Bisyinshanic Acids A and B, Two Novel Diterpene Dimers from the Roots of *Euphorbia yinshanica*

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Two novel diterpene dimers with a bismagdalenic acid skeleton, bisyinshanic acids A and B (**1** and **2**, resp.), along with eight known diterpenoids (**3**–**10**), were isolated from the roots of *Euphorbia yinshanica*. Their structures were elucidated on the basis of spectroscopic evidence.

Introduction. – *Euphorbia*, the largest genus in the family Euphorbiaceae, consists of *ca.* 2000 known species, more than 80 of which are distributed in China [1] and range from annual plants to trees. All contain latex and have characteristic flower structures [2]. Many secondary metabolites with unique diterpenoid skeletons in the genus have been found to display a number of interesting biological activities [3–5]. *Euphorbia yinshanica* S.Q. spreading in Tianjun, Xunhua, Minhe in Qinghai Province, China, is a traditional Tibetan medicine used for curing furuncles, exanthema, cutaneous anthrax, and acts as a purgative [6]. Its chemical constituents have not been investigated so far. Detailed studies on the profile of all secondary metabolites could contribute to a taxonomic subdivision of this complex genus. Herein, we report the isolation and structure elucidation of the chemical composition of the roots of *E. yinshanica*.

The EtOH extract of the roots afforded two novel dimeric diterpenes with bismagdalenic acid skeleton, **1** and **2**, and eight known diterpenoids, including *ent*-(13*S*)-13-hydroxyatis-16-ene-3,14-dione (**3**) [3], *ent*-(13*R*,14*R*)-13,14-dihydroxyatis-16-en-3-one (**4**) [7], *ent*-(3*β*,13*S*)-3,13-dihydroxyatis-16-en-14-one (**5**) and *ent*-atis-16-ene-3,14-dione (**6**) [3] with an *ent*-atisane skeleton, *ent*-(3*S*,16*S*)-3,16,17-trihydroxykauran-2-one (**7**), and *ent*-(16*R*)-16,17-dihydroxykauran-3-one (**8**) [8], possessing an *ent*-kaurane skeleton, and helioscopinolides A and E (**10** and **9**, resp.) [9] with an abietane skeleton (*Fig. 1*).

Results and Discussion. – Repeated column chromatography of the EtOH extract from the roots of *E. yinshanica* yielded compounds **1**–**12**.

Compound **1** was obtained as a colorless oil with $[\alpha]_D^{26} = -73.8$ ($c = 0.3$, $CHCl_3$), and the molecular formula was determined as $C_{40}H_{56}O_6$ by high-resolution electro-

