Alboatisin A, a new diterpenoid from *Euphorbia fischeriana*

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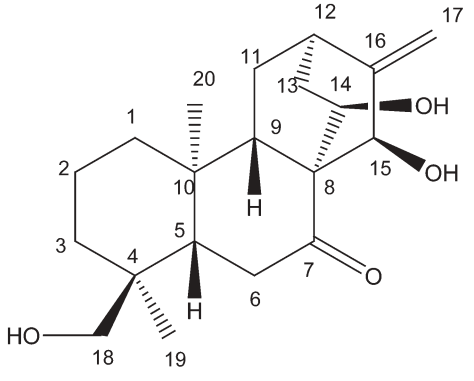
A new diterpenoid, 18-hydroxy-14,15-dihydroxy-7-oxo-*ent*-atis-16-ene, and another kown diterpenoid, *ent*-atis-16(17)-ene-3,14-dione, were isolated from the roots of *Euphorbia fischeriana*. The structures of those compounds were elucidated by spectroscopic methods and literatura data.

Keywords: *Euphorbia fischeriana*, diterpenoid, Chinese medicine

The roots of *Euphorbia fischeriana* Steudis is one of the traditional Chinese medicines named "Lang-Du", which is used mainly in the Northwest and Yunnan Province of P.R. China. It is used as a traditional remedy for the treatment of oedema, ascites and cancer.[1] Prior research has shown that the main chemical constituents of the roots are diterpenoids,[2–4] and these diterpenoids showed cytotoxic activity towards sarcoma 180, Ehrlich ascites and Hela cells.[5,6] In our chemical investigation of this plant, we have isolated a new diterpene, alboatisin A (**1**), and one known diterpene, *ent*-atis-16(17)-ene-3,14-dione (**2**). We now report the elucidation of the structures of these two diterpenoids.

Compound **1** was isolated as a white powder. Its HRESIMS spectrum exhibited a quasi molecular ion peak at *m/z* 357.2045 ([M + Na]$^+$, Calcd 357.2047), which indicated that the molecular formula was $C_{20}H_{30}O_4$. This was further confirmed by the 20 resonances observed in the ^{13}C and DEPT NMR spectra of **1** (Table 1). The ^{1}H NMR spectrum of **1** showed two methyl signals at δ 0.78 and 1.07, a pair of oxygenated methylene signals at δ 3.48 and 3.24, and two methine signals at δ 4.79 and 5.93. The ^{13}C NMR and^{13}C NMR (DEPT) spectra displayed 20 carbon signals including two methyls, eight methylenes, five methines, and five quaternary carbon atoms. Since diterpenoids have been isolated from this species,[7–10] **1** was presumed to be a diterpenoid. ^{1}H–^{1}H COSY correlations of H-11 with a methine proton (H-12), instead of H-11 with an oxymethine proton, suggested that was an atisane diterpenoid. Comparison of **1** with eriocatisin A (**3**)[11] revealed the ^{1}H and ^{13}C NMR spectra of **1** were similar to those of **3** with only one obvious difference being one oxygenated carbon δ_C 70.7 in **1** instead of a carbonyl carbon signal in **3**. This indicated the location of the hydroxyl is C-18 or C-19 in **1**. On the basis of the key ROESY correlations between H-18/H-5 (Fig. 2), the position of hydroxyl of **1** was confirmed on C-18. The HMBC



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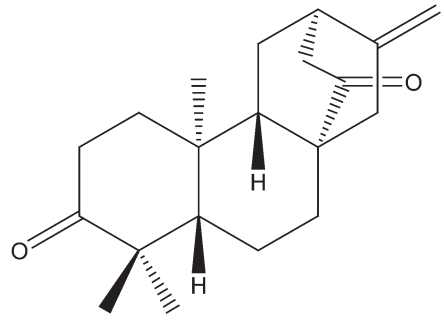

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Table 1 ^{1}H and ^{13}C NMR data (in $CDCl_3$) for compounds **1** and **2** (δ in ppm, *J* in Hz)

