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Two new 18-en-oleane derivatives from marine mangrove plant, *Barringtonia racemosa*

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Two new triterpenoids, olean-18-en-3 β -O-E-coumaroyl ester (**1**) and olean-18-en-3 β -O-Z-coumaroyl ester (**2**), were isolated from the stem bark of marine mangrove plant *Barringtonia racemosa*, along with five known compounds, germanicol, germanicone, betulinic acid, lupeol, and taraxerol. Their structures were determined mainly by spectroscopic methods.

1. Introduction

Barringtonia (Lecythidaceae) is a genus of medicinal mangrove plants widely distributed in the mangrove forest of Southeastern Asia, such as South China and India (Huang et al. 2004; Hasan et al. 2000; Khan et al. 2001; Thomas et al. 2002). Indian used the seeds of *B. racemosa* as a folk medicine to treat certain tumours (Thomas et al. 2002). The roots of *B. racemosa* are used to treat cinchona, and the fruits are effective in cough, asthma, and diarrhea (Huang et al. 2004). The seeds and bark of this plant are used as fish poison, and the extracts of the seeds were tested for their anti-Dalton's Lymphoma Ascitic (anti-DLA) activity in mice (Khan et al. 2001; Thomas et al. 2002). Previous chemical investigation on the plant *B. racemosa* indicated that it mainly contained polyhydroxylated triterpenoids, saponins, and diterpenoids (Hasan et al. 2000). In the course of examination of the secondary metabolites of this plant growing in the coastline of South China, the stem bark of *B. racemosa* at the mangrove garden in Hainan Island was investigated. In this paper, structure elucidation of two new compounds is reported.

2. Investigations, results and discussion

From the EtOH extract, seven triterpenoids were isolated, five of which were identified as germanicol (Gonzalez et al. 1981), germanicone (Gonzalez et al. 1981), betulinic acid (Siddiqui et al. 1988), lupeol (Yan et al. 2001) and taraxerol (Laphookhieo et al. 2004), and two were determined as new olean-18-ene derivatives, olean-18-en-3 β -O-E-coumaroyl ester (**1**) and olean-18-en-3 β -O-Z-coumaroyl ester (**2**).

Compound **1** was obtained as a white solid, and its molecular formula, C₃₉H₅₆O₃, was determined on the basis of the HRFABMS. The IR absorptions at 3368, 1708, 1681, 1632, and 1605 cm⁻¹ suggested the presence of hydroxyl, unsaturated carbonyl, and aromatic groups. The ¹H NMR

