Two New Compounds from the Bark of *Dysoxylum hainanense*

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Two new compounds, *ent*-8(9)-pimarene-20-hydroxy-16-nor-15-oic acid (**1**) and 20S,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28),22-dien-3-oic acid (**2**), along with four known compounds have been isolated from the bark of *Dysoxylum hainanense*. The new structures were elucidated by spectroscopic techniques (NMR, MS, UV, IR and optical rotation). The known compound, *ent*-8(9)-pimarene-15,16-diol (**3**), was reported from *D. hainanense* for the first time.

Key words: Dysoxylum hainanense, *ent*-8(9)-Pimarene-20-hydroxy-16-nor-15-oic acid,
20S,24-Epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28),22-dien-3-oic acid

Introduction

Plants of the Meliaceae family are rich sources of structurally diverse and biologically significant limonoids [1]. Different types of limonoids, tirucallanes, apo-tirucallols, dammaranes, and *ent*-pimaranes were isolated from *Dysoxylum hainanense* collected from Yunnan province in our previous studies [2 – 7]. However, two novel rearranged oleanane-type triterpenes [8] and six ring A-modified novel triterpenoids [9] were reported from *D. hainanense* collected from Hainan province recently, which prompted us to re-investigate chemical constitutents of *Dysoxylum hainanense* in Yunnan. As a result, neither rearranged oleananes nor ring A-modified triterpenoids were found in this experiment, but two new compounds, *ent*-8(9)-pimarene-20-hydroxy-16-nor-15-oic acid (**1**) and 20S,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28),22-dien-3-oic acid (**2**), along with four known compounds, *ent*-8(9)-pimarene-15,16-diol (**3**) [10], richenoic acid (**4**) [11], *ent*-19-nor-4,16,18-trihydroxy-8(14)-pimaren-15-one (**5**) [5], and methyl richenoate (**6**) [11], structurally similar to those described in our previous report, were isolated (Fig. 1). Two independent investigations and an evaluation of the literature indicate that the chemical constitutents of *Dysoxylum hainanense* might be affected by its distribution. In this paper, we report the isolation and structural elucidation of these compounds from the bark of *D. hainanense*.

Results and Discussion

Compound **1** was found to have a molecular formula of $C_{19}H_{30}O_3$ on the basis of the HREIMS molecular ion at m/z = 306.2200 (calcd. 306.2195 for $C_{19}H_{30}O_3$), accounting for five degrees of unsaturation. The IR data showed the presence of OH (3453 cm^{-1}) and C=O (1695 cm^{-1}) groups. In the ^{1}H NMR spectrum, signals were observed for one olefinic proton at δ_H = 5.56 (1H, s) and three methyls (singlets at δ_H = 1.26, 0.84, 0.80). A combined analysis of the ^{13}C NMR (Table 1) and HSQC data revealed 19 carbon resonances due to one carbonyl carbon (δ_C = 181.1), two olefinic carbons (δ_C = 138.9 and 125.1), three methyls (δ_C = 24.9, 18.0 and 15.7), eight methylenes (δ_C = 71.6, 39.1, 35.8, 35.6, 31.9, 22.5, 18.7, and 18.6), two methines (δ_C = 50.6 and 47.8), and three quaternary carbons (δ_C = 42.9, 38.3 and 38.0). All these data suggested that **1** was similar to *ent*-16,18-dihydroxy-8(14)-pimaren-15-one [5], with the exception of a carbonyl carbon (δ_C = 181.1, s) present in **1** instead of an oxygenated methylene group (δ_C = 64.7, t) and a carbonyl carbon (δ_C = 214.4, s). The HMBC (Fig. 2) correlations from δ_H = 5.56 (H-14) to δ_C = 181.1 (C-15) assigned the carbonyl carbon (δ_C = 181.1, s) to C-15. Other

Table 1. ^1H and ^{13}C NMR data of compounds **1**[a] and **2**[a] at 400 MHz (δ_H) or 100 MHz (δ_C).

