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Volvalerelactones A and B, Two New Sesquiterpenoid Lactones with an Unprecedented Skeleton from *Valeriana officinalis* var. *latifolia*

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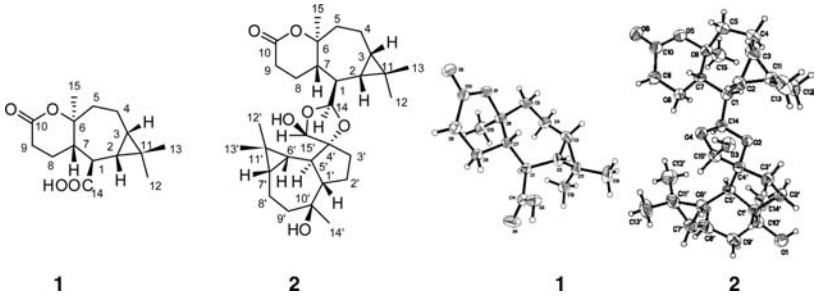
ABSTRACT



Volvalerelactones A and B (1 and 2), two new sesquiterpenoid lactones with an unprecedented 3/7/6 tricyclic ring system, were isolated from the roots of *Valeriana officinalis* var. *latifolia*. Their structures and relative configurations were elucidated by spectroscopic data and single-crystal X-ray diffraction crystallography, and the absolute configuration was assigned by computational methods. The possible biosynthetic pathways of 1 and 2 were also proposed.

The genus *Valeriana* consists of about 200 species and belongs to the family of Valerianaceae which is widely distributed throughout the world.[1] Valerianaceae (Valerianaceae) is a perennial herb native to Europe, Asia, and North America and has been widely used as a mild sedative and sleep aid for centuries.[2] *Valeriana officinalis* is the official species used in Europe and is commonly referred to as valerian, and the rhizomes and roots of this plant exhibit anxiolytic, antidepressant, antispasmodic, sedative, and anti-HIV activities.[3] Previous phytochemical investigations,[4] including our recent work,[5] of *V. officinalis* have resulted in the identification of iridoids, sesquiterpenoids, flavone glycosides, lignans, and alkaloids.

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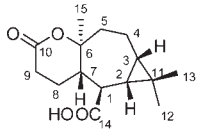
Valeriana officinalis var. *latifolia* has been used as an alternative species for *V. officinalis* in mainland China. Previously, we reported 12 germacrane-type sesquiterpenoids isolated from the roots of this plant.[6] Our further chemical investigation of *V. officinalis* var. *latifolia* resulted in the isolation of two novel sesquiterpenoid lactones, volvalerelactones A and B (**1** and **2**). In this paper, the isolation and structural elucidation of compounds **1** and **2** are described, as well as their AChE inhibitory and neurite outgrowth enhancing activities.



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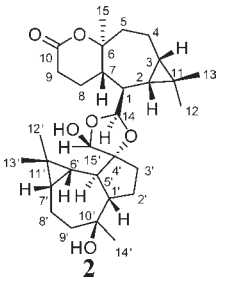

2

The air-dried powder of the roots of *Valeriana officinalis* var. *latifolia* (14 kg) was extracted by 95% EtOH at room temperature. The combined EtOH solution was concentrated under reduced pressure to give the crude extract (3 kg) followed by suspension in water, and then the extract was partitioned successively with $CHCl_3$ (3 × 4 L) and *n*-BuOH (3 × 4 L). The $CHCl_3$ extract (800 g) was first subjected to silica gel column chromatography (CC) eluted with petroleum ether−acetone (from 100:1 to 1:1) to afford fractions A−H. Fraction H (10 g) was then subjected to CC over silica gel (200−300 mesh) eluted with petroleum ether−acetone (from 5:1 to 1:1) to give four fractions Ha−Hd. Fraction Hb was chromatographed over a Sephadex LH-20 column, using $CHCl_3$/MeOH (1:1) as solvent to yield **1** (10 mg) and **2** (15 mg).

