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Two new diterpenes from *Euphorbia kansuensis*

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Abstract

Two new lathyrane diterpenes, 3β,5α-dihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E -diene (**1**) and 3β,5α,20-trihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene (**2**), were isolated from the roots of *Euphorbia kansuensis*. Their structures were determined by spectroscopic methods.

Keywords: *Euphorbia kansuensis*; 3β,5α-Dihydroxy-15β-cinnamonyloxy-14-oxolathyra-6Z,12E-diene; 3β,5α,20-Trihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene

1. Introduction

Euphorbia is the largest one in the family of Euphorbiaceae, comprising about 2000 species. More than 80 of them are distributed in China [1]. Detailed study of the profile of all secondary metabolites could contribute to taxonomic subdivision of this complex genus. And many secondary metabolites with specific types of diterpenes skeleton in the genus have been found to possess a number of interesting biological activities [2–4]. *Euphorbia kansuensis* Proch., a perennial herbaceous plant, with a milky juice in the aerial part and a yellow juice in the roots, is distributed mainly in Qinghai, Gansu, Tibetan and Sichuan provinces of China [1]. As a Tibetan medicine, the roots of this plant have been used as pyretolysis, cholagogue, apocenosis and purgative [5]. Its chemical constituents have not been reported previously. In our continuing search for biologically active compounds from the Chinese Euphorbiaceae [6–8], we report two new diterpenes (**1** and **2**) isolated from the roots of *E. kansuensis*. We herein describe the isolation and structural elucidation of these compounds.

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2. Experimental

2.1. General

Melting points: XRC-1 micro-melting apparatus. Optical rotations: Perkin-Elmer 341. IR: Perkin-Elmer FTIR. UV: Perkin-Elmer Lambda 35. NMR:Avance 600. HR-ESI-MS Bruker BioTOF Q.

2.2. Plant

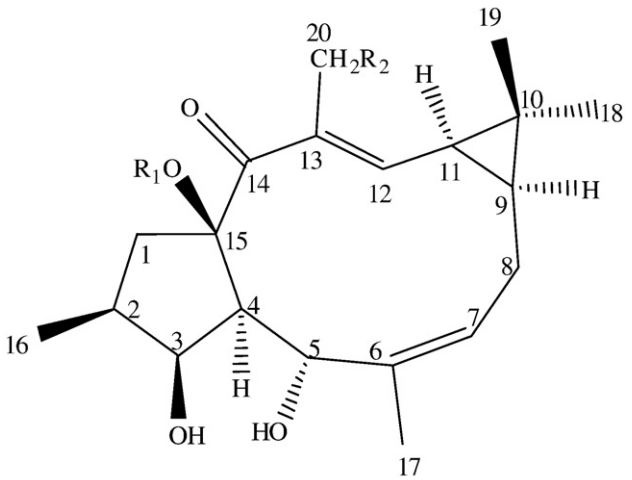
E. kansuensis roots (Euphorbiaceae), collected from Maqin county, Qinghai province of China, in July 2005 were identified by Prof. Xuefeng Lu, the Northwest Institute of Plateau Biology, Chinese Academic of Science, Xining, Qinghai, China, where a voucher specimen was deposited.

2.3. Extraction and isolation

The air-dried roots of *E. kansuensis* (15 kg) were extracted with 85% EtOH at 85 °C and evaporated *in vacuo*. The residue suspended in water was extract with $CHCl_3$. The $CHCl_3$ extract (200 g) was Si-gel CC eluting with petroleum ether, petroleum ether-EtOAc (96:4 to 36:64) and EtOAc. The portion (1 g) eluted with Petroleum ether-EtOAc (68:32) was Si-gel CC eluting with Petroleum ether-EtOAc (75:25) to afford compound **1** (20 mg). The portion (2 g) eluted with Petroleum ether-EtOAc (36:64) was fractionated on RP-18 CC eluting with H_2O-MeOH (4:1 to 1:4) and MeOH. Sediment from the fractionation eluted with H_2O–MeOH (2:3) was recrystallized in MeOH to afford compound **2** (15 mg).

3β,5α-Dihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene (**1,** Fig. 1), White needles; mp 198–199 °C; $[\alpha]^{20}_D$-330 (*c* 0.60, MeOH); UV max (MeOH): 318 (log ε 3.04) nm; IR bands (KBr): 3434, 2932, 1717, 1634, 1452, 1332, 1314, 1279, 1159, 1130, 1011, 770, 712 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data: see Table 1; HRESIMS *m/z*: 487.2454 $[M+Na]^+$ Calc. for $C_{29}H_{36}O_5Na$ 487.2455.

