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Cytotoxic Diterpenoids from the Roots of *Euphorbia ebracteolata*

Abstract

Three new diterpenoids, yuexiandajisu D (**1**), E (**2**) and F were isolated from the roots of *Euphorbia ebracteolata*, along with eight known diterpenoids, jolkinolide B (**4**), jolkinolide A, *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**6**), *ent*-(13*S*)-hydroxyatis-16-ene-3,14-dione, *ent*-3β,(13*S*)-dihydroxyatis-16-en-14-one, *ent*-3-oxokaurane-16α,17-diol, *ent*-16α,17-dihydroxyatisan-3-one and *ent*-atisane-3β,16α,17-triol. The structures of all compounds were deduced using spectroscopic methods and confirmed for **1** and **2** by single-crystal X-ray diffraction. A biogenetic pathway for the formation of **1** and **2** is proposed briefly. Cytotoxic activities were evaluated against ANA-1, B 16 and Jurkat tumor cells. Jolkinolide B (**4**) displayed modest activity on ANA-1, B 16 and Jurkat tumor cells with IC_{50} values 4.46×10^{-2}, 4.48×10^{-2}, $6.47\times10^{-2}\mu$M, and *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**6**) showed significant activity against ANA-1 and Jurkat cells with IC_{50} values 7.12×10^{-3} and 1.79×10^{-2} μM. Compound **1** was found to be slightly active against ANA-1 cells with an IC_{50} value $2.88\times10^{-1}\mu$M. Structure-activity relationships of isolated compounds are also discussed.

Key words
Euphorbiaceae · *Euphorbia ebracteolata* · yuexiandajisus D – F · diterpenoids · cytotoxic activity

Introduction

As a perennial herbage widely distributed in Qingzhou, Shangdong Province of the People's Republic of China, *Euphorbia ebracteolata* Hayata (Euphorbiaceae) has long been used in folk medicine for treatment of pulmonary tuberculosis and chronic tracheitis [1]. Earlier investigators have reported the isolation of abietane diterpene lactones [2], casbane-type diterpenes [3], isopimarane diterpene [4], acetophenones [5], [6], [7] and flavonoids [8], [9], [10], [11] from this plant. As part of our research on bioactive diterpenoids from euphorbiaceous plants, we have investigated the roots of *E. ebracteolata* and isolated three new diterpenoids (**1 – 3**), along with eight known diterpenoids (**4 – 11**). To the best of our knowledge, this is the first report on a rosane-type diterpenoid from a euphorbiaceous species. Compounds **4 – 11** were isolated from this plant for the first time. We describe herein the isolation and structure elucidation of these compounds and their cytotoxic activity against tumor ANA-1, B 16 and Jurkat cells.

Materials and Methods

Apparatus

The melting points were measured on a Leica Galen III apparatus and are uncorrected. Optical rotations were measured on a Per-

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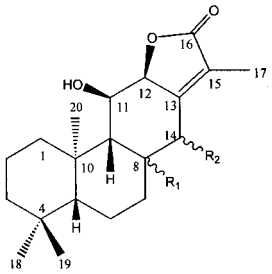
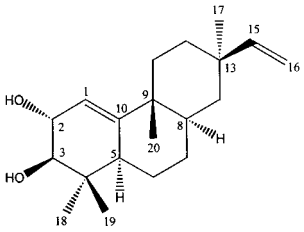


1 R_1 = β-OH, R_2 = α-OH
2 R_1 = α-OH, R_2 = β-OH



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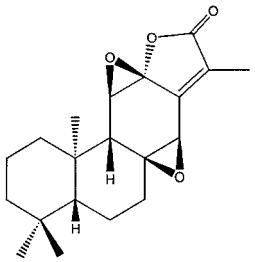

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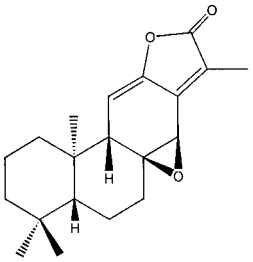

