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A new carotane sesquiterpene from *Walsura robusta*

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[ABSTRACT] AIM: To study the chemical constituents from the leaves of *Walsura robusta*. **METHODS**: The leaves of *W. robusta* were extracted with MeOH and compounds isolated by silica gel, Rp-C_{18}, Sephadex LH-20 and semipreparative HPLC. These compounds were elucidated by extensive spectroscopic analysis (MS, NMR, IR, and UV). **RESULTS**: Two sesquiterpenoids were obtained and their structures were identified as 10β-nitro-isodauc-3-en-15-al (**1**) and 10-oxo-isodauc-3-en-15-al (**2**). **CONCLUSIONS**: Compound **1** was new with a nitro group. **1** and **2** were isolated from genus *Walsura* for the first time.

[KEY WORDS] Meliaceae; *Walsura robusta*; Sesquiterpenoid; Nitro compound

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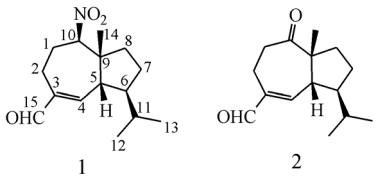
1 Introduction

The genus *Walsura* (Meliaceae), comprising 16 species, is naturally distributed in subtropical regions such as Southern China, India, and Indonesia[1]. In the previous literature, triterpenoids, phenols, and steroids were isolated from this genus, which exhibited cell protective, antioxidant and antimalarial activities [2-10]. The extracts of the leaves and twigs of *W. robusta* Roxb., with the Chinese name "gesheshu" was used as an insecticide in Xishuangbanna[5]. In order to seek new natural products with bioactivities, the chemical constituents of the leaves of *W. robusta* were studied. In this paper, one new sesquiterpenoid 10β-nitro-isodauc-3-en-15-al (**1**) with a rare nitro group, along with one known compound, 10-oxo- isodauc-3-en-15-al (**2**)[11] were isolated. The structure of the new carotane sesquiterpenoid was elucidated on the basis of spectroscopic analysis and comparison with the related compounds reported in the literature. Compounds **1** and **2** showed no antimicrobial activities against *Staphylococcus aureus*, MRSA 92# (MRSA, methicillin-resistant *S. aureus*), MRSA 98#, and MRSA 111#.



1 2

Fig. 1 Structures of compounds 1 and 2

2 Results and Discussion

10β-Nitro-isodauc-3-en-15-al (**1**) was obtained as a colorless oil, displaying a molecular formula of $C_{15}H_{23}NO_3$ with five degrees of unsaturation as determined by HR-EI-MS at *m/z* 265.167 8 $[M]^+$ (Calcd. for 265.167 8). The IR spectrum showed the presence of aldehyde (1685 cm^{-1}) and nitro (1547 cm^{-1}, 1 385 cm^{-1}) groups. The ^1H NMR and ^{13}C NMR of **1** (Table 1) showed signals of three methyls (δ_H 0.93, d, J = 6.7 Hz, 6H and 1.23, s, 3H), a trisubstituted double bond moiety (δ_H 6.53, J = 4.5 Hz, δ_C 157.7 and 141.2), and an aldehyde group (δ_H 9.41, δ_C 192.1). These data suggested that **1** had the same skeletal structure as aphanamol II[12], except for the substituent at C-10. As is well known, the carbon signal of the methine at δ_C 92.6 is at rather lower field comparison of that of ordinary hydroxymethine in the same skeletal sesquiterpene, and the methine attached a nitro group was therefore suggested[13]. The molecular formula ($C_{15}H_{23}NO_3$)

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also confirmed the presence of a nitro group (NO_2) in **1**. The methine was assigned at C-10 by means of the HMBC correlation between H-14 with C-5, C-8, C-9, and δ_C 92.6. Detailed analysis of the 2D NMR data, including the HSQC, ^{1}H-^{1}H COSY, and HMBC data (Table 1 and Fig. 2), confirmed the planar structure of compound **1**.

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compound 1 in $CDCl_3$

Carbon No.	δ_H (multi, *J* in Hz)	δ_C	HMBC
1	2.24 (m), 1.90 (m)	23.4 CH_2	C-2, C-3, C-9, C-10
2	2.79 (m), 2.20 (m)	18.9 CH_2	C-1, C-3, C-4, C-10, C-15
3		141.2 C	
4	6.53 (d, 4.5)	157.7 CH	C-2, C-3, C-5, C-9, C-15
5	2.37 (dd, 8.4, 4.5)	54.4 CH	C-14, C-3, C-4, C-6, C-9, C-10, C-11, C-15
6	1.88 (m)	54.9 CH	C-4, C-7, C-11, C-12
7	1.89 (m), 1.47 (m)	27.1 CH_2	C-6, C-8
8	1.67 (m), 1.47 (m)	39.2 CH_2	C-6, C-7, C-10, C-14
9		45.7 C	
10	4.27 (dd, 11.7, 4.6)	92.6 CH	C-5, C-8, C-9, C-14
11	1.62 (m)	32.6 CH	C-6, C-7, C-12
12	0.93 (d, 6.7)	19.6 CH_2	C-6, C-11, C-13
13	0.93 (d, 6.7)	22.0 CH_3	C-6, C-11, C-12
14	1.23 (s)	21.0 CH_3	C-5, C- 8, C-9, C-10
15	9.41 (s)	192.1 CH	C-2, C-3, C-4

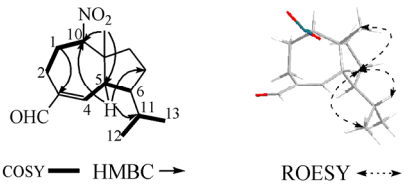

Fig. 2 Key COSY, HMBC, and ROESY correlations of compound 1

The relative configuration of **1** was established by the ROESY correlations, as well as the coupling constants. Key ROESY cross-peaks of H-5/H-14 and H-5/H-11 were observed, which indicated that H-5 and the isopropyl group were co-facial, arbitrarily assigned as *β*-orientated, while H-6 was *α*-oriented. The coupling constants of H-10 (dd, *J* = 4.6 and 11.7 Hz) indicated that the proton was *α*-orientated. Accordingly, compound **1** was deduced as 10*β*-nitro-isodauc-3-en-15-al.