Volvalerelactone A (**1**)[7] was isolated as a colorless prism ($CHCl_3$/CH_3OH). Its molecular formula, $C_{15}H_{22}O_4$, with five degrees of unsaturation, was established on the basis of HRESIMS. The IR spectrum indicated the presence of hydroxyl (3424 cm^{-1}) and carbonyl (1726, 1683 cm^{-1}) groups. The ^1H NMR spectra (Table 1) of compound **1** revealed three singlet methyl [δ_H 1.05 (3H, s, H-13), 1.07 (3H, s, H-12), and 1.35 (3H, s, H-15)] signals. The ^{13}C NMR and DEPT spectroscopic data (Table 1) displayed 15 carbon signals in accordance with the molecular formula $C_{15}H_{24}O_4$ obtained from HRESIMS, including three methyl, four methylene, four methine, and two quaternary carbons (one oxygenated) and two carbonyls, indicative of a sesquiterpenoid skeleton. The ^{13}C NMR data of compound **1** also indicated the presence of carboxylic moiety (δ_C 179.4, C-14) and ester carbonyl group (δ_C 171.0, C-10). The signals at δ_C 15.5 (C-12, $-CH_3$), δ_C 28.2 (C-13, $-CH_3$), and δ_C 20.0 (C-11, qC) in the ^{13}C NMR spectrum were characteristic for a dimethylcyclopropane ring,[6,8] which was further confirmed by the HMBC correlations (Figure S4, Supporting Information) from H-12 to C-2 (δ_C 29.5), C-3 (δ_C 27.5), C-11, and C-13 and correlations from H-13 to C-2, C-3, C-11, and C-12. 15-CH_3 was established to be connected to C-6, an oxygenated quaternary carbon, by the HMBC correlations from H-15 to C-5 (δ_C 43.0), C-6 (δ_C 86.7), and C-7 (δ_C 44.4). The correlations from H-1 (δ_H 1.93) and H-2 (δ_H 1.01) to C-14 suggested the −COOH group was linked to C-1 (δ_C 43.2). The ester carbonyl group was assigned to C-10 on the basis of the correlations from H-9 (δ_H 2.57, 2.63) to C-10, C-7, and C-8 (δ_C 21.1). The $^1H-^1H$ COSY cross peaks (Figure S4, Supporting Information) of H-1/H-2, H-2/H-3, H-3/H-4, H-4/H-5, H-1/H-7, H-7/H-8, and H-8/H-9 also supported the above propositions. Apart from two degrees of unsaturation occupied by the carboxylic moiety and ester carbonyl group, the remaining three degrees of unsaturation indicated that **1** should possess tricyclic system, and thus C-10 and C-6 should have formed a lactone moiety.

The relative configurations of the stereogenic centers (C-1, C-2, C-3, C-6 and C-7) of **1** were assigned by a ROESY experiment and comparison of its NMR data with those of the related compounds. H-2 and H-3 were found to be on the same side and possess β-orientation based on the ROESY correlation of H-2/H-3 (Figure S5, Supporting Information) and by comparing the ^{13}C NMR data with those of volvalerenal A-D[6] and isobicyclogermacrenal.[8] H-7 was determined to be β-oriented by the key correlation of H-7/H-2, and the α-orientations of H-1 and 15-CH_3 were established by the correlations (Figure S5, Supporting Information) of H-7/H-9β, H-9α/15-CH_3, and H-1/15-CH_3. The X-ray structure of **1** (Figure 1)[9] verified not only the planar structure but also the relative configuration of **1**.

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(7) Volvalerelactone A (**1**): colorless prisms ($CHCl_3$/MeOH); mp = 196−199 °C, $[\alpha]^{22.4}_D$ −15.45 (*c* 0.098, MeOH); IR (KBr) ν_{max} 3424, 2985, 2925, 2865, 1726, 1683, 1462, 1389, 1314, 1234, 1189, 1107, 1072, 996 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESI-MS *m*/*z* 289 [M + Na]$^+$; HREI-MS *m*/*z* 266.1525 [M]$^+$ (calcd for $C_{15}H_{22}O_4$, 266.1518).