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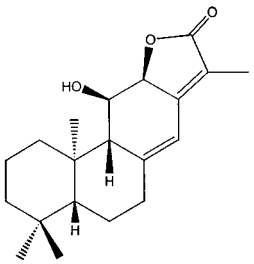

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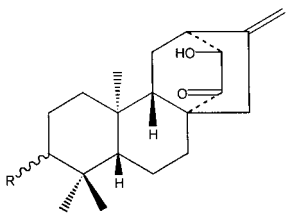
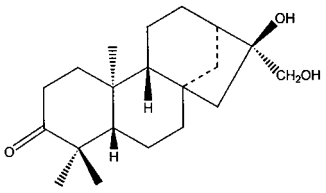


7 R = =O
8 R = α-OH



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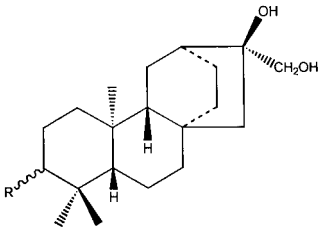

10 R = =O
11 R = α-OH

kin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 16 PC FT-IR spectrometer. UV spectra were obtained on a Beckman DU® 650 spectrophotometer. 1D and 2D NMR spectra were run on Bruker DRX-500 and JEOL JNM-EX 400 spectrometers. HR-ESI-MS were obtained on a PE Biosystems Mariner System 5140 LC/MS spectrometer. Silica gel (Merck, Kieselgel 60, 70 ~ 230 mesh) was used for column chromatography.

Plant material

The roots of *Euphorbia ebracteolata* Hayata were collected from Qingzhou, Shangdong Province, People's Republic of China, in October 1998, and were identified by Dr Minjian Qin (Division of Pharmacognosy, China Pharmaceutical University, Nanjing, China). A voucher specimen (No.98056) was deposited in the herbarium of China Pharmaceutical University.

Extraction and isolation

Dried roots of *E. ebracteolata* (5.0 kg) were milled and extracted three times with 95% EtOH (3 × 30 L) for 2 h each time, with the solvent removed under reduced pressure. The 95% ethanolic extract was suspended in water, then partitioned with petroleum ether (3 × 4 L) and EtOAc (3 × 4 L) successively. The EtOAc-soluble fraction (185.0 g) was concentrated and subjected to silica gel (2.0 kg) column (10 × 130 cm) chromatography eluting with a $CHCl_3$-MeOH (1 : 0, 99 : 1, 98 : 2, 95 : 5, 9 : 1, 8 : 2, 6 : 4, each 25.0 L) gradient system to yield frs. 1 – 7. TLC inspection indicated that frs. 1, 6 and 7 were free of diterpenoids which were not investigated further. Fr. 2 (36.0 g) was further subjected to silica gel column (6 × 80 cm, 500 g) chromatography with a gradient (5.0 L each eluent) of petroleum ether (60 – 90 °C) (PE)-acetone (AC) (95 : 5, 90 : 10, 85 : 15, 80 : 20) to afford frs. 2 – 1, 2 – 2, 2 – 3 and 2 – 4. Fr. 2 – 1 (3.2 g) was chromatographed on a silica gel column (2 × 60 cm, 60 g) eluting with PE-AC (95 : 5, 4.0 L) to give **5** (28 mg). Fr. 2 – 2 (5.0 g) was chromatographed on a silica gel column (2 × 80 cm, 100 g) eluting with PE-AC (90 : 10, 4.0 L) to give **4** (33 mg, 2400 – 2550 mL) and **7** (23 mg, 2800 – 2900 mL). After recrystallization of fr. 2 – 3 (4.1 g) with PE-AC, **6** (50 mg) was obtained. Fr. 3 (25.0 g) was further purified over silica gel column (5 × 80 cm, 400 g) chromatography developing with PE-AC (8 : 2, 10.0 L) to afford frs. 3 – 1 (1.0 L), 3 – 2 (2.0 L), 3 – 3 (3.0 L), 3 – 4 (2.0 L) and 3 – 5 (2.0 L). Fr. 3 – 3 (5.8 g) was chromatographed on a silica gel column (4 × 70 cm, 200 g) eluting with PE-EtOAc (75 : 25, 6.0 L) to give **8** (26 mg, 2900 – 3000 mL) and **9** (30 mg, 3300 – 3450 mL). Fr. 3 – 5 (1.8 g) was further purified by recrystallization from PE-AC, to afford **3** (5 mg). Fr. 4 (12 g) was purified by silica gel column (4 × 100 cm, 240 g) chromatography developing with PE-AC (8 : 2, 8 L) to afford frs. 4 – 1 (1.0 L), 4 – 2 (2.0 L), 4 – 3 (3.0 L) and 4 – 4 (2.0 L). Fr. 4 – 3 (4.3 g) was chromatographed on a silica gel column (4 × 70 cm, 160 g) eluting with PE-EtOAc (7 : 3, 5 L) to give **1** (15 mg, 2000 – 2100 mL) and **2** (30 mg, 2200 – 2400 mL). Fr. 5 (15 g) was purified by silica gel column (4 × 100 cm, 300 g) chromatography developing with PE-AC (8 : 2, 6 L) to afford frs. 5 – 1 (1.0 L), 5 – 2 (1.0 L), 5 – 3 (2.0 L) and 5 – 4 (2.0 L). Fr. 5 – 3 (3.5 g) was chromatographed on a silica gel column (4 × 60 cm, 120 g) eluting with $CHCl_3$-MeOH (95 : 5, 3.5 L) to afford **10** (35 mg). Fr. 5 – 4 (3.0 g) was chromatographed on a silica gel column (4 × 60 cm, 120 g) eluting with $CHCl_3$-MeOH (95 : 5, 2.5 L) to afford **11** (28 mg).

