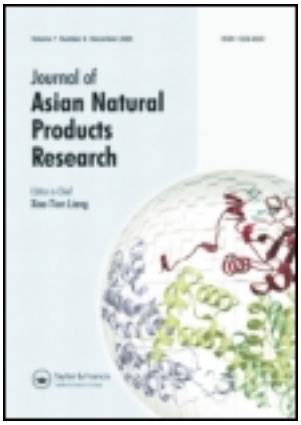

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A new diterpenoid from *Rhizophora apiculata*

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A new diterpenoid, 15(*S*)-isopimar-7-en-1-oxo-15,16-diol (**1**), was isolated from the stems of mangrove plant *Rhizophora apiculata*. The structure of the new compound was elucidated by MS, IR, 1D, and 2D NMR techniques, including HMQC, HMBC, and NOESY correlations. In addition, seven known constituents were isolated from this plant for the first time.

Keywords: *Rhizophora apiculata*; mangrove plant; diterpenoid

1. Introduction

Mangroves are composed of a large group of different salt-tolerant plants growing in tropical and subtropical intertidal estuarine areas. These plants, constantly subjected to tidal flushing with the ability to live in salt water, have specially adapted their own morphological structures and physiological mechanisms to the harsh natural surrounding [1]. In addition to the ecological and social–economic values, *Rhizophora apiculata*, a typical species of mangrove plants, has been used as an astringent for diarrhea, nausea, and vomiting, and as an sterilizing agent, deodorizer, and growth-promoting agent in China, India, Thailand, and Malaysia for ages [2,3]. It was reported that the extract of *R. apiculata* showed antioxidant and antiviral activities [3–6]. But only a few compounds such as triterpenes and phenolic compounds had been isolated from this plant [2,6]. To reveal the novel chemical constituent, a two-step preparative chromatography combining moderate resolution normal phase chromatography with high-resolution reverse-phase chromatography was applied to separate compounds from the extract of *R. apiculata*. A new diterpenoid compound, named 15(*S*)-isopimar-7-en-1-oxo-15,16-diol (**1**), was isolated from the stems of *R. apiculata*. Seven other known constituents, lupeol (**2**), ergosta-7,22-dien-3β-ol (**3**), methyl-ent-16β,17-dihydroxy-9(11)-kauren-19-oate (**4**), ent-12,17-epoxy-16β-hydroxy-9(11)-kauren-19-oate (**5**), methyl-ent-kaur-9(11)-ent-13,17-expoxy-16-hydroxy-19-oate (**6**), 16(*R*)-13,17-epoxy-16-hydroxy-ent-kaur-9(11)-en-19-al (**7**), and 13,16α,17-trihydroxy-ent-9(11)-kauren-19-oic acid (**8**), were isolated for the first time from the stems of *R. apiculata*.

2. Results and discussion

Compound **1** was separated as white powder and its molecular formula was determined as $C_{20}H_{32}O_3$ by means of

