Chinese Journal of Chemistry, **2004**, *22*, 199—202 **Article**

Three New Diterpenoids from *Euphorbia wallichii*

WANG, Huan[a,b](王环) ZHANG, Xiao-Feng[a](张晓峰) CAI, Xiang-Hai[b](蔡祥海)
MA, Yun-Bao[b](马云保) LUO Xiao-Dong*[,b](罗晓东)

[a] *Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810001, China*
[b] *State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, China*

Three new abietane diterpene lactones, 3*α*-hydroxyjolkinolide A (**1**), *ent*-8*α*,14*β*-dihydroxy-13(15)-ene-16(12*α*)-abietanolide (**2**) and *ent*-8*α*,14*α*-dihydroxy-13(15)-ene-16(12*α*)-abietanolide (**3**) as well as a known abietane diterpene jolkinolide A (**4**) were isolated from the roots of *Euphorbia wallichii*. Their structures were elucidated on the basis of spectroscopic analysis.

Keywords *Euphorbia wallichii*, Euphorbiaceae, abietane, 3α-hydroxyjolkinolide A, *ent*-8*α*,14*β*-dihydroxy-13(15)-ene-16(12*α*)-abietanolide, *ent*-8*α*,14*α*-dihydroxy-13(15)- ene-16(12*α*)-abietanolide

Introduction

In the Euphorbiaceae, the genus *Euphorbia* comprises about 2000 species. More than 80 of them are distributed in China. Various groups have been working on the chemical constituents of this genus, and many biologically active diterpenes were found.[1] *Euphorbia wallichii*, used as a traditional Chinese medicine, is distributed mainly in Qinghai, Tibet and Yunnan province of China. It has been used by Tibetan to cure furuncle, exanthema and cutaneous anthrax.[2] Its chemical constituents have not been reported before. From the alcohol extract of the roots of this plant, three new abietane diterpene lactones, together with a known abietanolide were obtained. In this paper the isolation and structure elucidation of these compounds were described.

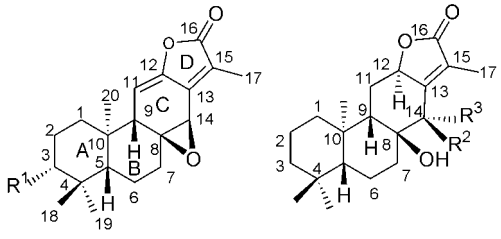

Figure 1 Structures of compounds **1**—**4**.

Results and discussion

The known compound was identified to be Jolkinolide A (**4**) based on comparing its spectral data with those reported in literature.[2]

Compound **1** has a molecular formula of $C_{20}H_{26}O_4$ as determined by HR-EI-MS and ^{13}C NMR spectra (Table 1). The UV and IR spectra showed strong absorp-

Table 1 ^{13}C NMR spectral data of compounds **1**—**4** (δ_C 100 MHz)[a]

Carbons	**1**	**2**	**3**	**4**
1	37.6 (t)	43.2 (t)	40.5 (t)	39.8 (t)
2	27.0 (t)	20.1 (t)	18.7 (t)	18.4 (t)
3	78.2 (d)	43.2 (t)	43.1 (t)	41.4 (t)
4	39.2 (s)	34.2 (s)	34.1 (s)	33.4 (s)
5	52.9 (d)	56.6 (d)	57.0 (d)	53.4 (d)
6	20.5 (t)	22.0 (t)	19.2 (t)	20.8 (t)
7	33.9 (t)	43.1 (t)	36.3 (t)	34.1 (t)
8	61.0 (s)	75.7 (s)	77.6 (s)	61.1 (s)
9	51.6 (d)	58.0 (d)	47.4 (d)	51.8 (d)
10	41.1 (s)	40.3 (s)	39.0 (s)	41.5 (s)
11	103.4 (d)	29.9 (t)	29.0 (t)	104.0 (d)
12	147.6 (s)	79.0 (d)	79.9 (d)	147.4 (s)
13	144.8 (s)	165.3 (s)	166.5 (s)	145.0 (s)
14	54.3 (d)	73.5 (d)	74.3 (d)	54.4 (d)
15	125.4 (s)	123.2 (s)	125.5 (s)	125.1 (s)
16	170.4 (s)	178.0 (s)	177.8 (s)	170.6 (s)
17	8.6 (q)	8.2 (q)	9.4 (q)	8.6 (q)
18	28.3 (q)	34.6 (q)	34.1 (q)	33.4 (q)
19	15.5 (q)	22.4 (q)	22.4 (q)	21.9 (q)
20	15.0 (q)	18.1 (q)	15.4 (q)	15.0 (q)