	1		**2**			**1**		**2**	
No.	δ_C [a]	δ_H *J* (Hz) [b]	δ_C [a]	δ_H *J* (Hz) [b]	No.	δ_C [a]	δ_H *J* (Hz) [b]	δ_C [a]	δ_H *J* (Hz) [b]
1α	38.8	1.43 [c]	37.2	1.88 [c]	11α	28.7	1.66 [c]	27.9	1.59 [c]
1β		0.77 [c]		1.35 (m)	11β		1.38 (m)		1.88 [c]
2α	17.9	1.59 (m)	34.1	2.56 (m)	12α	37.3	2.49 (br.s)	38.3	2.73 (m)
2β		1.33 (m)		2.28 [c]	13α	38.1	1.80 (br.d, 13.3)	44.6	2.28 [c]
3α	35.8	1.67 [c]	216.4		13β		2.26 (m)		2.28 [c]
3β		1.41 (m)			14	67.4	4.79 (dd, 9.6, 4.0)	216.1	
4	38.0		47.7		15α	66.3	1.31 [c]	42.6	2.28 [c]
5	46.1	2.18 (dd, 2.9, 14.0)	55.3	1.27 (m)	15β				2.28 [c]
6α	38.5	2.59 (dd, 14.0, 15.9)	20.2	1.59 [c]	16α	155.8		147.1	
6β		2.76 (dd, 2.9, 15.9)		1.59 [c]	17α	108.6	5.49 (br.s)	107.2	4.89 (br.s)
7α	213.4		31.2	2.28 [c]	17β		5.18 (br.s)		4.68 (br.s)
7β				0.91 (m)	18α	70.7	3.48 (d, 12.5)	25.9	1.08 s
8	60.0		47.6		18β		3.24 (d, 12.5)		
9	44.6	2.56 [c]	51.8	1.59 [c]	19	15.0	0.78 (s)	21.7	1.01s
10	36.9		37.3		20	17.3	1.07 (s)	12.8	0.87s

[a] At 125 MHz; [b] At 500 MHz; [c] overlap.

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correlations δ_H 3.48 (d, 12.5) with signals at δ 35.8 (C-3), 38.0 (C-4), and 15.0 (C-19) (Fig. 1) further supported the placement of hydroxyl at C-18 (δ_C 70.7, d). Key HMBC correlations between H-14/C-12 and C-13, and H-15/C-8 and C-16 confirmed the position of other two hydroxyl groups at C-14 (δ_C 67.4, d) and C-15 (δ_C 66.3, d), respectively. The position of one carbonyl group at C-7 was deduced from correlation between the protons at δ_H 2.18 (H-5), 2.59 (H-6), 2.56 (H-9), 1.31 (H-15) and a carbonyl carbon signal at δ_C 213.4 in the HMBC spectrum. The signals at δ_C 155.8 and 108.6 indicated the presence of a typical isolated double bond which was located at C-16 (C-17) according to the signal at δ_H 5.49 (H-17) correlated with signals at δ_C 66.3(C-15), and 37.3 (C-12) in the HMBC spectrum. Thus the positions of all of the functional groups were located.

Since the atisane diterpenoids isolated from the genus *Euphorbia* possess an *ent*-configuration, **1** was presumed to be an *ent*-atisane diterpenoid. The relative stereochemistry of compound **1** was established by analyzing information from the ROESY spectrum. The β-orientation for OH-14 was confirmed by the ROESY correlation of H-14 with H_3-20 as shown in a computer-generated 3D model (Fig. 2). The relative configuration of OH-15 was deduced to be β-orientated by the correlation of H-15 with H-13β (Fig. 2). Thus, the structure of **1**, named alboatisin A, was determined as 18-hydroxy-14,15-dihydroxy-7-oxo-*ent*-atis-16-ene.

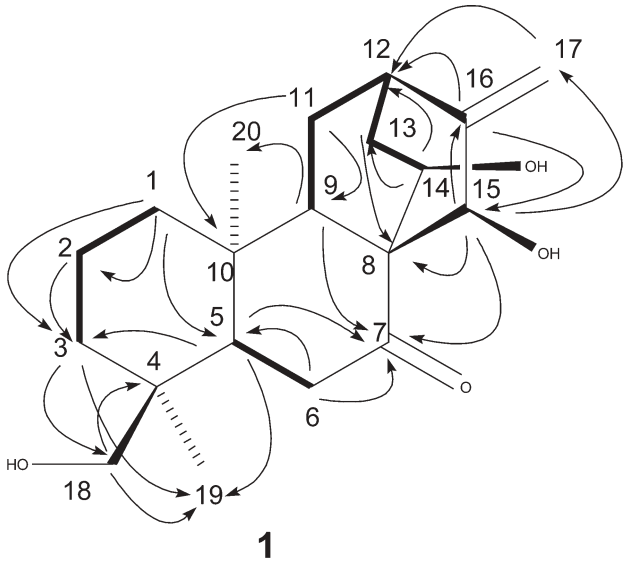

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Fig. 1 The key HMBC (H→C) and COSY (H—H) correlations of **1**.