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(9) Crystallographic data of volvalerelactone A (**1**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 819825). Copies of these data can be obtained free of charge via www.ccdc.cam.an.uk/conts/retrieving.html.

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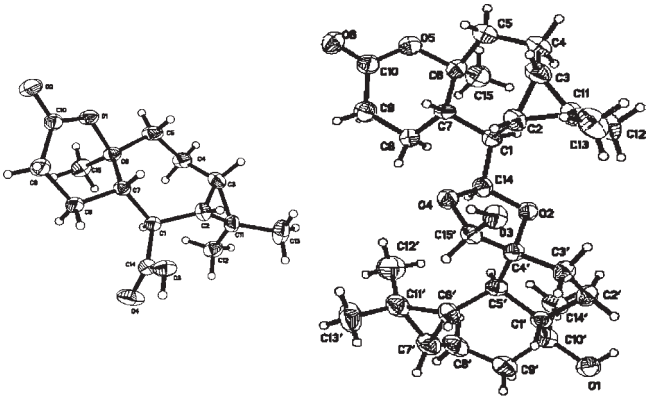
Table 1. ^{1}H and ^{13}C NMR[a] Data of **1** ($CDCl_3$) and **2** (CD_3OD) (δ in ppm, J in Hz)

volvalerelactone A (**1**)			volvalerelactone B (**2**)					
position	δ_C, mult	δ_H (J in Hz)	position	δ_C, mult	δ_H (J in Hz)	position	δ_C, mult	δ_H (J in Hz)
1	43.2, CH	1.93, m	1	39.9, CH	1.33, m	1′	59.4, CH	1.97, m
2	29.5, CH	1.01, m	2	28.2, CH	0.98, m	2′a	33.9, CH_2	1.76, m
3	27.5, CH	0.89, m	3	27.5, CH	0.68, m	2′b		1.85, m
4a	18.8, CH_2	1.94, m	4a	20.4, CH_2	1.90, m, 2H	3′a	25.6, CH_2	1.64, m
4b		1.98, m	4b			3′b		1.75, m
5a	43.0, CH_2	1.85, m,	5a	45.2, CH_2	1.56, m	4′	94.5, qC	
5b		1.99, m,	5b		1.76, m	5′	45.3, CH	1.34, m
6	86.7, qC		6	90.2, qC		6′	31.3, CH	0.52, m
7	44.4, CH	2.13, m	7	46.6, CH	2.00, m	7′	27.3, CH	0.73, m
8a	21.1, CH_2	1.77, m,	8a	21.9, CH_2	2.02, m	8′a	21.1, CH_2	0.94, m
8b		1.88, m,	8b		2.17, m	8′b		1.84, m
9a	28.4, CH_2	2.57, m, (α-H)	9a	30.0, CH_2	2.50, m	9′a	45.1, CH_2	1.83, m
9b		2.63, m, (β-H)	9b		2.59, m	9′b		1.94, m
10	171.0, qC		10	174.4, qC		10′	75.3, qC	
11	20.0, qC		11	20.5, qC		11′	21.3, qC	
12	15.5, CH_3	1.07, s	12	17.1, CH_3	1.00, s	12′	16.3, CH_3	1.08, s
13	28.2, CH_3	1.05, s	13	29.0, CH_3	1.05, s	13′	29.1, CH_3	1.07, s
14	179.4, qC		14	102.9, CH	5.09, d (2.9)	14′	20.0, CH_3	1.14, s
15	20.3, CH_3	1.35, s	15	20.9, CH_3	1.36, s	15′	97.1, CH	5.49, s

[a] ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and multiplicities inferred from DEPT and HSQC experiments.



1 **2**

Figure 1. ORTEP view of **1** and **2**.

Thus, the structure of **1** was established as shown, named volvalerelactone A (**1**).

To assign the absolute configuration, the optical rotation (OR) value of **1** was calculated by density functional theory (DFT) methods[5a,10] in the Gaussian 03 program package.[11] The "self-consistent reaction field" method (SCRF) was employed to perform the OR calculation of the most stable conformer of **1** at the B3LYP/6-311G ++ (d, p)//B3LYP/6-311G (d, p) level. The calculated OR value (−11.03) for **1** is close to its experimental values (−15.45) (for more details, see the Supporting Information), indicating that the absolute configuration of volvalerelactone A (**1**) was elucidated as shown in Figure 1, with a configuration of 1*R*,2*S*,3*S*,6*S*,7*S*.

