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Volvalerenone A, a new type of mononorsesquiterpenoid with an unprecedented 3,12-oxo bridge from *Valeriana officinalis*

Peng-Cheng Wang [a,c], Xin-Hui Ran [a], Rui Chen [a], Liang-Chun Li [d], Shan-Shan Xiong [e], Yu-Qing Liu [a], Huai-Rong Luo [a], Jun Zhou [a,*], You-Xing Zhao [a,b,*]

[a] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, PR China*
[b] *Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agriculture Sciences, Haikou 571101, PR China*
[c] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China*
[d] *Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China*
[e] *Department of Chemistry, Yunnan University, Kunming 650091, PR China*

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ABSTRACT

Volvalerenone A (**1**), a new type of mononorsesquiterpenoid with an unprecedented 5/6/6 tricyclic ring system, was isolated from the roots of *Valeriana officinalis*, the official species of valerian used in Europe. The structure of volvalerenone A was elucidated based on its spectroscopic and single-crystal X-ray crystallography data. The absolute configuration was assigned by the computational method. A possible biosynthetic pathway of volvalerenone A was also proposed. Preliminary biological studies showed that volvalerenone A had weak inhibitory activity on acetylcholine esterase (AChE), and no enhancing activity on nerve growth factor (NGF)-mediated neurite outgrowth in PC12 cells was observed at 50 μM.

Valerian is the most commonly used herb medicine which acts as a mild sedative and tranquilizer in Europe, Asia, and North America. There are at least 25 valerian-containing products in the United Kingdom and over 400 in Germany.[1] In fact, nearly all sleep-aid herb dietary supplements contain valerian.[2] The genus *Valeriana* consists of about 200 species and belongs to the family of Valerianacae which is widely distributed throughout the world.[3] *Valeriana officinalis* is the official species used in Europe and is commonly referred to as valerian. From the chemical point of view, there are two main groups of compounds, volatile oil fraction (containing monoterpenes and sesquiterpenoids) and valepotriates (represented by valtrate and isovaltrate) isolated from *V. officinalis*.[1,4] The valerane-type sesquiterpenoid (valerenic acid, acetoxyvalerenic acid, and valerenal) has been reported to be present only in *V. officinalis*, allowing its distinction from the other species of this genus.[5] *V. officinalis* shows a lot of pharmacological properties including sedative, anxiolytic, antidepressant, antispasmodic, and anti-HIV activities,[1,6] and it is still an object of research aimed at establishing the chemical and pharmacological basis of these activies.[7] We previously reported a series of valerane-type sesquiterpenoids and iridoids from *V. officinalis*.[8] Our continuous phytochemical investigation of this plant has led to the isolation of a new type of mononorsesquiterpenoid, volvalerenone A (**1**) (Fig. 1A). This Letter reports the isolation and structural elucidation, as well as the AChE inhibitory and neurite outgrowth-enhancing activities of volvalerenone A (**1**).

The air-dried powder of the roots of *V. officinalis* (5 kg) was extracted by 95% EtOH (3 × 5 L) at room temperature. The combined EtOH solution was concentrated under reduced pressure to give the crude extract (1 kg) and followed by suspension in water, then partitioned successively with petroleum ether (3 × 2 L), EtOAc (3 × 2 L), and *n*-BuOH (3 × 2 L). The EtOAc extract (80 g) was first subjected to silica gel column chromatography and eluted with petroleum ether–acetone (from 100:1 to 1:1) to afford fractions A–H. Fraction B (10 g) was then subjected to CC over silica gel (200–300 mesh) and eluted with petroleum ether–EtOAc (from 50:1 to 1:1) to give four fractions Ba–Bd. Fraction Bb was chromatographed over a Sephadex LH-20 column, using $CHCl_3$/MeOH (1:1) as solvent to yield **1** (10 mg).

Volvalerenone (**1**)[9] was isolated as a colorless prism ($CHCl_3$/MeOH). High resolution ESI-MS (HRESIMS) analysis of **1** gave an $[M+Na]^+$ ion peak at *m/z* 275.1259 consistent with a molecular formula of $C_{14}H_{20}O_4$ (calcd for $C_{14}H_{20}O_4Na$, 275.1259), with five degrees of unsaturation. The IR spectrum indicated the presence of hydroxy groups (3491 cm^{-1}), and an α,β-unsaturated ketonic group (1681, 1636 cm^{-1}). The ^1H NMR spectrum (Table 1) of

* Corresponding authors. Tel.: +86 871 5223264; fax: +86 871 5223261 (J.Z.).
E-mail addresses: jzhou@mail.kib.ac.cn (J. Zhou), yxzhao@mail.kib.ac.cn (Y.-X. Zhao).