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HR-ESI-MS at *m/z* 321.2424 $[M + H]^+$. In the positive- and negative-ion ESI-MS of **1**, quasi-molecular ion peaks were observed at *m/z* 321 $[M + H]^+$ and 319 $[M - H]^-$, respectively. Other peaks at *m/z* 343 $[M + Na]^+$, 375 $[M + CH_3OH + Na]^+$, and 351 $[M + CH_3OH - H]^-$ were observed in the positive- and negative-ion ESI-MS of **1**. The major IR absorption bands indicated a double bond (1661 cm^{-1}), a carbonyl group (1716 cm^{-1}), and hydroxyl groups (3418 cm^{-1}). Unambiguous assignments for the ^1H and ^{13}C NMR (see Table 1) signals were completed with HMQC, HMBC, and NOESY experiments to confirm the configuration of compound **1**. The ^1H NMR spectrum showed signals for an olefinic proton at δ 5.45 (1H, m, H-7) on a triple-substituted double bond, an oxygenated methine at δ 3.71 (1H, dd, $J = 9.8, 2.9$ Hz, H-15), one oxygen-bearing methylene at δ 3.66 (1H, dd, $J = 10.9$, 2.9 Hz, H-16β) and 3.49 (1H, dd, $J = 10.9$, 9.8 Hz, H-16α), and four methyl groups at δ 0.83 (3H, s, H_3-17), 0.95 (3H, s, H_3-18), 1.13 (3H, s, H_3-19), and 1.14 (3H, s, H_3-20). ^{13}C NMR exhibited signals for a keto signal at δ 216.8 (C-1), two olefinic carbons at δ 121.0 (C-7) and 136.0 (C-8), two oxygenated carbons at δ 72.8 (C-15) and 62.4 (C-16). The ^1H and ^{13}C NMR spectral data were very close to those of 15(*S*)-isopimar-7-en-15,16-diol [7], with the exception of the keto carbon at C-1 which could be confirmed by the HMBC correlations between C-1 and H_2-2 at δ 2.76 and 2.16, H_2-3 at δ 1.77 and 1.66, and H_3-20 (see Figure 1). The location of carbon–carbon double bond was deduced to be at C-7 and C-8 based on HMBC correlations between H-7 and C-5 at δ 52.5, C-6 at δ 23.4, C-9 at δ 44.0, and C-14 at δ 45.8. The relative configuration of **1** was determined by the NOESY experiment and comparison of its NMR spectral data with those of 15(*S*)-isopimar-7-en-15,16-diol. The NOESY correlations between H-15 and H-12α at δ 1.55, H_3-18, and H_3-20 revealed H-15, H_3-18, and H_3-20 at α orientation. The correlations between H-9 at δ 2.41 and H-5 at δ 1.52, H-12β at δ 1.38, and H-14β at δ 2.16 indicated H-5 and H-9 at β-orientation. The absolute configuration of asymmetric center of C-15 was determined as *S* due to the chemical

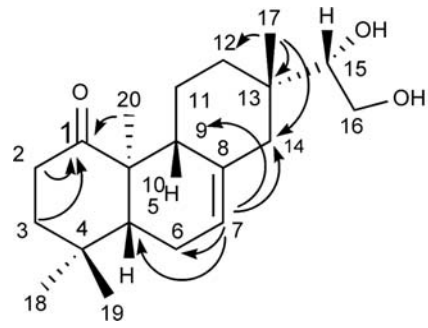

Figure 1. Structure and key HMBC correlations of compound **1**.

Table 1. ^1H and ^{13}C NMR spectral data of **1** (recorded at 500/125 MHz in $CDCl_3$, *J* in Hz).

Position	δ_H	δ_C
1		216.8
2	α 2.76 (1H, m)	35.9
	β 2.16 (1H, m)	
3	α 1.77 (1H, m)	42.4
	β 1.66 (1H, m)	
4		32.4
5	1.52 (1H, m)	52.5
6	α 2.08 (1H, m)	23.4
	β 1.92 (1H, m)	
7	5.45 (1H, m)	121.0
8		136.0
9	2.41 (1H, m)	44.0
10		49.4
11	α 1.96 (1H, m)	22.1
	β 1.86 (1H, m)	
12	α 1.55 (1H, m)	35.1
	β 1.38 (1H, m)	
13		36.8
14	α 2.76 (1H, m)	45.8
	β 2.16 (1H, m)	
15	3.71 (1H, dd, 9.8, 2.9)	72.8
16	α 3.49 (1H, dd, 10.9, 9.8)	62.4
	β 3.66 (1H, dd, 10.9, 2.9)	
17	0.83 (3H, s)	22.7
18	0.95 (3H, s)	32.8
19	1.13 (3H, s)	22.7
20	1.14 (3H, s)	14.3

shift of C-15 at δ 72.8 whereas chemical shift at δ 78 indicated *R* configuration [7]. On the basis of all the above evidence, the structure of **1** was established as 15(*S*)-isopimar-7-en-1-oxo-15,16-diol.

