Eight New Diterpenoids from the Roots of *Euphorbia nematocypha*

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From the dried roots of *Euphorbia nematocypha*, eight new diterpenoids, with *ent*-atisane (*i.e.*, **1**–**5**) and isopimarane (*i.e.*, **6**–**8**) type skeletons, together with five known compounds, were isolated. The structures of these new compounds were elucidated by spectroscopic data. Compounds **1**–**8** were evaluated for their cytotoxicity against a small panel of human cancer cell lines.

Introduction. – The genus *Euphorbia*, with over 2000 species, is the largest in the plant family Euphorbiaceae and has attracted considerable attention as a rich source of diterpenoids with diverse structures and interesting biological properties [1][2]. In connection with an ongoing search for biologically active metabolites from herbs used in Traditional Chinese Medicine, we have investigated *Euphorbia nematocypha* HAND.-MAZZ. (Chinese name '*Da Lang Du*'). This is a perennial herbaceous plant widely distributed in the middle and northwest Yunnan Province, especially in the alpine grasslands of Zhongdian (Shangrila) County at altitudes between 2500 and 3700 m. Previous studies on *E. nematocypha* led to the isolation of some triterpenoids and macrocyclic diterpenoids [3][4]. In order to further study its constituents, we have reinvestigated this plant. The present paper describes the isolation and structure elucidation of eight new diterpenoids, including *ent*-atisane (*i.e.*, **1**–**5**) and isopimarane (*i.e.*, **6**–**8**) type, and five known compounds were also identified. Compounds **1**–**8** were evaluated for their cytotoxicity against the HL-60, A-549, MOLT-4, and BEL-7402 human cancer cell lines.

Results and Discussion. – Compound **1** was assigned the molecular formula $C_{20}H_{28}O_3$ through an analysis of its HR-ESI-MS (*m/z* 339.1930, $[M+Na]^+$), requiring seven degrees of unsaturation. Its IR spectrum showed absorptions at 3406, 1707, and 1635 cm^{-1}, which were attributed to OH, C=O, and C=C functional groups. From the ^1H- and ^{13}C-NMR spectra (*Tables 1* and *2*), two C=O groups (δ(C) 217.0, 216.0) and an exocyclic $C=CH_2$ group (δ(C) 144.2 (*s*), 109.5 (*t*)), which accounted for three of seven degrees of unsaturation. Therefore, **1** was proposed to be tetracylic. The three diagnostic tertiary Me *singlet*s (δ(H) 1.02, 1.06, 1.17; δ(C) 21.8, 26.4, 15.6), the negative

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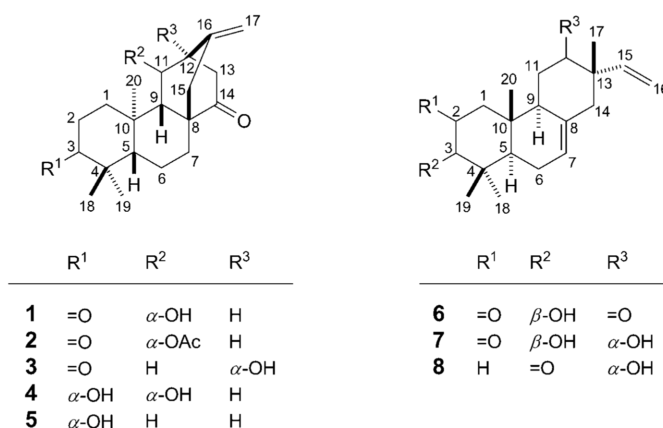

	R^1	R^2	R^3
1	=O	α-OH	H
2	=O	α-OAc	H
3	=O	H	α-OH
4	α-OH	α-OH	H
5	α-OH	H	H

	R^1	R^2	R^3
6	=O	β-OH	=O
7	=O	β-OH	α-OH
8	H	=O	α-OH

optical rotation, and chemotaxonomic considerations suggested that compound **1** belongs to the *ent*-atisane diterpenoid family [5][1]).

The positions of two C=O groups and one OH substituent of **1** were determined on the basis of the observed HMBC correlations from H−C(9), H−C(12), H−C(13), and H−C(15) to C(11) (δ(C)70.7), from H−C(1), H−C(2), Me(18), and Me(19) to C(3) (δ(C) 217.0), and from H−C(7), H−C(12), H−C(13), and H−C(15) to C(14) (δ(C) 216.0) (*Fig. 1*). The relative configuration of **1** was deduced from its ROESY spectrum and the coupling constants of H−C(9) (δ(H) 1.76 (*d*, J = 10.0)) and H−C(11) (δ(H) 4.52 (*dd*, J = 4.0, 10.0)), which indicated that H−C(11) adopts a β-orientation, while OH was α-oriented (*Fig. 2*). Thus, the structure of **1** was determined as *ent*-(5β,8α,9β,10α,11α,12α)-11-hydroxyatis-16-ene-3,14-dione.

