Three New Sesquiterpenes from *Laggera pterodonta*

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A new eremophilane sesquiterpene, (2β)-2-deoxo-2-methoxytessaric acid (**1**), and two new eudesmane sesquiterpenes, (3β,10α)-3-methoxyleudesma-4,11(13)-dien-12-oic acid (**2**) and (3α,4β,8α)-4-(acetyloxy)-3-(2,3-dihydroxy)-2-methyl-1-oxobutoxy-8-hydroxyeudesm-7(11)-eno-12,8-lactone (**3**), along with the ten known compounds **4**–**13** were isolated from the aerial parts of *Laggera pterodonta*. The structures of the new compounds were elucidated by spectroscopic analyses, including 2D-NMR data.

Introduction. – *Laggera pterodonta* (DC.) BENTH. (Compositae), widely distributed in the southwest of China, especially in Yunnan Province, has been used as folk medicine since ancient times. Pharmacological investigations have indicated that the extract of *L. pterodonta* has antileukaemia, antibacterial, anti-inflammatory, and antimalarial activities [1]. Previous research has also revealed that flavonols, which account for the anti-inflammatory activity [2], and eudesmane sesquiterpenes represent the main secondary metabolites of this plant [3][4]. Until now, more than 60 sesquiterpenoids were isolated from *L. pterodonta* [5]. In the course of our systematic search for bioactive compounds from Chinese medicinal herbs, the aerial parts of *L. pterodonta* were studied, and a new eremophilane sesquiterpene, (2β)-2-deoxo-2-methoxytessaric acid[1]) (**1**), and two new eudesmane sesquiterpenes, (3β,10β)-3-methoxyeudesma-4,11(13)-dien-12-oic acid[1]) (**2**) and (3α,4β,8α)-4-(acetyloxy)-3-(2,3-dihydroxy-2-methyl-1-oxobutoxy)-8-hydroxyeudesm-7(11)-eno-12,8-lactone[1]) (**3**), along with the ten known compounds **4**–**13** were isolated (eremophilane = (1*S*,4a*R*,7*R*,8a*R*)-decahydro-1,8a-dimethyl-7-(1-methylethyl)naphthalene; eudesmane = (1*R*,4a*R*,7*R*, 8a*S*)-decahydro-1,4a-dimethyl-7-(1-methylethyl)naphthalene). Compounds **1**–**13** were tested for anti-inflammatory and cytotoxic activity, but none of them showed any activity. Herein, we report the isolation and structural elucidation of these secondary metabolites.

Results and Discussion. – Purification of the AcOEt extract led to the isolation of the three new compounds **1**–**3**, together with the ten known analogues 14,5α-

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

epoxyeudesm-11(13)-en-12-oic acid (**4**) [6], 5β-hydroxycostic acid (**5**) [6], 5α-hydroxycostic acid (**6**) [6], 5α-hydroxy-4α,14-dihydrocostic acid (**7**) [6], 4α,5α-dihydroxyeudesm-11(13)-en-12-oic acid (**8**) [1], ilicic acid (**9**) [7], 4-*O*-acetyl-3-*O*-(2,3-expoxy-2-methylbutanoyl)cuauthemone (**10**) [8], 4-*O*-acetyl-3-*O*-[3-(acetyloxy)-2-methyl-2-hydroxybutanoyl)cuauthemone (**11**) [9], 3-*O*-(2,3-expoxy-2-methylbutanoyl)cuauthemone (**12**) [8], and 3-*O*-(2,3-dihydroxy-2-methylbutyroyl)cuauthemone (**13**) [10] (*Fig. 1*).