Volvalerelactone B (**2**),[12] colorless crystal (MeOH), possessed a molecular formula of $C_{30}H_{46}O_6$ as established by the HRESIMS at m/z 525.3192 $[M + Na]^+$, indicating eight degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl (3440 cm^{-1}) and carbonyl (1638 cm^{-1}) groups. The ^{13}C and DEPT NMR spectra of compound **2** revealed 30 carbon resonances attributed to six methyl, eight methylene, 10 methine, and six quaternary carbons. Among them, one ester carbonyl and five oxygen-bearing carbons (two acetal methine) were detected. Compound **2** appeared to be a dimeric sesquiterpenoid because its characteristic pair of signals with close chemical shifts in the ^{1}H and ^{13}C NMR spectra (Table 1) were reminiscent of the presence of two sesquiterpenoid moieties, referred to as parts **a** and **b** in the following discussion. Careful analysis of the ^{13}C NMR data of **2** indicated that 15 carbon signals (part **a**) were highly similar to **1** except that the carboxyl group (C-14) in **1** was replaced by a ketal methine (δ_C 102.9) in **2**, which was established from the HMBC correlations (Figure 1) from H-14 (δ_H 5.09) to C-1 (δ_C 39.9) and C-2 (δ_C 28.2) and the $^{1}H-^{1}H$ COSY correlation of H-1/H-14. The remaining NMR data (part **b**) were characterized for an aromadendrane sesquiterpenoid and similar to those of (+)-3α, 9β-aromadendranediol,[13] which was supported by the

(11) Frisch, M. J. *Gaussian 03*, revision E. 01; Gaussian, Inc.: Wallingford, CT, 2004 (see the Supporting Information for the complete reference).

(12) Volvalerelactone B (**2**): mp = 260−263 °C; colorless crystal (MeOH); $[\alpha]^{21.6}_D$ −30.39(*c* 0.102, MeOH); IR (KBr) ν_{max} 3440, 2926, 2859, 1638, 1461, 1383, 1104, 1076 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EI-MS m/z 502 $[M]^+$; HRESI-MS m/z 525.3192 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_6Na$, 525.3192).

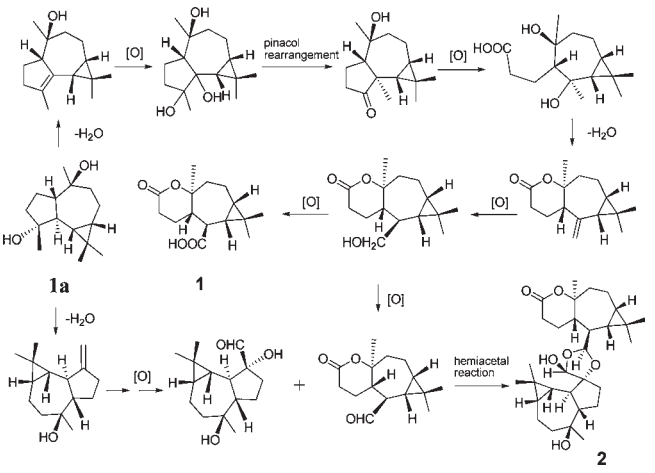
(13) Sun, Z. H.; Zhang, S.; Shi, J.; Hu, C. Q. *Chinese J. Org. Chem.* **2004**, *24*, 806–810.