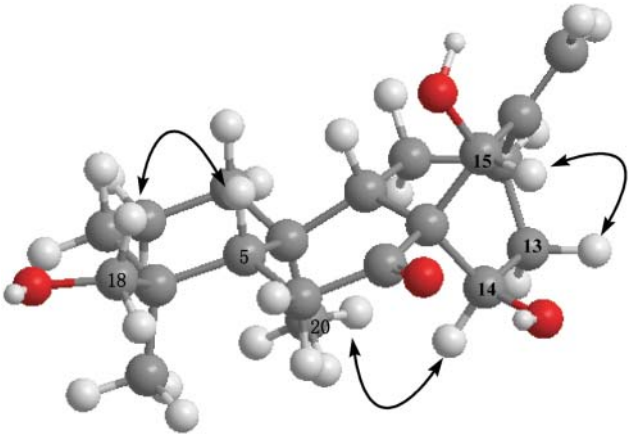

Fig. 2 Key ROESY (H↔H) correlations of **1**.

Compound **2** was obtained as needles; m.p. 160–163 °C; The molecular formula was assigned as $C_{20}H_{28}O_2$ on the basis of HRESIMS [M + Na]$^+$ *m/z* 323.1975 (Calcd for 323.1977). The ^{1}H and ^{13}C NMR spectra of **2** revealed two carbonyl carbons (δ_C 216.4 and 216.1), one olefinic carbons (δ_C 147.1, 107.2), and three methyl groups (δ_C 25.9, 21.7, and 12.8) signals (Table 1), which indicated **2** was an atisane diterpenoid substituted by two carbonyl groups. The structure of compound **2** (Fig. 1) was identified as *ent*-atis-16(17)-ene-3,14-dione based on the literature comparisons.[7]

Experimental

Melting points of the natural products were determined on an XRC-1 apparatus and were uncorrected. IR spectra were recorded as KBr pellets on a Perkin-Elmer 599B spectrophotometer. MS were determined on a Bruker Daltonics Apex III mass spectrometer. NMR spectra were measured on Bruker DRX-500 spectrometers with TMS as internal standard and $CDCl_3$ as solvent. Silica gel (200–300 mesh) was used for column chromatography and silica gel GF254 for TLC (Qingdao Marine Chemical Co., China). Sephadex LH-20 (GE), reversed-phase C-18 silica gel (60 mesh, Merck). Dionex P680ALPC equipped with an Alltima C-18 column (4.6 × 250 mm) was used for HPLC analysis and a semi-preparative Alltima C-18 column (10 × 250 mm) was used in sample preparation.

Plant material

The roots of *Euphorbia fischeriana* were collected in Xining, Qinghai Province, China. The plant material was identified by Prof. SUO You-Rui, Northwest Institute of Plateau Biology, Chinese Academy of Sciences. A voucher specimen (No.Xiyi-2010110) was deposited in the Herbarium of University of Xi'an Medicine, China.

Extraction and isolation

Air-dried roots of *E. fischeriana* (1.0 kg) were extracted by 75% ethanol three times (each 3 days and 5 L) at room temperature. The extracts were concentrated *in vacuo* to afford a crude residue (210 g) which was suspended in water (600 mL) and subsequently partitioned successively with EtOAc to afford EtOAc fraction. The EtOAc fraction (16 g) was subjected to column chromatography on silica gel using a step gradient elution of petroleum ether-EtOAc The fractions obtained from petroleum ether-EtOAc (1:1) elution were combined and subjected to repeated column chromatography and further purified by HPLC to yield pure compounds **1** (5 mg) and **2** (3 mg).

18-Hydroxy-14,15-dihydroxy-7-oxo-ent-atis-16-ene (**1**): $C_{20}H_{30}O_4$, white solid; m.p. 156–158 °C; $[\alpha]^{19}_D$.–6.8° (C 0.20, MeOH); UV ($CHCl_3$): λ_{max} (log ε) 241 (1.81), 267 (1.75) nm; IRν_{max} (KBr. cm^{-1}): 3442, 2929, 2865, 1686, 1658, 1638, 1463, 1442, 1388, 1240, 1076 and 1055; HRESIMS [M + Na]$^+$ *m/z* 357.2045 (Calcd for 357.2047) for $C_{20}H_{30}O_4Na$); ^{1}H and ^{13}C NMR data see Table 1.

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