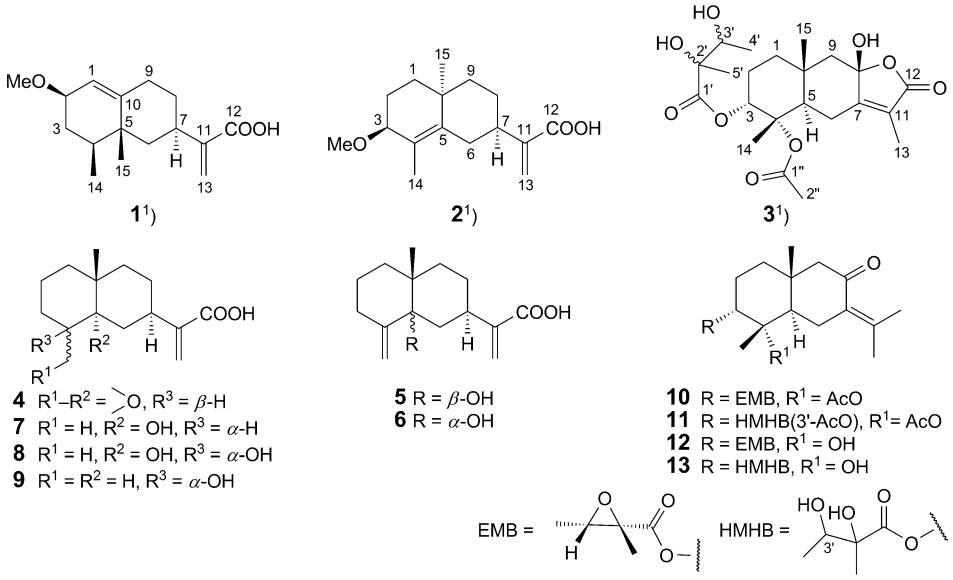

Fig. 1. *Compounds* **1**–**13**, *isolated from* Laggera pterodonta

Compound **1**, an optically active, colorless oil, had the molecular formula $C_{16}H_{24}O_3$ as established by HR-ESI-MS ($[M+Na]^+$ at m/z 287.1627). The IR absorption bands at 3432 and 1692 cm^{-1} implied the presence of a conjugated carboxylic acid group. In addition to a MeO group (δ(H) 3.43; δ(C) 56.1), 15 C-atoms were observed in the ^{13}C-NMR spectrum (*Table 1*), arising from two Me groups (δ(C) 15.5 and 19.1), five CH_2 groups (δ(C) 28.1, 29.5, 31.9, 40.3, and 124.5), four CH groups (δ(C) 31.7, 32.8, 73.2, and 120.4), and four quaternary C-atoms (δ(C) 38.6, 144.9, 149.3, and 172.1). Correlations in the ^{1}H,^{1}H-COSY and HSQC plots revealed the presence of two proton-bearing structural fragments, CHCHCH$_2$CHMe (**a**) and CH$_2$CHCH$_2$CH$_2$ (**b**; *Fig. 2*). Detailed HMBC analysis suggested that the structure of **1** was very similar to that of tessaric acid (=(2*R*,8*S*,8a*R*)-1,2,3,4,6,7,8,8a-octahydro-8,8a-dimethyl-α-methylene-6-oxonaphtalene-2-acetic acid), a known eremophilane derivative [11]. The main difference between the two compounds was that the C=O group in tessaric acid was replaced by an oxygenated methine group in **1** as deduced from the HMBC cross-peaks of δ(H) 5.56 (*d*, $J=3.7$ Hz, H–C(1)), 1.69 (H$_\beta$–C(3)), and 1.83 (H–C(4)) (see *Table 2*) with δ(C) 73.2 (*d*, C(2)) (*Fig. 2*). In addition, the MeO signal at δ(H) 3.43 correlated with C(2), suggesting that the MeO group is located at C(2). The relative configuration of **1** was determined by a

Table 1. *^{13}C-NMR Data* (100 MHz, $CDCl_3$, 27°) *of* **1**–**3**[1]). δ in ppm.

C-Atom	**1**	**2**	**3**
C(1)	120.4 (*d*)	34.2 (*t*)	33.7 (*t*)
C(2)	73.2 (*d*)	21.9 (*t*)	23.6 (*t*)
C(3)	31.9 (*t*)	79.2 (*d*)	78.2 (*d*)
C(4)	31.7 (*d*)	124.7 (*s*)	71.9 (*s*)
C(5)	38.6 (*s*)	140.1 (*s*)	51.1 (*d*)
C(6)	28.1 (*t*)	31.2 (*t*)	21.1 (*t*)
C(7)	32.8 (*d*)	39.9 (*d*)	161.1 (*s*)
C(8)	29.5 (*t*)	27.9 (*t*)	103.3 (*s*)
C(9)	40.3 (*t*)	41.9 (*t*)	53.7 (*t*)
C(10)	149.3 (*s*)	34.2 (*s*)	34.9 (*s*)
C(11)	144.9 (*s*)	145.3 (*s*)	121.9 (*s*)
C(12)	172.1 (*s*)	170.4 (*s*)	172.3 (*s*)
C(13)	124.5 (*t*)	123.9 (*t*)	8.1 (*q*)
C(15)	15.5 (*q*)	22.9 (*q*)	21.0 (*q*)
C(14)	19.1 (*q*)	17.3 (*q*)	18.7 (*q*)
MeO or C(1′)	56.1 (*q*)	56.8 (*q*)	170.1 (*s*)
C(2′)			76.3 (*s*)
C(3′)			74.5 (*d*)
C(4′)			13.2 (*q*)
C(5′)			21.5 (*q*)
MeCO			174.7 (*s*), 22.0 (*q*)