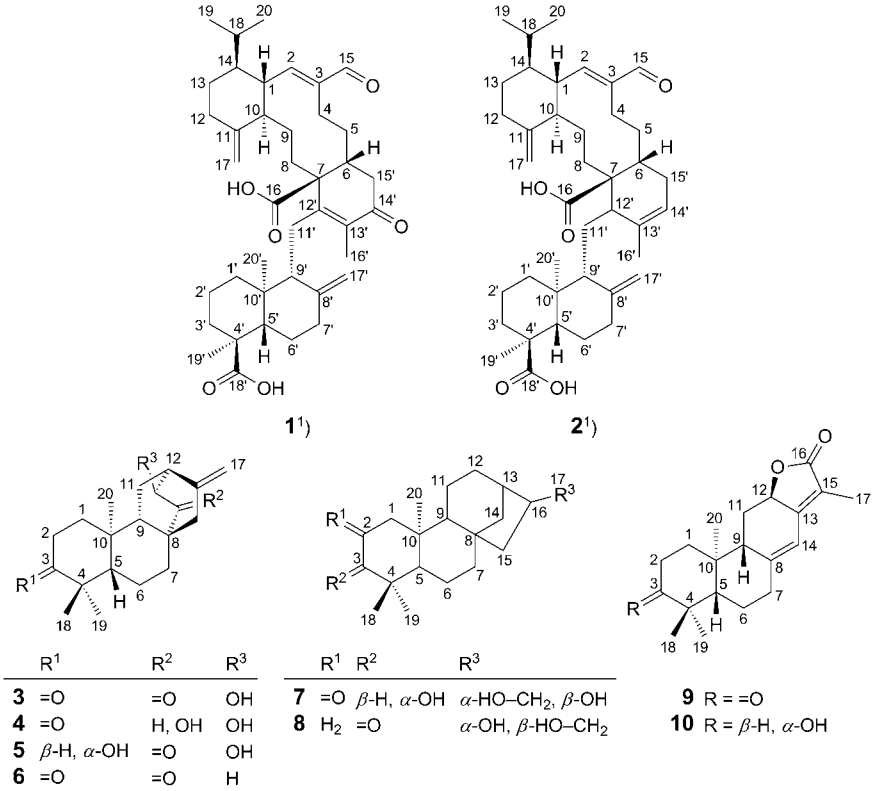

Fig. 1. *Structures of compounds* **1**–**10**

spray-ionization mass spectroscopy (HR-ESI-MS) at *m*/*z* 631.3998 ($[M-H]^-$), indicating 13 degrees of unsaturation. The IR spectrum of **1** exhibited absorption bands ascribable to COOH groups (3432 and 1721 cm^{-1}), an α,β-unsaturated ketone (1643 cm^{-1}), an α,β-unsaturated aldehydic CO group (1669 cm^{-1}), and C=C bonds (1600 cm^{-1}). The ^{13}C- and DEPT NMR spectra of **1** (*Table 1*) permitted the differentiation of the 40 resonances into five Me groups, fifteen CH_2 groups, nine CH groups, and eleven quaternary C-atoms, indicating the presence of four CO groups including an α,β-unsaturated ketone (δ(C) 197.5), a tetrasubstituted C=C bond (δ(C) 136.9 and 151.6), and two exocyclic C=C bonds. The ^1H-NMR spectrum of **1** (*Table 1*) pointed to the presence of five olefinic H-atoms (δ(H) 6.58, 4.80, 4.68, 4.62, 4.34), one aldehyde H-atom (δ(H) 10.13), and five Me groups (δ(H) 0.71, 0.74, 0.96, 1.07, 1.94). Considering the structures of diterpenoids from the genus *Euphorbia*, these spectral data suggested that **1** was a dimeric diterpenoid consisting of two different units, one moiety possessing a magdalenic acid skeleton (**1a**) [10], while the other (**1b**) is derived from an *ent*-labdane nucleus [11]. The ^1H-NMR spectrum of **1b** exhibited signals for two Me groups at δ(H) 0.74 (*s*) and 1.07 (*s*), as well as one characteristic exocyclic CH_2

[1]) The configuration of the C(2)=C(3) bond is (*Z*) (*cf. Fig. 3*).

