SHORT REPORT

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Two New Dammarane Triterpenes from the Leaves of *Ceriops tagal*

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Abstract: Two new dammarane triterpenes, cereotagalol C (**1**) and cereotagalol D (**2**), were isolated from the leaves of *Ceriops tagal*. Their structures were elucidated by spectroscopic and mass-spectrometric analyses, including 1D-, 2D-NMR and HR-ESI-MS. Their cytotoxic activities against A549, HepG2, HCT-116 and CNE-2 cell lines were also evaluated, which revealed that compounds **1** and **2** had selected moderate cytotoxicity against HCT-116 and CNE-2 with IC_{50} values of 29.7 and 37.2 μM, respectively.

Keywords: Dammarane triterpenes; *Ceriops tagal*; spectroscopic analyses; cereotagalol C; cereotagalol D.

1. Plant Source

As a part of our continuing studies on anti-proliferative active natural products, we herein reported the isolation and structural elucidation of two new dammarane triterpenes, cereotagalol C (**1**) and cereotagalol D (**2**) (Figure 1), as well as their cytotoxic activities against A549, HepG2, HCT-116 and CNE-2 cell lines.

The leaves of *Ceriops tagal* were collected in Haikou City, Hainan Province of P. R. China, during May 2013, and authenticated by Prof. Weidong Han (College of Agriculture, Guangdong Ocean University). A voucher specimen (No. 20130513) was deposited in the Guangdong Key Laboratory for Research and Development of Natural Drugs, Guangdong Medical College, Zhanjiang, P. R. China.

2. Previous Studies

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The Ceriops genus belonging to the Rhizophoraceae family contains three species, namely *C. tagal*, *C. decandra*, and *C. australis* [1]. *C. tagal* is the only species of this genus mainly distributed in Hainan Island of China [2]. It has been used for the treatment of malaria in China [2], infected wounds in Thailand and obstetric and hemorrhagic conditions in the Philippines [3]. Previous phytochemical studies revealed that it was rich of diterpenoids and triterpenoids [4].

3. Present Study

The air dried leaves of *Ceriops tagal* (10.0 kg) were powdered and extracted three times with 95% EtOH at room temperature (3×30 L). The extracts were combined and then kept standing for 48 hours at room temperature to precipitate the tannins. The upper solution was concentrated under reduced pressure to yield the crude extract (800 g). The residue was suspended in water, and then partitioned with EtOAc. After removing the solvent, the EtOAc extract (300 g) was separated by D101 macroporous resin column chromatography (CC) using gradient ethanol aqueous solutions (60%, 80% and 95%) as eluent to give three fractions (A-C). Fraction B (80% ethanol, 100g) was then subjected to silica gel CC eluting with *n*-hexane-ethyl acetate (H:E 100:0→1:1) to yield seven subfractions B1-B7. Subfraction B4 (H:E 9:1, 7.0 g) was chromatographed on ODS column to afford eight subfractions B4a-B4h. B4e (CH_3CN:H_2O 6:4, 1.5 g) was purified by prepared HPLC to yield compound **2** (10 mg). Subfraction B5 (H:E 8:2, 5.0 g) was subjected to ODS CC with CH_3CN and H_2O mixture from 50:50 to 0:100 as elution to afford eleven subfractions B5a-B5k. The crystalline of compound **1** (1132.0 mg) was precipitated from the methanol solution of subfraction B5k (CH_3CN:H_2O 7:3, 2.1 g).

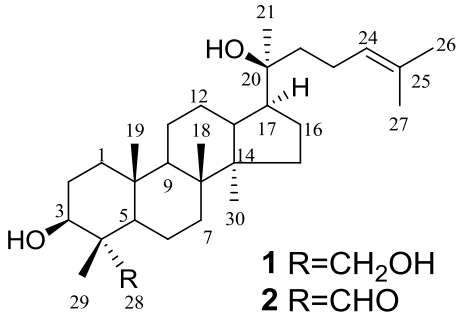

Figure 1. The structures of compounds **1** and **2**.

Cereotagalol C ***(1)****:* White powder; ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$): see Table 1; HRESIMS: *m/z* 483.3856 (calcd. 483.3809 for $C_{30}H_{52}O_3Na$).

Cereotagalol D ***(2)****:* White powder; ^1H and ^{13}C NMR (500 and 125 MHz, $CDCl_3$): see Table 1; HRESIMS: *m/z* 481.3692 (calcd. 481.3652 for $C_{30}H_{50}O_3Na$).

Cytotoxicity activity assay [5]*:* The cytotoxicity effects of compounds **1** and **2** were determined against the cancer cell lines A549, HepG2, HCT-116 and CNE-2 by the MTT method. The cell suspensions were plated into 96-well plates and cultured in RPMI-1640 at 37 °C, with 5% CO_2 in incubator overnight. The test compound solutions (in 0.1% DMSO) at different concentrations were added to the corresponding wells. After exposure for 68 h, MTT was added to each well and the plates were incubated for 4 h. Finally, the supernatant was discarded and 200 μL of DMSO was added to the well to dissolve the blue-violet crystal, then the optical density (OD) values were read on the microplate reader at 570 nm. All tests and analyses were carried out in triplicate. DMSO and doxorubicin were applied as the blank control and positive control, respectively.

