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A New Lathyrane Diterpenoid from the Whole Plant of *Euphorbia altotibetica*

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The Euphorbiaceae family is one of the largest families in the plant kingdom. It comprises 263 genera and about 7300 species of almost cosmopolitan distribution. *Euphorbia*, the largest genus of Euphorbiaceae, with about 1600 species is characterized by the presence of milky latex.[1] This genus has been the subject of numerous chemical studies.[2] *E. altotibetica* is a perennial herb mainly growing in northwestern China and is often used for the treatment of curing skin tinea and tumefaction in folk.[3] To our best knowledge, only a few phytochemical studies have been published on *E. altotibetica* to date.[4] As a part of systematic research on *Euphorbia* species, a new lathyrane-type diterpenoid, named altotibetol (**1**), was isolated from the whole plant of *E. altotibetica*. This paper describes the isolation, structure elucidation, and cytotoxic activities of this new compound.

Compound **1** was isolated as a colorless oil with an $[\alpha]_D^{24}$ value of +6.25 (*c* 0.40, $CHCl_3$). A molecular formula of $C_{22}H_{32}O_5$ and seven degrees of unsaturation were established for **1** on the basis of the observed sodiated molecular ion peak at *m/z* 399.2140 $[M + Na]^+$ in its HR-ESI-MS (calcd. for $C_{22}H_{32}O_5Na$, 399.2142). The IR absorption bands at 3414, 1740, 1646, and 1615 cm^{-1}, respectively, indicated the presence of hydroxyl, carbonyl, and double bond groups in the molecule. The ^1H and ^{13}C-NMR spectra (Table 1) exhibited typical resonances for one acetoxy group [δ_H 2.04 (3H, s); δ_C 169.7, 21.6]. In addition, with the aid of the ^{13}C-NMR and DEPT spectra, five quaternary carbons, eight methines, two methylenes, and five methyl groups were detected, accounting for a 20-carbon-containing diterpene skeleton. The carbon resonance at δ_C 194.7 (C-14) demonstrated the presence of one α,β-unsaturated carbonyl group in the molecule. Furthermore, two trisubstituted olefins were evident from the carbon resonances at δ_C 120.3 (C-5), 145.9 (C-6), 145.6 (C-12), and 132.9 (C-13) and the protons resonances at δ_H 6.11 (1H, brd, *J* = 10.8, H-5) and 6.57 (1H, brd, *J* = 11.2, H-12). The above functionalities accounted for four degrees of unsaturation, and the remaining three degrees of unsaturation required the presence of three additional rings in the molecule. The characteristic ^1H-NMR signals at δ_H 1.15 (1H, m, H-9), 1.42 (1H, dd, *J* = 8.0, 11.2, H-11), 1.19 (3H, s, H-18), 1.09 (3H, s, H-19) and ^{13}C-NMR signals at δ_C 30.7 (C-11), 29.5 (C-9), 29.1 (C-18), 24.4 (C-10), and 16.2 (C-19) indicated a gem-dimethyl-substituted cyclopropane ring,[5-7] which was further confirmed by HMBC spectrum (Fig. 2). The above information suggested that the basic structure of **1** was a lathyrane diterpenoid skeleton. Analysis of the ^1H-^1H COSY spectrum (Fig. 2) provided a little information on the partial structures. The following structural fragments were elucidated on the basis of the correlated proton sequences: –CH_2–CH(CH_3)–CH(OH)–CH–CH= [unit A, δ_H 2.25 (1H, m, H-1α), 2.67 (1H, m, H-1β), 2.18 (1H, m, H-2β), 3.84 (1H, dd, *J* = 4.0, 6.8, H-3), 2.62 (1H, dd, *J* = 6.8, 10.8, H-4), 6.11 (1H, brd, *J* = 10.8, H-5)], and –CH(OH)–CH_2–CH–CH–CH= [unit B, δ_H 4.04 (1H, dd, *J* = 2.8, 10.8, H-7α), 2.37 (1H, m, H-8α), 1.70 (1H, m, H-8β), 1.15 (1H, m, H-9), 1.42 (1H, dd, *J* = 8.0, 11.2, H-11), 6.57 (1H, brd, *J*