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 125 MHz, resp) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	2.84–2.89 (*m*)	49.9	2.86–2.90 (*m*)	50.7
H–C(2)	6.58 (*d*, J = 6.0)	153.0	6.50 (*d*, J = 12.2)	153.9
C(3)		141.6		141.5
H_α–C(4)	2.03–2.07 (*m*)	22.2	1.98–2.02 (*m*)	24.4
H_β–C(4)	2.79–2.83 (*m*)		2.81–2.85 (*m*)	
H_α–C(5)	1.33–1.38 (*m*)	30.5	1.46–1.49 (*m*)	28.7
H_β–C(5)	1.71–1.74 (*m*)		1.91–1.95 (*m*)	
H–C(6)	1.82–1.86 (*m*)	33.8	1.30–1.32 (*m*)	38.1
C(7)		53.0		51.9
H_α–C(8)	1.58–1.62 (*m*)	22.6	0.97–1.01 (*m*)	23.2
H_β–C(8)	1.69–1.72 (*m*)		1.67 (*d*, J = 12.0)	
H_α–C(9)	1.70–1.74 (*m*)	31.4	1.23–1.28 (*m*)	29.7
H_β–C(9)	2.15–2.19 (*m*)		1.92–1.94 (*m*)	
H–C(10)	1.92–1.97 (*m*)	50.2	2.86–2.90 (*m*)	50.6
C(11)		151.4		152.6
H_α–C(12)	1.26–1.28 (*m*)	36.9	2.12–2.16 (*m*)	37.0
H_β–C(12)	1.73–1.75 (*m*)		2.47 (*d*, J = 12.3)	
H_α–C(13)	1.20–1.25 (*m*)	26.2	1.21–1.25 (*m*)	26.2
H_β–C(13)	1.84–1.87 (*m*)		1.82–1.87 (*m*)	
H–C(14)	1.61 (br. *s*)	48.4	1.53–1.59 (*m*)	48.3
H–C(15)	10.13 (*s*)	191.0	10.11 (*s*)	190.9
C(16)		180.0		187.2
H_a–C(17)	4.62 (*s*)	105.7	4.60 (*s*)	105.4
H_b–C(17)	4.80 (*s*)		4.79 (*s*)	
H–C(18)	1.23–1.28 (*m*)	30.1	1.23–1.27 (*m*)	29.9
Me(19)	0.96 (br. *s*)	21.5	0.93 (*d*, J = 7.0)	21.6
Me(20)	0.71 (br. *s*)	15.7	0.69 (br. *s*)	15.6
H_α–C(1′)	1.73 (br. *s*)	37.0	1.76 (*d*, J = 12.7)	36.9
H_β–C(1′)	2.12–2.15 (*m*)		2.13–2.16 (*m*)	
H_α–C(2′)	1.58–1.63 (*m*)	18.4	1.57–1.62 (*m*)	18.4
H_β–C(2′)	1.58–1.63 (*m*)		1.57–1.62 (*m*)	
H_α–C(3′)	1.62–1.64 (*m*)	35.5	1.57–1.62 (*m*)	35.9
H_β–C(3′)	2.15–2.20 (*m*)		2.01 (br. *s*)	
C(4′)		48.9		47.9
H–C(5′)	1.77–1.83 (*m*)	51.2	1.80–1.82 (*m*)	50.7
H_α–C(6′)	1.21–1.24 (*m*)	26.6	1.42–1.47 (*m*)	27.2
H_β–C(6′)	1.40–1.46 (*m*)		1.83–1.87 (*m*)	
H_α–C(7′)	1.61–1.65 (*m*)	38.6	1.68 (br. *s*)	38.4
H_β–C(7′)	2.18 (*d*, J = 11.5)		2.25 (*d*, J = 11.8)	
C(8′)		145.6		146.2
H–C(9′)	2.12–2.16 (*m*)	53.8	1.83–1.87 (*m*)	52.4
C(10′)		39.9		39.5
H_α–C(11′)	2.32 (br. *d*, J = 14.0)	27.3	1.65–1.69 (*m*)	29.3
H_β–C(11′)	2.67 (*t*, J = 14.0)		1.92–1.95 (*m*)	
C(12′) or H–C(12′)		151.6	2.45 (*d*, J = 12.0)	38.9
C(13′)		136.9		135.7
C(14′) or H–C(14′)		197.5	5.78 (br. *s*)	128.4
H_α–C(15′)	2.74–2.79 (*m*)	38.3	1.83–1.87 (*m*)	27.5
H_β–C(15′)	2.45–2.50 (*m*)		2.08–2.13 (*m*)	
Me(16′)	1.94 (*s*)	13.3	1.71 (*s*)	20.2
H_a–C(17′)	4.34 (*s*)	108.5	4.35 (*s*)	107.6
H_b–C(17′)	4.68 (*s*)		4.82 (*s*)	
C(18′)		186.1		186.3
Me(19′)	1.07 (*s*)	16.1	1.08 (*s*)	16.2
Me(20′)	0.74 (*s*)	14.3	0.70 (*s*)	14.1

group at δ(H) 4.34 (*s*) and 4.68 (*s*). The remaining exocyclic CH_2 group (δ(H) 4.62 (*s*) and 4.80 (*s*)) was deduced to be located in subunit **1a**, consistent with the implied structure of magdalenic acid.

A close comparison of the spectroscopic data with those of bismagdalenic acid revealed that the two compounds shared the same carbon skeleton [10]. Assignments of all H- and C-atoms in **1** can be made by ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra (*Fig. 2*). For subunit **1a**, a series of HMBCs between the signal of H–C(1) and those of C(18), C(2), and C(3), between the signal of H–C(14) and those of C(19) and C(20), the one of H–C(15) and those of C(2) and C(4), the one of H–C(17) and those of C(1), C(10), and C(12), and the signal of H–C(6) and those of C(7), C(8), and C(16) was observed, as well as ^{1}H,^{1}H-COSY correlations between the signals of H–C(1) and H–C(2) and H–C(14), between those of H–C(5) and H–C(4) and H–C(6), between the signals of H–C(9) and H–C(8) and H–C(10), and between those of CH_2(13) and CH_2(12) and H–C(14). These correlations indicated that an aldehyde group (δ(C) 191.0) was located at C(15), and a COOH group (δ(C)180.0) was assigned to C(16). The aldehyde group was suggested to be conjugated with the C(2)=C(3) bond (δ(C) 153.0 and 141.6, resp.), as the signal of C(2) was significantly shifted downfield. Therefore, subunit **1a** was unambiguously confirmed to have a magdalenic acid skeleton. For subunit **1b**, in the HMBC spectrum correlations of the signal of Me(19′) with those of C(3′), C(5′), and C(18′), of the signal of Me(20′) with those of C(1′), C(5′), and C(9′), of the signals of CH_2(17′) with those of C(7′) and C(9′), and of the signal of H–C(9′) with those of C(11′) and C(12′) were observed, and in the ^{1}H,^{1}H-COSY spectrum, correlations between the signals of CH_2(2′) and CH_2(1′) and CH_2(3′),