Yuexiandajisu D (**1**): colorless crystals ($CHCl_3$-CH_3OH); m.p. 246 – 247 °C; $[\alpha]_D^{25}$: –139.3° (*c* 0.15, $CHCl_3$ + CH_3OH); UV ($CHCl_3$): λ_{max} (log ε) = 225(1.23) nm; IR (KBr): ν_{max} = 3489 (brs), 2932, 1769, 1389, 1245, 1170, 1051 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Table **1**; HR-ESI-MS: *m/z* = 373.1998 $[M + Na]^+$ (calcd. for $C_{20}H_{30}O_5Na$: 373.1992).

Yuexiandajisu E (**2**): colorless crystals ($CHCl_3$-CH_3OH); m.p. 259 – 260 °C; $[\alpha]_D^{25}$: –104.1° (*c* 0.71, $CHCl_3$ + CH_3OH); UV ($CHCl_3$): λ_{max} (log ε) = 221(1.20) nm; IR (KBr): ν_{max} = 3341 (brs), 2927, 1737, 1694, 1425, 1310, 1102, 1046 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Table **1**; HR-ESI-MS: *m/z* = 373.1999 $[M + Na]^+$ (calcd. for $C_{20}H_{30}O_5Na$: 373.1992).

Yuexiandajisu F (**3**): white powder; $[\alpha]_D^{25}$: + 36.7° (*c* 0.09, $CHCl_3$); IR (KBr): ν_{max} = 3340 (brs), 2933, 1642, 1045, 1020, 770 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR, see Table **2**; HR-ESI-MS: *m/z* = 327.2301 $[M + Na]^+$ (calcd. for $C_{20}H_{32}O_2Na$: 327.2302).