HMBC correlations (Figure S4, Supporting Information) from H-12′ (δ_H 1.08) and H-13′ (δ_H 1.07) to C-6′ (δ_C 31.3), C-7′ (δ_C 27.3), C-11′ (δ_C 21.3); H-14′ (δ_H 1.14) to C-1′ (δ_C 59.4), C-9′ (δ_C 45.1), and C-10′ (δ_C 75.3); and H-15′ (δ_H 5.49) to C-4′ (δ_C 94.5) and C-5′ (δ_C 45.3). The $^1H-^1H$ COSY correlations (Figure S4, Supporting Information) of H-1′/H-2′, H-2′/H-3′, H-1′/H-5′, H-5′/H-6′, H-6′/H-7′, H-7′/H-8′ and H-8′/H-9′ also supported this proposition. The two sesquiterpenoid units (parts **a** and **b**) were connected via a five-membered ring containing two oxygen atoms, which was determined by the key HMBC correlation from H-15′ to C-14 (δ_C 102.9) combined with the molecular formula and one residual degree of unsaturation.

The relative configuration of **2** was assigned by a ROESY experiment and X-ray diffraction.[14] The β-orientations of H-2, H-3, H-6′, H-7′, 13-CH_3, and 13′-CH_3 and α-orientations of 12-CH_3 and 12′-CH_3 were assigned by comparing the ^{13}C NMR data with those of the structurally related compounds.[6,8] H-7 and H-1′ were determined to be β-oriented, and H-1, H-14, 15-CH_3, and H-5′ were established to be α-oriented by the ROESY correlations (Figure S5, Supporting Information) of H-7/H-2, H-1′/H-6′, H-1/12-CH_3, H-1/H-14, 15-CH_3/12-CH_3, and H-5′/12′-CH_3. In addition, the key ROE between H-14/H-15′ indicated the α-orientation of the H-15′ in the five-membered ring as shown in Figure 1. The X-ray diffraction study was carried out to establish the relative configurations of C-4′ and C-10′ and the final structure of **2**. Biogenetically, the absolute configuration of part **a** in compound **2** was the same as those of compound **1**, with the configurations of 1*R*,2*S*,3*S*,6*S*,7*S*. 14*R* was also determined on the basis of its X-ray structure. Other stereogenic centers in part **b** were accordingly assigned to be 1′*S*,4′*R*,5′*S*,6′*S*,7′*S*,10′*S*,15′*S*. Thus, the absolute configuration of volvalerelactone B (**2**) was elucidated as shown in Figure 1, named volvalerelactone B, and an ORTEP drawing is shown in Figure 1.

Plausible biogenetic pathways of **1** and **2** were proposed as shown in Scheme 1. The new skeleton of sesquiterpenoid lactones might be derived from the natural armadendrane-type sesquiterpenoid (+)-3α,9β-aromadendranediol.[13] The key steps of this biogenetic pathway to the formation of **1** include the methyl migration and several oxidation steps. The formation of the dimer **2** involves the hemiacetal reaction of two aldehyde groups, creating a five-membered ring system possessing an acetal and a hemiacetal functionalities. Compound **1** is a new type of sesquiterpenoid lactone with an unprecedented 3/7/6 tricyclic ring system, and compound **2** represents an unprecedented carbon skeleton because of the unique five-member ring system connecting the two sesquiterpenoid units.

Scheme 1. Possible Biogenetic Pathways to **1** and **2** from 3α,9β-Aromadendranediol (**1a**)



The acetylcholine esterase (AChE) inhibitory activity of **1** and **2** was assayed using the Ellman method.[15] Compounds **1** and **2** showed no inhibitory activity at the concentration of 100 μM. Tacrine (0.33 μM) was used as the positive control and showed 48.9% inhibition. Compounds **1** and **2** were also evaluated for the enhancing activity on nerve growth factor (NGF)-mediated neurite outgrowth in PC12 cells.[16] The result indicated that the proportion of the NGF (5 ng/mL)-induced neurite-bearing cells was not enhanced by compounds **1** and **2** at 50 μM, respectively.

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Supporting Information Available. Experiment procedures, crystallographic data, key 2D correlations of **1** and **2**, optical rotation calculations, 1D and 2D NMR spectra, mass spectra, IR, $[\alpha]^D$, and X-ray crystallographic data (CIF) of **1** and **2**. This material is available free of charge via Internet at http://pubs.acs.org.

(14) Crystallographic data of volvalerelactone B (**2**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 819824). Copies of these data can be obtained free of charge via www.ccdc.cam.an.uk/conts/retrieving.html.

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