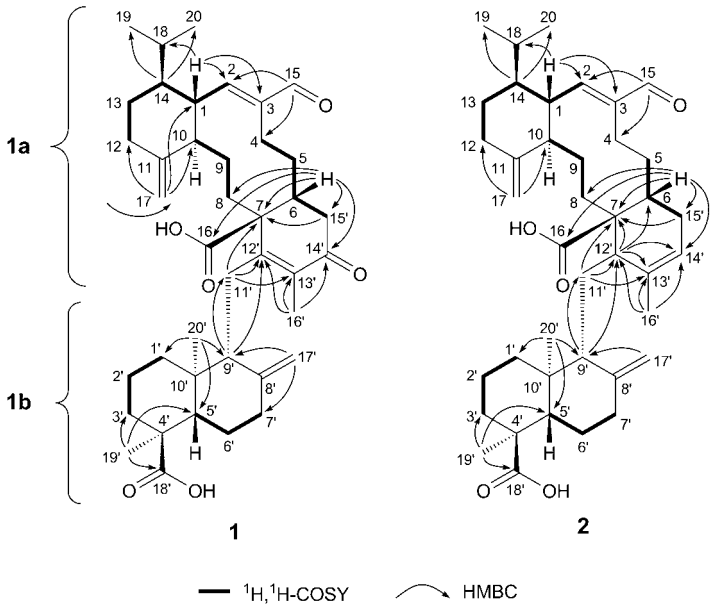

Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC correlations of* **1** *and* **2**

and between those of $CH_2(6')$ and H–C(5′) and $CH_2(7')$ confirmed the bicyclic labdane structure. The strong correlations in the HMBC spectrum between the signals at δ(H) 2.67 (t, J = 14.0, H_β–C(11′)) and δ(C) 151.6 (C(12′)) and 136.9 (C(13′)), and between the signals at δ(H) 2.12–2.16 (m, H–C(9′)) and δ(C) 151.6 (C(12′)) demonstrated that the C=C bond was located between C(12′) and C(13′). Furthermore, HMBCs between the signal of Me(16′) and those of C(12′), C(13′), C(14′) also confirmed the C(12′)=C(13′) position and the CO group attributable to C(14′). The two monomer units were connected through C(6) to C(15′) and C(7) to C(12′), which was supported by the evidence of the ^{1}H,^{1}H-COSY correlations between the signals of H–C(6) and H–C(15′), and the HMBCs between the signals of H–C(6) and C(14′) and C(15′), those of H_β–C(11′) and C(7), and those of H–C(15′) and C(7).

