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A novel sulfur-containing diterpenoid from *Fritillaria anhuiensis*

Qing-Yao Shou [a], Qing Tan [a], Zheng-Wu Shen [a,b,*]

[a] School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China
[b] Basilea Pharmaceutical China Ltd., Haimen, Jiangsu 226100, People's Republic of China

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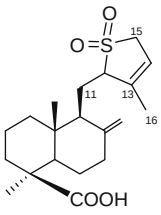
ABSTRACT

Two novel diterpenoids were isolated from the bulbs of *Fritillaria anhuiensis* S. C. Chen and S. E. Yin. Compound **1** was the first diterpenoid containing a sulfonyl group isolated from nature. Compound **2** was a novel kaurane-type diterpenoid. Their structures were determined by extensive spectroscopic analysis (IR, MS, NMR, and X-ray diffraction). Compound **1** significantly attenuated nitric oxide (NO) production of a macrophage cell line of Raw 264.7 cells stimulated with IFN-γ.

Fritillaria anhuiensis S. C. Chen and S. E. Yin is mainly distributed in Dabie mountains in Anhui province of China. The bulbs of this plant have been used in traditional Chinese medicine to treat cough, sputum, and asthma.[1] Previous chemical investigation revealed four isosteroidal alkaloids[2] and seven diterpenoids.[3,4] As a systematic approach to this species, we focused our studies on the non-alkaloid constituents of the bulbs of *F. anhuiensis*. Herein, we report on the isolation and structure elucidation of a novel sulfur-containing labdane-type diterpenoid as well as a novel kaurane-type diterpenoid. Compound **1** is the first diterpenoid containing a sulfonyl group isolated from nature, which significantly attenuated nitric oxide (NO) production of a macrophage cell line of Raw 264.7 cells stimulated with IFN-γ.



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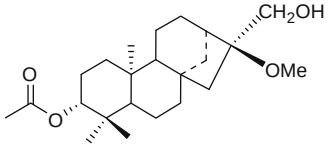

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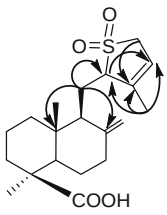
The chloroform fraction of the bulbs' ethanol extracts was purified by repeated column chromatography over silica gel eluted with petroleum ether/acetone, petroleum ether/ethyl acetate and Pharmadex LH-20 eluted with methanol to afford compounds **1** and **2**.

Compound **1** was obtained as colorless needles. Its molecular formula was established as $C_{20}H_{30}O_4S$ by HRESI-MS at *m/z* 367.1944 $[M+1]^+$ (calcd 367.1943). The IR spectrum showed absorption bands of a sulfonyl group (1304 and 1117 cm^{-1}) and a carboxylic group (3422 and 1693 cm^{-1}). The analysis of ^1H and ^{13}C NMR spectrum indicated the existence of two tertiary methyl groups [δ_H 0.62, δ_H 1.22 (3H each, s)], an olefinic methyl (δ_H 1.88, 3H, s), a methylene attached to a sulfur atom (δ_H 3.67, 2H, br s; δ_C 55.8), a methine attached to a sulfur atom (δ_H 3.67, 1H, br s; δ_C 65.7), a trisubstituted double bond (δ_H 5.66, br s; δ_C 117.0, 139.1), as well as an exocyclic double bond [δ_H 4.94, 4.46 (1H each, br s); δ_C 106.6, 147.9]. All those signals indicated that compound **1** had a labdane skeleton with a sulfonyl group. The sulfonyl group was located between C-12 (δ_C 65.7) and C-15 (δ_C 55.8) according to the HMBC correlations (Fig. 1). Thus a subunit of 3-methylsulfolene was established. HMBC correlations of δ_H 2.00 (H-11) with δ_C 65.7 (C-12), δ_C 40.9 (C-10) as well as δ_C 147.9 (C-8) supported the 3-methylsulfolene that was attached to C-9 through a methylene connection. Furthermore, the HSQC spectrum confirmed correla-



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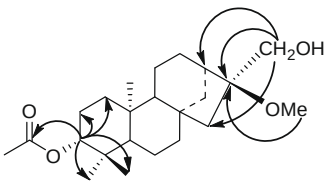

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Figure 1. Key HMBC correlations of compounds **1** and **2**.