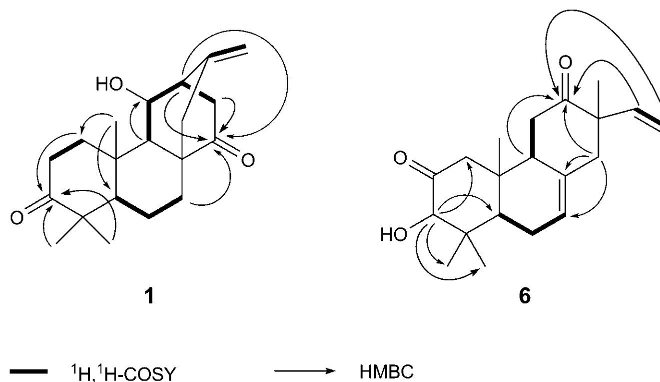

Fig. 1. *Key HMBC and COSY correlations for* **1** *and* **6**

Compound **2** was obtained as a white powder, for which the molecular formula was assigned as $C_{22}H_{30}O_4$ on the basis of the HR-ESI-MS, from a $[M+Na]^+$ ion at *m/z*

[1]) The same arguments apply to the proposed absolute configuration of compounds **2**–**5** as well.

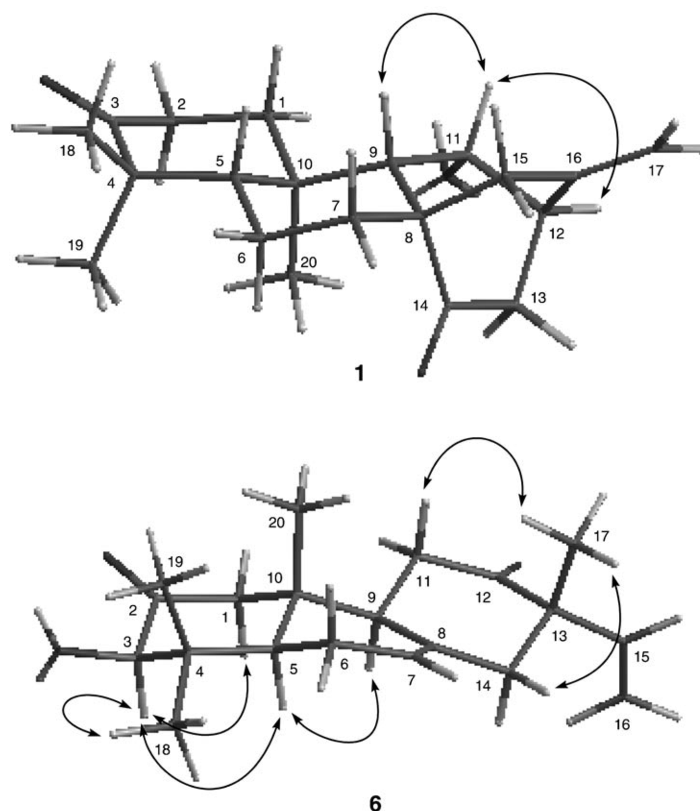

Fig. 2. *Key ROESY correlations for* **1** *and* **6**

381.2037 (calc. 381.2041). The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**, and differed only in the absence of the OH signal of **1** and the appearance of an AcO signal (δ(H) 2.11, and δ(C) 21.7, 169.6) in **2**. A difference in the chemical-shift value of C(12) (δ(C) 48.4 in **1**; δ(C) 42.7 in **2**) allowed the AcO group to be placed at C(11). This was confirmed by the HMBC spectrum. Comparison of the ROESY spectra of compounds **1** and **2** pointed to the same relative configuration for both compounds. Therefore, the structure of **2** was elucidated as *ent*-(5β,8α,9β,10α,11α,12α)-3,14-dioxoatis-16-en-11-yl acetate.

Compound **3**, an amorphous powder, exhibited the molecular formula $C_{20}H_{28}O_3$, as determined from the HR-ESI-MS (*m/z* 339.1942, $[M+Na]^+$). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) showed similarities with those of **1**, indicating two C=O groups (δ(C) 212.5, 215.8), an exocyclic $C=CH_2$ group (δ(C) 148.2 (*s*), 104.2 (*t*)), and three Me groups (δ(C) 12.4, 21.5, 25.6). However, in compound **3**, the presence of a quaternary C-atom (δ(C) 71.7) suggested the presence of a tertiary OH group. Further, an additional CH_2 group (δ(C) 34.7) was noticed. These observations, together with HMBC correlations (from the OH H-atom to C(11), C(12), C(13), and C(16), and from CH_2(11), CH_2(13), CH_2(15), and CH_2(17) to C(12)), indicated that this OH

Table 1. *^{1}H-NMR Chemical Shift Assignments for* **1**–**8**. δ in ppm, J in Hz; assignments were confirmed by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