[a] Compounds **1** and **4** were measured in $CDCl_3$, while **2** and **3** in CD_3OD.

* E-mail: xdluo@mail.kib.ac.cn; Tel.: +86-871-5223421; Fax: +86-871-5150227
Received January 26, 2003; revised and accepted September 15, 2003.
Project supported by the Foundation of the State Key Laboratory of Phytochemistry and Plant Resources in west China, Kunming Institute of Botany, Chinese Academy of Sciences and Knowledge Innovation Project from the Northwest Institute of Plateau Biology, Chinese Academy of Sciences (No. CXLY-2002-7).

Table 2 ^{1}H NMR spectral data of compounds **1—3** (δ_H, 400 MHz)[a]

H	1	2	3
1	1.77, 1.43 (m, each 1H)	1.53, 0.86 (m, each 1H)	1.49, 0.75 (m, each 1H)
2	1.60, 1.72 (m, each 1H)	1.37 (m, 2H)	1.28 (m, 2H)
3	3.38 (dd, J=4.2, 11.4 Hz, 1H)	1.36, 1.12 (m, each 1H)	1.28 (m, 1H); 1.08 (dd, J=4.0, 13.2 Hz, 1H)
5	1.23 (dd, J=2.3, 12.3 Hz, 1H)	0.90 (m, 1H)	0.78 (m, 1H)
6	1.61, 1.84 (m, each 1H)	1.47,,1.63 (m, each 1H)	1.31, 1.48 (m, each 1H)
7	2.14, 1.68 (m, each 1H)	2.17 (m, 1H); 1.86 (d, J=12.8 Hz, 1H)	1.95, 1.47 (m, each 1H)
9	2.62 (d, J=5.2 Hz, 1H)	1.54 (m, 1H)	1.37 (dd, J=7.5, 10.8 Hz, 1H)
11	5.44 (d, J=5.2 Hz, 1H)	1.53 (m, 1H); 2.37 (dd, J=6.8, 13.4 Hz, 1H)	1.22, 2.19 (m, each 1H)
12	—	5.12 (dd, J=6.8, 10.2 Hz, 1H)	5.37 (dd, J=8.8, 9.7 Hz, 1H)
14	3.74 (s, 1H)	4.28 (s, 1H)	3.97 (s, 1H)
17	2.08 (s, 3H)	1.71 (d, J=1.8 Hz, 3H)	1.80 (t, J=1.8 Hz, 3H)
18	0.85 (s, 3H)	0.78 (s, 3H)	0.80 (s, 3H)
19	1.09 (s, 3H)	0.83 (s, 3H)	0.80 (s, 3H)
20	0.75 (s, 3H)	1.16 (s, 3H)	1.02 (s, 3H)

[a] Compound **1** was measured in $CDCl_3$, while **2** and **3** in CD_3OD.

tions for α, β, γ, δ-unsaturated-γ-lactone [λ_{max} 283 nm; ν_{max} 1751 cm^{-1} (lactone), 1654 cm^{-1} (C=C)] and a hydroxyl group (ν_{max} 3445 cm^{-1}).

The ^{13}C NMR spectrum showed signals of four tertiary methyls, four methylenes, four methines, and three quaternary carbons, two double bonds [δ_C 130.4 (d), 147.6 (s); 125.4 (s), 144.8 (s)] and a lactone carbonyl [δ_C 170.4 (s)].

