

TRITERPENOIDS OF *ISODON LOXOTHYRSUS*

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(Received in revised form 8 February 1996)

Key Word Index—*Isodon loxothyrsus*; Labiatae; triterpenoid; ursane-type skeleton; diterpenoid.

Abstract—A new triterpenoid, 3 β ,13 β -dihydroxy-urs-11-en-28-oic acid, along with four known triterpenoids, oleanolic acid, ursolic acid, 2 α -hydroxy ursolic acid and 2 α ,19 α -dihydroxy ursolic acid, two known diterpenoids, rabdoloxin B and rabdokunmin D, were isolated and characterized from the aerial parts of *Isodon loxothyrsus*. The structures of these compounds were elucidated by spectral analyses. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Several cytotoxic diterpenoids having either the *ent*-kaurene or the 6,7-*seco-ent*-kaurene skeleton have been isolated from *Isodon loxothyrsus* [1, 2]. In the course of our systematic studies on the biologically active substances from *Isodon* plants, we investigated the chemical constituents of the title plant collected at Lijiang county, Yunnan province of China. The EtOAc-soluble part of the extract of the aerial parts has led to the isolation of one new triterpenoid (**1**), four known triterpenoids and two known diterpenoids. The known compounds, oleanolic acid (**2**) [3], ursolic acid (**3**) [4], 2 α -hydroxy ursolic acid (**4**) [5], 2 α ,19 α -dihydroxy ursolic acid (**5**) [6], rabdoloxin B (**6**) [1] and rabdokunmin D (**7**), were identified by comparison with authentic samples or literature data. In this paper, we report on the structural elucidation of the new compound (**1**).

RESULTS AND DISCUSSION

Compound **1** was shown to have the molecular formula C₃₀H₄₈O₄, by EI- and FAB-mass spectra, implying seven degrees of unsaturation. Absorptions for hydroxyl (3520, 3500 cm⁻¹) and carbonyl (1740 cm⁻¹) groups were observed in its IR spectrum. The ¹H NMR spectrum contained the signals for seven skeletal methyl groups, of which five were singlets (δ 0.84, 1.01, 1.19, 1.22 and 1.24) and two were doublets (δ 0.83, 0.97). These data, coupled with the presence of 30 carbon atom signals in its ¹³C NMR spectrum (Table 1) suggested that **1** was an ursane-type triterpenoid.

The ¹H NMR spectrum of **1** further revealed a secondary hydroxyl group (δ 3.44, 1H, *dd*, *J* = 9.0,

7.3 Hz), whose chemical shift and splitting pattern were typical of a 3 β -equatorial hydroxy in a conventional ursane-type triterpenoid nucleus [8, 9], and two olefinic protons which resonated at δ 5.65 (*dd*, *J* = 10.4, 3.0 Hz) and 6.03 (*br d*, *J* = 10.4 Hz). Placement of this olefinic function at C-11 and C-12 was established by analogy with that of the known C-I, previously isolated from the bark of *Pieris japonica* D. Don [10]. The ¹H and ¹³C NMR spectra of **1** were remarkably similar to those of C-I, suggesting a close similarity in the structure of these two compounds. The ¹H NMR spectrum of **1** differed from that of C-I in having a hydroxy at C-3 instead of an acetoxy group. There were also differences in the IR spectra. Furthermore, the EI-mass spectrum of **1** gave the molecular ion peak [M]⁺ at *m/z* 472, i.e. 18 amu higher than the corresponding 3 β -hydroxy-urs-11-en-13(28)-lactone [8], and thus having one degree less unsaturation.

Table 1. ¹³C NMR chemical shifts and assignments for compound **1**

C		C	
1	38.7, <i>t</i>	16	25.9, <i>t</i>
2	23.2, <i>t</i>	17	45.2, <i>s</i>
3	78.0, <i>d</i>	18	60.6, <i>d</i>
4	39.6, <i>s</i>	19	38.7, <i>d</i>
5	55.1, <i>d</i>	20	40.4, <i>d</i>
6	18.0, <i>t</i>	21	31.6, <i>t</i>
7	31.0, <i>t</i>	22	32.0, <i>t</i>
8	42.1, <i>s</i>	23	28.4, <i>q</i>
9	53.4, <i>d</i>	24	16.0, <i>q</i>
10	36.7, <i>s</i>	25	16.2, <i>q</i>
11	133.7, <i>s</i>	26	19.2, <i>q</i>
12	129.4, <i>d</i>	27	18.3, <i>q</i>
13	89.4, <i>s</i>	28	179.3, <i>s</i>
14	42.3, <i>s</i>	29	18.2, <i>q</i>
15	27.9, <i>t</i>	30	19.4, <i>q</i>