		1[a])	**2**[b])	**3**[a])[c])	**4**[b])	**5**[b])	**6**[b])	**7**[b])	**8**[b])
1	α	2.56–2.59 (*m*)	1.62–1.64 (*m*)	1.22–1.26 (*m*)	1.18–1.20 (*m*)	1.03–1.06 (*m*)	2.50 (*d*, *J* = 12.8)	2.30 (*d*, *J* = 12.9)	2.05–2.07 (*m*)
	β	2.69–2.71 (*m*)	1.92–1.94 (*m*)	1.68–1.72 (*m*)	2.44–2.46 (*m*)	1.49–1.51 (*m*)	2.22 (*d*, *J* = 12.8)	2.54 (*d*, *J* = 12.9)	1.50–1.52 (*m*)
2	α	2.28–2.32 (*m*)	2.38–2.41 (*m*)	2.18–2.22 (*m*)	1.53–1.56 (*m*)	1.47–1.49 (*m*)	–	–	2.65–1.69 (*m*)
	β	2.60–2.62 (*m*)	2.50–2.54 (*m*)	2.43–2.47 (*m*)	1.54–1.57 (*m*)	1.60–1.64 (*m*)	–	–	2.24–2.26 (*m*)
3		–	–	–	3.20 (*dd*, *J* = 3.6, 11.2)	3.19 (*dd*, *J* = 3.6, 11.2)	3.96 (*d*, *J* = 3.8)	3.94 (*d*, *J* = 4.4)	–
5		1.26 (*dd*, *J* = 1.6, 12.4)	1.37 (*dd*, *J* = 2.4, 12.0)	1.59 (*dd*, *J* = 7.5, 14.5)	0.70–0.78 (*m*)	0.79 (*dd*, *J* = 3.6, 10.8)	1.94 (*dd*, *J* = 5.2, 11.8)	1.60–1.63 (*m*)	1.55–1.59 (*m*)
6	α	1.66–1.70 (*m*)	1.67–1.70 (*m*)	1.49–1.53 (*m*)	1.50–1.52 (*m*)	1.55–1.58 (*m*)	2.25–2.30 (*m*)	2.03–2.06 (*m*)	1.89–1.93 (*m*)
	β	1.42–1.48 (*m*)	1.49–1.52 (*m*)	1.38–1.41 (*m*)	1.49–1.51 (*m*)	1.57–1.59 (*m*)	2.08–2.10 (*m*)	2.04–2.07 (*m*)	2.06–2.10 (*m*)
7	α	2.32–2.37 (*m*)	2.37–2.40 (*m*)	2.18–2.24 (*m*)	2.29–2.32 (*m*)	2.28–2.33 (*m*)	5.66 (br. *s*)	5.46 (br. *s*)	5.46 (br. *s*)
	β	0.85–0.90 (*m*)	0.87–0.95 (*m*)	0.82–0.90 (*m*)	0.79–0.86 (*m*)	0.88–0.91 (*m*)			
9		1.76 (*d*, *J* = 10.0)	1.95 (*d*, *J* = 10.0)	1.18 (*dd*, *J* = 3.0, 15.5)	1.64 (*d*, *J* = 9.6)	1.59*[d])	2.62*	2.40–2.41 (*m*)	2.19–2.20 (*m*)
11	α	–	–	1.69–1.71 (*m*)	–	1.60–1.62 (*m*)	2.30–2.33 (*m*)	1.62–1.63 (*m*)	1.79 (*d*, *J* = 13.9)
	β	4.52 (*dd*, *J* = 4.0, 10.0)	5.51 (*dd*, *J* = 1.0, 10.0)	1.77–1.79 (*m*)	4.63–1.65 (*m*)	1.80–1.84 (*m*)	2.30–2.33 (*m*)	1.54–1.57 (*m*)	1.57–1.60 (*m*)
12	α	2.65–2.67 (*m*)	2.80–2.83 (*m*)	–	2.59–2.61 (*m*)	2.67–2.69 (*m*)	–	–	–
	β	–	–	–	–	–	–	3.62 (br. *s*)	3.64 (br. *s*)
13	α	2.93 (*dd*, *J* = 1.6, 14.2)	2.70 (*dd*, *J* = 2.4, 19.2)	2.22–2.24 (*m*)	2.90 (*dd*, *J* = 2.0, 18.8)	2.28–2.32 (*m*)	–	–	–
	β	2.17–2.21 (*m*)	2.23–2.26 (*m*)	2.20–2.22 (*m*)	2.12–2.13 (*m*)	2.25–2.26 (*m*)	–	–	–
14	α	–	–	–	–	–	2.58 (*d*, *J* = 14.6)	2.58–2.60 (*m*)	2.54 (*d*, *J* = 8.0)
	β	–	–	–	–	–	2.41 (*d*, *J* = 14.6)	1.76–1.80 (*m*)	1.59–1.61(*m*)
15	α	2.11–2.17 (*m*)	2.19–2.21 (*m*)	2.19–2.20 (*m*)	2.07–2.11 (*m*)	2.13–2.16 (*m*)	5.87 (*dd*, *J* = 10.7, 17.5)	5.80 (*dd*, *J* = 10.7, 17.5)	5.80 (*dd*, *J* = 10.7, 17.5)
	β	2.31–2.34 (*m*)	2.23–2.26 (*m*)	2.43–2.45 (*m*)	2.28–2.31 (*m*)	2.19–2.22 (*m*)			
16		–	–	–	–	–	5.12 (*d*, *J* = 17.5)	5.05 (*d*, *J* = 17.5)	5.02 (*d*, *J* = 17.6)
		–	–	–	–	–	5.10 (*d*, *J* = 10.7)	5.15 (*d*, *J* = 10.7)	5.16 (*d*, *J* = 10.8)
17		4.98 (br. *s*)	5.07 (br. *s*)	5.06 (br. *s*)	4.94 (br. *s*)	4.85 (br. *s*)	1.17 (*s*)	0.86 (*s*)	0.90 (*s*)
		4.78 (br. *s*)	4.85 (br. *s*)	4.64 (br. *s*)	4.74 (br. *s*)	4.65 (br. *s*)			
18		1.06 (*s*)	1.08 (br. *s*)	0.84 (*s*)	0.95 (*s*)	0.97 (*s*)	0.76 (*s*)	0.76 (*s*)	1.12 (*s*)
19		1.02 (*s*)	1.04 (br. *s*)	0.85 (*s*)	0.98 (*s*)	0.75 (*s*)	1.17 (*s*)	1.15 (*s*)	1.07 (*s*)
20		1.17 (*s*)	1.13 (br. *s*)	0.76 (*s*)	0.76 (*s*)	0.67 (*s*)	0.82 (*s*)	0.86 (*s*)	1.07 (*s*)