Table **1** ^{1}H- and ^{13}C-NMR spectral data in ($CDCl_3$ + CD_3OD) at 500/125 MHz for compounds **1** and **2**[a]

	1			2		
No.	δ_C	δ_H	*HMBC*	δ_C	δ_H	*HMBC*
1	38.3	1.02 (m), 1.64 (m)	C-10, 20	40.9	1.09 (m), 1.87 (m)	C-10, 20
2	17.3	1.36 (m)	C-1	18.0	1.49 (m), 1.63 (m)	C-1
3	41.3	1.10 (m), 1.33 (m)	C-5, 18, 19	40.9	1.13 (m), 1.38 (m)	C-5
4	32.4			32.3		
5	55.3	0.81 (m)	C-1, 7, 20	54.7	0.95 (m)	C-4, 6, 7, 10, 20
6	16.7	1.54 (m), 1.60 (m)	C-7	19.8	1.56 (m)	C-8
7	34.9	1.44 (m), 1.93 (m)	C-5, 6, 8	39.9	1.57 (m), 2.13 (m)	C-5, 6, 8,14
8	74.4			75.0		
9	56.3	1.15 (d, 3.6)	C-10, 11, 20	62.6	1.81 (brs)	C-1, 8, 10, 11, 12, 14, 20
10	37.2			36.9		
11	65.0	4.14 (dd, 3.6, 5.2)	C-10, 13	67.3	4.32 (d, 3.6)	C-8, 9, 12
12	79.7	5.50 (dd, 2.0, 5.2)	C-13, 15	79.0	5.22 (dd, 2.0, 3.6)	C-13, 15
13	160.3			157.5		
14	71.8	4.00 (s)	C-8, 9, 13, 15	71.9	4.37 (s)	C-7, 8, 13, 15
15	125.9			124.2		
16	176.2			175.4		
17	7.9	1.87 (d, 2.0)	C-13, 15, 16	6.7	1.81 (d, 2.0)	C-13, 15, 16
18	32.4	0.81 (s)	C-3, 4, 5, 19	33.0	0.83 (s)	C-3, 4, 5, 19
19	20.6	0.79 (s)	C-3, 4, 5, 18	20.8	0.79 (s)	C-3, 4, 5, 18
20	15.7	1.02 (s)	C-1, 5, 9, 10	16.8	1.16 (s)	C-1, 5, 9, 10

[a] Chemical shifts in ppm, coupling constants in Hz.

Table **2** ^{1}H- and ^{13}C-NMR spectral data in ($CDCl_3$) at 500/125 MHz for compound **3**[a]

No.	δ_C	δ_H	*HMBC*
1	118.8	5.37 (d, 2.4)	C-3, 5, 9
2	72.3	3.95 (dd, 8.4, 2.4)	C-10
3	81.1	3.20 (d, 8.4)	C-2, 4, 5, 18, 19
4	38.4		
5	44.0	2.15 (dd, 13.8, 3.4)	C-3
6	18.2	1.65 (m)	C-8, 10
7	25.5	1.16 (m)	C-5, 14
8	31.2	1.65 (m)	C-10, 13
9	37.0		
10	151.8		
11a	35.0	1.67 (m)	C-20
11b		1.42 (m)	
12a	32.8	1.44 (m)	C-9, 11, 17
12b		1.20 (m)	
13	36.3		
14a	39.7	1.12 (m)	C-8, 13, 15, 17
14b		1.07 (m)	
15	151.1	5.74 (dd, 17.4, 10.8)	C-12, 13, 14, 17
16a	108.8	4.85 (dd, 17.4, 1.2)	C-13, 15
16b		4.78 (dd, 10.8, 1.2)	
17	22.3	0.91 (s)	C-12, 13, 14, 15
18	23.9	0.98 (s)	C-3, 4, 5, 19
19	13.9	0.66 (s)	C-3, 4, 5, 18
20	20.7	0.87 (s)	C-8, 9, 10, 11

[a] Chemical shifts in ppm, coupling constants in Hz.