Comparison of the ^{1}H NMR spectra data (Table 2) and ^{13}C NMR spectra data (Table 1) of **1** and **4** it is indicated that **1** possessed an abietanolide skeleton. The NMR spectral data of **1** were similar to those of Jolkinolide A ($C_{20}H_{26}O_4$) except for the obvious chemical shift differences in ring A. The ^{1}H NMR and ^{13}C NMR spectra showed that the C-3 methylene group was replaced by a secondary hydroxy group [δ_C 78.8 (d), δ_H 3.38 (dd, J=4.2, 11.4 Hz)] in **1**. In addition, the chemical shifts of C-2 and C-4 were shifted downfield yo δ 8.6 and δ 5.8 respectively, which may be due to β-effect from 3-OH. The structure was further supported by analyses of H-H COSY, HMQC and HMBC spectra. Because J values for H-3 were 4.2 Hz (J_{ae}) and 11.4 Hz (J_{aa}), 3-OH was placed at α position. It was also supported by ROE interaction between H-5 and H-3, H-5 and H-9. Other chiral carbons of **1** were the same as those of **4**, which was supported by RoEsy spectrum. Thus **1** was established to be 3α-hydroxyjolkinolide A.

The molecular formula of **2** was determined to be $C_{20}H_{30}O_4$ on the bases of HR-EI-MS and the ^{13}C NMR spectrum, which possessed six degrees of unsaturation. Compared with **4**, compound **2** also possessed an abietanolide skeleton. It showed the presence of hydroxyl(s) (ν_{max} 3445 cm^{-1}) and an α,β-unsaturated-γ-lactone carbonyl (λ_{max} 216 nm; ν_{max} 1748 cm^{-1}). The ^{13}C NMR spectrum showed signals for four tertiary methyls, six methylenes, four methines, three quaternary carbons, one double bond [δ_C 165.3 (s, C-13), 123.2 (s, C-15)] and a lactone carbonyl [δ 177.4 (s, C-16)]. Analysis of the 1D and 2D NMR spectral data indicated that the moieties of rings A, B and D in **2** were identical to those in Jolkinolide A. In ring C there was an oxymethine carbon [δ_C 79.0 (d); δ_H 5.12 (dd, J=6.8, 10.2 Hz, 1H)] and a methylene carbon [δ_C 29.9 (t); δ_H 2.37, 1.53 (2H)] instead of a double bond in **2**. In addition, the 8,14-epoxy group was cleaved and formed 8,14-dihydroxy, corresponding with the unsaturated degree. The above inference was confirmed by the following HMBC (t_m: 62.5 ms) correlations: H-11β with C-8, C-9, C-10, C-12 and C-13; H-12 with C-11, C-13 and C-15; H-14 with C-8, C-9, C-12, C-13 and C-15; H-1α with C-2, C-3, C-5, C-10 and C-20; H-1β with C-9, C-10 and C-20; H-5 with C-4, C-6, C-9, C-10, C-19 and C-20; H-9 with C-1, C-5, C-8, C-10, C-11, C-12, C-14 and C-20. Since the diterpenes of abietane skeleton found in *Euphorbia* were all *-ent* type, **2** was described as *ent*-8,14dihydroxy-13(15)-ene-16(12)-abietanolide. In the RoEsy spectrum (Table 3), ROE interaction between H-12 and H-20, H-12 and H-11α, H-14 and H-17, H-14 and H-7β, unambiguously determined H-12 at α position, and H-14 in β position.

The orientation of 8-OH was determined by the comparison of ^{13}C NMR spectra among Ebracteo- latanolide B,[9] compound **2**, and Gelomulide I.[13] In the former, 8-OH and 14-OH were both at α position, 11-OH was in axial bond at α position. If 8-OH in compound **2** was at α position, its $\delta_{C\text{-}8}$ should be bigger than that in the former compound due to losing big γ effect by 11-OH when they were detected in the same solvent. However, the chemical shifts of the similar carbon could be changed, when the concentration or solvent was changed. The above change was concerned with the hydrogen bond.[14] Among $CDCl_3$, CD_3OD and