[a]) At 500 MHz. [b]) At 400 MHz. [c]) Measured in $CDCl_3 + CD_3OD$ (1 : 1). [d]) Asterisks (*) denote overlapping signals.

Table 2. *^{13}C-NMR Chemical Shift Assignments for Compounds* **1**–**8**. δ in ppm; assignments were confirmed by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

	1[a])	**2**[b])	**3**[a])[c])	**4**[b])	**5**[b])	**6**[b])	**7**[b])	**8**[b])
1	38.6	38.6	36.7	38.3	36.7	50.7	51.4	37.7
2	34.4	33.9	33.7	27.1	26.8	210.0	210.9	34.6
3	217.0	216.4	215.8	79.0	78.9	82.4	82.3	216.7
4	47.9	47.7	47.1	39.2	38.7	45.1	45.2	47.4
5	56.7	56.0	51.3	55.6	54.6	48.4	49.1	51.7
6	20.2	20.2	19.6	19.0	18.9	23.7	23.6	23.8
7	31.0	30.8	30.3	31.4	31.5	122.6	121.6	121.3
8	48.7	48.5	47.5	48.8	47.8	131.7	134.5	134.9
9	57.1	56.1	54.6	58.1	52.7	49.0	45.9	44.5
10	38.9	38.5	37.1	39.0	37.8	41.7	41.9	34.8
11	70.7	71.4	34.7	70.8	27.8	37.4	25.9	25.6
12	48.4	42.7	71.7	48.1	38.4	213.3	72.3	72.6
13	38.1	38.2	41.5	38.0	44.5	50.5	41.7	41.7
14	216.0	214.8	212.5	216.5	216.8	41.7	37.1	37.3
15	42.9	42.7	50.6	43.1	42.8	141.1	145.6	145.7
16	144.2	142.2	148.2	144.6	147.5	114.5	114.1	114.0
17	109.5	111.0	104.2	109.1	106.8	23.8	23.0	23.0
18	26.4	26.8	25.6	28.5	28.3	28.1	28.7	25.6
19	21.8	21.5	21.5	16.0	15.5	15.8	16.4	22.5
20	15.6	16.1	12.4	15.5	13.2	14.4	15.4	14.6
AcO	–	21.7, 169.6	–	–	–	–	–	–

[a]) At 125 MHz. [b]) At 100 MHz. [c]) Measured in $CDCl_3 + CD_3OD$ (1 : 1).

group is positioned at C(12). Thus, compound **3** was determined as *ent*-(5β,8α,9β,10α,12α)-12-hydroxyatis-16-ene-3,14-dione.