Seven known constituents, lupeol (**2**) [8], ergosta-7,22-dien-3β-ol (**3**) [9], methyl-ent-16β,17-dihydroxy-9(11)-kauren-19-oate (**4**) [10], ent-12,17-epoxy-16β-hydroxy-9(11)-kauren-19-oate (**5**) [11], methyl-ent-kaur-9(11)-ent-13,17-expoxy-16-hydroxy-19-oate (**6**) [7], 16(*R*)-13,17-epoxy-16-hydroxy-ent-kaur-9(11)-en-19-al (**7**) [12] and 13,16α,17-trihydroxy-ent-9(11)-kauren-19-oic acid (**8**) [13], were identified by comparing their NMR spectral data with those reported in the literature. To our knowledge, this is the first report of compounds **2**–**8** from *R. apiculata.*

3. Experimental

3.1 General experimental procedures

Melting point was determined on an X-4 melting point apparatus and is uncorrected (Beijing Tech instrument, Beijing, China). Optical rotation was measured on Jasco-P-1010 spectrophotometer (Jasco, Tokyo, Japan). IR spectra were obtained on Perkin-Elmer 983G spectrometer (Perkin-Elmer, Tucson, AZ, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance-500 spectrometer (Bruker, Fallanden, Switzerland) using $CDCl_3$ as a solvent and TMS as an internal reference. HR-ESI-MS was recorded on Waters Q-TOF premier MS spectrometer (Waters, Milford, MA, USA) and ESI-MS was recorded using Finnigan TSQ instrument (Thermo, San Jose, CA, USA). SiO_2 (100–200 mesh, Qingdao Marine Chemical Ltd, Qingdao, China) was used for normal phase column chromatography. Reverse-phase preparative chromatography was recorded on Waters Delta Prep 4000 HPLC system equipped with Water 2996 diode array detector and Waters Nova-Pak ODS column (250 × 20 mm, 5 μm). HPLC grade methanol was purchased from Yuwang Chemical (Shandong, China) and other analytical grade chemical solvents were provided by Kemio Chemical (Tianjing, China).

3.2 Plant material

The stems of *R. apiculata* were collected from Hainan Province, China, in June 2009, and identified by Prof. Shi-Xiang Bao. A voucher specimen (No. 090601) has been deposited at Dalian Institute of Chemical Physics, Chinese Academy of Sciences. The collected materials were dried and stored in a dark room.

3.3 Extraction and isolation

The air-dried stems of *R. apiculata* (1 kg) were cut into small pieces and ultrasonically extracted with EtOAc (3 × 4 l). After removal of the solvent by rotating evaporation, the residue (3 g) was subjected to silica gel column chromatography and eluted with petroleum ether:EtOAc:MeOH gradient system from 100:0:0 to 0:0:100. Eighteen fractions (Fr. 1–18) were obtained according to the TLC analysis result. Preparative reverse-phase high-performance liquid chromatography was used for further separation of the fractions. Compounds **2** (12 mg) and **3** (8 mg) were obtained from Fr. 3 (120 mg) with 100% methanol eluting at 16 ml/min. Fr. 14 (95 mg) was separated with 75% methanol eluting at 16 ml/min to give compounds **1** (8 mg), **4** (5 mg), and **5** (3 mg). Fr. 17 (160 mg) afforded compounds **6** (6 mg), **7** (5 mg), and **8** (4 mg) with 70% methanol eluting at 12 ml/min.

3.3.1 Compound *1*

White amorphous solid, mp: 68–70°C; $[\alpha]_D^{20}$ 32.4 (*c* 0.48, CH_3Cl); IR (film): ν_{max} 3418, 2930, 2869, 2706, 1716, 1661, 1447 cm^{-1}; ^{1}H and ^{13}C NMR spectral data: see Table 1; ESI-MS: *m/z* 321 $[M + H]^+$, 343 $[M + Na]^+$, 375 [M +

$CH_3OH + Na]^+$, 319 $[M - H]^-$, $[M + CH_3OH - H]^-$; HR-ESI-MS: *m/z* 321.2424 (calcd for $C_{20}H_{33}O_3$, 321.2430).

3.4 Bioassay

Inhibition of cell growth activity was determined by an MTT assay using human alveolar basal epithelial cells (A549). *Cis*-Diaminedichloroplatinum was used as a positive control. None of these compounds showed remarkable cytotoxic activity against cancer cell A549 at the concentration of 40 μM.

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