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A NEW TRITERPENOID ESTER FROM *Lobelia sessilifolia*

Jiaming Sun,[1] Xiuli Wang,[2] Hui Zhang,[1*] and Junshan Yang[3*]

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*One novel triterpenoid ester, oleanol 28-aldehyde 3-*O*-β-palmitate (**1**), was isolated from the aerial part of* Lobelia sessilifolia *Lamb. along with seven known compounds, namely two triterpenoid esters (**2**, **3**), one sterol (**4**), two coumarins (**5**, **6**), and two triterpenes (7, **8**). The structure of **1** was established on the basis of spectroscopic and chemical data.*

Keywords: *Lobelia sessilifolia*, triterpenoid ester, oleanol 28-aldehyde 3-*O*-*β*-palmitate.

Lobelia is a genus of about 380 species, several members of which are known for their medicinal application in Chinese folklore. About 20 species of *Lobelia* have been reported to occur in China [1]. It is used in Chinese medicine for the treatment of phlegm, cough, cirrhosis ascites, abscess, and snakebite [2]. Chemical studies on the genus have been reported and have revealed the presence of piperidine alkaloids, polyacetylenes, triterpenes, and flavonoid glucosides [3–6].

To search for biologically active compounds, we studied the aerial part of *Lobelia sessilifolia* Lamb. This paper reports the isolation and structural elucidation of one novel triterpenoid ester, oleanol 28-aldehyde 3-*O*-*β*-palmitate (**1**), along with seven known compounds, namely *β*-amyrin 3-*O*-*β*-palmitate (**2**), maniladiol 3-*O*-*β*-palmitate (**3**), stigmasterol (**4**), limettin (**5**), scoparone (**6**), oleanolic acid (**7**), and ursolic acid (**8**). All of these known compounds were isolated from *L. sessilifolia* for the first time.

Compound **1** was obtained as a colorless powder. Its molecular formula was established as $C_{46}H_{78}O_3$ by HR-EI-MS at *m/z* 678.5958 $[M]^+$ (calcd 678.5951). The IR absorption bands at 1730 and 1146 cm^{-1} were consistent with the presence of carboxyl and ester groups, respectively. The ^{13}C NMR spectrum of **1** displayed 46 carbon signals, of which 30 were assigned to the triterpene. In the ^{13}C NMR spectrum, seven methyl carbons at δ 15.4, 16.8, 17.0, 22.1, 23.4, 28.1, and 33.3, as well as two olefinic carbons at δ 123.2 and 143.0, coupled with information from the ^1H NMR spectrum (seven tertiary methyl groups at δ 0.93, 0.86, 0.92, 1.14, 0.92, 0.85, and 0.86, one olefinic proton at δ 5.34, and one hydroxymethyl proton at δ 4.49) (Table 1), indicated that **1** had a 3-hydroxyolean-12-ene skeleton. Furthermore, the NMR spectra showed one aldehyde group at δ_H 9.40 and the corresponding carbon δ_C 207.5 (C-28). These signal patterns were similar to those of oleanol 28-aldehyde 3-*O*-*β*-acetate except for the fatty acid moiety [7]. Alkaline hydrolysis of **1** afforded a triterpene aldehyde and a fatty acid. The fatty acid was esterified with methanol and analyzed as a methyl ester using GC-MS and identified as palmitic acid. The position of the palmitic acid residue was elucidated by the heteronuclear multibond correlation (HMBC) spectrum. The proton of the esterified position at δ_H 4.49 (H-3) was correlated with the esteric carbonyl carbon at δ_C 173.7; thus palmitic acid was linked at 3-OH of oleanol 28-aldehyde through an ester bond. The fragment pathway of compound **1** showed the existence of oleanol 28-aldehyde and palmitic acid, which was analyzed by EI-MS (Fig. 1). On the basis of the above evidence, compound **1** was determined to be oleanol 28-aldehyde *3-O-β*-palmitate.

1) Development Center of Traditional Chinese Medicine and Bioengineering, Changchun University of Chinese Medicine, 130117, Changchun, P. R. China, fax: +86 0431 86172080, e-mail: zhanghui-8080@163.com; 2) Development Center of Daxinganling Vocational College, 165000, Jiagedaqi, P. R. China; 3) Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, 100094, Beijing, P. R. China, e-mail: junshanyang@hotmail.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 376–378. Original article submitted April 21, 2011.