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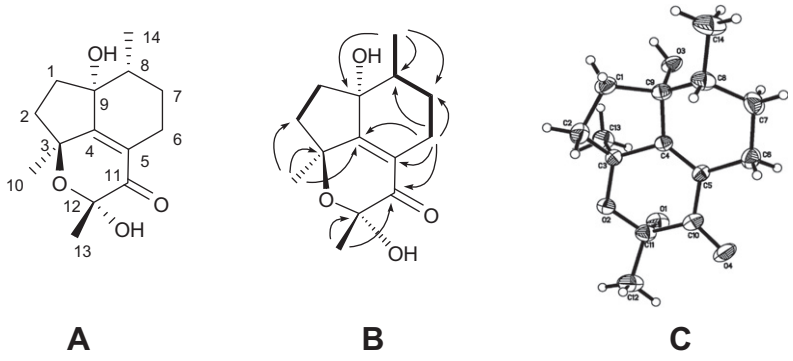


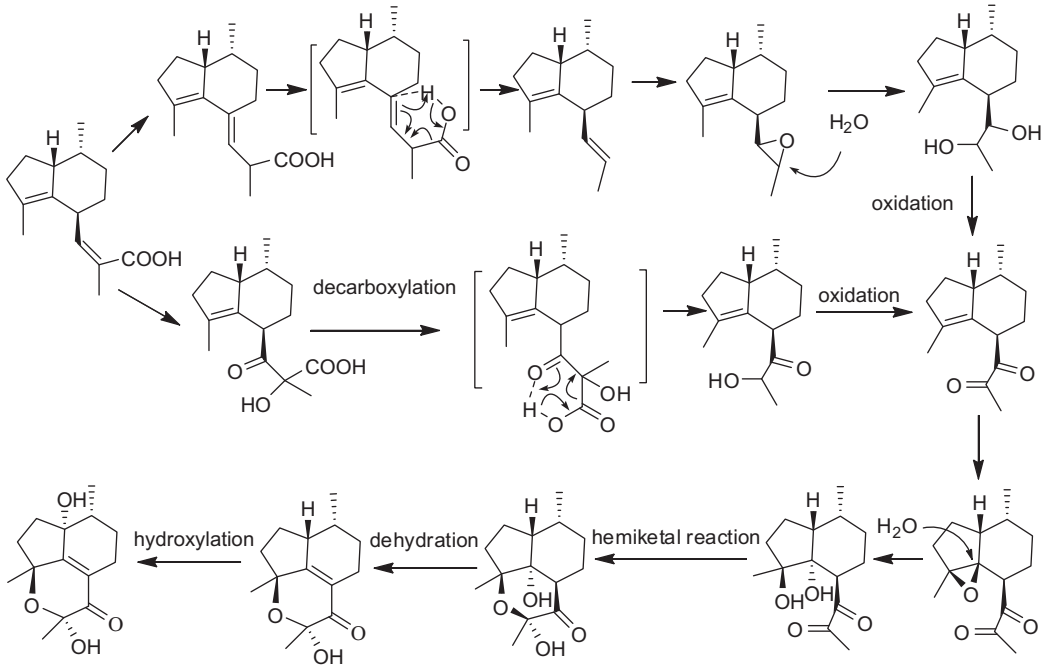
Figure 1. (A) The structure of compound **1**, (B) THE key HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **1**, (C) the ORTEP view of **1**.

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

Position	δ_H (J in Hz)	δ_C, mult
1	1.93 (2H, m)	36.3 t
2a	1.68 (1H, m)	39.5 t
2b	2.06 (1H, m)	
3		80.7 s
4		166.2 s
5		125.9 s
6a	2.10 (1H, m)	22.5 t
6b	2.35 (1H, m)	
7	1.60 (2H, m)	26.2 t
8	1.33 (1H, m)	39.6 d
9		76.2 s
10	1.78 (3H, s)	28.9 q
11		195.4 s
12		97.0 s
13	1.42 (3H, s)	26.0 q
14	1.02 (3H, d, 6.7)	15.0 q

[a] ^{1}H NMR at 500 MHz, ^{13}C NMR at 100 MHz, and multiplicities inferred from DEPT and HSQC experiments.

compound **1** revealed two singlet methyl (δ_H 1.42, 1.78) and a doublet methyl (δ_H 1.02, d, J = 6.7 Hz) signals. The ^{13}C NMR and DEPT spectra (Table 1) displayed a total of 14 carbon signals, which was in accordance with the molecular formula of $C_{14}H_{20}O_4$. More specifically, three methyl, four methylene, one methine, and three oxygenated quaternary carbons, two olefinic carbons, and one carbonyl carbon were observed, indicative of a mononorsesquiterpenoid skeleton. In the ^{13}C NMR spectrum, the carbon signals at δ_C 195.4 (C-11), 166.2 (C-4), and 125.9 (C-5) were assigned to the α,β-unsaturated ketone group, and the quaternary carbon signal at δ_C 97.0 was proposed to be assigned to C-12 bonded to two oxygen atoms. In the ^{1}H–^{1}H COSY spectrum, the cross signals between H-6/H-7, H-7/H-8, and H-8/H-14 indicated the moiety showed in bold bands in Figure 1B. In the HMBC spectrum (Fig. 1B), the correlations from H-14 (δ_H 1.02) to C-7 (δ_C 26.2), C-8 (δ_C 39.6), and C-9 (δ_C 76.2) suggested that the 14-CH_3 was connected to C-8, and C-9 was oxygenated. The HMBC correlations from H-10 (δ_H 1.78) to C-2 (δ_C 39.5), C-3 (δ_C 80.7), and C-4 indicated that the 10-CH_3 was attached to C-3, which was an oxygenated quaternary



