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Volvalerenol A, a New Triterpenoid with a 12-Membered Ring from *Valeriana hardwickii*

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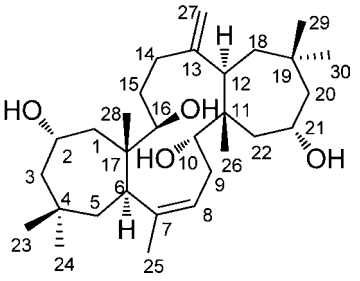
ABSTRACT



Volvalerenol A (1), an unprecedented type of triterpenoid with a 7/12/7 tricyclic ring system, was obtained from the ethanol extracts of the roots of *Valeriana hardwickii*. The structure and relative configurations were established by comprehensive analysis of MS and NMR spectroscopic data. The possible biogenetic pathway of 1 was also deduced.

Triterpenoids are a group of secondary metabolites containing a large number of compounds, and interest in the biological activity of triterpenoids and triterpenoid saponins is continuously increasing.[1] The genus *Valeriana* (Valerianaceae) contains about 200 species and has a wide distribution all over the world.[2] *Valeriana officinalis*, commonly denoted valerian, has been used as a perennial herb by local people in Europe, Asia, and North America. *V. officinalis* is the official species and has been used as a mild sedative and sleep aid for centuries.[2] Previous phytochemical studies revealed that sesquiterpenoids and iridoids were the characteristic constituents in the plants of this genus,[3] and the pharmacological investigation of the extracts and compounds from these plants were proposed to possess various pharmacological properties, including sedative, anxiolytic, and antidepressant. The iridoids from Valerianae Radix was reported to have anti-HIV activities.[4]

V. hardwickii Wall is a medical herb that is widely distributed in south and southwest China, and it is used to promote blood circulation, regulate menstruation, and

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(1) (a) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2010**, *27*, 79–132. (b) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2011**, *28*, 1087–1117.

(2) (a) Houghton, P. J. *J. Ethnopharmacol.* **1988**, *22*, 121–142. (b) Houghton, P. J. *J. Pharm. Pharmacol.* **1999**, *51*, 505–512. (c) Yager, J.; Siegfreid, S. L.; DiMattero, T. L. *Am. J. Psychiat.* **1999**, *156*, 1432–1438.

(3) (a) WHO monographs on selected medicinal plants: *Radix valerianae*; WHO; Geneva, Switzerland, 1999; Vol. 1, pp 267– 76. (b) Tang, Y. P.; Liu, X.; Yu, B. *J. Nat. Prod.* **2002**, *6*, 1949–1952. (c) Bos, R.; Hendriks, H.; Bruins, A. P.; Kloosterman, J.; Sipma, G. *Phytochemistry* **1985**, *25*, 133–135. (d) Ming, D. S.; Yu, D. Q.; Yang, Y. Y.; He, C. M. *Tetrahedron Lett.* **1997**, *38*, 5205–5208. (e) Lin, S.; Shen, Y. H.; Li, H. L.; Yang, X. W.; Chen, T.; Lu, L. H.; Huang, Z. S.; Liu, R. H.; Xu, X. K.; Zhang, W. D.; Wang, H. *J. Nat. Prod.* **2009**, *72*, 650–655. (f) Yu, L. L.; Han, C. R.; Huang, R.; Lv, Y. P.; Gui, S H.; Chen, Y. G. *Pharmazie* **2006**, *61*, 486–488. (g) Wang, R.; Xiao, D.; Bian, Y. H.; Zhang, X. Y.; Li, B. J.; Ding, L. S.; Peng, S. L. *J. Nat. Prod.* **2008**, *71*, 1254–1257.