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TABLE 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
1	0.91 (1H, m); 1.44 (1H[a])	33.2	20	–	30.6
2	1.88 (1H[a]); 1.98 (1H, m)	23.6	21	1.36 (1H, m); 1.89 (1H, td, J = 12.6, 4.3)	32.7
3	4.49 (1H, t, J = 8.0)	80.5	22	1.38 (2H, m)	38.2
4	–	37.7	23	0.85 (3H, s)	28.1
5	1.83 (1H, d, J = 2.9)	55.3	24	0.86 (3H, s)	16.8
6	1.34 (1H, m); 1.50 (1H[a])	18.2	25	0.93 (3H, s)	15.4
7	1.50 (1H[a]); 1.58 (1H[a])	34.8	26	0.92 (3H, s)	17.0
8	–	39.6	27	1.14 (3H, s)	22.1
9	2.62 (1H, dd, J = 9.6, 2.0)	47.5	28	9.40 (1H, s)	207.5
10	–	36.9	29	0.86 (3H, s)	33.3
11	1.88 (2H, m)	23.4	30	0.91 (3H, s)	23.4
12	5.34 (1H, br.s)	123.2	1′	–	173.7
13	–	143.0	2′	2.29 (2H, t, J = 7.2, 7.6)	34.9
14	–	41.7	3′	1.19 (2H, m)	25.2
15	1.86 (1H, m); 2.31 (1H[a])	27.7	4′–13′	1.25 (20H, br.s)	29.3
16	1.01 (1H, m); 1.96 (1H, m)	26.7	14′	1.22 (2H, m)	29.7
17	–	33.1	15′	1.18 (2H, m)	31.9
18	2.01 (1H, m)	47.5	16′	0.88 (1H, t, J = 6.8)	22.7
19	1.77 (1H, br.s); 1.30 (1H[a])	45.6			14.1

[a]Overlapped signals.

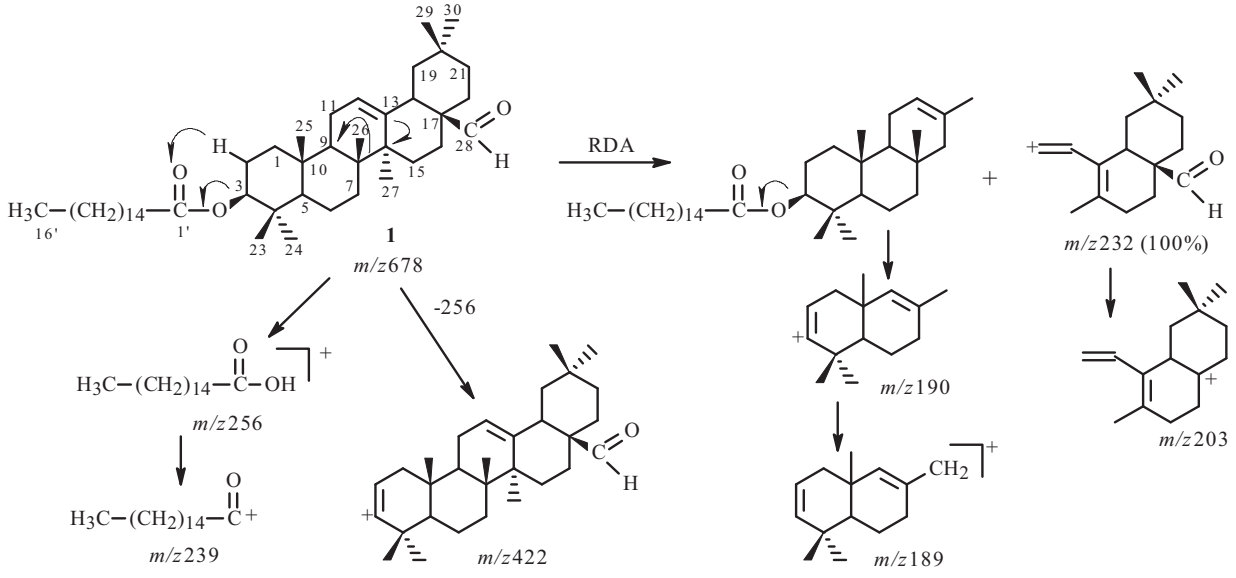

Fig. 1. EI-MS Fragment pathway of compound **1**.