Table 1. NMR spectral data of compound **1**

Position	δ_H mult (*J* in Hz)	δ_C	DEPT
1α	2.25 m	40.9	CH_2
1β	2.67 m		
2	2.18 m	40.9	CH
3	3.84 dd (4.0, 6.8)	81.8	CH
4	2.62 dd (6.8, 10.8)	48.8	CH
5	6.11 brd (10.8)	120.3	CH
6		145.9	C
7	4.04 dd (2.8, 10.8)	75.2	CH
8α	2.37 m	36.5	CH_2
8β	1.70 m		
9	1.15 m	30.7	CH
10		24.4	C
11	1.42 dd (8.0, 11.2)	29.5	CH
12	6.57 brd (11.2)	145.6	CH
13		132.9	C
14		194.7	C
15		96.0	C
16	1.10 d (7.2)	18.3	CH_3
17	1.49 brs	18.9	CH_3
18	1.19 s	29.1	CH_3
19	1.09 s	16.2	CH_3
20	1.83 brs	12.2	CH_3
15-OAc	2.04 s	21.6	CH_3
		169.7	C

Notes: NMR data were measured in $CDCl_3$ at 400 MHz for ^1H or 100 MHz for ^{13}C, respectively.

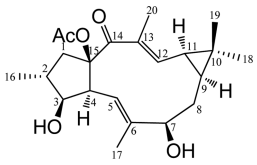

Figure 1. The structure of compound **1**.

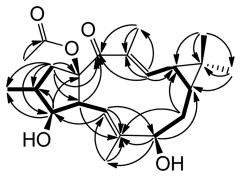

Figure 2. ^{1}H–^{1}H COSY (bold lines) and key HMBC correlations (H → C) of compound **1**.

= 11.2, H-12)], which represented the structure moieties C-1–C-5 and C-7–C-12 of a lathyrane diterpenoid, respectively. The HMBC correlations enable assembly of units A and B with the quaternary carbons and other functionalities. The HMBC correlations of H-5 with C-3, C-6, C-7, and C-17, correlations of H-12 with C-11, C-13, C-14, and C-20, and correlations of H_2-1 with C-2, C-3, C-4, C-14, C-15, and C-16 established the connectivity of units A and B. The clear HMBC correlation of H-4 with C-14 indicated that a carbonyl group must be sited at C-14. The significant HMBC correlations of H_3-17 with C-5, C-6 and correlations of H_3-20 with C-12, C-13, indicated two trisubstituted olefins should be sited at C-5, C-6 and C-12, C-13, respectively. The oxygenated methine signal H-3 showed HMBC correlations with C-1, C-2, C-15, and C-16, suggested one hydroxyl group should be attached to C-3. The proton signal H-7 showed HMBC correlations with C-5, C-6, C-9, and C-17, indicated another hydroxyl group should be linked to C-7. The downfield shifted ^{13}C-NMR chemical shift value of C-15 suggested the acetoxy group was located at C-15.[7] Therefore, the planar structure of compound **1** was determined as depicted in Figure 1.

The relative stereochemistry of **1** was predicted by analysis of its NOESY (Fig. 3) and ^{13}C-NMR spectra. Since the angular proton H-4 was assumed to be α-oriented on a biogenetic basis,[5,8-11] the NOESY cross peaks between H-4 and H-1α, H_3-16, H-3, indicated that Me-16 at C-2 was in α-configuration and the hydroxyl at C-3 was in β-configuration. The absence of a cross peak between H-4 and Me-OAc at C-15 supported a *trans*-fused cyclopentane ring which is usual in other lathyrane derivatives.[7,12,13] The strong NOE effect between H-4 and H_3-17 indicated the relative *cis*-orientation of H-4 and the vinylic methyl group. In addition, the NOESY correlation of H-5 and H-8β indicated an *E*-geometry for the Δ^5 double bond. The α-oriented H-7, H-8α, and H-9 were consequently deduced from the distinct NOE interactions of H-7 with H_3-17, H-8α, and H-9. NOESY correlations of H_3-18 with H-9, H-11 established a *cis*-orientation for H-9 and H-11. The upfield-shifted carbon signal of C-20 at δ_C 12.2 revealed an *E*-geometry for the Δ^{12} double bond,[14] and this was confirmed by NOESY correlations of H-11 with H_3-20, and of H-12 with H_3-19. A computer-modeled structure of **1** (CS ChemBio 3D Ultra Version 11.0 using MM2 force field calculation for energy minimization), on which the NOESY correlations were depicted in Figure 3, further supported the stereochemistry assignments. Thus, the structure of compound **1** was assigned as shown in Figure 1, and this compound was named altotibetol.