3β,5α,20-Trihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene(**2,**Fig. 1), White needles, mp 207–208 °C; $[\alpha]^{20}_D$-295 (*c* 0.56, MeOH); UV max (MeOH): 311 (log ε 3.35) nm; IR bands (KBr): 3435, 2919, 1715, 1635, 1451,



1 R_1=Cinnamoyl; R2=H

2 R_1=Cinnamoyl;R2=OH

Fig. 1. Structures of compounds **1** and **2.**

Table 1
^{1}H-NMR and ^{13}C-NMR data for **1** and **2** ($CDCl_3$ for **1** and CD_3OD for **2,** *J* in Herz and δ in ppm)

	1	1	2	2
C	δ_H	δ_C	δ_H	δ_C
1α	3.41 (1H, dd, *J* 7.8, 13.3)	47.4	3.29 (1H, dd, *J* 7.1, 13.0)	46.1
1β	1.81 (1H, d, *J* 13.3)		1.75 (1H, t, *J* 13.0)	
2	2.12 (1H, m)	37.7	2.00 (1H, m)	37.9
3	4.39 (1H, t, *J* 3.3)	79.7	4.35 (1H, t, *J* 3.6)	79.2
4	2.21 (1H, dd, *J* 3.3, 6.2)	54.8	2.17 (1H, dd, *J* 3.6, 5.4)	53.9
5	5.47 (1H, d *J* 6.2)	65.7	5.54 (1H, d, *J* 5.4)	65.0
6	–	135.3	–	135.9
7	5.27 (1H, dd, *J* 3.8, 11.9)	126.7	5.28 (1H, dd, *J* 3.3, 12.3)	125.8
8α	2.33 (1H, m)	23.8	2.34 (1H, m)	23.3
8β	2.40 (1H, m)		2.52 (1H, m)	
9	1.24 (1H, m)	31.8	1.42 (1H, ddd, 4.2, 7.9, 12.5)	33.7
10	–	25.6	–	26.9
11	1.39 (1H, dd *J* 8.6, 11.6)	27.8	1.66 (1H, dd, *J* 7.9, 11.8)	27.6
12	6.53 (1H, d, 11.6)	143.3	6.79 (1H, d, 11.8)	148.2
13	–	133.1	–	135.5
14	–	197.7	–	197.3
15	–	92.6	–	92.1
16	1.09 (3H, d, *J* 6.7)	13.4	1.07 (3H, d, *J* 6.7)	12.1
17	1.67 (3H, s)	17.7	1.70 (3H, t, *J* 1.3)	16.4
18	1.05 (3H, s)	28.6	1.09 (3H, s)	27.4
19	1.04 (3H, s)	16.8	1.06 (3H, s)	16.0
20	1.78 (3H, s)	12.0	4.23, 4.27 (2H, AB q, *J* 11.7)	55.5
1′	–	165.9	–	166.1
2′	6.50 (1H, d, *J* 16.0)	117.9	6.66 (1H, d, *J* 16.0)	117.8
3′	7.70 (1H, d, *J* 16.0)	146.3	7.72 (1H, d, *J* 16.0)	146.2
4′	–	134.1	–	134.1
5′	7.51 (1H, m)	128.2	7.61 (1H, m)	128.0
6′	7.40 (1H, m)	129.0	7.43 (1H, m)	128.7
7′	7.40 (1H, m)	130.7	7.43 (1H, m)	130.5
8′	7.40 (1H, m)	129.0	7.43 (1H, m)	128.7
9′	7.51 (1H, m)	128.2	7.61 (1H, m)	128.0

1314, 1280, 1158, 1127, 1060, 1012, 768 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data: see Table 1; HRESIMS *m/z*: 503.2425 $[M+Na]^+$ Calc. for $C_{29}H_{36}O_6Na$ 503.2404.

3. Results and discussion

Compound **1** (Fig. 1) was assigned the molecular formula of $C_{29}H_{36}O_5$ by HRESIMS. The ^{1}H-NMR and ^{13}C-NMR spectra (Table 1) revealed the presence of a cinnamoyl group, and indicated that the remaining moiety of **1** consisted of 20 carbons: five methyls (two tertiary, one secondary and two allylic methyls), two methylenes, eight methines, and five quaternary carbons. Among them there were two double bonds (both trisubstituted), one conjugated carbonyl, two oxymethines and one oxygenated quaternary carbon. These features were similar to those of lathyrane type diterpene jolkinol B [7]. Compared with jolkinol B, **1** contained one more double bond, but lacking of a methylene and an oxygenated quaternary carbon, which indicated the 5, 6 epoxy moieties was cleaved and formed a $\Delta^{6(7)}$ double bond. The above inference was confirmed by the 2D-NMR spectra (HMQC, COSY and HMBC). Correlations in HMBC from H-1 to C-2, 3, 4, 14, 15 and 16, H-3 to C-1 and 15, H-4 to C-5, 6 and 14, H-5 to C-4, 6, 7, 15 and 17, H-12 to C-13, 14 and 20, and the absence of HMBC cross-signal between H-3 and C-1′, H-5 and C-1′ revealed that the structure of **1** was 3,5-dihydroxy-15-cinnamoyloxy-14-oxolathyra-6,12-diene. In the lathyrane skeleton, H-4 was used as a reference point and assumed to have α orientation. NOE cross peaks between H-4 and H-2, H-3, and H-17, H-12 and H-5, H-8β and H-19, H-19 and H-3′, H-5′ and H-9′ revealed that Me-16, 3-OH and 15-cinnamoyloxy were in β orientation, while 5-OH in α orientation. NOE correlations between H-7 and H-8α, H-9 and H-17, H-11 and H-9, H-18 and H-20 indicated that $\Delta^{6(7)}$ was in *cis*- configuration and Δ^{12} was in *trans*-configuration. All relevant NOE effects were in