X-ray crystallographic analysis data of yuexiandajisu D (1) and yuexiandajisu E (2)

Diffraction intensity data were acquired with a Bruker APEX CCD single crystal X-ray diffractometer with Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator. Crystal data for **1**: $C_{20}H_{30}O_5$ (350.44 g/mol), crystal size 0.15 × 0.10 × 0.10 mm^3, orthorhombic, space group $P2_12_12_1$, T = 100(2) K, a = 10.150(2) Å, b = 10.483(2) Å, c = 16.890(3) Å, V = 1797.2(6) Å^3, D_c = 1.295 Mg/m^3, Z = 4, $F_{(000)}$ = 760, $\mu_{(Mo\text{-}Ka)}$ = 0.092mm^{-1}. A total of 7203 reflections were collected in the range 2.29° < Θ < 24.98°, with 2785 independent reflections [R(int) = 0.1059], completeness to Θ max was 88.8 %; absorption correction was by SADABS with max. and min. transmission, 1.00 and 0.72; refinement method (Bruker AXS Shelxtl 6.10), full-matrix least-squares on F^2, the number of data/restraints/parameters were 2785/0/239; goodness-of-fit on F^2 = 0.88; final R indices [I >2σ (I)], R_1 = 0.0466, wR_2 = 0.0793; R indices (all data), R_1 = 0.0938, wR_2 = 0.0878, largest difference peak and hole, 0.21 and –0.21 e/Å^3. Crystal data for **2**: $C_{20}H_{30}O_5$ (350.44 g/mol), crystal size 0.20 × 0.15 × 0.12 mm^3, monoclinic, space group $P2_1$, T = 100(2) K, a = 7.1849(13) Å, b = 9.8081(18) Å, c = 12.590(2) Å, β = 99.425(4)°, V = 875.2(3) Å^3, D_c = 1.330 Mg/m^3 , Z = 2, $F_{(000)}$ = 380, $\mu_{(Mo\text{-}K\alpha)}$ = 0.094 mm^{-1}. A total of 4471 reflections were collected in the range 2.65° < Θ < 24.99°, with 2962 independent reflections [R(int) = 0.0398], completeness to Θ max. 99.5 %; max. and min. transmission 1.00 and 0.74; data/restraints/parameters 2962/1/238; goodness-of-fit on F^2 = 0.99; final R indices [I >2σ (I)], R_1 = 0.0550, wR_2 = 0.1074; R indices (all data), R_1 = 0.0751, wR_2 = 0.1156, largest difference peak and hole, 0.25 and –0.24 e/Å^3. All hydrogen atoms were located in Fourier difference maps and refined with idealized geometries and riding con-

straints. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (Accession No. CCDC 226211 for **1** and 226212 for **2**).

Cytotoxicity experiments

Cytotoxicity was measured by the improved MTT method for the tumor cell lines B16 (mouse melanoma), ANA-1 (mouse macrophage) and Jurkat (human T lymphoma). Briefly, 5×10^4 cells were added to each well (100 μL/well), and incubated with various concentrations of drugs (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} mol/L) or without drugs in four replicates for 72 h at 37 °C in a humidified atmosphere of 5 % CO_2. After 72 h, 40 μL of MTT solution (2 mg/mL) were added to each well, which were incubated for another 4 h. Then DMSO was added to each well (200 μL/well). After 15 minutes at room temperature, the OD value of each well was recorded on an ELISA reader (TECAN 500) at 540 nm.