The isolated sesquiterpenoids were screened for their antimicrobial activity against *Staphyloccocus aureus*, MRSA 92$^{\#}$ (MRSA, methicillin-resistant *S. aureus*), MRSA 98$^{\#}$, and MRSA 111$^{\#}$ using the agar plate punch assay. The minimum inhibitory concentrations (MICs) were determined by the two-fold dilution method[14]. The results revealed that compounds **1** and **2** showed no antimicrobial activities against *Staphylococcus aureus*, MRSA 92$^{\#}$, MRSA 98$^{\#}$, and MRSA 111$^{\#}$.

3 Experimental

3.1 *General experimental procedures*

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were detected on a Shimadzu UV-2401 spectrophotometer. IR spectra were determined on a Tenor 27 spectrophotometer with KBr pellet. ESI-MS and HR-EI-MS were measured on a Finnigan MAT 90 instrument and Waters AutoSpec Premier P776, respectively. 1D and 2D NMR spectra were recorded on Bruker AM-400 and Bruker DRX-500 spectrometers with TMS as internal standard. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 column. Column chromatography was performed with silica gel (38−48 μm, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), and MCI gel (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

3.2 *Plant material*

The leaves of *W. robusta* were collected in Hainan Province, People's Republic of China in December 2010. The plant was authenticated by Dr. HU Guang-Wan, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. H20101202) was deposited in the State Key Laboratory of Photochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS.

3.3 *Extraction and isolation*

The dried and powdered leaves (12 kg) of *W. robusta* were extracted with MeOH three times under reflux, and the solvent was evaporated *in vacuo*. The residue was partitioned in water and extracted successively with petroleum ether and EtOAc. The EtOAc fraction (200 g) was separated by silica gel column chromatography (CC) eluted with a gradient of

petroleum ether/Me_2CO (50 : 1 to 1 : 1) and $CHCl_3$/MeOH in a gradient (15 : 1 to 3 : 1), to obtain eight fractions (Fr. A−H) according to TLC monitor. Fr. E (57 g) was subjected to MCI-gel column (MeOH/H_2O, 6 : 4 to 9 : 1) to give eighteen sub-fractions (E1−E18). Fr. E14 (8 g) was subjected to CC eluted with $CHCl_3$-Me_2CO (200 : 1→100 : 1→50 : 1→25 : 1→15 : 1→10 : 1→5 : 1→1 : 1) to yield eleven fractions (E14A−E14K. Fraction E14F was applied to a Sephadex LH-20 column and then purified by HPLC to obtain **1** (17 mg) and **2** (11 mg).

3.4 *Antimicrobial and insecticidal assays*

Antimicrobial assays were performed according to the previously described protocols[14].

4 Identification

10*β*-Nitro-isodauc-3-en-15-al (**1**) Colorless oil; $[\alpha]_D^{19}$ −10.1 (*c* 0.14, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.28) nm; ^{1}H NMR and ^{13}C NMR data (see Table 1); IR ν_{max}: 2 959, 2 837, 1 685, 1 547 cm^{-1}; HR-EI-MS *m/z* 265.167 8 $[M]^+$ (Calcd. for $C_{15}H_{23}NO_3$, 265.1678).

10-Oxo-isodauc-3-en-15-al (**2**) Colorless oil; $C_{15}H_{24}O_2$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.28 (1H, s, H-15), 6.57 (1H, d, 5.3 Hz, H-4), 1.58 (1H, m, H-11), 1.26 (3H, s, H-14), 0.88 (3H, s, H-12), 0.86 (3H, s, H-13); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 212.3 (C, C-10), 192.7 (CH, C-15), 158.7 (CH, C-4), 143.7 (C, C-3), 59.6 (C, C-9), 55.4 (CH, C-6), 53.1 (CH, C-5), 38.9 (CH_2, C-8), 35.1 (CH_2, C-7), 32.4 (CH, C-11), 26.8 (CH_2, C-1), 25.0 (CH_3, C-14), 22.0 (CH_3, C-13), 19.4 (CH_3, C-12), 19.6 (CH_2, C-2).

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割舌树中一个新的胡萝卜烷型倍半萜

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【摘 要】 目的:研究割舌树叶子的化学成分。方法:利用正相硅胶、反相 Rp-C_{18}、凝胶 Sephadex LH-20、HPLC 等色谱方法对割舌树的甲醇提取部分进行分离纯化, 运用 MS、NMR、IR 和 UV 来鉴定化合物的结构。结果:从割舌树中分离得到两个倍半萜 10-nitro-isodauc-3-en-15-al (**1**) 和 10-oxo-isodauc-3-en-15-al (**2**)。结论:化合物 **1** 为新的硝基胡萝卜烷型倍半萜,化合物 **1** 和 **2** 均为首次从该属植物中分离得到。

【关键词】 楝科; 割舌树; 倍半萜; 硝基化合物

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