Compound **4** was attributed the molecular formula $C_{20}H_{30}O_3$ from its HR-ESI-MS at *m*/*z* 341.2097 ($[M+Na]^+$, calc. 341.2092). The presence of the OH groups (3485 cm^{-1}), C=O group (1695 cm^{-1}), and a C=C bond (1654, 1446 cm^{-1}) were apparent from its IR spectrum. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **4** with those of **1** revealed that one C=O group of **1** had disappeared in **4** and was replaced by an oxygenated CH group (δ(C) 79.0), indicating that **4** was a reduced derivative of **1**. The HMBC spectrum showed correlations from CH_2(7), H–C(12), CH_2(13), and CH_2(15) to C(14) (δ(C) 216.5), and from CH_2(2), H–C(5), Me(18), and Me(19) to C(3), which indicated that the C=O and OH groups were placed at C(14) and C(3), respectively. The relative configuration of HO–C(3) and HO–C(11) were assigned using coupling constant data, as well as the NOEs in the ROESY spectrum. NOE Effects between H–C(3) and H–C(5), Me(18), and H_β–C(1), as well as of H–C(11) and H–C(9), H_β–C(1), H_α–C(12), and H–C(11) had no correlation with H–C(20) which demonstrated the α-orientation of both HO–C(3) and HO–C(11). Compound **4** was thus assigned as *ent*-(3α,5β,8α,9β,10α,11α,12α)-3,11-dihydroxyatis-16-en-14-one.

The HR-ESI-MS of compound **5** gave a *quasi*-molecular ion peak at *m*/*z* 325.2133 ($[M+Na]^+$, calc. 325.2143), in agreement with a molecular formula of $C_{20}H_{30}O_2$. On analysis of its ^{1}H- and ^{13}C-NMR spectra, similar features to that of **4** were evident, but with only one OH group in **5**. The oxygenated CH signal (δ(H) 3.19 (*dd*, $J = 3.6, 11.2$))

correlated with C(18) (δ(C) 28.3), C(19) (δ(C) 15.5), and C(5) (δ(C) 54.6) in its HMBC spectrum, revealing the position of a OH group at C(3). Comparison of the ROESY spectra of compounds **5** and **4** enable us to assume the same relative configuration for **5** as that of **4**. Finally, the structure of **5** was assigned as *ent*-(3α,5β,8α,9β,10α,12α)-3-hydroxyatis-16-en-14-one.

Compound **6** was isolated as white powder ($[\alpha]^{25}_{D} = -21.9$ ($c = 0.57$, $CHCl_3$)) and exhibited a $[M + Na]^+$ ion peak at *m/z* 339.1934 (calc. 339.1936) in the HR-ESI-MS, associated with a molecular formula of $C_{20}H_{28}O_3$, indicating seven degrees of unsaturation. The IR absorptions at 3382, 1738, 1711 and 1640 cm^{-1} revealed the presence of OH, C=O, and C=C functional groups. The ^{1}H-NMR signals of **6** were distributed mainly in the upfield region around δ(H) 0.7–1.5 ppm, wherein four tertiary Me groups at δ(H) 0.76, 0.82, 1.17, 1.17 (each *s*, 3 H) were recognized. In addition, resonances for H-atoms of an *ABX* system at δ(H) 5.12 (*d*, $J = 17.5$), 5.10 (*d*, $J = 10.7$), and 5.87 (*dd*, $J = 10.7$, 17.5), and an additional olefinic H-atom at δ(H) 5.66 (br. *s*) and an oxygenated CH group at δ(H) 3.96 (*d*, $J = 3.8$), were observed. The ^{13}C-NMR and DEPT spectra showed signals corresponding to four Me groups (δ(C) 14.4, 15.8, 23.8, 28.1), two C=O groups (δ(C) 210.0, 213.3), four olefinic C-atoms (δ(C) 114.5, 122.6, 131.7, 141.1), and one oxygenated CH group (δ(C) 82.4) (see *Tables 1* and *2*). According to the molecular formula and the presence of four degrees of unsaturation inferred from the ^{1}H- and ^{13}C-NMR data, it was apparent that three rings were present in the molecule. From the above observations and by comparison with the NMR data from closely related structures, it was evident that compound **6** belongs to the isopimarane family of diterpenoids [6–8]. The value of its specific rotation was close to that of linifoliol [9]. In the HMBC spectrum, the signal at δ(H) 3.96 showed correlations (*Fig. 1*) with signals at δ(C) 15.8 (C(19)), 28.1 (C(18)), 45.1 (C(4)), and 210.0 (C(2)), supporting the placement of the OH group at C(3). The position of one C=O group at C(2) was deduced from correlation between the H-atoms at δ(H) 3.96 (H–C(3)), 2.22 (H_β–C(1)), and 2.50 (H_α–C(1)) and a C=O signal at δ(C) 210.0. Similarly, the location of the other C=O group was assigned to C(12), wherein correlations between δ(C) 213.4 and each of the H-atoms resonating at δ(H) 5.87 (H–C(15)), 5.12 and 5.10 (CH_2(16)), 2.58 (H–C(14)), 2.62 (H–C(9)), and 1.17 (Me(17)) were observed. Because of the weak correlations of H–C(7) in the HMBC spectrum, we used the ^{1}H,^{1}H-COSY correlations (*Fig. 1*) between H–C(5)/H–C(6) and H–C(6)/H–C(7) to determine the location of the C=C bond as C(7)=C(8).