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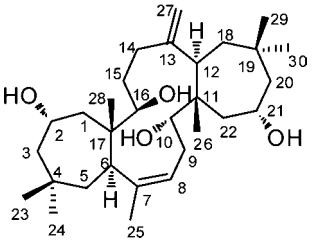
invigorate the spleen in Chinese folk medicine.[5] During our continuous investigation of the sedative and antidepressant activities secondary metabolites from the genus *Valeriana*, the chemical constituents investigation of the root of *V. officinalis* and *V. officinalis var. latifolia* have resulted in the discovery of some sesquiterpenoid monomers and iridoids in previously studies,[6] including a new type of bisesquiterpenoid.[7] In our search for bioactive constituents from *V. hardwickii*, a new type of triterpenoid, volvalerenol A (**1**), with a 12-membered macro-ring and a unique 7/12/7 tricylic ring system, was discovered. In this paper, the structural elucidation of compound **1**, representing the first example of a new class of triterpenoid, was reported.

The roots of *V. hardwickii* (8.0 kg) were powdered and extracted by 95% EtOH at room temperature three times (3 × 20 L), once per week. The EtOH solution was collected together and evaporated under reduced pressure to give a residue (1.5 kg), suspended in water, and partitioned by $CHCl_3$ (3 × 5 L) and *n*-BuOH (3 × 5 L). The $CHCl_3$ residue (215 g) was chromatographed over silica gel eluted with petroleum ether−acetone gradients (from 100:1 to 1:1) to afford four fractions (A−D), based on their TLC characteristics. Fraction C (15 g) was further separated by silica gel column eluted with $CHCl_3$−CH_3OH gradients (from 50:1 to 1:1) to afford five fractions (Ca−Ce). Fraction Cd was purified repeatedly by Sephadex LH-20 gel column ($CHCl_3$−MeOH, 1:1) to yield **1** (5 mg).



Volvalerenol A (**1**)[8] was isolated as a white amorphous solid (CH_3OH). Its molecular formula was assigned as $C_{30}H_{52}O_4$ on the basis of HRESIMS (*m*/*z* 499.3772 $[M + Na]^+$, calcd for $C_{30}H_{52}O_4Na$, 499.3763), with 5 degrees of unsaturation. The IR spectrum displayed the presence of hydroxyl (3433 cm^{-1}) and double bond (1630 cm^{-1}) groups. The ^1H NMR spectra (Table 1) of compound **1** exhibited signals of seven single methyl [δ_H 0.94 (3H, s, H-23), 0.88 (3H, s, H-24), 1.56 (3H, s, H-25), 0.78 (3H, s, H-26), 0.71 (3H, s, H-28), 0.95 (3H, s, H-29), 0.83 (3H, s, H-30)], one sp^2 methine proton (δ_H 5.26, d, 4.7, H-8), and two terminal olefinic protons (δ_H 4.78, s; 4.56, s, H-27). The ^{13}C NMR and DEPT spectroscopic data (Table 1) showed 30 carbon resonances in accordance with the molecular formula $C_{30}H_{52}O_4$ deduced from HRESIMS, including seven methyl, 10 methylene (one sp^2 methylene), seven methine (one sp^2 methine and four oxygenated ones), and six quaternary carbons (two sp^2 quaternary carbons), suggesting a triterpenoid skeleton.

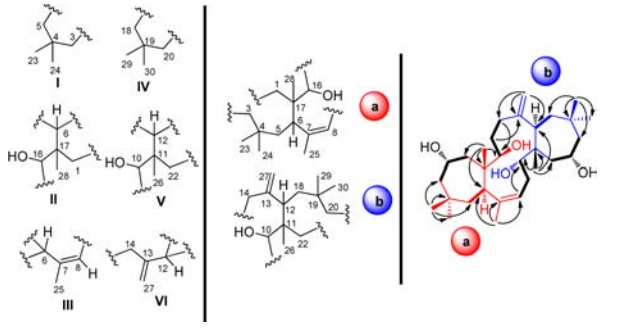


Figure 1. Key HMBC (→) and ^1H−^1H COSY (—) correlations of **1**.