Scheme 1. Possible biogenetic pathway to **1** from valerenic acid.

carbon. The 13-CH_3 was determined to be linked to C-12 as supported by the HMBC correlations from H-13 (δ_H 1.42) to C-12 and C-11. The linkage between C-6 and C-5 was established by the HMBC correlations from H-6 (δ_H 2.10, 2.35) to C-4, C-5, and C-11. The above HMBC correlations and ^1H–^1H COSY cross signals established the main connections of the carbons in compound **1**. Apart from two degrees of unsaturation occupied by the double bond and the ketonic group, the remaining three degrees of unsaturation indicated that **1** should possess a tricyclic system while the usual valerane-type sesquiterpenoids only contained two rings.[1,4c,4f] In addition, there were only three oxygen atoms besides the ketonic carbonyl oxygen; therefore, compound **1** must contain an oxobridge between C-3 and C-12 or C-9, which formed another ring.

It is impossible to determine the location of the oxobridge and the relative configurations of all stereogenic centers in **1** based on NMR spectroscopic data alone. Thus we turned our attention to molecular modeling and X-ray crystallographic analysis. The oxobridge was established to be between C-3 and C-12 forming a six ring system by the X-ray crystallographic analysis (Fig. 1C).[10] The X-ray crystallography data also establish the relative configurations at C-3, C-8, C-9, and C-12 of volvalerenone (**1**). Therefore, the structure of **1** was established as shown in Figure 1A and the compound was named as volvalerenone A (**1**).

To assign the absolute configuration, the optical rotation (OR) value of **1** was calculated by density functional theory (DFT) methods[11] in the GAUSSIAN 03 program package.[12] The X-ray crystallography data were used to act as the initial-optimized structure, and the Self-Consistent Reaction Field (SCRF) model was employed to perform the OR calculation using the B3LYP/6-311++G (d,p) method in MeOH. The calculated OR value (+24.5) for **1** is close to its experimental value (+13.9) (for more details see Supplementary data), which indicated the absolute configuration of volvalerenone (**1**) as shown in Figure 1A. The absolute configurations at C-3, C-8, C-9, and C-12 were assigned to be (3*S*), (8*R*), (9*R*), and (12*R*), respectively. Furthermore, the absolute configuration of (8*R*) is consistent with that of valerenic acid, the structurally related valerane-type sesquiterpenoid, whose configuration was determined by the total synthesis.[13]

Biogenetically, **1** might be derived from valerenic acid, which was the main constituent in *V. officinalis* and was recorded as the standard component in the United States Pharmacopeia.[14] A plausible biogenetic pathway of **1** was proposed as shown in Scheme 1.

The acetylcholine esterase (AChE) inhibitory activity of volvalerenone A (**1**) was assayed using the Ellman method.[15] At the concentration of 100 μM, **1** inhibited acetylcholine esterase activity by 11.8%. As the positive control, tacrine showed an inhibition rate of 48.9% at 0.33 μM. Compound **1** was 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 compound **1** at 50 μM.

Acknowledgments

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Supplementary data

Experiment procedures, optical rotation calculation, 1D and 2D NMR spectra, mass spectra, and X-ray crystallographic data in CIF format of volvalerenone (**1**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.08.023.

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9. Volvalerenone (**1**): colorless prisms ($CHCl_3$/MeOH); $[\alpha]_D^{18.2}$ +13.93 (*c* 0.223, MeOH); UV (MeOH) λ_{max} (log ε): 241 (3.98); IR (KBr) ν_{max} 3491, 2961, 2927, 2856, 1681, 1636, 1460, 1369, 1188, 1118 cm^{-1}; ESI-MS *m*/*z* 275 $[M+Na]^+$; HRESI-MS *m*/*z* 275.1259 (calcd for $[M+Na]^+$ 275.1259).
10. Crystallographic data of compound **1**: $C_{14}H_{20}O_4$, MW = 252.30; monoclinic, space group *P*2(1); *a* = 8.2702 (16), *b* = 8.0789 (16), *c* = 10.279 (2) Å, α = 90.00, β = 97.024 (3), γ = 90.00, *V* = 681.7 (2) Å^3, *Z* = 2, *d* = 1.229 g/cm^3, crystal dimensions 0.19 × 0.16 × 0.10 mm were used for measurement on a SHELXL-97 with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 4463, of which 3060 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0555, wR_2 = 0.1357. The crystal structure of compound **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using the difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997), and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 776982). These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.
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