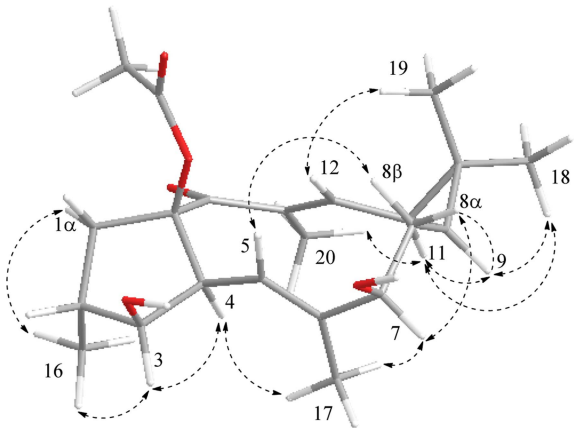

Figure 3. Energy minimized structure and key NOESY correlations of compound **1**.

The cytotoxicity of compound **1** was evaluated against selected cancer cell lines, including human leukemia (K562), human gastric carcinoma (SGC-7901), human hepatocarcinoma (SMCC-7721) cell lines using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) colorimetric assay as previously reported.[15] Compound **1** was inactive against SGC-7901 and SMCC-7721 cell lines, but exhibited moderate activity against K562 cell lines (IC_{50} 34.32 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin Elmer 341 polarimeter. IR spectra were taken on a Nicolet NEXUS 670 FT-IR spectrometer. NMR spectra were recorded on a Bruker AVANVCE III-400 and a Varian Mercury-600BB NMR spectrometers with TMS as internal standard. LR-ESI-MS data were obtained on a Bruker Daltonics Esquire 6000 mass spectrometer. HR-ESI-MS data were recorded on a Thermo LTQ Orbitrap Elite mass spectrometer. Sephadex LH-20 were supplied by Amersham Pharmacia Biotech. Silica gel (200-300 mesh) used for column chromatography and silica gel GF_{254} (10-40 μM) used for TLC were supplied by the Qingdao Marine Chemical Factory, Qingdao, China. Spots were detected on TLC under UV light or by heating after spraying with 5% H_2SO_4 in C_2H_5OH (v/v).

Plant Material. The whole plant of *E. altotibetica* were collected from Qinghai Province, China, in August 2010 and identified by Prof. Hui-Yan Xiong, Qinghai University. A voucher specimen (No. 201008EA) was deposited at the School of Pharmacy, Lanzhou University.

Extraction and Isolation. The shade dried plant material (9.0 kg) was powdered and extracted three times (7 days in each case) with 95% EtOH at room temperature. After evaporation of solvent in vacuo, the residue (400 g) was suspended in H_2O and partitioned with EtOAc and n-BuOH successively. The EtOAc-soluble fraction (293 g) was first subjected to column chromatography on silica gel eluted with petroleum ether-acetone (40:1 → 0:1) to afford seven fractions A–G according to TLC analysis. Fraction F (petroleum ether-acetone, 1:1) was further CC on silica gel by gradient elution with $CHCl_3$-acetone (20:1 → 1:1) to give five subfractions. Subfractions A ($CHCl_3$-acetone, 20:1) was subjected to CC on Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) and silica gel ($CHCl_3$-acetone, 3:1) to yield compound **1** (4 mg).

Altotibetol (1): Colorless oil; $[\alpha]_D^{24}$ +6.25 (*c* 0.40, $CHCl_3$); IR (film) ν_{max} cm^{-1}: 3414, 2953, 2924, 2870, 1740, 1646, 1615, 1454, 1371, 1266, 1063, 1046, 736; ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) see Table 1; LR-ESI-MS *m/z* 399.4 $[M + Na]^+$; HR-ESI-MS *m/z* 399.2140 $[M + Na]^+$ (calcd. for $C_{22}H_{32}O_5Na$, 399.2142)

Cytotoxicity Assay. Cytotoxicity against the K562, SGC-7901, and SMCC-7721 cell lines was evaluated by using the MTT method according to the protocols described in the previous literature.[15]

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Supporting Information. The spectral data of compound **1** are available on request from the correspondence author.

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