Results and Discussion

Yuexiandajisu D (**1**) was obtained as block-shaped colorless crystals from a mixture of 1 : 1 (v/v) chloroform and methanol. The molecular formula of **1** was deduced to be $C_{20}H_{30}O_5$ by HR-ESI-MS (*m/z* = 373.1998 $[M + Na]^+$) and ^{13}C NMR data (Table **1**). The IR spectrum of **1** indicated the presence of hydroxy (3489 cm^{-1}) and α,β-unsaturated lactone (1769 cm^{-1}) groups. The ^1H- and ^{13}C-NMR spectra (Table **1**) displayed signals for three methyl groups (δ_H = 0.79, s, δ_C = 20.6; δ_H = 0.81, s, δ_C = 32.4; δ_H = 1.02, s, δ_C = 15.7), one vinylic methyl group (δ_H = 1.87, d, J = 2 Hz, δ_C = 7.9), three hydroxymethines (δ_H = 4.00, s, δ_C = 71.8; δ_H = 4.14, δ_C = 65.0; δ_H = 5.50, δ_C = 79.7), one α,β-unsaturated lactone (δ_C = 160.3, 125.9, 176.2), and one quaternary carbon connected with an oxygen atom (δ_C = 74.4). Considering the structure of diterpenoids isolated from the genus *Euphorbia*, all the spectral data showed that the structure of compound **1** was an abietane-type diterpenoid. Comparison of the ^{13}C NMR data of **1** with those of **4** [2] suggested that compound **1** is similar to **4** except for the C ring and lactone ring. The characteristic ^1H NMR signal at δ_H 5.50 (dd, J = 2.0, 5.2 Hz), which showed coupling with H-11 and long-range (W-type) coupling with 17-CH_3, suggested that an epoxy unit between C-11 and C-12 (present in **4**) was replaced by one hydroxy group at C-11 (present in **1**). On comparing the ^{13}C NMR spectrum of **1** with that of **4**, the downfield signals of C-8 (δ_C = 74.4 in **1** compared to δ_C = 66.1 in **4**) and C-14 (δ_C = 71.8 in **1** compared to δ_C = 55.3 in **4**), indicated that the 8,14-epoxy ring was opened to form one secondary hydroxy group and one tertiary hydroxy group at C-14 and C-8, respectively. The above conclusions were further supported by the observation that **1** has 20 mass units more than **4**. Complete assignments of proton and carbon signals were performed by HMQC, ^1H-^1H COSY and HMBC experiments. Consequently, **1** was identified as a 8,11,14-trihydroxy derivative of an *ent*-13(15)-abieten-16,12-olide. The availability of suitable single crystals allowed for an X-ray structure analysis (Fig. **1**), which verified compound **1** as *ent*-8β,11β,14α-trihydroxy-13(15)-abieten-16,12-olide.

Yuexiandajisu E (**2**) was obtained as rod-shaped colorless crystals from a mixture of 1 : 1 (v/v) chloroform and methanol. The molecular formula of **2** was deduced to be $C_{20}H_{30}O_5$ by HR-ESI-MS (*m/z* = 373.1999 $[M + Na]^+$) and ^{13}C-NMR data (Table **1**). The IR spectrum of **2** indicated the presence of hydroxy (3341 cm^{-1}) and α,β-unsaturated lactone (1737 cm^{-1}) groups. Comparison of the ^1H- and ^{13}C-NMR spectra of **1** and **2** indicated that these two compounds possess the same basic skeleton and functionalities except for the stereochemistry on some carbon atoms. X-ray crystallography (Fig. **1**) provided unequivocal evidence of the structure and relative stereochemistry of **2** as *ent*-8α,11β,14β-trihydroxy-13(15)-abieten-16,12-olide.

The biotransformations to give **1** and **2** were proposed to start from **6**. By the enzyme-catalyzed epoxidation, the double bond between C-8 and C-14 could be converted to an epoxide ring. Nucleophilic attack of H_2O, either on C-8 or on C-14, and epoxide opening would then lead to compounds **1** and **2**.

Yuexiandajisu F (**3**) was isolated as a white powder, whose molecular formula was established as $C_{20}H_{32}O_2$ from HR-ESI-MS and ^{13}C-NMR (Table **2**). The IR spectrum showed the presence of hy-