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These findings substantiated that the γ -lactone moiety, as in C-I and 3 β -hydroxy-urs-11-en-13(28)-lactone, was opened in compound **1** to give a free 13-hydroxy and a free 28-carboxy group, consistent with the seven degrees of unsaturation required by the molecular formula. From the above analyses, it follows that the new compound is 3 β ,13 β -dihydroxy-urs-11-en-28-oic acid (**1**).

The new triterpenoid (**1**) is also the first example isolated from the *Isodon* genus of a triterpenoid possessing an ursane-type skeleton with free 13 β -hydroxy and 28-carboxy groups and a double bond in the 11,12-position.

EXPERIMENTAL

General. Mps: uncorr; NMR: 400 MHz (^1H) and 100 MHz (^{13}C , DEPT) using TMS as int. standard; FAB-MS and EIMS: ZAB-HS mass spectrometer.

Plant material. *Isodon loxothyrsus* (Hand.-Mazz) Hara was collected in October 1993 at Lijiang, Yunnan, P.R. China. The species was authenticated by Prof. Li Xiwen, Kunming Institute of Botany, Academia Sinica, where a voucher specimen is deposited.

Extraction and isolation. Dried and finely powdered aerial parts of *I. loxothyrsus* (2.4 kg) were extracted with hot EtOH (5×31) for 10 days. Filtration and evapn of the solvent yielded 620 g of residue which was dissolved in EtOH–H₂O (1:9) and partitioned with petrol, EtOAc and *n*-BuOH to give frs P (80 g), E (190 g) and N (190 g), respectively. Fr. E was chromatographed over silica gel (2 kg), using petrol with increasing proportions of CHCl₃, CHCl₃–Me₂CO and Me₂CO, to give **1** (50 mg), oleanolic acid (**2**, 1 g), ursolic acid (**3**, 100 mg), 2 α -hydroxy ursolic acid (**4**, 50 mg), 2 α , 19 α -dihydroxy ursolic acid (**5**, 80 mg), rabdoloxin A (**6**, 65 mg) and rabdokunmi D (**7**, 50 mg). Compounds **2**–**7** were identified by direct comparison with authentic samples through mixed mp., TLC, IR and ^1H NMR determinations.

Compound 1. Needles, mp 238°. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3520, 3500, 2980, 2850, 1740, 1450, 1380 and 1350; ^1H NMR (C₅D₅N): δ 0.83 (3H, *d*, *J* = 6 Hz, 30-H),

0.84 (3H, *s*, 23-H), 0.97 (3H, *d*, *J* = 6 Hz, 29-H), 1.01 (3H, *s*, 24-H), 1.19 (3H, *s*, 25-H), 1.22 (3H, *s*, 26-H), 1.24 (3H, *s*, 27-H), 3.44 (1H, *dd*, *J* = 90, 7.3 Hz, 3 α -H), 5.65 (1H, *dd*, *J* = 10.4, 3.0 Hz, 11-H), 6.01 (1H, *d*, *J* = 10.4 Hz, 12-H); ^{13}C NMR (C₅D₅N): Table 1; EI-MS (70 eV) *m/z*: 472 [M]⁺ (8), 454 (66), 439 (10), 426 (44), 410 (100), 340 (16), 300 (24), 290 (32), 255 (22), 215 (43), 201 (51), 189 (46), 131 (50), 105 (60), 91 (58), 81 (64), 65 (12), 57 (90); FAB-MS (neg.) *m/z*: 471 [M – H] (45), 454 (100), 439 (12), 409 (15).

Acknowledgements—The authors are grateful to the analytical group of the Laboratory of Phytochemistry, KIB, for the spectral measurements and to Mr Lin Zhong-wen, Laboratory of Phytochemistry, KIB, for the collection of the plant material.

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