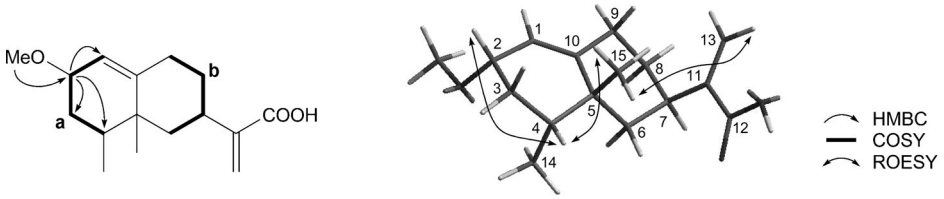

Fig. 2. *Key 2D correlations of* **1**[1])

ROESY analysis (*Fig. 2*); the correlations H–C(2)/H–C(4), H_b–C(13)/Me(14), H_b–C(13)/Me(15), and H_a–C(13)/H–C(7) indicated the α-orientation of H–C(2) and H–C(4), and β-orientation of Me(14) and Me(15). Since **1** had a specific rotation ($[\alpha]_{20}^{D} = -166.2$) similar to that of tessaric acid, ($[\alpha] = -156.2$) whose absolute configuration was established by X-ray single-crystal diffraction [12], the absolute configuration of **1** should be the same as that of tessaric acid. Thus, the structure of **1** was deduced as (2β)-2-deoxo-2-methoxytessaric acid[1]).

The HR-ESI-MS of **2** gave a $[M + Na]^+$ peak at *m*/*z* 287.1618, corresponding to the molecular formula $C_{16}H_{24}O_3$. The IR absorption at 1706 cm^{-1} indicated the presence of a C=O group. The ^{1}H-NMR spectrum (*Table 2*) revealed the presence of two olefinic H-atoms (δ(H) 5.58 and 6.28 (2 br. s)), one MeO group (δ(H) 3.35 (*s*)), and two Me groups (δ(H) 1.02 and 1.72 (2 *s*)). The ^{13}C-NMR data of **2** (*Table 1*) showed close resemblance to those of eudesma-4,11(13)-dien-12-oic acid [13], but with an additional MeO group (δ(H) 3.35 and δ(C) 56.8). The MeO group was connected to C(3) as

Table 2. *^{1}H-NMR Data* (400 MHz, $CDCl_3$, 27°) *of* **1**–**3**[1]). δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**
H–C(1)	5.56 (*d*, *J* = 3.7)	1.26–1.58, 1.60–1.68 (2*m*)	1.25–1.39 (*m*)
H–C(2) or CH_2(2)	3.57 (br. *s*)	1.60–1.68, 1.89–1.92 (2*m*)	1.88–1.96, 1.70–1.75 (2*m*)
CH_2(3) or H–C(3)	1.52 (*ddd*, *J* = 18.8, 17.9, 7.0), 1.69–1.76 (*m*)	3.41 (*s*)	4.90 (*t*, *J* = 2.2)
H–(4)	1.78–1.90 (*m*)	–	–
CH_2(6)	1.66–1.75 (*m*)	1.72–1.79 (*m*), 2.65 (*d*, *J* = 13.2)	2.16–2.50 (*m*), 2.97 (*dd*, *J* = 13.3, 2.5)
H–C(7)	2.61–2.63 (*m*)	2.45 (*t*, *J* = 11.6)	–
CH_2(8)	1.78–1.90 (*m*), 1.68 (*dd*, *J* = 23.3, 9.7)	1.26–1.58, 1.72–1.79 (2*m*)	–
CH_2(9)	2.45 (*dd*, *J* = 22.2, 9.6), 2.07 (*dd*, *J* = 13.5, 7.8)	1.26–1.58, 1.60–1.68 (2*m*)	1.52, 2.19 (2*d*, each *J* = 13.4)
CH_2(13) or Me(13)	5.64, 6.26 (2*s*)	5.58, 6.28 (2br. *s*)	1.99 (*s*)
Me(14)	0.84 (*s*)	1.72 (*s*)	1.20 (*s*)
Me(15)	0.86 (*s*)	1.02 (*s*)	1.11 (*s*)
MeO	3.43 (*s*)	3.35 (*s*)	–
H–C(3′)	–	–	5.16 (*q*, *J* = 6.3)
Me(4′)	–	–	1.30 (*d*, *J* = 5.6)
Me(5′)	–	–	1.28 (*s*)
MeCO	–	–	1.80 (*s*)

