

Diterpenoids from *Isodon lophanthoides*

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Abstract

A new diterpenoid was isolated from the leaves of *Isodon lophanthoides*, together with two known diterpenoids, lophanic acid and 8(17),12,14-labdatriene-19-oic acid. The structure of the new compound was determined to be 11 β -hydroxyisopimara-8,15-diene-3-one (**1**) on the basis of spectroscopic evidence. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: *Isodon lophanthoides*; Diterpenoids; 11 β -Hydroxyisopimara-8,15-diene-3-one

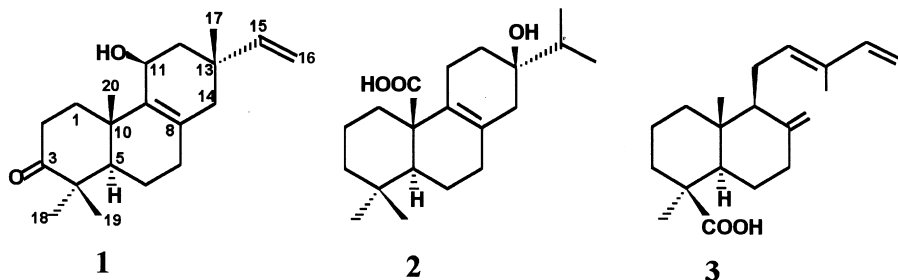
1. Introduction

Isodon lophanthoides var. *gerardianus* [Bentham] H. Hara (Lamiaceae) is widely distributed in south and south-east Asia. It has been used as a local folk medicine in China for treatment of enteritis, jaundice, hepatitis, laryngopharyngitis, lepromatous leprosy and ascariasis [1]. The leaves were reported to be rich in abietane quinones [2,3]. In order to isolate bioactive constituents from the *Isodon* genus, we reinvestigated the leaves of *I. lophanthoides* collected from the northern part of

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Guangxi province, China, which led to the isolation of a new diterpenoid **1** together with two known diterpenoids, lophanic acid (**2**) and 8(17),12,14-labdatriene-19-oic acid (**3**). This paper deals with the characterisation of these compounds and the revised assignments on the ^{13}C -NMR signals of compound **2** [4].



2. Experimental

2.1. Plant material

The leaves of *Isodon lophanthoides* var. *gerardianus* were collected in the Huaping Natural Reserve Area, Longsheng Prefecture of the Guangxi Zhuang Autonomous Region, between September and November 1996. They were authenticated by Prof. Li Xi-Wen at Kunming Institute of Botany, Chinese Academy of Science, where a voucher specimen (KIB 96-11-12 Lin) is deposited.

2.2. Extraction and isolation

The dried and powdered leaves (3.6 kg) were extracted with EtOH (7000 ml \times 3) under reflux for 2 h each time. The EtOH solution was treated with activated charcoal (100.0 g \times 3) and then filtered. The filtrate was concentrated in vacuo to give a residue which was dissolved in EtOH (500 ml) and then diluted with water to a solution containing 20% EtOH. The solution was partitioned with petrol (60–90°C) and EtOAc (2000 ml \times 3), respectively. The EtOAc extract was evaporated in vacuum to give a residue (164 g) which was chromatographed on a Si gel column (200–300 mesh, 1.5 kg) with CHCl_3 containing increasing amounts of Me_2CO . The fractions were combined by monitoring with TLC. Fractions III and VII (obtained from CHCl_3 – Me_2CO 9:1) were decoloured again using MCI-gel CHP-20P CC, and MeOH and Me_2CO as solvents. The residues were submitted to Si gel CC. Elution with cyclohexane and increasing amount of *i*-PrOH yielded compounds **1** (266 mg), **2** (16.7 g) and **3** (1.2 g).

11β-Hydroxy-8,15-isopimaradiene-3-one (**1**). C₂₀H₃₀O₂, resinous substance; [α]_D²²: +22.8° (c 0.34 CHCl₃); IRmax (KBr): 3444, 3079, 2950, 2871, 1703, 1470, 1435, 1273, 1251, 1207, 1115, 1037, 998, 974, 929, 912 and 880 cm⁻¹; ¹H-NMR (500 MHz, Py-*d*₅): 5.82 (1H, *dd*, *J* 17.5 and 10.7 Hz, H-15), 5.28 (1H, *dd*, *J* 17.5 and 1.5 Hz, H-16a), 5.01 (1H, *dd*, *J* 10.7 and 1.5 Hz, H-16b), 4.13 (1H, *br s*, H-11α), 2.80 (1H, *d*, *J* 17.0 Hz, H-12β), 2.58 (1H, *m*, H-2β), 2.45 (1H, *m*, H-2α), 2.38 (1H, *dd*, *J* 12.0 and 2.3 Hz, H-5α), 1.97 (2H, *m*, H₂-6), 1.94 (1H, *m*, H-7β), 1.88 (1H, *m*, H-1β), 1.85 (1H, *m*, H-7α), 1.82 (1H, *m*, H-12α), 1.58 (1H, *m*, H-14β), 1.54 (1H, *m*, H-1α), 1.35 (1H, *d*, *J* 16.8 Hz, H-14α), 1.16 (3H, *s*, Me-17), 1.09 (3H, *s*, Me-20), 1.04 (3H, *s*, Me-18) and 1.03 (3H, *s*, Me-19); ¹³C-NMR: see Table 1; EI-MS (70 eV) *m/z*: 302 [M]⁺(43), 284 [M-H₂O]⁺(14), 274 [M-CO]⁺(5), 256 [M-H₂O-CO]⁺(23), 245 (8), 229 (19), 213 (15), 199 (12), 185 (17), 175 (25), 164 (33), 139 (32), 129 (35), 115 (34), 105 (49) and 55 (100).

