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Fitoterapia 74 (2003) 459–463

FITOTERAPIA

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Eudesmane derivatives from *Laggera pterodonta*

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Received 2 November 2002; accepted in revised form 18 April 2003

Abstract

A new eudesmane derivative, 4α,5α-dihydroxyeudesma-11(13)-en-12-oic acid, was isolated from *Laggera pterodonta*. Its structure was elucidated as (**1**) on the basis of spectroscopic evidence. Moreover, six known compounds were isolated: pterodontriol A (**2**), pterodontriol B, pterodonta acid (**3**), ilicic acid (**4**), costic acid and β-amyrin. Cytotoxic activity of compounds **2**, **3** and **4** was investigated.

Keywords: *Laggera pterodonta*; 4α,5α-Dihydroxyeudesma-11(13)-en-12-oic acid; Cytotoxic activity

1. Introduction

Laggera pterodonta is widely distributed in the South-West of China, especially in Yunnan. It has been traditionally used for its anti-inflammatory and antibacterial properties. In addition, *L. pterodonta* was reported to possess anti-leukemia activity [1]. Previous investigations of the EtOAc and BuOH extracts led to the conclusion that *L. pterodonta* is rich in eudesmane sesquiterpenes and eudesmane glycosides [2,3]. In the present study, we report an investigation of the petroleum ether extract of *L. pterodonta*.

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doi:10.1016/S0367-326X(03)00106-0

2. Experimental

2.1. General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were measured with Perkin-Elmer 577 spectrophotometer. MS were obtained with a Finnigan-4510 mass spectrometer using the EI mode. Optical rotations were measured with Perkin-Elmer 241 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-400 MHz spectrometer. Two-dimensional-NMR spectra were recorded with a Bruker AM-500 MHz spectrometer. TLC analysis was carried out on precoated Si-gel plates (Merck Kieselgel F_{254}).

2.2. Plant material

L. pterodonta (DC.) Benth. (Compositae), collected in November 1994 at Qiubei County, Yunnan Province of China, was identified by Prof. Zhong-Wen Lin. A voucher specimen (No. 9411122 CLD) was deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China.

2.3. Extraction and isolation

Powdered aerial parts of *L. pterodonta* (10.2 kg, dw) were extracted with 95% EtOH. The residue (486 g), obtained by removal of the solvent in vacuo, extracted with petrol ether gave a crude extract (108 g). A portion (98 g) of crude extract was Si-gel CC eluting with petroleum ether/acetone in increasing proportions. Eight crude fractions were collected (TLC monitoring). The fifth fraction was Sephadex LH-20-CC eluting with acetone. After concentration in vacuo, the residue was further purified by Si-gel CC eluting with i-PrOH/$CHCl_3$ gradient (1:20 to 1:5) and crystallized from aq MeOH to yield compound **1** (38 mg).

4α,5α-Dihydroxyeudesma-11(13)-en-12-oic acid (**1**): $C_{15}H_{24}O_4$, colorless needles, mp 183–185 °C (MeOH); $[\alpha]_D^{25}$ +13.4° (c 0.38, MeOH); IR bands (KBr): 3600, 3550, 1730, 1635 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.87 (1H, *m*, H-1α), 1.42 (1H, *m*, H-1β), 1.90 (1H, *m*, H-2α), 1.84 (1H, *m*, H-2β), 1.88 (1H, *ddd*, *J* 13.5, 12.8, 4.2 Hz, H-3α), 1.40 (1H, *ddd*, *J* 13.5, 4.2, 4.5 Hz, H-3β), 1.62 (1H, *dd*, *J* 13.5, 4.5 Hz, H-6α), 1.57 (1H, *dd*, *J* 13.5, 12.0 Hz, H-6β), 2.90 (1H, *dddd*, *J* 12.0, 12.0, 4.5, 4.5 Hz, H-7α), 0.92 (1H, *m*, H-8α), 1.69 (1H, *m*, H-8β), 1.05 (1H, *m*, H-9α), 0.94 (1H, *m*, H-9β), 5.70 (1H, *bs*, H-13), 6.31 (1H, *bs*, H-13′), 1.17 (3H, *s*, H-14), 1.26 (3H, *s*, H-15); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 17.5 (C-1), 29.7 (C-2), 36.3 (C-3), 75.2 (C-4), 76.1 (C-5), 26.2 (C-6), 34.4 (C-7), 31.2 (C-8), 37.7 (C-9), 36.8 (C-10), 144.8 (C-11), 171.3 (C-12), 125.5 (C-13), 25.8 (C-14), 22.2 (C-15); EIMS *m/z*: 268 $[M]^+$ (13%), 250 (45), 232 (74), 192 (55), 180 (57), 111 (100), 84 (90), 70 (75), 55 (74).

2.4. Cells and treatment

Human oral epidermoid carcinoma cells (KB), human lung carcinoma epithelial cells (A549), human malignant melanona cells (SK-MEL) from ATCC, Rockville, USA. Cell culture flasks and 96 well microtitre plates from Nalge Nunc Int. (Naperville, IL, USA). Culture medium, other medium constituents and phosphate buffered saline (PBS) from Gibco BRL (Gaithersburg, MD, USA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pyruvic acid, β-nicotinamide adenine dinucleotide, reduced form (NADH) from Sigma (St. Louis, MO, USA). KB and SK-MEL were maintained in minimum essential medium (MEM). A549 were grown in Dulbecco's modified Eagle's medium (DMEM)/F-12 medium. All media were supplemented with 10% Fetal Bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Cultures were incubated at 37 °C in a 5% CO_2/95% air humidified atmosphere.