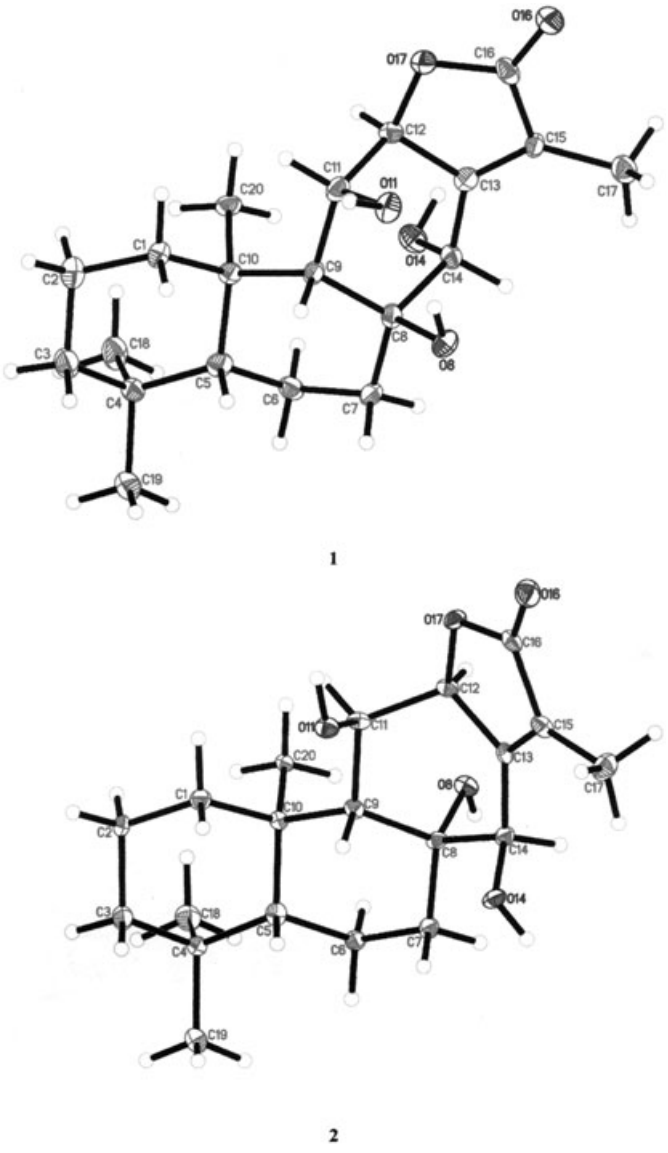

Fig. **1** Thermal ellipsoid plots of the X-ray structures of compounds **1** and **2**.

droxy (3340 cm^{-1}) and double bond (1642 cm^{-1}) groups in its molecular structure. The ^{1}H-NMR spectrum (Table **2**) showed the presence of four olefinic protons at δ_H = 5.37 (1H, d, J = 2.4 Hz); δ_H = 5.74 (1H, dd, J = 17.4, 10.8 Hz); δ_H = 4.85 (1H, dd, J = 17.4, 1.2 Hz); δ_H = 4.78 (1H, dd, J = 10.8, 1.2 Hz), while two proton signals at δ_H = 3.95 (1H, dd, J = 8.4, 2.4 Hz) and δ_H = 3.20 (1H, d, J = 8.4 Hz) can be assigned to two oxygen-bearing methines, respectively. Four methyl groups attached to quaternary carbons were observed at δ_H = 0.98 (3H, s), δ_H = 0.91 (3H, s), δ_H = 0.87 (3H, s), δ_H = 0.66 (3H, s). The ^{13}C-NMR spectra (Table **2**) indicated the presence of four olefinic carbons, two oxygenated methines, and four methyls. In the HMBC spectrum of **3**, ^{13}C-^{1}H long-range correlation signals were found for H-1 (δ = 5.37) with C-9 (δ = 37.0), C-5 (δ = 44.0) and C-3 (δ = 81.1); H-3 (δ = 3.20) with C-18 (δ = 23.9), C-19 (δ = 13.9), C-2 (δ = 72.3) and C-5 (δ = 44.0); H-15 (δ = 5.74) with C-17 (δ = 22.3), C-12 (δ = 32.8), C-13 (δ = 36.3) and C-14 (δ = 39.7); H-20 (δ = 0.87) with C-10 (δ = 151.8), C-8 (δ = 31.2) and C-11 (δ 35.0). The above data mentioned enabled the establishment of a rosane-type diterpenoid skeleton for compound **3** [12]. Furthermore, these data suggested that two hydroxy groups were located at C-2, C-3, and one double bond was between C-1 and C-10, while the other double bond was between C-15 and C-16. The relative stereochemistry of **3** was determined on the basis of the results of the NOESY spectrum. The observed NOE correlations between H-2/H-19, H-3/H-18 and H-3/H-5 indicated that the hydroxy groups attached to C-2 and C-3 are α-oriented and β-oriented, respectively. The CH_3–20 showed NOE with CH_3–19 and H-15, indicating that CH_3–20 was β-configured. Furthermore, the strong NOE between H-8/H-17 and H-8/H-5 suggested an α-orientation for H-8. On the basis of these observations, the structure of **3** was assigned as 2α,3β-dihydroxy-1(10),15-rosadiene.