DMSO, DMSO has the highest ability to form hydrogen bond with the solute. Ability of CD_3OD is almost as big as that of $CDCl_3$. In DMSO, the hydrogen bond made the electron of S=O move to the hydrogen of the molecular solute. It increased the electron density of the carbon connected with the hydrogen or hydroxyl which could form hydrogen bond with DMSO, and reduced the chemical shift of that carbon. The former compound was detected in $CDCl_3$+DMSO, and **2** was in CD_3OD. So $\delta_{C\text{-}8}$ in **2** should be bigger than that in the former ($\delta_{C\text{-}8}$=75.0). On the contrary, the $\delta_{C\text{-}8}$ in **2** was 75.7, which was almost as big as that in the former. So, 8-OH in **2** was not at α position but at β position, which was similar to that in the latter compound. And it was further proved by the similar chemical shifts of C-8 in **2** and the latter compound (in $CDCl_3$) (Figue 2). In ring B of **2**, 8-OH was in equatorial bond, which made less α-effect than that in axial bond (at α position). That explained why the chemical shift of C-8 in **2** was not bigger than that in the former. Therefore, **2** was assigned as *ent*-8α,14β-dihydroxy-13(15)-ene-16(12α)-abietanolide. The ^{13}C NMR data of rings C and D in **2** were similar to those in the latter compound. In the latter compound, ring C was conformed to be of chair conformation by the X-ray crystallographic analysis. Thus ring C in **2** was assumed to be chair conformation (Figure 3).

Compound **3** gave the same molecular formula $C_{20}H_{30}O_4$ as **2** in its HR-EI-MS. The ^1H NMR and ^{13}C NMR spectra of **3** were similar to those of **2**. In the HMBC spectrum of **3**, the following long couplings:

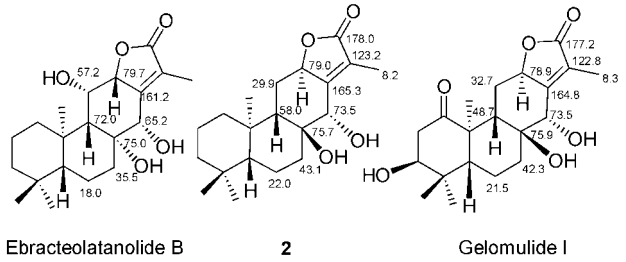

Figure 2 ^{13}C NMR shifts of Ebracteolatanolide B ($CDCl_3$+DMSO), **2** (CD_3OD) and Gelomulide I ($CDCl_3$).

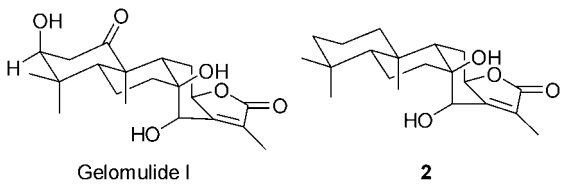

Figure 3 Stero-structure of Gelomulide I and **2**.

H-11β with C-9, C-10, C-12 and C-13; H-12 with C-11, C-13 and C-15; H-14 with C-8, C-9, C-13 and C-15; H-1α with C-3, C-5, C-9 and C-10; H-1β with C-2, C-3, C-5, C-9, C-10 and C-20; H-5 with C-4, C-6, C-9, C-10, C-18, C-19 and C-20; H-9 with C-8, C-10, C-11 and C-20, showed that **3** was 14-epimer of **2**. Obviously, the chemical shifts of C-9 and C-7 were shifted upfield with δ 10.6 and 6.8 as compared with those of **2**. It is suggested that 14-hydroxyl of **3** was placed at β position, and located on the same side with H-9 and axial bond of H-7, which made bigger γ-effect than that of **2**. So 14-hydroxyl in **3** was regarded as β-orientation instead of α-orientation in **2**. Moreover, the above inference was proved by the NOE interaction in the RoEsy spectrum of **3** (Table 3). Thus **3** was elucidated as *ent*-8α,14α-dihydroxy-13(15)-ene-16(12α)-abietanolide.

Experimental

General procedures

All melting points were measured on an XRC-1 micro-melting apparatus and uncorrected. Optical rotations were measured with a Horbia SEAP-300 spectropolarimeter. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer with KBr pellets. UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer. MS spectra were obtained with a VG Auto Spec-3000 spectrometer, at 70 eV for EI. 1D and 2D NMR spectra were recorded on a Bruker AM-400 or a DRX-500 MHz spectrometer with TMS as internal standard. Silica gel (200—300 mesh) for CC and GF254 for analytical TLC were from the Qingdao Marine Chemical Factory, China.