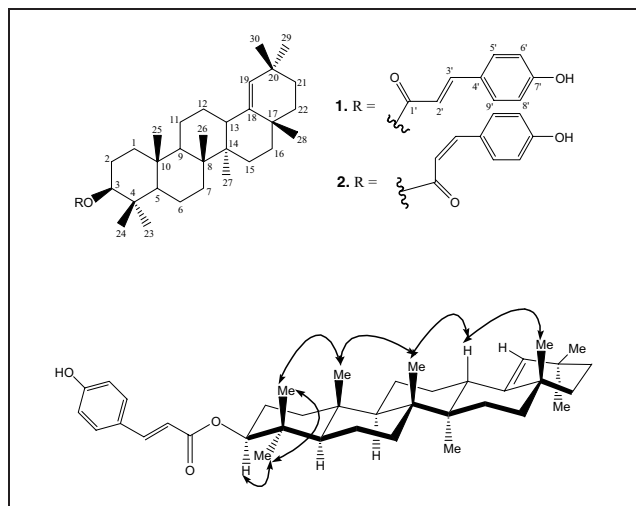
spectrum showed eight methyl singlets at δ 0.77 (3H, s), 0.92 (3H, s), 0.94 (3H, s), 0.95 (3H, s), 0.96 (3H, s), 0.97 (3H, s), 1.05 (3H, s), and 1.10 (3H, s), an oxygenated methine at δ 4.65 (1H, dd, J = 6.0, 11.5 Hz), an olefinic singlet at δ 4.89 (1H, s), two *trans*-coupled olefinic protons at δ 6.34 (1H, d, J = 16.0 Hz) and 7.64 (1H, d, J = 16.0 Hz), and the 1,4-disubstituted aromatic protons at δ 6.87 (2H, d, J = 8.5 Hz) and 7.46 (2H, d, J = 8.5 Hz). The ¹³C NMR spectrum exhibited 39 signals, attributable to eight methyls, ten methylenes, eleven methines and ten quaternary carbons, as determined by a DEPT experiment. The HMQC spectrum allowed to assign protons and the protonated carbons in the molecule (Table). The ¹H NMR data in association with the carbon signals at δ 167.3 (s, C-1'), 116.4 (d, C-2'), 144.0 (d, C-3'), 127.4 (s, C-4'), 129.9 (d, C-5', 9'), 115.8 (d, C-6', 8'), and 157.6 (s, C-7') indicated the presence of a *trans*-coumaroyl moiety, which was further supported by HMBC correlations. The remaining ¹H and ¹³C NMR signals were attributed to an olean-18-ene by HMQC and HMBC analysis, and by directly comparison of the NMR data of **1** with those reported (Gonzalez et al. 1981, 1989; Majumder et al. 1979). The HMBC correlations between the methyl protons at δ 0.96 (3H, s, H-29) and 0.97 (3H, s, H-30) and the carbons at δ 129.8 (d, C-19), 32.4 (s, C-20), and 33.4 (t, C-21), between the methyl protons at δ 1.05 (3H, s, H-28) and δ 142.7 (s, C-18), between the methyl protons at δ 0.92 (3H, s, H-23), 0.94 (3H, s, H-24) and δ 81.0 (d, C-3), confirmed the positions of double bond at C-18 and oxygenated methine at C-3. The coumaroyl unit was determined to form an ester with OH-3 on the basis of HMBC correlation between H-3 and the carbonyl carbon at δ 167.3 (s). The *trans*-conjunctions of the pentacyclic core and a α -configuration of H-3 were deduced through NOESY correlations as shown in the Fig. Accordingly, the structure of **1** was determined as olean-18-en-3 β -O-E-coumaroyl ester.

The molecular formula of **2** was the same as that of **1**, as determined by HRFABMS. The IR, ¹H and ¹³C NMR

Table 1: ^1H and ^{13}C NMR data of **1** and **2**

	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	38.7 t	1.10 m, 1.76 m	38.7 t	1.10 m, 1.80 m
2	23.6 t	1.79 m, 1.72 m	23.7 t	1.70 m, 1.78 m
3	81.0 d	4.65 dd 6.0, 11.5	81.0 d	4.57 dd 5.0, 11.5
4	38.1 s		37.9 s	
5	55.6 d	0.86 m	55.7 d	0.85 m
6	18.2 t	1.38 m, 1.50 m	18.2 t	1.38 m, 1.55 m
7	34.6 t	1.40 m, 1.54 m	34.5 t	1.40 m, 1.52 m
8	40.8 s		40.8 s	
9	51.1 d	1.33 m	51.1 d	1.30 m
10	37.2 s		37.2 s	
11	21.2 t	0.94 m, 1.57 m	21.1 t	0.95 m, 1.59 m
12	26.2 t	1.30 m, 1.55 m	26.2 t	1.30 m, 1.53 m
13	38.4 d	2.30 brd 11.0	38.4 d	2.29 brd 12.0
14	43.4 s		43.3 s	
15	27.5 t	1.00 m, 1.10 m	27.5 t	1.02 m, 1.13 m
16	37.4 t	1.20 m, 1.40 m	37.4 t	1.18 m, 1.39 m
17	34.4 s		34.4 s	
18	142.7 s		142.7 s	
19	129.8 d	4.89 s	129.8 d	4.89 s
20	32.4 s		32.4 s	
21	33.4 t	1.32 m, 1.50 m	33.4 t	1.32 m, 1.50 m
22	37.7 t	1.20 m, 1.30 m	37.7 t	1.20 m, 1.30 m
23	28.0 q	0.92 s	28.0 q	0.90 s
24	15.5 q	0.94 s	16.1 q	0.95 s
25	16.1 q	0.95 s	16.5 q	0.96 s
26	16.7 q	1.10 s	16.8 q	1.11 s
27	14.6 q	0.77 s	14.6 q	0.76 s
28	25.3 q	1.05 s	25.3 q	1.03 s
29	31.4 q	0.96 s	31.4 q	0.96 s
30	29.2 q	0.97 s	29.2 q	0.97 s
1'	167.3 s		166.5 s	
2'	116.4 d	6.34 d 16.0	118.0 d	5.87 d 12.5
3'	144.0 d	7.64 d 16.0	143.0 d	6.86 d 12.5
4'	127.4 s		127.7 s	
5', 9'	129.9 d	7.46 d 8.5	132.3 d	7.67 d 8.5
6', 8'	115.8 d	6.87 d 8.5	114.9 d	6.82 d 8.5
7'	157.6 s		156.5 s	