2.5. Cells proliferation assays

Assessment of cell viability was carried out using a modified method of Mosmann [4] based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). At the end of each incubation period, 20 μl of MTT (5 mg/ml in PBS) were added into each well and the cultures were further incubated for 4 h at 37 °C in a 5% CO_2/95% air humidified atmosphere. Then 100 μl of stop solution (10% SDS in 1:1, v/v isobutanol: 0.02 M) were added into the wells to lyse the cells and solubilize the formazan crystals formed. The microtitre plates were maintained in a dark, humidified chamber overnight. The formation of formazan was measured with a microtitre plate reader at 570 nm. Cell viability was estimated as the percentage absorbance of sample relative to control.

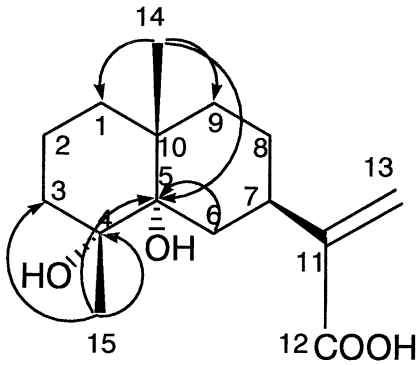

Fig. 1. Significant correlations in HMBC spectrum of **1**.

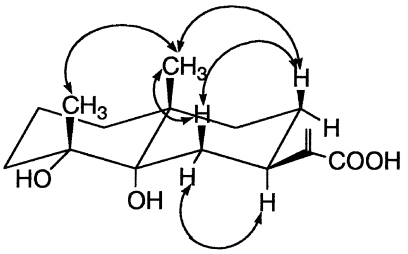

Fig. 2. Significant correlations in NOESY spectrum of **1**.

3. Results and discussion

A new compound 4α,5α-dihydroxyeudesma-11(13)-en-12-oic acid (**1**) was isolated as colorless needles. The EIMS and FABMS gave an $[M]^+$ ion at m/z 268 consistent with a molecular formula of $C_{15}H_{24}O_4$.

The ^{13}C-NMR spectrum revealed the presence of 15 carbons, including two methyl, six methylene, one methine, one double bond, three quaternary carbons and one C=O. Two oxygen-bearing quaternary carbon signals appearing at δ 75.2 and 76.1 ppm, along with two fragments due to the successive loss of water appearing in EIMS at m/z 250 and 232, suggested the presence of two hydroxy groups, further on, supported by the IR spectrum with bands at 3600 and 3500 cm^{-1}. Two protons at δ 5.70 and 6.31 attributable to an olefinic methylene together with the α,β-unsaturated carboxylic acid resonance at δ 171.7, indicated that **1** possesses a typical allylic acid moiety [5]. Both of the ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of ilicic acid [3]. However, the H-5 signal of the ilicic acid was absent in the ^{1}H-NMR spectrum of **1**. Instead, a quaternary carbon signal appeared at δ 76.1, which showed respective correlations with Me-14, Me-15 and H-6 in the HMBC spectrum (Fig. 1). Therefore, **1** is deduced to be a 5α-hydroxy-ilicic acid. The α-orientation of H-7 was deduced from the observed coupling constants at δ 2.90 (1H, *dddd*, *J* 12.0, 12.0, 4.5, 4.5 Hz), which agreed with an axial–axial and axial–equatorial pattern. Whereas the signal of 7β-H has been reported as a broad singlet [6]. The relative configurations of the substituents of **1** were revealed by the analysis of the NOESY spectrum (Fig. 2), and correlations of Me-14 with Me-15, H-6α with H-7α, H-6β with Me-14 and H-8β and H-8β with H-6β could be clearly

Table 1
Inhibition ratios of compounds **2**, **3** and **4** on KB, SK-MEL and A549 cell lines

Tumor cell lines	KB	SK-MEL	A549
Compound **2**[*]	12.4	10.9	0
Compound **3**[*]	5.0	1.6	6.8
Compound **4**[*]	7.0	23.6	10.3

[*] 20 μg/ml.

observed from the spectrum, suggesting that the A/B rings in the skeleton were *trans*-fused. Both hydroxy-groups located at C-4 and C-5 possess an α-orientation, and Me-14, Me-15 and allylic acid moiety at C-7 possesses a β-orientation. Thus, **1** was elucidated as 4α,5α-dihydroxyeudesma-11(13)-en-12-oic acid.

Pterodontriol A (**2**), pterodonta acid (**3**) and ilicic acid (**4**) were investigated for cytotoxic activities on three tumor cell lines. All compounds exhibited a weak cytotoxicity on KB, SK-MEL and A549 cell lines as reported in Table 1.

Acknowledgments

This work was financed by the Life Sciences Special Fund of the Chinese Academy of Sciences supported by the Chinese Ministry of Finance (STZ-00-24), the Yunnan Province Foundation of Applied Basic Research (2000C0072M), the opening foundation of KLPPRWC, KIB, CAS and the China–France PRA (BT01-02). The authors thank Christiana Gaspard (ISCN, CNRS) for helping measuring KB cell cytotoxicity, Prof. Dr Joachim Stöckigt (University of Mainz) for SK-MEL and A549 cell lines screening. We are grateful to Prof. Dr C.H.K. Cheng (The Chinese University of Hong Kong) for his comments and revision in improving this report.

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