The relative configuration of **1** was established on the basis of a Rotational nuclear *Overhauser* Effect Spectroscopy (ROESY) experiment (*Fig. 3*). The partial structure **1b** was deduced to possess an *ent*-configuration by the comparison of the optical-rotation value with those of *ent*-labdane and labdane diterpenoids, respectively [12–17]. Indeed, negative optical rotations were reported for *ent*-labdane diterpenenes, while the positive sign of the optical rotation was found for labdane. Since the dimer **1** originated from **1a** and **1b**, their configuration could be the same [11]. The observations of $[\alpha]_D = -40$ (c = 0.16, $CHCl_3$) for magdalenic acid (**1a**) [10] and $[\alpha]_D^{26} = -73.8$ (c = 0.3, $CHCl_3$) for dimer **1** suggested that subunit **1b** is an *ent*-labdane unit. The relative configuration of H–C(5′) was assumed to be β. The strong ROESY correlations between signals at δ(H) 1.07 (Me(19′)) and δ(H) 0.74 (Me(20′)), and those at 1.77–1.83 (H–C(5′)) and δ(H) 2.12–2.16 (H–C(9′)), and the absence of correlations between δ(H) 0.74 (Me(20′)) and 1.77–1.83 (H–C(5′)), indicated that H–C(9′) and COOH(18′) were in β-orientation, and that Me(19′) and Me(20′) were α-oriented. The ROESY correlations between the signals at δ(H) 2.84–2.89 (H–C(1)) and 10.13 (H–C(15)) and 0.71 (Me(20)), and between the signals at δ(H) 6.58 (H–C(2)) and 1.92–1.97 (H–C(10)) and 1.61 (H–C(14)), confirmed the *trans*-diaxial bridgehead configuration (C(1) to C(10)) and also illustrated that H–C(1), H–C(15) and Me(20) were on top of the molecule (β-face), while H–C(2), H–C(10), and H–C(14) were underneath (α-face).

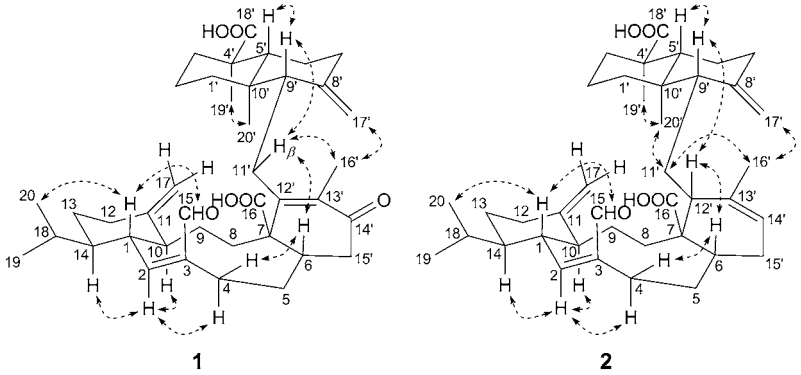

Fig. 3. *Key ROESY correlations of* **1** *and* **2**

The correlations between the signals at δ(H) 2.67 (*t*, $J = 14.0$, H_β–C(11′)) and those at 1.94 (*s*, Me(16′)), 2.12 – 2.16 (*m*, H–C(9′)), and 1.82 – 1.86 (*m*, H–C(6)), and between the one at 1.82 – 1.86 (*m*, H–C(6)) and those at 2.79 – 2.83 (*m*, H_β–C(4)), and 2.67 (*t*, $J = 14.0$, H_β–C(11′)) observed in ROESY spectrum indicated that H–C(6) was in β-orientation. The relative configuration of C(7) could not be determined because of decomposition of **1** after purification, but it is assumed to be β as in bismagdalenic acid [10]. Thus, the structure of **1** was established as presented in *Fig. 1*. Compound **1** was new and given the trivial name bisyinshanic acid A.

Compound **2** was obtained as a colorless oil, and the molecular formula was determined to be $C_{40}H_{58}O_5$ by the HR-ESI-MS (negative-ion mode; $[M-H]^-$, *m*/*z* found 617.4201, calc. 617.4206). It was suggested to be an analogue of **1** on the basis of characteristic ^{13}C- and ^{1}H-NMR data (*Table 1*). The IR spectrum of **2** indicated the presence of COOH groups (3380 cm^{-1} and 1729 cm^{-1}), an α,β-unsaturated aldehydic CO group (1694 cm^{-1}), and of C=C bonds (1646 cm^{-1}). The ^{1}H-NMR spectrum of **2** (*Table 1*) showed the same spectral features as compound **1**, except for the appearance of the signals at δ(H) 2.45 (*d*, $J = 12.0$, 1 H) and 5.78 (br. *s*, 1 H), which were assigned to a H–C(12′) group and an olefinic H–C(14′), respectively. The signal of the C(14′)=O group in **1** was not observed in the ^{13}C-NMR spectrum of **2**.