Table 3 Key ROE correlations of compounds **1—3** (500 MHz)[a]

H	1	2	3
H-12	—	H-11α, H-20	H-11α
H-14	H-6α, H-7α	H-7β, H-17	H-7α
H-5	H-3, H-9, H-18	H-3β, H-9, H-18	H-3β, H-6β, H-7β, H-9
H-9	H-1β, H-5	H-1β, H-5, H-7β, H-17, H-18,	H-1β, H-5, H-7β, H-18
H-20	H-6α, H-7α, H-19	H-1α, H-12, H-11α, H-19	H-1α, H-3α, H-19, H-11α
H-1β	H-2β, H-3, H-6β, H-9, H-18	H-3β, H-9	H-3β, H-6β, H-7β, H-9
H-11α	—	H-6α, H-7α, H-12, H-20	H-6α, H-7α, H-12, H-20
H-7β	H-2β	H-6β, H-9, H-11β, H-14	H-1β, H-5, H-9

[a] Compound **1** was measured in $CDCl_3$, while **2** and **3** were in CD_3OD. Their mixing time (t_m) were 0.3, 0.8 and 1.0 s, respectively.

Plant material

Euphorbia wallichii hook. f. was collected from Xinghai county, Qinghai province, China, in July 2001. It was identified by Prof. Zhang Xiao-Feng, Northwest Plateau Institute of Biology, Chinese Academy of Science, Xining, Qinghai, China, where a voucher specimen (no. 1002) was deposited.

Extraction and isolation

The air-dried roots (10 kg) of *Euphorbia wallichii* were extracted with EtOH (95%) four times at room temperature, the combined extracts were evaporated *in vacuo*. The residue was suspended in H_2O and then extracted with $CHCl_3$ for three times. The $CHCl_3$ layer was concentrated *in vacuo* to give 200 g of residue, which was chromatographed on silica gel. The column was eluted with petroleum ether-EtOAc (from petroleum ether to petroleum-EtOAc 1∶1). According to differences in composition monitored by TLC (GF_{254}), 17 fractions were obtained. Sediment from fraction 3 (9.9 g) was washed intensively with petrol-acetone (10∶1) to afford **4** (100 mg). Fraction 10 (5.2 g) was chromatographed repeatedly on silica gel column eluted with petrol-Me_2CO (from 22∶3 to 7∶3) to give three subfractions (A—C). Fraction B (1.1 g) was subjected to CC on silica gel with $CHCl_3$-Me_2CO (94∶6) to afford **1** (40 mg). Fraction 11 (6.8 g) was subjected to CC on silica gel with petrol-Me_2CO (from 17∶3 to 7∶3). Five subfractions (a—e) were collected. Fraction c (1.8 g) was subjected to CC on silica gel with $CHCl_3$-Me_2CO (from 95∶5 to 4∶1) to give three subfractions (I—III). Sediment from fraction II (120 mg) was washed intensively with petrol-acetone (10∶1) and recrystallized by MeOH, then it was washed intensively again to afford **3** (6 mg). Fraction III (700 mg) was chromatographed on silica gel column eluting with $CHCl_3$-Me_2CO (9∶1) to afford **2** (15 mg).

3α-Hydroxyjolkinolide A (1) White powders, m.p. 228—230 ℃; $[\alpha]_D^{23}$ +115.2 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max}: 283 (log ε 4.06) nm; ^{1}H NMR ($CDCl_3$) spectral data see Table 2; ^{13}C NMR ($CDCl_3$) spectral data see Table 1; IR (KBr) *v*: 3576, 3445, 2930, 2866, 1751, 1654, 1220, 1072, 1033, 881, 847, 765 cm^{-1}; EIMS *m/z* (%): 330 (M^+, 5), 312 (24), 297 (3), 176 (100), 164 (35), 149 (20), 135 (27), 107 (17), 91 (18), 81 (23), 69 (23), 55 (20); HREIMS calcd for $C_{20}H_{26}O_4$ 330.1831, found 330.1831 (error: δ 0.0).

