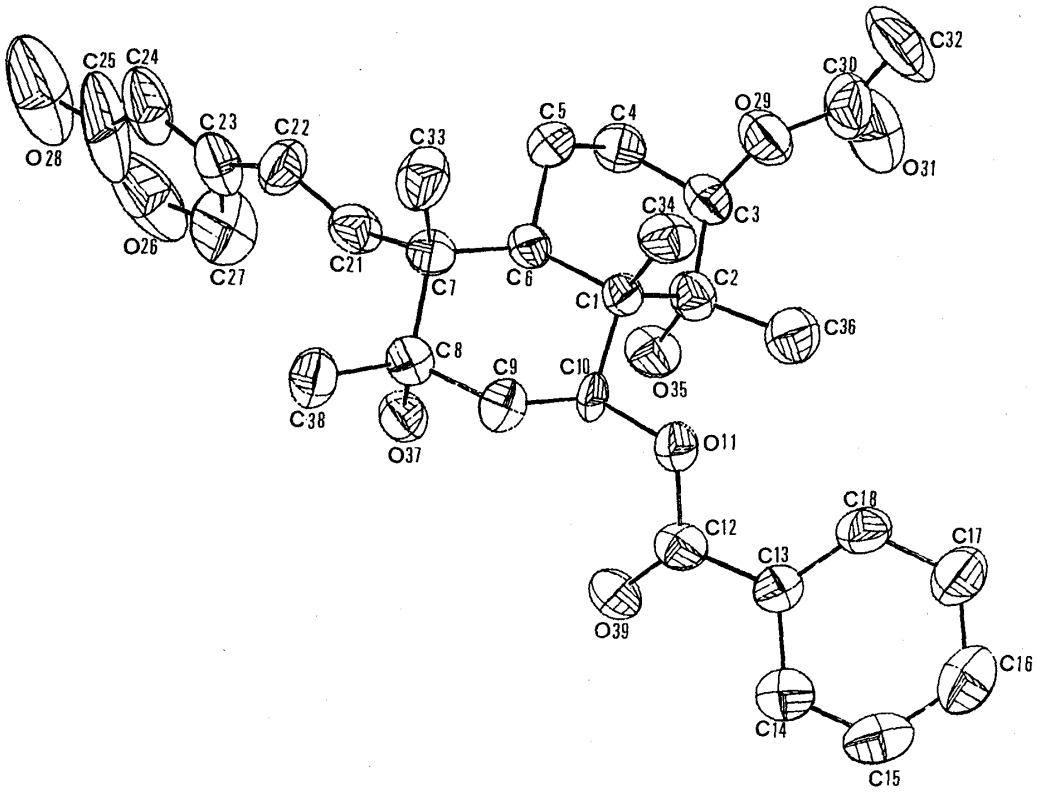



Fig. 1. Molecular Structure and Atomic Numbering of **3b**

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) | | |

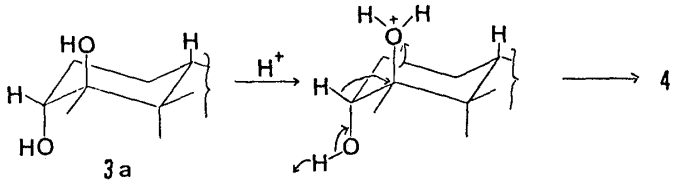
$M^+$ peak at *m/z* 452 and fragment ion peaks at *m/z* 330 ($M^+ - C_6H_5COOH$), 312 ($M^+ - H_2O - C_6H_5COOH$), 204 ($M^+ - H_2O - C_6H_5COOH -$  $-C\equiv C$, 100%), 122

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 |

($C_6H_5COOH$) and 105 ($C_6H_5CO$). The fragment ion peak at *m/z* 204 was also present in **3a** with 67% relative intensity. The $^1$H- and $^{13}$C-NMR spectra were as follows: $^1$H-NMR ($CDCl_3$) $\delta$: 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-$d_6$) $\delta$: 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); $^{13}$C-NMR (by DEPT method) ($CDCl_3$) $\delta$: 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 $CHCl_3$ 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. $^1$H- and $^{13}$C-NMR spectra were run on a Brucker AM 300 at 300 MHz in $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in $\delta$-values and coupling constants ($J$) are given in hertz (Hz). El-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 $C_{27}H_{34}O_7$: C, 68.92; H, 7.28. Found: C, 68.67; H, 7.24. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3460, 3370, 1775, 1740, 1665, 1635, 1600, 1585, 1490. EI-MS $m/z$: 470 ($M^+$), 452 ($M^+ - H_2O$), 348 ($M^+ - PhCOOH$), 330 (100%, $M^+ - H_2O - PhCOOH$), 287, 204, 180, 122, and 105. $^1$H- 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 $C_{27}H_{32}O_6$: C, 71.66; H, 7.13. Found: C, 71.98; H, 7.10. IR $\nu_{max}^{KBr}$ 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 $\nu_{max}^{KBr}$ cm$^{-1}$: 3500, 3420, 1775, 1745, 1665, 1630, 1600, 1485, 1275, 1125, 715. $^1$H-NMR (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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