* Corresponding author. Tel.: +86 513 82198001; fax: +86 513 82198003.
E-mail address: jeff_shen_1999@yahoo.com (Z.-W. Shen).

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tions between each proton and its related carbon signal. The stereochemistry of **1** was finally confirmed unambiguously by X-ray diffraction analysis of a single crystal obtained from acetone (Fig. 2).[5] Thus compound **1** was concluded to be 12,15-sulfonyl-8(17),13-labdadien-19-oic acid. All the ^{1}H and ^{13}C NMR spectroscopic signals of **1** were assigned based on the ^{1}H–^{1}H COSY, HSQC, and HMBC spectra.

The origin of compound **1** could be rationalized biogenetically and traced back to compound **3** (Scheme 1), a known labdane-type diterpenoid, which was also isolated from this plant.[6] We suggested that the sulfur donor might be sulfide derived from sulfate activation, sulfate reduction, and ferredoxin-linked sulfite reduction in plants.[7] After sulfuration, dehydration, and oxidation, compound **1** could be formed, finally.

Compound **2** was obtained as colorless needles. The HRESI-MS exhibited an ion peak at *m/z* 401.2663 $[M+Na]^+$ (calcd 401.2668), corresponding to the molecular formula of $C_{23}H_{38}O_4$. The presence of four rings in the structure of **2** was deduced from the molecular formula and the ^{13}C NMR spectrum. Analysis of NMR spectroscopic data revealed that three tertiary methyl groups [δ_H 0.84, 0.85, 1.03 (3H each, s)], and a methylene attached to a hydroxy group (δ_H 3.73, 2H, brs; δ_C 60.6). Thus, compound **2** appeared to be a diterpenoid belonging to kaurane analog. The NMR signals at δ_H 2.04 (3H, s), δ_C 21.6, 171.3 as well as δ_H 3.15 (3H, s), δ_C 49.2 indicated the presence of an acetoxy group and a methoxy group. The HMBC spectrum (Fig. 2) exhibited correlations among the proton at δ_H 4.45 (H-3) and the carbons at δ_C 171.3 (C-21), δ_C 38.5 (C-1), δ_C 23.8 (C-2), δ_C 28.5 (C-18), and δ_C 16.8 (C-19), thus the acetoxy group was assigned to C-3 position. HMBC correlation of the singlet at δ_H 3.15 with δ_C 87.1 allowed us to assign the methoxy group at C-16. The relative stereochemistry of **2** was established by analysis of its NOESY spectrum (Fig. 3). The NOESY correlations between δ_H 2.17 (H-13) and δ_H 3.15 (H-23) indicated that the methoxy group was α orientated. The coupling constants between H-2 and H-3 were 9.6 Hz and 6.4 Hz, which indicated the 1,3-diaxial connection.[8] Hence, the acetoxy group was β orientated with the equatorial conformation. Based on all of the spectroscopic evidence, the structure of compound **2** was established as *ent*-3β-acetoxy-16α-methoxykauran-17-ol.

Compound **1** colorless needles (acetone); mp: 159.6–160.3 °C; $[\alpha]^{24}$ +53.2 (*c* 0.23, $CHCl_3$); IR (KBr) ν_{max} 2934, 2872, 2853, 1717, 1693, 1447, 1437, 1304, 1117, 887 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79 (m, H-1a), 1.41 (m, H-1b), 1.80 (m, H-2a), 1.53 (m, H-2b), 2.12 (m, H-3a), 1.08 (m, H-3b), 1.48 (m, H-5), 1.88 (m, H-6a), 2.02 (m, H-6b), 1.99 (m, H-7a), 2.46 (m, H-7b), 2.20 (m, H-9), 1.75 (m, H-11a), 2.00 (m, H-11b), 3.67 (m, H-12), 5.66 (br s, H-14), 3.67 (br s, H-15), 1.89 (s, H-16), 4.94 (s, H-17a), 4.46 (s, H-17b), 1.22 (s, H-18), 0.62 (s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.5, C-1; 20.0, C-2; 37.9, C-3; 44.4, C-4; 55.9, C-5; 26.3, C-6; 38.7, C-7; 147.9, C-8; 52.5, C-9; 40.9, C-10; 22.3, C-11; 65.7, C-12; 139.1, C-13; 117.0, C-14; 55.8, C-15; 18.3, C-16; 106.6, C-17; 29.2, C-18; 184.1, C-19; 12.8, C-20; ESI-MS 367.2 $[M+1]^+$; HRESI-MS *m/z* 367.1944 $[M+1]^+$ (calculated for $C_{20}H_{31}O_4S$, 367.1943).