	— 1 —		— 2 —	
Position	δ_H	δ_C	δ_H	δ_C
1	1.04 (1H, m) 1.73 (1H, m)	39.1 (t)	1.58 (2H, m)	35.5 (t)
2	1.53 (2H, m)	18.6 (t)	2.26 (1H, m) 2.34 (1H, m)	28.6 (t)
3	1.19 (1H, m) 1.38 (1H, m)	35.6 (t)		175.1 (s)
4		38.0 (s)		148.5 (s)
5	1.34 (1H, m)	47.8 (d)	2.15 (1H, m)	51.1 (d)
6	1.28 (1H, m) 1.44 (1H, m)	22.5 (t)	1.46 (2H, m)	22.5 (t)
7	2.27 (1H, m) 2.11 (1H, m)	35.8 (t)	1.19 (2H, m)	34.5 (t)
8		138.9 (s)		51.2 (s)
9	1.82 (1H, m)	50.6 (d)	1.61 (1H, m)	41.6 (d)
10		38.3 (s)		39.7 (s)
11	1.67 (2H, m)	18.7 (t)	1.86 (2H, m)	25.4 (t)
12	1.69 (1H, m)	31.9 (t)	1.33 (1H, m)	25.6 (t)
12	1.79 (1H, m)		1.49 (1H, m)	
13		42.9 (s)	1.56 (1H, m)	44.2 (d)
14	5.56 (1H, s)	125.1 (d)		40.7 (s)
15		181.1 (s)	1.13 (1H, m)	31.8 (t)
15		181.1 (s)	1.51 (1H, m)	
16			1.75 (2H, m)	27.6 (t)
17	1.26 (3H, s)	24.9 (q)	2.18 (1H, m)	48.2 (d)
18	3.06 (1H, s)	71.6 (t)	0.98 (3H, s)	15.6 (q)
18	3.34 (1H, s)			
19	0.80 (3H, s)	18.0 (q)	0.84 (3H, s)	20.6 (q)
20	0.84 (3H, s)	15.7 (q)		92.6 (s)
21			1.44 (3H, s)	23.8 (q)
22			7.75 (1H, d, 5.7)	161.0 (d)
23			6.08 (1H, d, 5.7)	121.7 (d)
24				172.6 (s)
28			4.70 (1H, s)	113.7 (t)
28			4.84 (1H, s)	
29			1.74 (3H, s)	24.2 (q)
30			0.94 (3H, s)	16.4 (q)

[a] Compound **1** was measured in $CDCl_3$ + CD_3OD, and **2** was measured in CD_3COCD_3; (*J* in Hz and δ in ppm.)

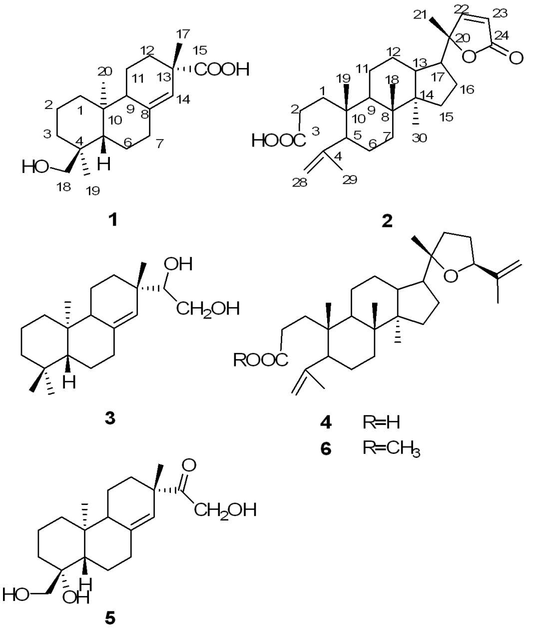

Fig. 1. Structures of compounds **1**–**6**.

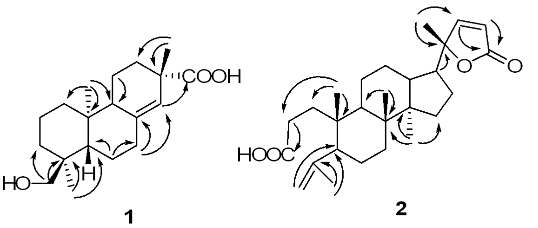

Fig. 2. Key HMBC correlations of compounds **1** and **2**.

parts of the structure were identical to those of *ent*-16,18-dihydroxy-8(14)-pimaren-15-one according to a detailed analysis of 2D NMR spectral data. Because of the co-occurrence in the same plant as well as a similar negative optical rotation value like *ent*-16,18-dihydroxy-8(14)-pimaren-15-one [5], the structure of **1** was elucidated as *ent*-8(9)-pimarene-20-hydroxy-16-nor-15-oic acid (Fig. 1).