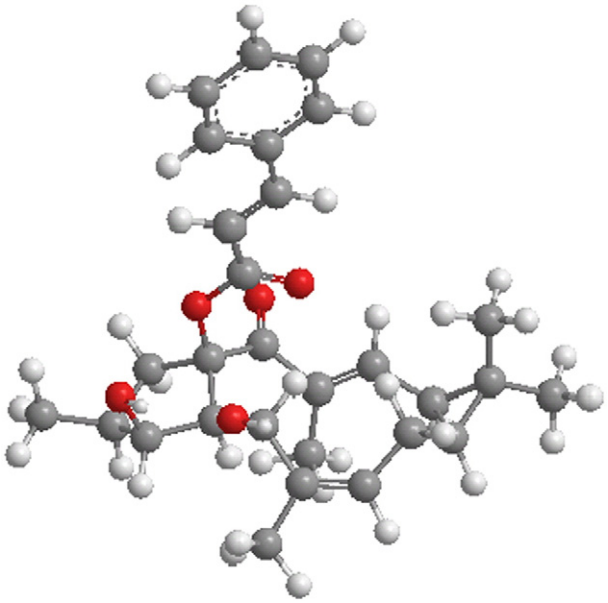


Fig. 2. Calculated conformation of **1.**

agreement with the energy minimized conformation calculated using Chem3D software (Fig. 2). Thus **1** was elucidated as 3β,5α-dihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene.

Compound **2** was assigned the molecular formula $C_{29}H_{36}O_6$ by HRESIMS. The NMR spectral data of **2** were similar to those of **1**. The major difference between compounds **2** and **1** was the replacement of an allylic methyl of **1** by an oxymethylene [δ_C 55.5 (CH_2), δ_H 4.23, 4.27 (2H, AB q, 11.7)]. This suggested that **2** was the oxygenate derivative of **1**. The connectivities of these oxo-groups' position were established by the following long range C–H correlations: H-1 with C-2, 3, 4, 14, 15 and 16, H-3 with C-1 and 15, H-4 with C-3, 5, 6, 14 and 15, H-5 with C-4, 6, 7, 15 and 17, H-12 with C-9, 10, 13, 14 and 20, and H-20 with C-12, 13 and 14. And correlations in HMBC between H-3 and C-1′, H-5 and C-1′ were not observed. In addition, the NOE data of **2** were similar to those of **1**. Therefore, **2** was elucidated as 3β,5α,20-trihydroxy-15β-cinnamoyloxy-14-oxolathyra-6Z,12E-diene.

Few lathyrane compounds of the $\Delta^{6(7),\,12}$ types have so far been isolated as natural products. Although compound **1** and other seven lathyranes of the $\Delta^{6(7),\,12}$ type were synthesized [9,10], the stereochemistry of lathyranes of the $\Delta^{6(7),\,12}$ type was tangled. According to Uemura et al. [9], **1** was figured as 5α-OH-6E,12E-diene. While E. Hecker considered **1** and seven others as 5α-H-6,12E-diene with the unestablished conformation of $\Delta^{6(7)}$, and named one basic structure of the seven compounds as isolathyrol [10]. In 1994, Z. J. Jia reported the isolation of a diterpene with this skeleton from *Euphorbia micractina*, characterizing it as 15β-*O*-benzoyl-5α-hydroxyl-isolathyrol [11]. Despite the 2D NMR spectra data given in the reference [11], the reported stereochemistry of 5α-OH or 5β-OH in lathyranes of the $\Delta^{6(7),12}$ type were both roughly deduced on the basis of the compound lathyrol. Later, Appendino et al. discussed the stereochemistry of lathyrol in detail, determining 5-OH in α orientation [12]. Thus the conclusion of 5β-OH in isolathyrol deduced from 5β-OH in lathyrol by E. Hecker was erroneous.

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