The relative configuration of **6** was deduced from the ROESY spectrum (*Fig. 2*). NOE effects were observed between H–C(3)/Me(18), H–C(3)/H–C(5), and H–C(3)/H_α–C(1), supporting an α-orientation of H–C(3). Moreover, Me(17) has no correlations with other H-atoms except with H_β–C(14) and H_β–C(11), which indicated that Me(17) has β-orientation. Thus, the structure of compound **6** was determined as (3β,13α)-3-hydroxypimara-7,15-diene-2,12-dione.

Compound **7** was shown to have the molecular formula $C_{20}H_{30}O_3$ as deduced from HR-ESI-MS, in which a *pseudo*-molecular ion $[M + Na]^+$ was observed at *m/z* 341.2102 (calc. 341.2093). The ^{1}H- and ^{13}C-NMR spectra of **7** revealed one C=O group (δ(C) 210.9), four olefinic C-atoms (δ(C) 114.1, 121.6, 134.5, 145.6) and two oxygenated CH (δ(C) 72.3, 82.3) signals (see *Tables 1* and *2*). The ^{1}H- and ^{13}C-NMR data on **7** were assigned by analysis of its ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra, and it became

clear that compounds **6** and **7** possess the same parent skeleton, but differ mainly in ring *C*. The absence of a C=O signal (δ(C) 213.3 in **6**) and the appearance of one oxygenated C-atom (δ(C) 72.3 in **7**) indicated the replacement of one C=O with one OH group. Further, key HMBC correlations between H−C(12) and C(9), C(14), and C(15), as well as between CH_2(16), CH_2(11), and Me(17) and C(12) confirmed the position of a OH-group at C(12). The relative configuration of the OH group was elucidated from the ROESY spectrum where correlations between H−C(12) and Me(17) and H_β−C(11) were detected. The structure of **7** was thus defined as (3β,12α,13α)-3,12-dihydroxypimara-7,15-dien-2-one.

The HR-ESI-MS suggested the molecular formula $C_{20}H_{30}O_2$ for compound **8**, from the $[M+Na]^+$ ion at *m/z* 325.2148. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of **7**, indicating the presence of Δ^{15}-isopimarene skeleton for compound **8**, which contains a secondary OH group (δ(H) 3.64, br. *s*; δ(C) 72.6) and one C=O group (δ(C) 216.7). The location of these functional groups was corroborated by 2D-NMR studies (COSY, HSQC, HMBC). The relative configuration of **8** was identical to that of **7** (ROESY evidence). On the basis of these data, compound **8** was shown to be (12α,13α)-12-hydroxypimara-7,15-dien-3-one.

The five known compounds were identified by comparison of their spectroscopic data with those reported in the literature as euphol [4], *ent*-atis-16-ene-3,14-dione [6], 3-oxoatisane-16α,17-diol [5], *ent*-atisane-3β,16α,17-triol [5], and nepehinol acetate [4].

Compounds **1**–**8** were evaluated for their cytotoxicity against the HL-60, A-549, MOLT-4, and BEL-7402 human cancer cell lines. They showed only weak cytotoxicity in these tested systems ($IC_{50} > 100$ μM).

Financial support for this study was provided by the *National Natural Science Foundation of China* (No.20502026 to *Q.-B. H.*), the *Young Academic and Technical Leader Raising Foundation of Yunnan Province* (2006PY01-47), and the *Natural Science Foundation of China* (30772637).

Experimental Part

General. Semi-prep. HPLC: *Agilent 1100* liquid chromatograph with a *Zorbax SB-C$_{18}$*, 9.4 mm × 25 cm column. Prep. HPLC: *Shimadzu LC-8A* prep. liquid chromatograph with a *Shimadzu PRC-ODS (K)* column (34 mm × 15 cm). Column chromatography (CC) was performed on silica gel (SiO_2; 200–300 mesh; *Qingdao Marine Chemical, Inc.*, Qingdao, P. R. China), *Lichroprep RP-18* gel (40–63 μm, *Merck*, Darmstadt, Germany), *MCI* gel (75–150 μm, *Mitsubishi Chemical Corporation*, Tokyo, Japan), or *Sephadex LH-20* (*Pharmacia*). Fractions were monitored by TLC, and spots were visualized by heating SiO_2 plates sprayed with 5% H_2SO_4 in EtOH. Optical rotations: *Perkin-Elmer Model 241* polarimeter. UV Spectra: *UV 210A* spectrometer. IR Spectra: *Bio-Rad FTS-135* spectrometer. 1D- and 2D-NMR: *Bruker AM-400* or a *Bruker DRX-500* NMR spectrometer with TMS as internal standard. MS: *VG Auto spec-3000* spectrometer or *Finnigan MAT 90* instrument.

Plant Material. The roots of *E. nematocypha* HAND.-MAZZ. were collected in Zhongdian County, Yunnan Province, P. R. China, on August 2006. The sample was identified by Prof. *Xi-Wen Li*, and a voucher specimen (KIB 06081305) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and milled roots of *E. nematocypha* (2 kg) were extracted by maceration with 70% acetone (4 × 10 l, each time 4 d) at r.t. After filtration and evaporation of the solvent under reduced pressure, the combined acetone extract (125 g) was extracted in H_2O (2.5 l) and then partitioned with $CHCl_3$ (4 × 2 l) to afford dried $CHCl_3$- (68 g) and H_2O-soluble residues.