spectra of **2** virtually resembled those of **1**, except for the proton signals assigned for H-2' and H-3' of coumaroyl moiety shifted to δ 5.87 (d, $J = 12.5$ Hz, H-2') and 6.86 (d, $J = 12.5$ Hz, H-3'). The coupling constant between H-2' and H-3' indicated a Z-geometry of the double bond. The structure of **2** was thus identified as olean-18-en-3 β -O-Z-coumaroyl ester.

Fig: Key NOE correlations of **1**

3. Experimental

3.1. General

Melting points were measured on a XT-4A micromelting point apparatus without correction. The IR spectra were determined on a Nexus 470 FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance-500 FT NMR spectrometer using TMS as internal standard. EIMS was performed with a Bruker APEII mass spectrometer, and HRFABMS data were recorded by using a VG Atospec spectrometer. Column chromatography was carried with silica gel (200–300 mesh), and HF254 silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, People's Republic of China.

3.2. Plant material

The specimen of *Barringtonia racemosa* was collected at the mangrove garden in Dongzhaigan of Hainan Island, People's Republic of China in 2002. The species was identified by Prof. Lin Peng of Xia Men University. A voucher specimen was deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

3.3. Extraction and isolation

The stem bark of *B. racemosa* (3.2 kg) were air-dried and then ground. The sample was percolated with 95% EtOH twice at room temperature and then extracted with hot 95% EtOH at 60 °C. Both extracts were combined and concentrated *in vacuo* to afford a black residue (300 g). The residue was dissolved in water and partitioned against petroleum ether, EtOAc, and n-BuOH, successively. The petroleum ether extract (29 g) was subjected to a silica gel column and eluted with petroleum ether-EtOAc as gradient to obtain six fractions A–F. Fraction C (4.3 g, 5:1) was subjected to a silica gel column and eluted with petroleum ether-EtOAc (10:1) to yield compounds **1** (12 mg) and **2** (8 mg). Fraction B (1.1 g, 10:1) was followed by the same method as fraction C over a silica gel column and eluted with petroleum ether-chloroform (10:1) to obtain germanicol (800 mg) and germanicone (150 mg). Fraction D (4.3 g, 2:1) was separated into four additional fractions (D-1, D-2, D-3, and D-4) on a silica gel column eluting with petroleum ether-EtOAc (5:1). D-3 (2.3 g) was further chromatographed on a silica gel column by eluting with petroleum ether-EtOAc (5:1) to afford taraxerol (50 mg), lupeol (30 mg) and betulinic acid (18 mg).

3.3.1. Olean-18-en-3 β -O-E-coumaroyl ester (**1**)

White solid, $[\alpha]_{\text{D}}^{20} + 71.15$ (c, 0.02, CHCl_3); UV (CHCl_3) λ_{max} 309, 298 nm; IR (KBr) ν_{max} 3368, 2932, 2859, 1708, 1681, 1632, 1605, 1514, 1451, 1263, 1167 cm^{-1} ; ^1H and ^{13}C NMR data, see Table; HRFABMS m/z 571.4164 $[\text{M}-1]^-$ (calcd for $\text{C}_{39}\text{H}_{55}\text{O}_3$, 571.4156).

3.3.2. Olean-18-en-3 β -O-Z-coumaroyl ester (**2**)

White solid, $[\alpha]_{\text{D}}^{20} + 44.62$ (c, 0.02, CHCl_3); UV (CHCl_3) λ_{max} 309, 298 nm; IR (KBr) ν_{max} 3369, 2925, 2854, 1707, 1680, 1632, 1605, 1514, 1460, 1265, 1166 cm^{-1} ; ^1H and ^{13}C NMR data, see Table; HRFABMS m/z 571.4168 $[\text{M}-1]^-$ (calcd for $\text{C}_{39}\text{H}_{55}\text{O}_3$, 571.4156).

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