The HMBCs between the signals of H–C(11′) and C(7), C(12′), and C(13′), those of H–C(12′) and C(6), C(7), C(13′), and C(14′), those of Me(16′) and C(13′) and C(14′) demonstrated that compound **2** possessed the same linkage between its two monomer units as compound **1**. The ^{1}H,^{1}H-COSY spectrum displayed correlations between the signals of H–C(6) and H–C(5) and H–C(15′) in accordance with the above deduction (*Fig. 2*). The major differences between **1** and **2** were that the conjugated CO group located at C(14′) in **1** was absent in **2**, and that the location of the C=C bond was changed from C(12′)=C(13′) in **1** to C(13′)=C(14′) in **2**, which was deduced from the HMBCs of the signals of H–C(12′) and Me(16′) with those of C(13′) and C(14′) each, as shown in *Fig. 2*.

The relative configuration of **2** was determined through a ROESY experiment, which showed that **2** has the same relative configuration as **1** (*Fig. 3*). Strong ROE correlations between the signals of H–C(9′) and H–C(12′) and those of H–C(6) and H–C(12′) indicated the β-configuration of H–C(12′) and H–C(6). The relative configuration of C(7) in **2** was not determined for the same reason as in compound **1**. Hence, the structure of **2** was elucidated as presented in *Fig. 1*. Compound **2** was also new and given the trivial name bisyinshanic acid B.

In addition, *ent*-(13*S*)-13-hydroxyatis-16-ene-3,14-dione (**3**), *ent*-(13*R*,14*R*)-13,14-dihydroxyatis-16-en-3-one (**4**), *ent*-(3β,13*S*)-3,13-dihydroxyatis-16-en-14-one (**5**), *ent*-atis-16-ene-3,14-dione (**6**), *ent*-(3*S*,16*S*)-3,16,17-trihydroxykauran-2-one (**7**), *ent*-(16*R*)-16,17-dihydroxykauran-3-one (**8**), and helioscopinolides A (**10**) and E (**9**) were identified by comparision of their ^{1}H- and ^{13}C-NMR and MS spectroscopic data with those reported in the literature. The pertinent ^{13}C-NMR data of all of these diterpenoids are included in *Table 2*.

Table 2. *^{13}C-NMR* (125 MHz) *Data of Compounds* **3**–**10**

C-Atom	**3**[a])	**4**[b])	**5**[a])	**6**[a])	**7**[c])	**8**[a])	**9**[a])	**10**[a])
C(1)	36.6 (*t*)	37.7 (*t*)	36.4 (*t*)	37.1 (*t*)	53.3 (*t*)	39.2 (*t*)	37.3 (*t*)	37.4 (*t*)
C(2)	34.0 (*t*)	32.2 (*t*)	26.8 (*t*)	34.1 (*t*)	211.3 (*s*)	34.0 (*t*)	34.4 (*t*)	27.5 (*t*)
C(3)	216.0 (*s*)	219.2 (*s*)	78.8 (*d*)	216.4 (*s*)	82.6 (*d*)	218.6 (*s*)	215.6 (*s*)	78.5 (*d*)
C(4)	47.4 (*s*)	48.5 (*s*)	38.6 (*s*)	47.5 (*s*)	45.2 (*s*)	47.1 (*s*)	47.5 (*s*)	39.1 (*s*)
C(5)	55.0 (*d*)	55.8 (*d*)	54.6 (*d*)	55.3 (*d*)	54.1 (*d*)	54.2 (*d*)	54.7 (*d*)	54.3 (*d*)
C(6)	19.8 (*t*)	19.9 (*t*)	18.8 (*t*)	20.0 (*t*)	19.7 (*t*)	21.1 (*t*)	24.5 (*t*)	23.4 (*t*)
C(7)	30.3 (*t*)	29.0 (*t*)	30.8 (*t*)	31.4 (*t*)	40.9 (*t*)	40.5 (*t*)	36.5 (*t*)	36.9 (*t*)
C(8)	47.2 (*s*)	47.2 (*s*)	47.4 (*s*)	47.6 (*s*)	43.3 (*s*)	43.2 (*s*)	150.2 (*s*)	151.4 (*s*)
C(9)	51.0 (*d*)	53.3 (*d*)	51.9 (*d*)	51.8 (*d*)	56.0 (*d*)	55.6 (*d*)	50.6 (*d*)	51.5 (*d*)
C(10)	37.4 (*s*)	38.6 (*s*)	37.8 (*s*)	37.6 (*s*)	45.0 (*s*)	38.4 (*s*)	40.9 (*s*)	41.2 (*s*)
C(11)	25.2 (*t*)	27.6 (*t*)	25.2 (*t*)	27.8 (*t*)	18.4 (*t*)	19.2 (*t*)	27.7 (*t*)	27.5 (*t*)
C(12)	44.7 (*d*)	44.4 (*d*)	44.8 (*d*)	38.3 (*d*)	26.2 (*t*)	26.5 (*t*)	75.6 (*d*)	75.9 (*d*)
C(13)	75.0 (*d*)	75.9 (*d*)	75.1 (*d*)	44.5 (*t*)	40.3 (*d*)	40.7 (*d*)	155.6 (*s*)	156.0 (*s*)
C(14)	218.0 (*s*)	79.6 (*d*)	218.3 (*s*)	216.6 (*s*)	37.4 (*t*)	37.8 (*t*)	114.7 (*d*)	114.2 (*d*)
C(15)	43.5 (*t*)	39.9 (*t*)	43.9 (*t*)	42.6 (*t*)	51.3 (*t*)	52.0 (*t*)	117.0 (*s*)	116.5 (*s*)
C(16)	142.2 (*s*)	144.7 (*s*)	142.7 (*s*)	147.0 (*s*)	79.1 (*d*)	79.7 (*d*)	175.1 (*s*)	175.2 (*s*)
C(17)	110.9 (*t*)	110.8 (*t*)	110.7 (*t*)	107.1 (*t*)	69.0 (*t*)	69.6 (*t*)	8.3 (*q*)	8.2 (*q*)
C(18)	26.1 (*q*)	26.7 (*q*)	28.4 (*q*)	25.9 (*q*)	29.1 (*q*)	27.3 (*q*)	26.5 (*q*)	28.6 (*q*)
C(19)	21.8 (*q*)	16.4 (*q*)	15.6 (*q*)	21.8 (*q*)	15.9 (*q*)	20.9 (*q*)	21.8 (*q*)	15.6 (*q*)
C(20)	13.6 (*q*)	14.5 (*q*)	14.0 (*q*)	12.7 (*q*)	18.1 (*q*)	17.6 (*q*)	16.2 (*q*)	16.7 (*q*)