The $CHCl_3$-soluble extract (68 g) was subjected to CC over SiO_2 (200–300 mesh), using a mixture of petroleum ether (PE) and acetone (from 1:0 to 0:1). According to the differences in composition

indicated by TLC, seven crude fractions (*A – G*) were obtained. *Fr. A*, *B*, and *C* contained mainly triterpenoids and lipids, and were not further investigated. *Fr. D* (14.2 g) with various kinds of diterpenoids as suggested by TLC was subjected to SiO_2 (200 – 300 mesh) CC (4.5 × 70 cm) and eluted with PE/AcOEt (100 : 0 to 70 : 30) to give ten subfractions (*Fr. D.01 – Fr. D.10*). *Fr. D.02*, containing one major compound, was further purified by *Sephadex LH-20* ($CHCl_3$/MeOH, 1 : 1) to yield compounds **1** (120 mg) and **2** (43 mg). Compounds **5** (5 mg) and nepehinol acetate (650 mg) were obtained from *Fr. D.01* (1.5 g) by repeated CC over SiO_2 (1.5 × 16 cm) with hexane/AcOEt (97 : 3). *Fr. D.03* and *Fr. D.04* were combined and further separated by SiO_2 CC (2 × 25 cm) and eluted with hexane/AcOEt (gradient from 95 : 5 to 90 : 10), to give five subfractions *Fr. D.03.01 – Fr. D.03.05*. *Fr. D.03.02* (720 mg) was subjected to SiO_2 CC (1.5 × 15 cm) with hexane/AcOEt (95 : 5) repeatedly and gave **3** (23 mg), **4** (4 mg), euphol (5 mg), and *ent*-atisane-3β,16α,17-triol (2 mg), resp. *ent*-Atis-16-ene-3,14-dione (18 mg) and 3-oxoatisane-16α,17-diol (26 mg) were relatively more polar compounds isolated from subfraction *Fr. D.05*.

Fr. F (4.3 g) contained more polar constituents than those of *Fr. D*, as indicated by TLC. *Fr. F* was then decolored (*MCI* gel) and further separated over SiO_2 with hexane/AcOEt (from 95 : 5 to 80 : 20), yielding subfractions *Fr. F.01 – Fr. F.04*. *Fr. F.03* (370 mg) was dissolved in acetone and subjected to purification by *RP18*-HPLC (MeOH/H_2O, 8 : 2, 25 ml/min, detector 210 nm) to afford compound **6** (15 mg, t_R 38 min), **8** (7 mg, t_R 52 min), and subfraction *Fr. F.03.01*. *Fr. F.03.01* was further separated by semi-prep. HPLC (MeOH/H_2O, 7 : 3, 3 ml/min, detector 210 nm) and afforded 12 mg of **7**.

Frs. E (7.5 g) and *G* (2.3 g) contained mostly the compounds mentioned above and were not further investigated.

ent-*(5β,8α,9β,10α,11α,12α)-11-Hydroxyatis-16-ene-3,14-dione* (**1**). White powder. $[\alpha]_D^{25} = -45.14$ ($c = 0.29$, $CHCl_3$). UV ($CHCl_3$): 225 (2.31). IR (KBr): 3406, 3354, 2967, 2865, 1707, 1635,1477, 1388, 1096,1042. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS (pos.): 317 ($[M+H]^+$), 633 ($[2M+H]^+$). HR-ESI-MS (pos.): 339.1930 ($C_{20}H_{28}NaO_3^+$, $[M+Na]^+$; calc. 339.1936).

ent-*(5β,8α,9β,10α,11α,12α)-3,14-Dioxoatis-16-en-11-yl Acetate* (**2**). White powder. $[\alpha]_D^{25} = -31.25$ ($c = 0.18$, $CHCl_3$). UV ($CHCl_3$): 216 (2.68). IR (KBr): 3678, 3449, 2871, 1740, 1711, 1655, 1438, 1376, 1135, 1081,1012. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS (pos.): 381 ($[M+Na]^+$). HR-ESI-MS (pos.): 381.2037 ($C_{22}H_{30}NaO_4^+$, $[M+Na]^+$; calc. 381.2041).

ent-*(5β,8α,9β,10α,12α)-12-Hydroxyatis-16-ene-3,14-dione* (**3**). White powder. $[\alpha]_D^{25} = 22.31$ ($c = 0.13$, $CHCl_3$). UV ($CHCl_3$): 215 (2.41). IR (KBr): 3525, 3314, 2950, 1706, 1672, 1618, 1459, 1389, 1133, 1078. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS: 339 ($[M+Na]^+$), 655 ($[2M+Na]^+$). HR-ESI-MS (pos.) 339.1942 ($C_{20}H_{28}NaO_3^+$, $[M+Na]^+$; calc. 339.1936).

