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Nor-lupane triterpenoid and guaiane sesquiterpenoids from *Schefflera venulosa*

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ABSTRACT

A novel nor-lupane triterpenoid, 3-oxo-29α-hydroxy-17β,20-epoxy-28-norlupane (**1**), and two new guaiane sesquiterpenoids, schvenols A–B (**2**–**3**), has been isolated from leaves of *Schefflera venulosa*. Structures of the new compounds were elucidated on the basis of their spectroscopic methods, including 1D and 2D NMR techniques. And the structure of **1** was further confirmed by the X-ray diffraction analysis. None of the compounds showed inhibitory effects on NO release in LPS-stimulated RAW 264.7 macrophage cell line.

1. Introduction

Schefflera venulosa (Wight & Arn.), a plant of the Araliaceae family, is widely distributed in Yunnan, Guizhou, and Hunan provinces of China [1]. The stems and leaves of it have been used as folk medicines for the treatment of rheumatic arthritis and headache for a long time. In partial area of Yunnan province, it has also been used for the treatment of certain cancers. Phytochemistry research indicated that triterpenoids were the main constituents of *S. venulosa* [2–4], while some of them possessed spermicidal activity and anticancer activities [5,6]. As a part of our effort to discover naturally bioactive metabolites from traditional medicines [7,8], a phytochemical investigation of the leaves of *S. venulosa* was carried out, which resulted the isolation of a novel nor-lupane triterpenoid, 3-oxo-29α-hydroxy-17β,20-epoxy-28-norlupane (**1**) (Fig. 1), and two new guaiane sesquiterpenoids, schvenols A–B (**2**–**3**) (Fig. 1), together with seven known triterpenoids (**4**–**10**) and three known sesquiterpenoids (**11**–**13**). Reported herein are the isolation, structure elucidation, and the inhibitory effects on NO release of the new compounds.

2. Experimental

2.1. Gernal

Melting points were obtained on an X-4 micromelting point apparatus. Optical rotations were measured on a JASCO-20C digital polarimeter. IR spectra were obtained on a Tenor 27 spectrometer with KBr pellets. ^{1}H and ^{13}C NMR spectra were performed on a Bruker AM-400 or DRX-500 spectrometer with TMS as internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EIMS and HRESMS were taken on a VG Auto Spec-3000 mass spectrometer. The X-ray diffraction was performed on a Bruker APEX DUO diffractometer using graphite-monochromated Mo Kα radiation. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, People's Republic of China) or silica gel H (10–40 μm, Qingdao Marine Chemical Co. Ltd.), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). MPLC was performed on a Lisui EZ Purify III System including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Li Sui Chemical Engineering Co., Ltd., China) and columns packed with MCI gel (75–150 μm; Mitsubishi Chemical Corporation, Japan). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

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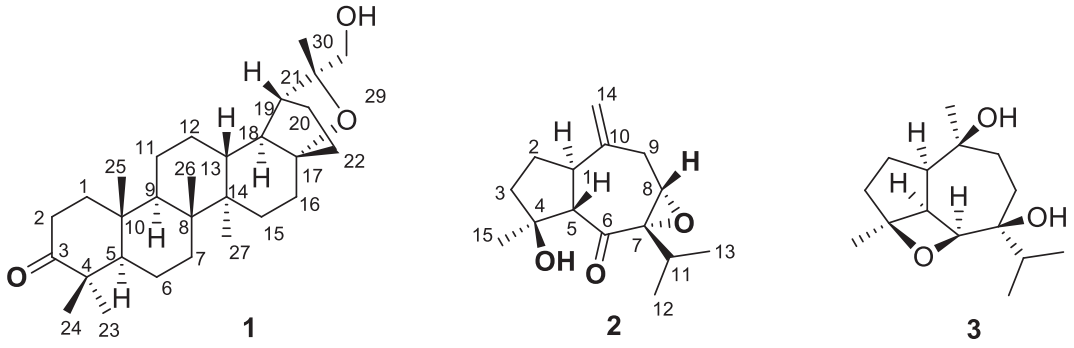

Fig. 1. Structures of compounds **1–3**.

2.2. Plant material

The leaves of *S. venulosa* was collected from Yunnan Institute of Materia Medica, People's Republic of China, in September 2009, and identified by Prof. X. Cheng (one of the authors), Kunming Institute of Botany. A voucher specimen (KIB20090900s01) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried and powdered leaves of *S. venulosa* (10 kg) were extracted three times with 95% EtOH at room temperature (3 × 24 h). After concentrated under reduced pressure, the water soluble residue was partitioned successively by petroleum ether and EtOAc. The petroleum ether fraction (165 g) was chromatographed on a silica gel column with a gradient elution of petroleum ether–EtOAc (9:1 to 5:5) to give three fractions (1–3). Fraction 2 (35 g) was applied to silica gel CC eluted with petroleum ether–EtOAc (from 20:1 to 7:3) to afford three subfractions (2a–2c). Subfraction 2a was further purified by recrystallization to obtain **4** (70 mg). Subfraction 2b was further purified silica gel CC eluted with petroleum ether–acetone (from 50:1 to 9:1) to yield **11** (6 mg) and **2** (7 mg). Fraction 3 (52 g) was separated by MPLC eluting with MeOH–H_2O (from 75:25 to 100:0) to provide five subfractions, 3a–3e. Subfraction 3b was subjected to silica gel CC ($CHCl_3$–acetone, 9:1), followed by Sephadex LH-20 (MeOH) to yield **5** (100 mg) and **7** (7 mg). Subfraction 3c was separated by silica gel CC eluted with ($CHCl_3$–acetone) (from 9:1 to 7:3) to afford **1** (10 mg). Compound **3** (20 mg) was obtained from subfraction 3d by repeated silica gel CC and Sephadex LH-20.