[a]) Measured in $CDCl_3$. [b]) Measured in CD_3OD. [c]) Measured in $CDCl_3/CD_3OD$ 1:1.

Experimental Part

General. Silica gel (SiO_2; 100–200 and 200–300 mesh), silica gel *H* (*Qingdao Marine Chemical Ltd.*, P. R. China), and *Sephadex LH-20* (*Amersham Biosciences*, Germany) were used for column chromatography (CC). MPLC was performed on a *Büchi Chromatography System* including pump module *C-605*, columns packed with *RP-18* silica gel (40–60 μm, *Amersham Biosciences*, Germany). TLC was carried out on precoated silica gel *GF254* plates (*Qingdao Marine Chemical Ltd.*, P. R. China), and the TLC spots were viewed at 254 nm and visualized using 5% H_2SO_4 in alcohol. Optical rotations: *Horiba SEPA-300* polarimeter. UV/VIS Spectra: *Shimadzu UV-2401PC* spectrophotometer. IR Spectra: *Tensor 27* FT-IR spectrometer with KBr pellets. NMR Spectra: *Bruker AV-400* and *DRX-500* spectrometers with TMS as an internal standard at r.t. (δ in ppm, *J* in Hz). ESI-MS and HR-ESI-MS: *API QSTAR Pulsar I* mass spectrometers.

Plant Material. The roots of *Euphorbia yinshanica* were collected in July 2008 at Xunhua, Qinghai Province of P. R. China. The plant was authenticated by Prof. *Shang-Wu Liu* (Northwest Institute of Plateau Biology, Chinese Academy of Sciences), and a voucher specimen (EY2008072103) was deposited at the Northwest Institute of Plateau Biology, Chinese Academy of Sciences.