Table 1. NMR data of compounds **1** and **2** (at 500 or 125 MHz in $CDCl_3$, δ in ppm, J in Hz)[a].

position	**1**		**2**	
	δ_C	δ_H	δ_C	$\delta_{H,}$
1	38.7 (CH_2)	1.69 (1H, m), 0.98 (1H, m)	38.6 (CH_2)	1.76 (1H, m), 1.06 (1H, m)
2	26.9 (CH_2)	1.61 (1H, m)	26.4 (CH_2)	1.62 (1H, m)
3	76.6 (CH)	3.63 (1H, t, 8.5)	71.8 (CH)	3.76 (1H, dd, 11.7, 4.6)
4	41.9 (C)	---	55.5 (C)	---
5	50.6 (CH)	1.34 (1H, m)	48.9 (CH)	1.29 (1H, m)
6	18.3 (CH_2)	1.49 (1H, m), 1.32 (1H, m)	21.0 (CH_2)	1.49 (1H, m), 1.32 (1H, m)
7	34.9 (CH_2)	1.51 (1H, m), 1.25 (1H, m)	34.6 (CH_2)	1.57 (1H, m), 1.23 (1H, m)
8	40.3 (C)	---	40.8 (C)	---
9	50.4 (CH)	0.88 (1H, m)	50.6 (CH)	1.43 (1H, m)
10	36.9 (C)	---	36.1 (C)	---
11	21.5 (CH_2)	1.52 (1H, m), 1.25 (1H, m)	21.6 (CH_2)	1.53 (1H, m), 1.27 (1H, m)
12	24.7 (CH_2)	1.52 (1H, m), 1.44 (1H, m)	24.8 (CH_2)	1.52 (1H, m), 1.44 (1H, m)
13	42.2 (CH)	1.62 (1H, m)	42.2 (CH)	1.63 (1H, m)
14	50.3 (C)	---	50.3 (C)	---
15	31.1 (CH_2)	1.44 (1H, m), 1.05 (1H, m)	31.1 (CH_2)	1.44 (1H, m), 1.06 (1H, m)
16	27.5 (CH_2)	1.84 (1H, m), 1.24 (1H, m)	27.4 (CH_2)	1.84 (1H, m), 1.23 (1H, m)
17	49.8 (CH)	1.73 (1H, m)	49.8 (CH)	1.74 (1H, m)
18	15.5 (CH_3)	0.96 (3H, s)	15.5 (CH_3)	0.96 (3H, s)
19	16.4 (CH_3)	0.88 (3H, s)	16.4 (CH_3)	0.89 (3H, s)
20	75.3 (C)	---	75.4 (C)	---
21	25.4 (CH_3)	1.14 (3H, s)	25.5 (CH_3)	1.14 (3H, s)
22	40.4 (CH_2)	1.47 (2H, t, 8.5)	40.4 (CH_2)	1.47 (2H, t, 8.5)
23	22.5 (CH_2)	2.05 (2H, m)	22.5 (CH_2)	2.05 (2H, m)
24	124.7 (CH)	5.11 (1H, m)	124.6 (CH)	5.12 (1H, m)
25	131.6 (C)	---	131.7 (C)	---
26	25.7 (CH_3)	1.62 (3H, d, 1.0)	25.7 (CH_3)	1.62 (3H, d, 1.0)
27	17.7 (CH_3)	1.68 (3H, d, 1.0)	17.7 (CH_3)	1.69 (3H, d, 1.0)
28	71.9 (CH_2)	3.71 (1H, d, 10.4) 3.42 (1H, d, 10.4)	207.0 (CH)	9.38 (1H, s)
29	11.3 (CH_3)	0.87 (3H, s)	8.6 (CH_3)	1.05 (3H, s)
30	16.5 (CH_3)	0.88 (3H, s)	16.4 (CH_3)	0.89 (3H, s)

[a] Assignments were established by interpretation of the ^{1}H-^{1}H COSY, HSQC, and HMBC spectra.

Compound **1** was obtained as colorless needle crystals by recrystallization in methanol. The molecular formula was established as $C_{30}H_{52}O_3$ by its HR-ESI-MS at *m/z* 483.3856 $[M+Na]^+$ (calcd for $C_{30}H_{52}O_3Na$: 483.3809). The ^{13}C NMR and DEPT-135 spectra of **1** showed 30 carbons including seven methyls, eleven methylenes, six methines, and six quaternary carbons. The ^{1}H NMR spectrum of **1** showed seven methyls at δ_H 0.87 (3H, s), 0.88 (3H, s), 0.88 (3H, s), 0.95 (3H, s), 1.13 (3H, s), 1.62 (3H, d, J = 1.0 Hz), 1.69 (3H, d, J = 1.0 Hz), a pair of oxy-methenyl protons at δ_H 3.42 (1H, d, J = 10.0 Hz) and 3.71 (1H, d, J = 10.0 Hz), a oxy-methine at δ_H 3.63 (1H, t, 8.5) and a alkene proton at δ_H 5.11 (1H, m). The above structural features revealed that compound **1** was a triterpenoid. The ^{13}C NMR and ^{1}H NMR spectra of compound **1** were very similar to those of cereotagalol A [6] except for the positions from 22 to 26, which suggested that their A/B/C/D rings were the same, while the side chain was different. With the aid of ^{1}H-^{1}H COSY, HSQC and HMBC experiments, all the ^{1}H and ^{13}C NMR signals of **1** were assigned as shown in Table 1. The side chain on C-17 of **1** was determined to