The total ^1H and ^{13}C NMR spectroscopic data of **1** were assigned by a combined analysis of 1D and 2D NMR spectrum including HSQC, HMBC, and ^1H−^1H COSY spectra. Extensive analyses of the 1D and 2D NMR spectra resulted in the elucidation of six fragments **I**−**VI** (Figure 1), the connectivity and relative stereochemistry of compound **1** were also established. In the HMBC spectrum (Figure 1), the correlations from 23-CH_3 and 24-CH_3 to C-3 (δ_C 50.9), C-4 (δ_C 30.8), and C-5 (δ_C 35.6), from 29-CH_3 and 30-CH_3 to C-18 (δ_C 38.2), C-19 (δ_C 30.8), and C-20 (δ_C 51.4) suggested the presence of two gem-dimethyl moieties in the molecule (fragments **I** and **IV**). The partial structures of **II** and **V** were established by the HMBC correlations from 28-CH_3 to C-1 (δ_C 53.7), C-6 (δ_C 43.3), C-16 (δ_C 80.5), and C-17 (δ_C 42.5) and the correlations from 26-CH_3 to C-10 (δ_C 76.9), C-11 (δ_C 39.7), C-12 (δ_C 43.1), and C-22 (δ_C 51.7), respectively. Fragments **III** and **IV** were identified on the basis of the HMBC correlations from 25-CH_3 to C-6, C-7 (δ_C 136.1), and C-8 (δ_C 120.3) and the correlations from H-27 to C-12, C-13 (δ_C 150.4), and C-14 (δ_C 35.6). The connections of fragments **I**, **II**, and **III** were assigned by the key HMBC correlations from both

(4) (a) Leathwood, P. D.; Chauffard, F.; Heck, E.; Munoz-Box, R. *Pharmacol., Biochem. Behav.* **1982**, *17*, 65–71. (b) Morazzoni, P.; Bombardelli, E. *Fitoterapia* **1995**, *66*, 99–112. (c) Miguel, H.; Feistelb; Hartwig, S.; Romanus, L.; Mirjam, H.; Hilke, W. *Phytomedicine* **2008**, *15*, 2–15. (d) Hazelhoff, B.; Smith, D.; Malingre, T. M. *Arch. Int. Pharmacodyn* **1982**, *257*, 274–287. (e) Murakami, N.; Ye, Y.; Kawanishi, M.; Aoki, S.; Kudo, N.; Yoshida, M.; Nakayama, E.; Shiodac, T.; Kobayashia, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2807–2810.

(5) *Chinese Materia Medica (Zhonghua Benchao)*; Editorial Committee of Chinese Materia Medica; Shanghai Science and Technology: Shanghai, 1999; Vol. 7, pp 6625–6626.

(6) (a) Wang, P. C.; Ran, X. H.; Chen, R.; Li, L. C.; Xiong, S. S.; Liu, Y. Q.; Luo, H. R.; Zhou, J.; Zhao, Y. X. *Tetrahedron Lett.* **2010**, *51*, 5451–5453. (b) Wang, P. C.; Hu, J. M.; Ran, X. H.; Cheng, Z. Q.; Jiang, H. Z.; Liu, Y. Q.; Zhou, J.; Zhao, Y. X. *J. Nat. Prod.* **2009**, *72*, 1682–1685. (c) Wang, P. C.; Ran, X. H.; Chen, R.; Luo, H. R.; Liu, Y. Q.; Zhou, J.; Zhao, Y. X. *J. Nat. Prod.* **2010**, *73*, 1563–1567. (d) Wang, P. C.; Ran, X. H.; Chen, R.; Luo, H. R.; Ma, Q. Y.; Liu, Y. Q.; Hu, J. M.; Huang, S. Z.; Jiang, H. Z.; Chen, Z. Q.; Zhou, J.; Zhao, Y. X. *Chem. Biodivers* **2011**, *8*, 1908–1913. (e) Zhao, Y. X.; Wang, P. C.; Ran, X. H.; Ma, Q. Y.; Liu, Y. Q.; Zhou, J. *J. Chin. Chem. Soc.* **2011**, *58*, 659–662.

(7) Wang, P. C.; Ran, X. H.; Luo, H. R.; Hu, J. M.; Chen, R.; Ma, Q. Y.; Dai, H. F.; Liu, Y. Q.; Xie, M. J.; Zhou, J.; Zhao, Y. X. *Org. Lett.* **2011**, *13*, 3036–3039.