Compound **2** has the molecular formula $C_{27}H_{40}O_4$ as evidenced by its HREIMS spectrum at m/z = 428.2926 (calcd. 428.2927 for $C_{27}H_{40}O_4$), accounting for eight degrees of unsaturation. The IR data showed the presence of C=O (1756, 1709 cm^{-1}) and C=C (1638 cm^{-1}) groups. Its UV spectrum showed the existence of conjugated groups based on the maximum absorption at 204 nm. The ^1H NMR spectrum (Table 1) showed signals of four olefinic protons at δ_H = 7.75 (1H, d, *J* = 5.7 Hz), 6.08 (1H, d, *J* = 5.7 Hz), 4.84 (1H, s), and 4.70 (1H, s), and five tertiary methyl groups at δ_H = 1.74 (3H, s), 1.44 (3H, s), 0.98 (3H, s), 0.94 (3H, s), and 0.84 (3H, s). The ^{13}C NMR spectrum (Table 1) indicated 27 carbon signals, including five methyls (δ_C = 24.2, 23.8, 20.6, 16.4, 15.6), nine methylenes (δ_C = 113.7, 35.5, 34.5, 31.8, 28.6, 27.6, 25.6, 25.4, 22.5), six methines (δ_C = 161.0, 121.7, 51.1, 48.2, 44.2, 41.6), and seven quaternary carbons (δ_C = 175.1, 172.6, 148.5, 92.6, 51.2, 40.7, 39.7). The spectral data of **2** were similar to those of 20S,24-epoxy-25,26,27-trisnor-24-oxo-seco-4(28)-dammar-3,4-en-3-oic acid [12], except for one more double bond signal (δ_C = 161.0, 121.7). The HMBC (Fig. 2) correlations from δ_H = 7.75 (H-22) to δ_C = 172.6 (C-24), and δ_H = 6.08 (H-23) to

δ_C = 172.6 (C-24) placed the double bond at C-22/23 (Fig. 1). Accordingly, the structure of **2** was elucidated to be 20S,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28),22-dien-3-oic acid.

Experimental Section

General

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Tenor 27 spectrophotometer using KBr pellets. 1D and 2D spectra were run on Bruker DRX-500 or AV-400 spectrometers with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Autospec-3000 spectrometer or an API QSTAR Pulsar I spectrometer. Column chromatography was performed on silica gel (200 – 300 mesh, Qingdao Marine Chemical Ltd., Qingdao, P. R. China), RP-18 gel (20 – 45 μm, Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden). Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd., Qingdao, P. R. China), and spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in EtOH.

Plant material

Dysoxylum hainanense plants were collected in Xishuangbanna, Yunnan Province, P. R. China, and identified by Mr. J.-Y. Cui, Xishuangbanna Tropical Plant Garden. A voucher specimen (No. Cui20081122) has been deposited at the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered bark of *D. hainanense* (12.5 kg) was extracted with methanol at r. t. three times (3 × 2 d) and concentrated under reduced pressure to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (200 g) was subjected to silica gel (200 – 300 mesh, 2 kg) column chromatography (CC), eluting with $CHCl_3$-Me_2CO (1 : 0, 20 : 1, 12 : 1, 8 : 1, 4 : 1, 2 : 1, 1 : 2) to yield fractions 1 – 5. Fractions 1 – 5 were further subjected to chromatography over RP-18 (MeOH-H_2O, 30 – 95 %) followed by silica gel CC (petroleum ether-Me_2CO, 20 : 1 – 1 : 2), and finally on Sephadex LH-20 ($CHCl_3$-MeOH, 1 : 1) to yield **1** (13 mg), **2** (7 mg), **3** (4 mg), **4** (10 mg), **5** (11 mg), and **6** (6 mg).

ent-8(9)-Pimarene -20-hydroxy -16-nor -15-oic acid (1)

Colorless amorphous powder. – $[\alpha]_D^{22}$ = −10.3 ($CHCl_3$-MeOH = 1 : 1, *c* = 0.2). – IR (KBr): ν = 3453 (OH), 2935, 1695 (C=O) cm^{-1}. – HRMS (EI): m/z = 306.2200 (calcd. 306.2195 for $C_{19}H_{30}O_3$). – ^1H NMR (400 MHz, $CDCl_3$ + CD_3OD) and ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD) spectral data: see Table 1.

20S,24-Epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28),22-dien-3-oic acid (2)

Colorless amorphous powder. – UV (MeOH): $\lambda_{max}(\log\varepsilon)$ = 204 (2.99) nm. – $[\alpha]_D^{22}$ = −5.6 (MeOH, *c* = 0.2). – IR (KBr): ν = 3432, 2938, 1756 (C=O), 1709 (C=O), 1638 (C=C) cm^{-1}. – HRMS (EI): m/z = 428.2926 (calcd. 428.2927 for $C_{27}H_{40}O_4$). – ^1H NMR (400 MHz, CD_3COCD_3) and ^{13}C NMR (100 MHz, CD_3COCD_3) spectral data: see Table 1.

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