the same as dammarenediol-Ⅱ [7] by comparison their carbons data, which was further supported by the HMBC correlations between H-21 (δ_H 1.14) and C-17 (δ_C 49.8)/C-22 (δ_C 40.4), between H-23 (δ_H 2.05) and C-20 (δ_C 75.3)/C-25 (δ_C 131.6), as well as between H-24 (δ_H 5.11) and C-26 (δ_C 25.7)/C-27 (δ_C 17.7) (Fig. 2). The stereochemistry at C-20 was characterized as *S* configured by comparison of the carbon chemical shifts at C-21 and C-22 with the corresponding reported dammaranes [7,8], and the presence of ROESY correlation between H-17 (δ_H 1.73) and H-21 (δ_H 1.14). The equatorial (*β*-) orientation of the 3-OH group was deduced by the large coupling (8.5 Hz) of proton H-3 [8]. Therefore, the structure of **1** was elucidated as 20(*S*)-3*β*,20,28-trihydroxydammar-24-ene and named as cereotagalol C.

Compound **2** was isolated as an amorphous white powder. The molecular formula was established as $C_{30}H_{50}O_3$ by its HR-ESI-MS at *m/z* 481.3692 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3Na$: 481.3652). The ^{13}C NMR and ^1H NMR spectra of **2** strongly resembled those of **1**, except for the positions of 3/4/5/6/28/29. The presence of aldehyde group (δ_H 9.38, δ_C 207.0) in **2**, instead of oxymethylene group (δ_H 3.41/3.71, δ_C 71.9) in **1**, implied that the above distinctions could be induced by the oxydation of C-28, which was further confirmed by the HMBC correlations from H-29 (δ_H 1.05) to C-3 (δ_C 71.8)/C-5 (δ_C 48.9)/C-28 (δ_C 207.0) (Fig. 2). The *S* configuration of C-20 and equatorial (*β*-) orientation of H-3 in **2** were defined by the same method as **1** and the presence of ROESY correlation between H-17 (δ_H 1.74) and H-21 (δ_H 1.14). Hence, the structure of **2** as elucidated as 20(*S*)-3*β*,20-dihydroxy-dammar-24-en-28-al, and designated as cereotagalol D.

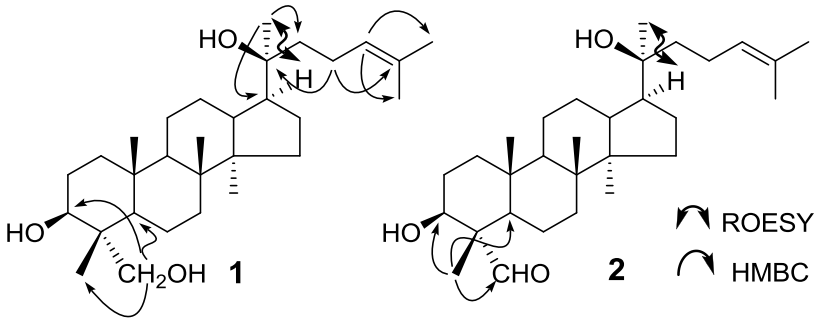

Figure 2. Key HMBC and ROESY correlations of compounds **1** and **2**.

As can be observed in the Table 2, compounds **1** and **2** showed selected moderate cytotoxicity against HCT-116 and CNE-2 with IC_{50} values of 29.7 and 37.2 μM, respectively. The oxidation degrees of C-28 in **1** and **2** maybe contribute to their cytotoxicity selectivity, but more cell lines should be tested in the future work. Compound **1** is an isomeride as well as a homologen of 20(*S*)-protopanaxadiol (PPD), which as a major active aglycon derivative of ginsenosides metabolized by human gastrointestinal microflora, exhibited extensive anticancer activities *in vitro* and *in vivo* [9]. The only structural difference between compound **1** and PPD is the –OH group attaching to C-28 of compound 1, locates at C-12 of PPD. Thus, although compound **1** only showed moderate cytotoxicity against HCT-116 in our four tested cell lines, it deserved further study on its anticancer potency.

Table 2. Cytotoxicity of compounds **1** and **2** against four cancer cell lines.

Compounds	IC_{50} μM [a]			
	A549	HepG2	HCT-116	CNE-2
1	＞50	＞50	29.7±0.25	＞50
2	＞50	＞50	＞50	37.2±0.20
Doxorubicin[b]	3.12±0.23	0.83±0.22	0.42±0.11	0.52±0.13

[a] All results are expressed as mean±SD, n=3 for each group.
[b] Positive control.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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