The EtOAc fraction (130 g) was chromatographed on a silica gel column with a gradient elution of petroleum ether–acetone (9:1 to 0:1) to give four fractions (4–7). Fraction 4 (15 g) was applied to silica gel CC eluted with petroleum $CHCl_3$–acetone (from 9:1 to 7:3) to afford three subfractions (4a–4c). Subfraction 4b was further purified by recrystallization to obtain **6** (30 mg). Subfraction 4c was subjected to silica gel CC ($CHCl_3$–acetone, 9:1), followed by Sephadex LH-20 (MeOH) to yield **9** (13 mg), **10** (10 m g). Fraction 5 was separated by silica gel CC eluted with ($CHCl_3$–acetone) (from 9:1 to 7:3) to afford **13** (18 mg). Fraction 6 (35 g) was chromatographed by MPLC eluting with MeOH–H_2O (from 30:70 to 100:0) to provide four subfractions, 6a–6f. Subfraction 6b was separated by silica gel CC (petroleum ether–acetone, 8:2) to yield **8** (10 mg). Subfraction 6c was subjected to silica gel CC eluted with $CHCl_3$–acetone (from 8:2 to 6:4) and Sephadex LH-20 (MeOH) to afford **12** (30 mg).

2.4. Spectroscopic data

3-Oxo-29α-hydroxy-17β,20-epoxy-28-norlupane (**1**): white crystals; $[\alpha]_D^{25}$ + 23.42 (c 0.19, MeOH); UV (MeOH) λmax (log ε): 224 (1.78), 233 (1.77), 250 (1.85) nm; IR (KBr) νmax: 3429, 2956, 2921, 2851, and 1706 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1;. HREIMS *m/z* 442.3444 $[M]^+$ ($C_{29}H_{46}O_3$ calcd 442.3447).

Crystal data for 3-oxo-29α-hydroxy-17β,20-epoxy-28-norlupane (**1**): $C_{29}H_{46}O_3$, M = 442.66; monoclinic, space group $P2_1$; a = 9.367(2) Å, b = 12.245(3) Å, c = 21.007(5) Å, $\alpha = \beta = \gamma = 90.00$, V = 2409.6 (10) $Å^3$, Z = 4, d = 50 mm, and crystal dimensions 0.04 × 0.10 × 0.22 nm was used for measurement on a Bruker APEX DUO with a graphite monochromater, Mo Kα radiation. The total number of reflections measured was 5597, of which 2355, were observed, $|F|^2 \geq 2\sigma|F|^2$. Final indices: R_1 = 0.0758, wR_2 = 0.1465 ($w = 1/\sigma|F|^2$), and S = 0.942. The crystal structure of **1** was solved by direct method SHLXS-97 and expanded using difference Fourier technique, refined by the program Bruker APEX DUO and the full-matrix least-squares calculations. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: 997341). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK.; fax: (+44) 1223-336-033; or desposit@ ccdc.cam.ac.uk).

Schvenol A (**2**): colorless oil; $[\alpha]_D^{25}$-5.73 (c 0.16, MeOH); UV (MeOH) λmax (log ε): 396 (0.88), 244 (2.42)nm; IR (KBr) νmax: 3432, 2966, 2931, 1722; ^1H and ^{13}C NMR data, see Table 2;. HREIMS *m/z* 250.1561 $[M]^+$ ($C_{25}H_{22}O_3$ calcd 250.1569).

Schvenol B (**3**): colorless oil; $[\alpha]_D^{25}$-5.34 (c 0.1, MeOH); UV (MeOH) λmax (log ε): 233 (1.77) nm; IR (KBr) νmax: 3432, 2921, 2851, 1680 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2;. HREIMS *m/z* 236.1815 $[M]^+$ ($C_{15}H_{24}O_2$ calcd 236.1822).

Table 1
^1H and ^{13}C NMR spectroscopic data for compound **1**.

No.	1		No.	1	
	δ_H	δ_C		δ_H	δ_C
1a	1.96 (*m*)	40.6	15b	1.31 (*overlapped*)	
1b	1.47 (*m*)		16a	1.74 (2H, *m*)	26.4
2a	2.48 (2H, *overlapped*)	34.1	17		85.5
3		218.1	18	1.82 (*m*)	50.2
4		47.3	19	2.04 (*br.s*)	44.8
5	1.38 (*m*)	54.8	20		83.0
6a	1.50 (2H, *overlapped*)	19.6	21a	1.78 (*m*)	21.3
7a	1.50 (2H, *overlapped*)	33.5	21b	1.55 (*overlapped*)	
8		40.6	22a	1.85 (*m*)	30.3
9a	1.45 (*m*)	49.9	22b	1.30 (*m*)	
10		36.9	23	1.05 (3H, *s*)	21.0
11a	1.50 (*m*)	26.4	24	1.10 (3H, *s*)	26.7
11b	1.22 (*m*)		25	0.96 (3H, *s*)	16.2
12a	1.55 (*overlapped*)	21.4	26	1.03 (3H, *s*)	15.6
12b	1.37 (*m*)		28	1.02 (3H, *s*)	13.5
13	1.47 (*m*)	34.7	29	3.64 (*d*, 8.4)	68.0
14		41.6		3.59 (*d*, 8.4)	
15a	1.53 (*m*)	28.1	30	1.24 (3H, *s*)	24.3