ent-*(3α,5β,8α,9β,10α,11α,12α)-3,11-Dihydroxyatis-16-en-14-one* (**4**). White powder. $[\alpha]_D^{25} = -29.35$ ($c = 0.32$, $CHCl_3$). UV ($CHCl_3$): 214 (2.41). IR (KBr): 3485, 2925, 1695, 1654, 1446, 1402, 1387, 1171, 1109, 1072. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS: 341 ($[M+Na]^+$), 659 ($[2M+Na]^+$). HR-ESI-MS (pos.): 341.2097 ($C_{20}H_{30}NaO_3^+$, $[M+Na]^+$; calc. 341.2092).

ent-*(3α,5β,8α,9β,10α,12α)-3-Hydroxyatis-16-en-14-one* (**5**). White powder. $[\alpha]_D^{25} = -6.48$ ($c = 0.18$, $CHCl_3$). UV ($CHCl_3$): 239 (2.23). IR (KBr): 3478, 2926, 1703, 1652, 1448, 1413, 1387, 1175, 1112, 1079. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS: 325 ($[M+Na]^+$), 627 ($[2M+Na]^+$). HR-ESI-MS (pos.): 325.2133 ($C_{20}H_{30}NaO_2^+$, $[M+Na]^+$; calc. 325.2143).

(3β,13α)-3-Hydroxypimara-7,15-diene-2,12-dione (**6**). White powder. $[\alpha]_D^{25} = -21.9$ ($c = 0.57$, $CHCl_3$). UV ($CHCl_3$): 213 (2.62). IR (KBr): 3496, 3382, 2930, 1738, 1711, 1640, 1432, 1379, 1228, 1157, 1063. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS (pos.): 339 ($[M+Na]^+$), 655 ($[2M+Na]^+$). HR-ESI-MS (pos.): 339.1934 ($C_{20}H_{28}NaO_3^+$, $[M+Na]^+$; 339.1936).

(3β,12α,13α)-3,12-Dihydroxypimara-7,15-dien-2-one (**7**). White powder. $[\alpha]_D^{25} = 0$ ($c = 0.57$, $CHCl_3$). UV ($CHCl_3$): 216 (2.47). IR (KBr): 3512, 3390, 2924, 1702, 1637, 1463, 1382, 1238, 1164, 1051. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS (pos.): 341 ($[M+Na]^+$), 659 ($[2M+Na]^+$). HR-ESI-MS (pos.): 341.2102 ($C_{20}H_{30}NaO_3^+$, $[M+Na]^+$; calc. 341.2093).

(12α,13α)-12-Hydroxypimara-7,15-dien-3-one (**8**). White powder. $[\alpha]_D^{25} = 0$ ($c = 1.12$, $CHCl_3$). UV ($CHCl_3$): 240 (2.28). IR (KBr): 3480, 3080, 2925, 1715, 1637, 1466, 1438, 1389, 1367, 1101, 1002. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. ESI-MS (pos.): 325 ($[M+Na]^+$), 627 ($[2M+Na]^+$). HR-ESI-MS (pos.): 325.2148 ($C_{20}H_{30}NaO_2^+$, $[M+Na]^+$; calc. 325.2143).

Cytotoxicity. MTT Assay (MOLT4, BEL7402). Cells were seeded in 96-well plates, treated with indicated concentrations of tested compounds or positive control compound (**VP-16**). After incubation for 24 h at 37°, 20 µl per well MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide; 5 mg/ml) was added to each well, and cells were incubated for additional 4 h. Then, 'triplex solution' (10% SDS, 5% i-BuOH, 12 mM HCl) was added to dissolve the formazan crystals overnight. Absorbance was measured at 570 nm by enzyme immunoassay instrument (*SpectraMax*, *Molecular Devices*, USA). The inhibition rate on cell proliferation was calculated as $(1 - A_{570\text{treated}}/A_{570\text{control}}) \times 100\%$ [10].

SRB Assay (HL-60, A549). Cells were seeded into 96-well plates and grown for 24 h. The cells were then treated with increasing concentrations of tested compounds or positive control compound (**VP-16**) and grown for a further 72 h. The cells were then fixed with 10% precooled trichloroacetic acid (TCA) for 1 h at 4° and stained for 15 min at r.t. with 100 µl of 4 mg/ml SRB (sulforhodamine B) soln. (*Sigma*) in 1% AcOH. SRB was then removed and cells were quickly rinsed five times with 1% AcOH. After air-drying, protein-bound dye was dissolved in 150 µl of 10 mM *Tris* base for 5 min and was measured at 515 nm using a multiwell spectrophotometer (*VERSAmax*, *Molecular Devices*, USA). The inhibition rate on cell proliferation was calculated as $(1 - A_{515\text{treated}}/A_{515\text{control}}) \times 100\%$.

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Received May 4, 2008