Along with the three new compounds, eight known diterpenoids were also isolated from the roots of *Euphorbia ebracteolata*. By comparison of physical and spectroscopic data (^{1}H-, ^{13}C-NMR and mass spectroscopic data) with the literature values, they were identified as jolkinolide B (**4**) ($[\alpha]_D^{25}$: +232, *c* 0.38, $CHCl_3$) [2], jolkinolide A (**5**) ($[\alpha]_D^{25}$: +120, *c* 0.24, $CHCl_3$) [13], *ent*-11α-hydroxyabieta-8(14),13(15)-dien-16,12α-olide (**6**) ($[\alpha]_D^{25}$: +210, *c* 0.32, $CHCl_3$) [13], *ent*-(13*S*)-hydroxyatis-16-ene-3,14-dione (**7**) ($[\alpha]_D^{25}$: +33, *c* 0.28, $CHCl_3$) [14], *ent*-3β,(13*S*)-dihydroxyatis-16-en-14-one (**8**) ($[\alpha]_D^{25}$: +36, *c* 0.25, $CHCl_3$) [14], *ent*-3-oxokaurane-16α,17-diol (**9**) ($[\alpha]_D^{25}$: –30, *c* 0.30, $CHCl_3$) [15], *ent*-16α,17-dihydroxyatisan-3-one (**10**) ($[\alpha]_D^{25}$: –32, *c* 0.29, $CHCl_3$) [14], and *ent*-atisane-3β,16α,17-triol (**11**) ($[\alpha]_D^{25}$: –80, *c* 0.35, $CHCl_3$) [16].

Abietane diterpenoids from the genus *Euphorbia* have been reported to have antitumor activity [17], [18]. Compounds **1**, **2** and **4**–**11** were tested for cytotoxicity against tumor ANA-1, B16 and Jurkat cells. As shown in Table **3**, compound **4** displayed modest activity in all tested tumor cells. Compound **6** showed significant activity against ANA-1 and Jurkat cells, while compound **1** was found to be slightly active against ANA-1.Compounds **1**, **4** and **6** also affected the morphological characters of these cells (data not shown). Cytotoxicity of the other compounds for these cells was not found in the experiments. The fact that compounds **1**, **4** and **6** have the same basic skeleton suggested some abietane diterpenoids showed activity against the tested tumor cells. The inactivity of **5** in the tested cell lines compared with **4** provided strong evidence for the necessity of the C-11/C-12 epoxy ring system in mediating these types of biological activity within the abietane-type diterpenoids. It is interesting to see that compounds **1** and **2** are diastereomers, the difference only being the stereochemistry at chiral centers C-8 and C-14. But only compound **1** showed activity, which suggested that the configuration of ring C is also crucial for the activity. From our results, it can be concluded that the α,β-unsaturated lactone is not the only necessary group for maintaining the cytotoxic effect, since the compounds **2** and **5** do not show cytotoxicity.

Table **3** Cytotoxic activity of compounds **1**, **4** and **6**

Tested compounds	*ANA-1*	*B 16*	*Jurkat*
1	2.88×10^{-1}	ND	4.48
4	4.46×10^{-2}	4.48×10^{-2}	6.47×10^{-2}
6	7.12×10^{-3}	23	1.79×10^{-2}
5-FU	1.12×10^{-8}	5.18×10^{-4}	7.48×10^{-3}

Half inhibition concentration IC_{50} (μM).
* ND: not determined.

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