(8) Volvalerenol A (**1**): white amorphous powder; $[\alpha]^{17.7}_D$ = +1.92 (*c* 0.23, CH_3OH); CD (0.00101 M, MeOH) λ_{nm} ($\Delta\varepsilon$) 213 (+ 3.71), 250 (− 1.24); UV (CH_3OH) λ_{max} (log ε) 202 (3.69); IR (KBr) ν_{max} 3433, 2951, 2928, 2864, 1630, 1461, 1385, 1272, 1157, 1072, 1036, 583 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; (+)-ESI-MS *m*/*z* 499 $[M + Na]^+$; HRESIMS *m*/*z* 499.3772 $[M + Na]^+$ (calcd for $C_{30}H_{52}O_4Na$, 499.3763).

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

position	δ_C, mult	δ_H (J in Hz)	position	δ_C, mult	δ_H (J in Hz)
1	53.7, CH_2	2.16, dt, 13.0, 2.4 (β-H)	16	80.5, CH	3.37, dd, 11.8, 4.4
		1.32, m (α-H)	17	42.5, qC	
2	66.1, CH	3.70, t, 10.6	18	38.2, CH_2	1.35, m (a-H)
3	50.9, CH_2	1.52, 2H, m			1.32, m (b-H)
4	30.8, qC		19	30.8, qC	
5	35.6, CH_2	1.30, 2H, m	20	51.4, CH_2	1.60, 2H, m
6	43.3, CH	1.94, m	21	66.5, CH	3.78, t, 10.2
7	136.1, qC		22	51.7, CH_2	2.06, m (β-H)
8	120.3, CH	5.26, d, 4.7			1.17, m (α-H)
9	31.2, CH_2	2.07, m (a-H)	23	31.5, CH_3	0.94, s
		1.91, m (b-H)	24	30.4, CH_3	0.88, s
10	76.9, CH	3.45, m	25	22.7, CH_3	1.56, s
11	39.7, qC		26	12.8, CH_3	0.78, s
12	43.1, CH	1.88, d, 9.5	27	109.0, CH_2	4.78, s (a-H)
13	150.4, qC				4.56, s (b-H)
14	35.6, CH_2	2.25, ddd, 13.0, 4.2, 3.0 (a-H)	28	12.2, CH_3	0.71, s
		2.01, m (b-H)	29	32.6, CH_3	0.95, s
15	32.1, CH_2	1.73, m (a-H)	30	30.6, CH_3	0.83, s
		1.37, m (b-H)			

[a] ^{1}H NMR at 600 MHz, ^{13}C NMR at 150 MHz in $CDCl_3$ and multiplicities inferred from DEPT and HSQC experiments.

25-CH_3 and 28-CH_3 to C-6 and the key $^{1}H-^{1}H$ COSY correlation (Figure 1) of H-5/H-6, forming part **a**. The formation of part **b** by fragments **IV**, **V**, and **IV** was also established from the key HMBC correlations of both 26-CH_3 and H-27 with C-12 and the key $^{1}H-^{1}H$ COSY correlation of H-12/H-18. The other two hydroxyl groups were determined to connect to C-2 and C-21 by the analyses of their low-field chemical shifts at δ_C 66.1 and 66.5 in ^{13}C NMR spectrum. The linkages of C-2 to C-3 and C-1 and of C-21 to C-20 and C-22 were deduced by the $^{1}H-^{1}H$ COSY correlations (Figure 1) of H-1/H-2, H-2/H-3, H-20/H-21, and H-21/H-22. Now, the two seven-membered rings were formed from the above HMBC and $^{1}H-^{1}H$ COSY cross peaks. Apart from the two rings and two double bonds, the remaining elements of the unsaturation in **1** were assumed to be another ring. The $^{1}H-^{1}H$ COSY correlations (Figure 1) of H-8/H-9, H-9/H-10, H-14/H-15, H-15/H-16 established the connection of part **a** and **b** as shown, forming a 12-membered ring, which was further confirmed by the HMBC correlations from H-8 to C-10 and H-15 (δ_H 1.37) to C-13, H-14 (δ_H 2.25) to C-16. Thus, the planar structure of **1** was assigned.