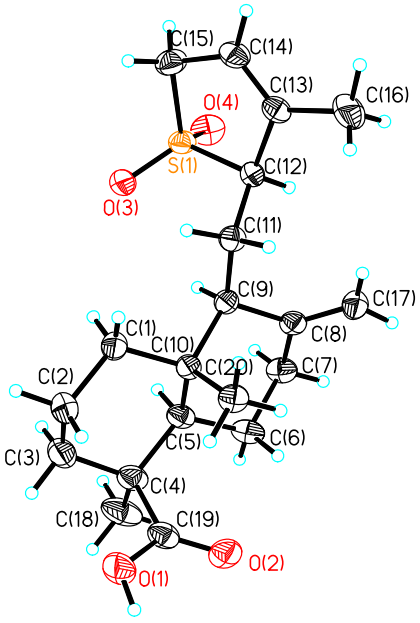
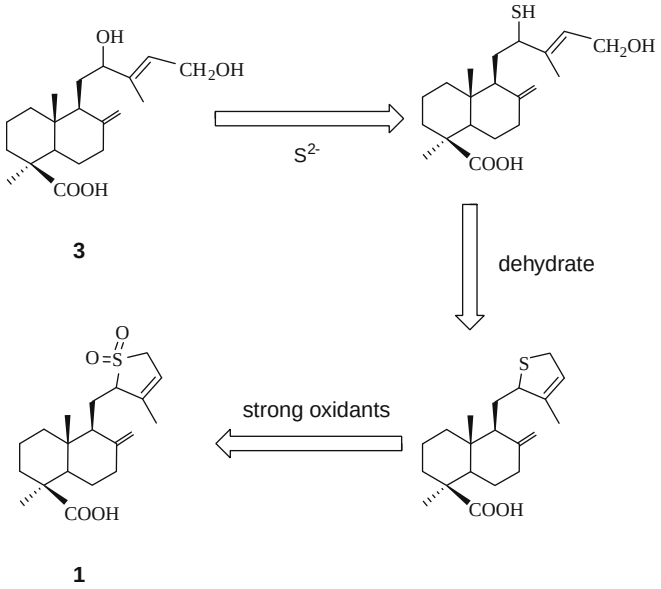


Figure 2. The ORTEP drawing of compound **1**.



Scheme 1. Proposed biogenesis of compound **1**.

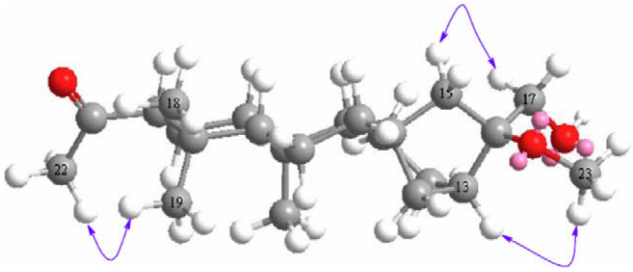


Figure 3. Key NOESY correlations of compound **2**.

Compound **2** colorless needles (acetone); mp: 222.1–223.5 °C; $[\alpha]^{24}$ −42.1 (*c* 0.51, $CHCl_3$); IR (KBr) ν_{max} 3402, 2923, 2961, 2851, 1722, 1645, 1447, 1375, 1242, 1045, 1022, 804 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79 (m, H-1a), 0.92 (m, H-1b), 1.68 (m, H-2), 4.45 (dd, *J* = 9.6, 6.4 Hz, H-3), 0.85 (m, H-5), 1.55 (m, H-6a), 1.33 (m, H-6b), 1.46 (m, H-7a), 1.87 (m, H-7b), 1.01 (m, H-9), 1.54 (m, H-11), 1.58 (m, H-12), 2.17 (m, H-13), 1.61 (m, H-14), 1.30 (m, H-15a), 1.57 (m, H-15b), 3.73 (s, H-17), 0.85 (s, H-18), 0.84 (s, H-19), 1.03 (s, H-20), 2.03 (s, H-22), 3.15 (s, H-23); ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.5, C-1; 23.8, C-2; 81.1, C-3; 39.2, C-4; 55.3, C-5; 20.3, C-6; 36.9, C-7; 44.4, C-8; 56.6, C-9; 37.9, C-10; 18.7, C-11; 26.1, C-12; 41.7, C-13; 42.1, C-14; 48.8, C-15; 87.1, C-16; 60.6, C-17; 28.5, C-18; 16.8, C-19; 18.1, C-20; 171.3, C-21; 21.6, C-22; 49.2, C-23; ESI-MS 401.3 $[M+Na]^+$; HRESI-MS *m/z* 401.2663 $[M+Na]^+$ (calculated for $C_{23}H_{38}O_4Na$, 401.2668).