deduced from HMBCs of δ(H) 1.72 (*s*, Me(14), 1.32 (*m*, H_α–C(1)), 1.57 (*m*, H_β–C(1)), and 3.35 (*s*, MeO) with 79.2 δ(C) (*d*, C(3)). In the ROESY plot, the correlations H–C(3)/Me(15) and H–C(7)/Me(15) were observed, which indicated α-orientation of H–C(3), H–C(7), and Me(15). Therefore, the structure of compound **2** was deduced as (3β,10α)-3-methoxyeudesma-4,11(13)-dien-12-oic acid.

Compound **3** had a molecular formula $C_{22}H_{32}O_9$ as inferred from the HR-ESI-MS (*m*/*z* 463.1951 $[M+Na]^+$). Its IR spectrum showed an α,β-unsaturated γ-lactone absorption (1739 cm^{-1}). Except for the presence of a-2,3-dihydroxy-2-methylbutanoyl group (δ(H) 1.30 (*d*, *J* = 5.6 Hz, Me(4′))), 1.28 (*s*, Me(5′))); and 5.16 (*q*, *J* = 6.3 Hz, H–C(3)); C(1′) to C(5′) at δ(C)) 170.1 (*s*), 76.3 (*s*), 74.5 (*d*), 13.2 (*q*), and 21.5 (*g*)) and an AcO group (δ(C) 22.0 (*q*) and 174.7 (*s*); δ(H) 1.80 (*s*) as deduced from HSQC and HMBC spectra, compound **3** possessed 15 other C-atoms signals. Detailed analysis of 2D-NMR data (*Fig. 3*) suggested that **3** should be an eudesmane sesquiterpene endowed with an α,β-unsaturated γ-lactone moiety (δ(C) 121.9, 161.1, and 172.3 (3 *s*)), as in the closely related (3α,4β,8α,-3,4,8-trihydroxyeudesm-7(11)-eno-12,8-lactone [14]. According to the HMBC cross-peak δ(H) 4.90 (*t*, *J* = 2.2 Hz, H–C(3))/δ(C) 170.1 (C(1′)), the (2,3-dihydroxy-2-methyl-butanoyl)oxy group should be located at C(3). In the ROESY plot, the correlations H–C(5)/MeC=O were observed. This suggested that the Ac group was bound to O–C(4). The relative configuration was established by the ROESY experiment, the correlations Me(14)/Me(15) and H–C(3)/Me(15) being observed, Thus, the (2,3-dihydroxy-2-methylbutanoyl)oxy and AcO groups are α-oriented. The configuration of OH–C(8) was thought to be β, because the chemical shift of Me(15) was influenced by a deshielding effect of OH–C(8), similar to

that of 8β-hydroxyixitlixochilin 4-*O*-acetate [8]. Therefore, the structure of **3** was determined as (3α,4β,8α)-4-(acetyloxy)-3-(2,3-dihydroxy-2-methyl-1-oxobutoxy)-8-hydroxyeudesm-7(11)-eno-12,8-lactone.

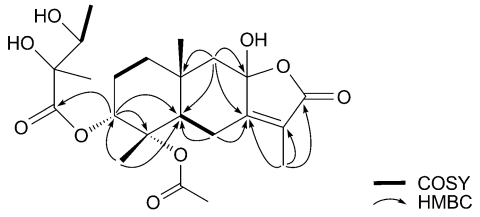

Fig. 3. *Key 2D correlations of* **3**

The presence of compounds **1**–**7** and **10**–**13** in *L. pterodonta* is reported for the first time. All compounds were tested for anti-inflammatory and cytotoxic activity *in vitro*. However, none of them exhibited such an activity.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Haiyang Chemical Co. Ltd.*), *RP-18* gel (20–45 μm, *Fuji Silysia Chemical Ltd.*), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*). TLC: SiO_2 *GF 254* (*Qingdao Haiyang Chemical Co., Ltd.*); detection by spraying with 10% H_2SO_4 in EtOH followed by heating. Optical rotations: *Horiba-SEPA-300* polarimeter. UV Spectra: *Shimadzu-UV-2401A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Tenor-27* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-DRX-500* and *-AM-400* spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *VG-Autospec-3000* spectrometer; in *m*/*z*. HR-ESI-MS: *API-QSTAR-Pulsar-1* spectrometer; in *m*/*z*.