The relative configuration of **1** was assigned by a ROESY experiment. In the ROESY spectrum (Figure 2), the correlations of 28-CH_3/H-2, H-2/23-CH_3, and H-6/24-CH_3 indicated that 28-CH_3, 23-CH_3, and H-2 were on the same side in the seven-membered ring and presumed to be β-oriented, and H-6 was deduced to be α-oriented. The β-orientation of 16-OH was deduced from the correlations of 28-CH_3/H-1β and H-1α/H-16. The $^{3}J_{15,16}$ values were 11.8 and 4.4 Hz, which further confirmed the β-orientation of 16-OH. The correlations of H-16/H-12, H-12/30-CH_3, 29-CH_3/H-21, 26-CH_3/H-21, 26-CH_3/H-22β, and H-10/H-22β indicated the α-orientations of H-12 and 30-CH_3 and β-orientations of H-21, 26-CH_3, H-10, and 29-CH_3. The geometry of the double bond between C-7 and C-8 was determined to be Z by the ROESY correlation of H-8/25-CH_3. Thus, the structure of compound **1** was established, named volvalerenol A.

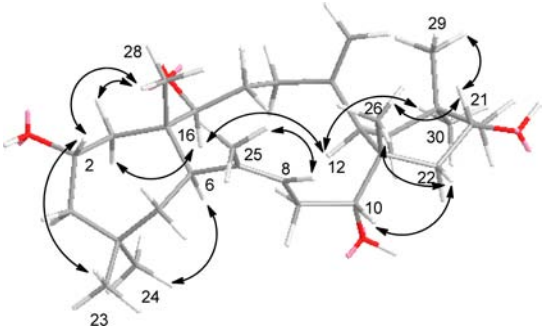
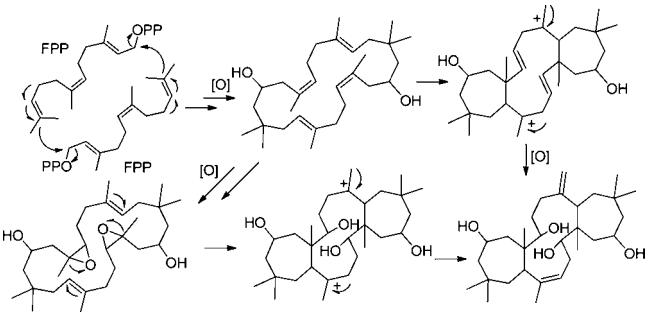


Figure 2. ROESY (↔) correlations of **1**.

The plausible biogenetic pathways of **1** were proposed as shown in Scheme 1. Compound **1** might be biogenetically derived from two molecules of FPP (farnesyl pyrophosphate) via electrophilic addition, charge-transfer reaction, and oxidation reactions or through epoxy intermediates, and electrophilic addition, and hydroxy transfer reactions. Compound **1** is a new type of triterpenoid, with a new carbon skeleton containing a 7/12/7 tricyclic ring system.

(9) (a) Rilling, H. *J. Bio. Chem.* **1966**, *241*, 3233–3236. (b) Niehaus, T. D.; Okada, S.; Devarenne, T. P.; Watt, D. S.; Sviripa, V.; Chappell, J. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 12260–12265.

Scheme 1. Possible Biogenetic Pathways to **1** from FPP (Farnesyl Pyrophosphate)



To the best of our knowledge, this triterpenoid with the 7/12/7 tricyclic ring system is reported for the first time.

In the biosynthesis pathways of usual triterpenes, squalene, derived from head-to-head condensation of two farnesyl pyrophosphates (FPP), is a general precursor.[9] However, the biosynthesis pathway of volvalerenol A (**1**) is proposed to be generated from two FPP molecules through a head-to-tail condensation.

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Supporting Information Available. Experiment procedures, 1D and 2D NMR spectra, mass spectra, IR, $[\alpha]_D$, and UV spectrum of **1**. This material is available free of charge via Internet at http://pubs.acs.org.

The authors declare no competing financial interest.