***ent*-8α,14β-Dihydroxy-13(15)-ene-16(12α)-abietan olide (2)** White powders, m.p. 336—338 ℃; $[\alpha]_D^{22}$ −56.1 (*c* 0.11, MeOH); UV (MeOH) λ_{max}: 216 (log ε 3.96) nm; ^{1}H NMR (CD_3OD) spectral data see Table 2; ^{13}C NMR (CD_3OD) spectral data see Table 1; IR (KBr) *v*: 3445, 2925, 1748, 1635, 1060, 1027 cm^{-1} EIMS *m/z* (%): 334 (M^+, 41), 316 (17), 301 (6), 194 (10), 179 (92), 161 (9), 151 (8), 137 (34), 123 (29), 109 (32), 95 (38), 81 (52), 69 (100), 55 (89); HREIMS calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2153 (error: δ −2.7).

***ent*-8α,14α-Dihydroxy-13(15)-ene-16(12α)-abietan olide (3)** White powders, m.p. 347—349 ℃; $[\alpha]_D^{18.1}$ −145.0 (*c* 0.19, $CHCl_3$); UV (MeOH) λ_{max}: 223 (log ε 3.94) nm; ^{1}H NMR (CD_3OD) spectral data see Table 2; ^{13}C NMR (CD_3OD) spectral data see Table 1; IR (KBr) *v*: 3444, 2927, 1760, 1634, 1054, 1035 cm^{-1}; EIMS *m/z* (%): 334 (M^+, 49), 316 (32), 301 (16), 194 (18), 179 (57), 161 (15), 151 (14), 137 (54), 123 (42), 109 (43), 95 (48), 81 (52), 69 (94), 55 (100); HREIMS calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2142 (error: δ 0.6).

References

1 Ng, A. S. *Phytochemistry* **1990**, *29*, 662.
2 Lal, A. R.; Cambie, R. C.; Rutledge, P. S.; Woodgate, P.D. *Phytochemistry* **1990**, *29*, 2239.
3 Borghi, D.; Baumer, L.; Ballabio, M.; Arlandini, E.; Perellino, N. C.; Minghetti, A.; Vincieri, F. F. *J. Nat. Prod.* **1991**, *54*, 1503
4 Jia, Z.-J.; Ding, Y.-L. *Planta Med.* **1991**, *57*, 569.
5 Shi, J.-G.; Jia, Z.-J.; Yang, L. *Chem. J. Chin. Univ.* **1994**, *15*, 861 (in Chinese).
6 Wu, D.-G.; Sorg, B.; Hecker, E. *J. Nat. Prod.* **1995**, *58*, 408.
7 Morgenstern, T.; Bittner, M.; Silva, M.; Aqueveque, P.; Jakupovic, J. *Phytochemistry* **1996**, *41*, 1149.
8 Appendino, G.; Jakupovic, S.; Tron, G. C.; Jakupovic, J.; Milon, V.; Ballero, M. *J. Nat. Prod.* **1998**, *61*, 749.
9 Wang, W.-X.; Ding, X.-B. *Acta Pharmaceutica Sinica* **1998**, *33*, 128 (in Chinese).
10 Shi, Y.-P.; He, Z.-J.; Jia, Z.-G. *Nat. Prod. Res. Devel.* **1999**, *11*, 85 (in Chinese).
11 Sutthivaiyakit, S.; Thapsut, M.; Prachayasittikul, V. *Phytochemistry* **2000**, *53*, 947.
12 North West Plateau Institute of Biology, Chinese Academy of Science In *Tibetan Medicine*, Ed.: Yang, Y.-C., Qinghai People Press, Qinghai, **1991**, pp. 145—46 (in Chinese).
13 Talapatra, B.; Das, G.; Das, A. K.; Biswas, K.; Talapatra, S. K. *Phytochemistry* **1998**, *49*, 1353.
14 Ning, Y.-C. *Structural Identification of Organic Compounds and Organic Spectroscopy*, Science Press, Beijing, **2000**, p. 118 (in Chinese).

(E0301266 SONG, J. P.)