3. Results and discussion

Careful column chromatography of the ethyl acetate extract of the leaves of *Isodon lophanthoides* resulted the isolation of three diterpene derivatives, **1**, **2** and **3**.

Compound **1** was obtained as a resinous substance, having the molecular formula C₂₀H₃₀O₂ (EIMS). The IR spectrum showed the presence of hydroxyl and keto groups. The ¹H- and ¹³C-NMR spectra indicated it to be composed of four methyl carbons, seven methylenes including one olefinic carbon, three methines including each one hydroxyl-bearing and olefinic carbons, and six quaternary carbons including one ketonic carbon and two olefinic carbons (Table 1). ¹H-NMR signals at δ 5.01 and 5.28 (each 1H, *dd*) and 5.82 (1H, *dd*) were assigned to a vinyl group. Two ¹³C-NMR singlets at δ 129.2 and 138.3 were due to a tetra-substituted double bond. All the above NMR signals were assigned by 2D COSY measurements.

Table 1
¹³C-NMR data of compounds **1** and **2** (125 MHz, Py-*d*₅)

C	1	2	C	1	2
1	35.1 <i>t</i>	35.3 <i>t</i>	11	68.0 <i>d</i>	23.0 <i>t</i>
2	34.7 <i>t</i>	21.0 <i>t</i>	12	39.5 <i>t</i>	33.7 <i>t</i>
3	216.3 <i>s</i>	42.6 <i>t</i>	13	35.6 <i>s</i>	71.2 <i>s</i>
4	47.0 <i>s</i>	34.2 <i>s</i>	14	35.1 <i>t</i>	42.6 <i>t</i>
5	45.5 <i>d</i>	52.8 <i>d</i>	15	146.2 <i>d</i>	34.2 <i>d</i>
6	21.7 <i>t</i>	18.9 <i>t</i>	16	112.1 <i>t</i>	17.5 <i>q</i>
7	30.5 <i>t</i>	32.7 <i>t</i>	17	28.7 <i>q</i>	17.3 <i>q</i>
8	129.1 <i>s</i>	129.8 <i>s</i>	18	26.8 <i>q</i>	32.5 <i>q</i>
9	138.3 <i>s</i>	131.2 <i>s</i>	19	21.6 <i>q</i>	20.9 <i>q</i>
10	38.1 <i>s</i>	49.2 <i>s</i>	20	18.0 <i>q</i>	178.3 <i>s</i>

Table 2
HMBC results of compound **1** (Py- d_5)

H	C	H	C
H ₂ -1	2, 3, 5, 9, 10, 20	H ₂ -14	7, 8, 9, 12, 13, 15, 17
H ₂ -2	1, 3, 4, 10	H-15	12, 13, 14, 17
H-5	1, 3, 4, 6, 7, 9, 10, 18, 19, 20	H ₂ -16	13, 15
H ₂ -6	4, 5, 7, 8	Me-17	12, 13, 14, 15
H ₂ -7	5, 8, 9, 14	Me-18	3, 4, 5, 19
H-11	8, 9, 12	Me-19	3, 4, 5, 18
H ₂ -12	9, 11, 13, 15, 17	Me-20	1, 5, 9, 10

Considering the presence of four methyls and a vinyl group, **1** might be a pimaradienonol.

This assumption was confirmed by analysis of its HMBC spectrum, in which cross-correlations were observed among Me-17 (with C-12, C-13, C-14 and C-15), Me-18 and Me-19 (with C-3, C-4 and C-5), Me-20 (with C-1, C-5, C-9 and C-10) and H-11 (with C-8, C-8 and C-12), besides the other significant correlations shown in Table 2. Thus, **1** was a pimarane diterpenoid with a hydroxyl group at C-11, a ketone at 3-position and a tetra-substituted double bond between C-8 and C-9, respectively. Compound **1** exhibited NOESY correlations for H-5 α (with Me-18), H-2 β (with Me-19 and Me-20), H-11 α (with H₂-12 and H-1 β) and Me-17 (with H-12 β and H-14 β). Therefore, the structure of **1** was determined as 11 β -hydroxy-isopimara-8,15-diene-3-one.

Compounds **2** and **3** were proved to be the known diterpene acids, lophanic acid [4] and 8(17),12,14-labdatriene-19-oic acid [5–8], respectively, as their physical and/or spectral data were in good agreement with those published in the literature.

The revised ¹³C-NMR data of compound **2** obtained from the ¹H-¹H COSY, HMQC and HMBC experiments are given in Table 1, since the signals corresponding to C-1, C-2, C-6 and C-14 were erroneously attributed to δ 40.4, 18.9, 20.9 and 35.3, respectively [4].

Tested in vitro against K562 cells [9], compounds **1–3** did not show any significant anti-tumour activity.

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