Extraction and Isolation. The fresh roots of *E. yinshanica* (10 kg) were extracted three times with 85% EtOH at r.t. The combined EtOH extracts were evaporated under reduced pressure to yield a residue, which was suspended in H_2O and extracted with AcOEt. The AcOEt extract (56 g) was separated with SiO_2 CC, successively eluting with $CHCl_3$, $CHCl_3$/acetone (from 40:1 to 1:1), and MeOH to give twelve fractions (*Frs. 1–12*) according to differences in compositions monitored by TLC (GF_{254}). *Fr. 6* (1.2 g) was divided into five subfractions (*Frs. 6A$_1$–6A$_5$*) by CC over *RP-18*, eluting with acetone/H_2O (from 60 to 100%). *Fr. 6A$_2$* (0.3 g, acetone/H_2O, 70%) and *Fr. 6A$_3$* (0.2 g, acetone/H_2O, 80%) were subjected to *Sephadex LH-20*, eluted with $CHCl_3$/MeOH 1:1, and purified with repeated SiO_2 CC eluting with petroleum ether (PE)/AcOEt (from 10:1 to 4:1) to afford compounds **1** (20 mg) and **2** (24 mg). *Fr. 8* (0.8 g) was eluted with $CHCl_3$/actone 10:1 over SiO_2 to give four further subfractions, *Frs. 8A$_1$–8A$_4$*. *Fr. 8A$_2$* (0.4 g) was then further subjected to MPLC to obtain two new subfractions, *Frs. A$_{21}$* (MeOH/H_2O, 70%) and *A$_{22}$* (MeOH/H_2O, 80%). *Fr. A$_{21}$* was purified by repeated SiO_2 CC eluted by PE/acetone (from 8:1 to 4:1) to afford compounds **3** (20 mg), **4** (12 mg), and **7** (22 mg). *Fr. A$_{22}$* was subjected to *Sephadex*

LH-20, eluted with MeOH, and then purified by repeated SiO_2 CC eluting with $CHCl_3$/acetone (from 15:1 to 10:1) to obtain compounds **5** (25 mg), **6** (11 mg), and **8** (19 mg). *Fr. 5* (0.9 g) was subjected to MPLC (MeOH/H_2O, from 70% to 100%) to obtain three subfractions, then further purification was carried out by repeated SiO_2 CC, eluting with PE/acetone (from 10:1 to 4:1) and PE/AcOEt (from 8:1 to 3:1), and *Sephadex LH-20*, eluting with $CHCl_3$/MeOH 1:1 to afford compounds **9** (21 mg) and **10** (29 mg).

Bisyinshanic Acid A (=*(4*S*,5*E*,12a*S*)-12-{[(1*R*,4a*S*,5*S*,8a*S*)-5-Carboxy-5,8a-dimethyl-2-methylidene-decahydronaphthalen-1-yl]methyl}-6-formyl-1,3,4,4a,7,8,8a,9,10,13,14,14a-dodecahydro-11-methyl-1-methylidene-10-oxo-4-(propan-2-yl)dibenzo[*a,e*][10]annulene-12a(2*H*)-carboxylic Acid*; **1**). Colorless oil. $[\alpha]_D^{26} = -73.8$ ($c = 0.3$, $CHCl_3$). UV ($CHCl_3$): 251 (3.80), 232 (3.57), 224 (3.56), 215 (3.51). IR (KBr): 3432, 2933, 2869, 1721, 1669, 1643, 1600, 1448, 1385, 1193, 980, 886, 768. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS (neg.): 631 ($[M-H]^-$). HR-ESI-MS (neg.): 631.3994 ($[M-H]^-$, $C_{40}H_{55}O_6^-$; calc. 631.3999).

Bisyinshanic Acid B (=*(4*S*,5*E*,12a*S*,14a*R*)-12-{[(1*R*,4a*S*,5*S*,8a*S*)-5-Carboxy-1,3,4,4a,7,8,8a,9,12,13,14,14a-dodecahydro-5,8a-dimethyl-2-methylidenedecahydronaphthalen-1-yl]methyl}-6-formyl-11-methyl-1-methylidene-4-(propan-2-yl)dibenzo[*a,e*][10]annulene-12a(2*H*)-carboxylic Acid*; **2**). Colorless oil. $[\alpha]_D^{26} = -94.7$ ($c = 0.5$, $CHCl_3$). UV ($CHCl_3$): 260 (3.76), 234 (3.58), 228 (3.57), 213 (3.51). IR (KBr): 3380, 2933, 2868, 1729, 1694, 1646, 1387, 1369, 1272, 1192, 1127, 980, 941, 890,758, 664. ^{1}H- and ^{13}C- NMR: see *Table 1*. ESI-MS (neg.): 617 ($[M-H]^-$). HR-ESI-MS (neg.): 617.4201 ($[M-H]^-$, $C_{40}H_{57}O_5^-$; calc. 617.4206).

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