Plant Material. The aerial parts of *L. pterodonta* were bought on the market of Chinese traditional medicine in Kunming,Yunnan Province, P. R. China, in April 2011, and identified by Prof. *Xiao Cheng*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20110704) has been deposited with the Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The aerial parts of *L. pterodonta* (9 kg) were extracted exhaustively with 95% EtOH at r.t. The EtOH extract was concentrated, and the residue extracted with AcOEt to yield a residue (400 g). This residue was subjected to CC (SiO_2 (2.5 kg, 150 × 12 cm), petroleum ether/acetone 20:1 → 1:1): *Fractions 1–5*. *Fr. 3* (80 g) was subjected to medium-pressure liquid chromatography (MPLC) (*MCI* (1 kg), H_2O, 60, 70, 80, 90, and 100% MeOH (4 l for each gradient): *Fr. 3a–Fr. 3e*. After repeated CC (SiO_2 (100 g, 60 × 4 cm), $CHCl_3$/acetone 100:1 → 1:1), *Fr. 3a* (4.8 g) afforded **12** (37 mg). *Fr. 3b* (17.4 g) was further applied to CC (SiO_2 (400 g, 120 × 4 cm), $CHCl_3$/AcOEt 100:1 → 1:1): **4** (81 mg), **5** (100 mg), **6** (580 mg), **7** (80 mg), **10** (2.6 g), and **11** (460 mg). *Fr. 4* (85 g) was separated by MPLC (*MCI* (1 kg), H_2O, 50, 60, 70, 80, 90, and 100% MeOH (4 l for each gradient)): *Fr. 4a–Fr. 4d*. *Fr. 4a* (9.4 g) was further applied to CC (SiO_2 (200 g, 70 × 4 cm), $CHCl_3$/MeOH 100:1 → 1:1): **8** (30 mg) and **9** (6.8 g). *Fr. 4b* (18.6 g) was subjected to CC (SiO_2 (400 g, 120 × 4 cm), petroleum ether/^{i}PrOH 100:1 → 1:1): **3** (5 mg), **13** (45 mg), and *Fr. 4b1* (90 mg). *Fr. 4b1* was further purified by prep. HPLC (*RP-18*, 58% MeOH/H_2O): **1** (8 mg) and **2** (6 mg).

(2β)-2-Deoxo-2-methoxytessaric Acid (=*(2*R,*6*R,*8*S,*8a*R*)-1,2,3,4,6,7,8,8a-Octahydro-6-methoxy-8,8a-dimethyl-α-methylenenaphtalene-2-acetic Acid*; **1**): Colorless oil ($CHCl_3$). $[\alpha]_D^{20} = -166.2$ (*c* 0.25,

$CHCl_3$). UV ($CHCl_3$): 239 (2.93), 222 (2.52). IR (KBr): 3432, 2933, 1692, 1625, 1461, 1190, 1080, 932. HR-ESI-MS (pos.): 287.1627 ($[M+Na]^+$; calc. 287.1623).

(3β,10β)-3-Methoxyeudesma-4,11(13)-dien-12-oic Acid (= rel-*(2*R,*4a*R,*7*S*)-1,2,3,4,4a,6,7-Octahydro-7-methoxy-4a,8-dimethyl-α-methylenenaphtalene-2-acetic Acid*; **2**): Colorless oil ($CHCl_3$). $[\alpha]_D^{20} = +54.5$ ($c = 0.20$, $CHCl_3$). UV ($CHCl_3$): 239 (3.30), 221 (2.87). IR (KBr): 3465, 2939, 1706, 1150, 1067, 864, 494, 442. HR-ESI-MS (pos.): 287.1618 ($[M+Na]^+$, calc. 287.1623).

(3a,4b,8a)-4-(Acetyloxy)-3-(2,3-dihydroxy)-2-methyl-1-oxobutoxy)-8-hydroxyeudesm-7(11)-eno-12,8-lactone (= rel-*(4a*R,*5*S,*6*R,*8a*R,*9a*S*)-5-(Acetyloxy)-9a-hydroxy-3,5,8a-trimethyl-2-oxo-2,4,4a,5,6,7,8,8a,9,9a-decahydronaphtho[2,3-*b*]furan-6-yl 2,3-Dihydroxy-2-methylbutanoate*; **3**). White, amorphous powder ($CHCl_3$). $[\alpha]_D^{20} = -19.9$ ($c = 0.19$, $CHCl_3$). UV ($CHCl_3$): 366 (1.41), 238 (2.97), 221 (2.54). IR (KBr): 3437, 2927, 1739, 1376, 1252. HR-ESI-MS (pos.): 463.1951 ($[M+Na]^+$; calc. 463.1944).

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