Nitric oxide (NO) is a short-lived free radical and signaling molecule that mediates many physiological and pathophysiological processes, including neurotransmission and inflammation.[9,10] Compounds **1** and **2** were evaluated for the activity of NO inhibition in a macrophage cell line of Raw 264.7 cells stimulated with IFN-γ according to a reported experimental procedure.[11,12] The inhibitory rate of compound **1** on NO production was 36.98% at the concentration of 0.27 μmol/mL, whereas, compound **2** was inactive at any concentration that was assessed.

The isolation of compound **1** adds to the structural diversity of diterpenoids from nature. As compound **1** was also the first compound with a sulfur atom from the genus of *Fritillaria*, it will be interesting to explore the true biogenetic origin and the biological role it plays in the life cycle of the plants.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.05.002.

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5. *X-ray crystal data of* **1**: $C_{20}H_{30}O_4S$; crystal size (mm) 0.10 × 0.10 × 0.15 colorless bulk crystal; orthorhombic with space group *C*2; unit cell dimensions $a = 31.0293$ (6) Å, $b = 10.3742$ (2) Å, $c = 7.3120$ (2) Å; volume 2313.95 (9) Å^3; $Z = 4$; formula weight 366.50; density (calcd) 1.052 g/cm^3; absorption coefficient 0.157 mm^{-1}; $F(0\,0\,0) = 792$. The reflection data were collected on a Bruker Smart Apex-11 diffractometer, using graphite-monochromated radiation Mo Kα $\lambda = 0.71073$ Å. A total of 5181 reflections were collected in the range of $2.80^\circ \leqslant \theta \leqslant 27.69^\circ$ of which 2920 unique reflections with $I > 2\sigma(I)$ were utilized for the analysis, and were used for refinement. The final R and R_w were 0.0626 and 0.1328, respectively, with goodness-of-fit of 0.991. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center, deposit No. CCDC 713572. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44-(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
6. Compound **3**, 12*R*,15-dihydroxy-8(17),13*E*-labdadien-19-oic acid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.80 (m, H-1a), 1.15 (m, H-1b), 1.90 (m, H-2a), 1.48 (m, H-2b), 2.12 (m, H-3a), 1.06 (m, H-3b), 1.44 (m, H-5), 1.90 (m, H-6a), 1.98 (m, H-6b), 2.38 (m, H-7a), 1.90 (m, H-7b), 2.12 (m, H-9), 1.62 (m, H-11a), 1.47 (m, H-11b), 3.92 (br d, H-12), 5.52(t, H-14), 4.11 (br d, H-15), 1.67 (s, H-16), 4.87 (s, H-17a), 4.49 (s, H-17b), 1.20 (s, H-18), 0.62 (s, H-20). ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.7, C-1; 21.6, C-2; 39.9, C-3; 45.7, C-4; 59.7, C-5; 28.1, C-6; 40.4, C-7; 150.8, C-8; 53.7, C-9; 41.7, C-10; 32.0, C-11; 76.4, C-12; 143.0, C-13; 125.2, C-14; 59.7, C-15; 14.0, C-16; 107.5, C-17; 28.1, C-18; 181.8, C-19; 12.6, C-20.
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12. Raw 264.7 cells (ATCC), a macrophage cell line, were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 10 μg/mL streptomycin. Raw 264.7 cells (5×10^4/well) were cultured in triplicate in 96-well plates and stimulated with 50 U/mL IFN-γ in the absence or presence of compound for 24 h. Cell viability was determined by MTT assays. The production of NO was determined by Griess reagent, the concentration was calculated from a $NaNO_2$ standard curve.