EXPERIMENTAL

General Comments. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 983G spectrometer. NMR spectra were measured in DMSO-d_6 on a Bruker AM-500 spectrometer using TMS as internal standard. NMR experiments included the HMQC and HMBC pulse sequences. Coupling constants (J values) are given in Hz. An Autospec Ultima ETOF spectrometer was used to record the EI-MS and HR-EI-MS. An Agilent 6890N-5973 GC-MS system was used to analyze the structure of the methyl ester. Column chromatography was performed on silica gel H (10–40 μm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People′s Republic of China) and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, U.S.A.)

Plant Material and Extraction and Isolation. The aerial parts of *Lobelia sessilifolia* Lamb. were collected in Yanji City, Jilin Province of People′s Republic of China, in August 2009 and authenticated by Prof. Minglu Deng of Changchun University of Chinese Medicine. A voucher specimen (CC-09-0825) was deposited at the Herbarium of Changchun University of Chinese Medicine. The aerial parts (3.8 kg) of *Lobelia sessilifolia* Lamb. were shade-dried, ground, and extracted with refluxing 95% EtOH successively (57 L, 2 h, 3 times). The EtOH extract was evaporated *in vacuo* to yield a semisolid (970 g), 700 g of which was column chromatographed over silica gel using petroleum ether and EtOAc step gradient as eluents to give several main fractions: A (125.7 g), B (85.3 g), C (21.2 g), D (12.7 g), and E (40.4 g). Fraction B was purified individually by repeated column cromatography over silica gel to yield **1** (12 mg), **2** (442 mg), and **3** (8 mg). Fractions C and D were purified successively with petroleum ether and EtOAc (8:2) over silica gel to afford **4** (10 mg), **5** (16 mg), and **6** (21 mg). Compounds **7** (11 mg) and **8** (15 mg) were isolated and purified from fraction E over Sephadex LH-20 with $CHCl_3$ and MeOH (1:1).

Hydrolysis of Compound 1. Hydrolysis of the fatty acid esters of the mixtures of **1** (1 mg) was performed with 5% KOH in MeOH (1.5 mL) under reflux for 2 h. Methyl ester derivatives of the fatty acid were prepared by the refluxing fatty acid with 1% H_2SO_4 in methanol (1.5 mL) for 1 h. The methyl ester was analyzed with GC-MS.

Gas Chromatography-Mass Spectrometry. The GC-MS analyses were carried out using an Agilent 6890N-5973 GC-MS system operating on electron impact mode (equipped with an HP 5ms capillary column 30 m × 0.25 mm, 0.25 mm film thickness). He (1.0 mL/min) was used as carrier gas. The initial temperature of the column was 60°C, and then it was heated to 260°C at a rate of 3°C/min. The identification of the fatty acid esters was based on comparison of its EI-mass spectra with the NIST/NBS and Wiley library spectra.

Oleanol 28-Aldehyde 3-*O*-*β*-Palmitate (1). $C_{46}H_{78}O_3$, white powder ($CHCl_3$); mp 85–86°C. IR (KBr, ν_{max}, cm^{-1}): 2891, 2852, 1730, 1468, 1367, 1248, 1220, 1196, 1175, 1146, 987. EI-MS *m/z*: 678 $[M]^+$, 422 (M – 256), 256, 239, 232, 203, 190, 189, 175, 135, 121, 95, 69, 57; HR-EI-MS *m/z* 678.7539 $[M]^+$ (calcd 678.7548 $[M]^+$). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm) and ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm), see in Table 1.

***β*-Amyrin 3-*O*-*β*-Palmitate (2)**. White powder ($CHCl_3$); mp 81–82°C. EI-MS *m/z* 664 $[M]^+$ [6].

Maniladiol 3-*O*-*β*-Palmitate (3). White powder ($CHCl_3$); mp 88–89°C. EI-MS *m/z* 680 $[M]^+$ [8].

Stigmasterol (4). White crystalline needles (MeOH); mp 178–180°C. EI-MS *m/z* 412 $[M]^+$ [9].

Limettin (5). White crystalline needles ($CHCl_3$); mp 147–148°C. FAB-MS *m/z* 206 $[M]^+$ [10].

Scoparone (6). White crystalline needles ($CHCl_3$); mp 144–145°C. EI-MS *m/z* 206 $[M]^+$ [11].

Oleanolic Acid (7). Colorless crystalline needles ($CHCl_3$); mp 291–292°C. EI-MS *m/z* 456 $[M]^+$ [12].

Ursolic Acid (8). Colorless crystalline needles ($CHCl_3$); mp 286–288°C. EI-MS *m/z* 456 $[M]^+$ [13].

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