Two New Rosane-Type Diterpenoids from *Euphorbia ebracteolata* HAYATA

by **Shu-Zhen Mu**[a]), **Chun-Rong Jiang**[a]), **Tao Huang**[a])[b]), and **Xiao-Jiang Hao***[a])

[a]) Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, P. R. China (phone: +86-851-3804492; fax: +86-851-3805081; e-mail: haoxj@mail.kib.ac.cn)

[b]) China College of Life Science, Guizhou University, Guiyang 550025, P. R. China

Two new diterpeniods, euphebracteolatins A and B (**1** and **2**, resp.) were isolated from *Euphorbia ebracteolata* HAYATA, along with two known ones (3*R*)-*ent*-rosa-1(10),15-dien-3α-ol (**3**) and yuexiandajisu D (**4**). The structures were established by extensive spectroscopic analyses and comparison with literature data.

Introduction. – The genus *Euphorbia* is the largest in the family Euphorbiaceae, comprising more than 2,000 species. About 80 species of the genus *Euphorbia* are distributed in China, with *Euphorbia ebracteolata* HAYATA widely occurring in China, Japan, Korea, and other countries. The root of *E. ebracteolata* is usually used to treat oedema, indigestion, bone tuberculosis, cough, and chronic bronchitis [1]. Previous chemical studies revealed the presence of terpenoids, acetophenone derivatives, flavonoids, tannins, steroids, and volatile oils in this genus [2], and some of them were found to exhibit several kinds of bioactivities [3]. Further investigation on the EtOH extract of this plant resulted in the isolation of two new diterpenoids, euphebracteolatins A and B (**1** and **2**, resp.), along with two known compounds, (3*R*)-*ent*-rosa-1(10),15-dien-3α-ol (**3**), yuexiandajisu D (**4**; *Fig. 1*). In this article, we describe the isolation and structural elucidation of these compounds.

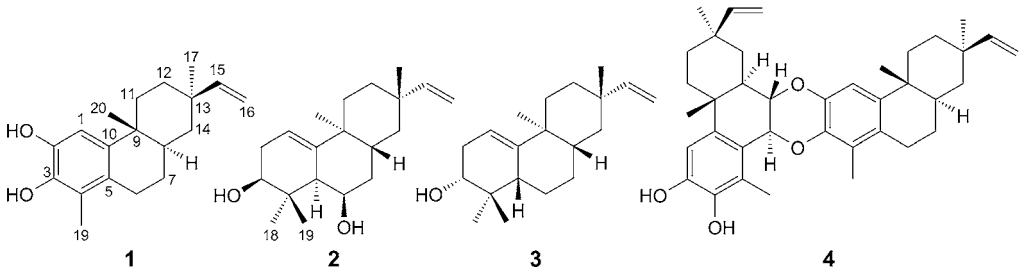

Fig. 1. *The structures of compounds* **1**–**4**

Results and Discussion. – Compound **1** was obtained as a light yellow oil. Its molecular formula was deduced as $C_{19}H_{26}O_2$ from its HR-EI-MS (*m*/*z* 286.1926 (M^+, calc. 286.1933)), with seven degrees of unsaturation. The IR spectrum of **1** showed the

absorptions of OH (3424 cm^{-1}) and phenyl (1613 and 1485 cm^{-1}) groups. The ^{13}C-NMR (*Table*) and DEPT spectra indicated that **1** contains 19 C-atoms, including three tertiary Me, six CH_2, three CH groups, and seven quaternary C-atoms. Among them, one monosubstituted olefinic C=C bond (δ(C) 151.1 and 108.8) and one polysubstituted aromatic ring (δ(C) 108.7, 140.9, 139.9, 122.7, 126.9, and 140.2) were indicated, which accounted for four degrees of unsaturation, the remaining three could be attributed to the presence of a three-ring system for **1**. The ^{1}H-NMR data (*Table*) exhibited characteristic signals for the aromatic H-atom (δ(H) 6.70 (*s*, 1 H) and the olefinic H-atoms (δ(H) 5.90 (*dd*, $J = 10.4, 17.2$, 1 H), 4.98 (*dd*, $J = 1.2, 17.2$, 1 H), and 4.90 (*dd*, $J = 1.2, 10.4$, 1 H).

Table. *^{1}H- and ^{13}C-NMR* (400 and 100 MHz, resp.) *Data of Compounds* **1** *and* **2** *in $CDCl_3$*. δ in ppm, J in Hz. Atom numbering as indicated in *Fig. 1*.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	6.70 (*s*)	108.7	5.45–5.42 (*m*)	116.2
2	–	140.9	2.31–2.27 (*m*, H_a), 1.96–1.89 (*m*, H_b)	31.8
3	–	139.9	3.53 (*dd*, $J = 6.0, 10.4$)	74.8
4	–	122.7	–	36.7
5	–	126.9	2.05–1.97 (*m*)	42.0
6	2.68–2.61 (*m*)	26.8	4.05–3.99 (*m*)	70.6
7	1.65–1.54 (*m*)	25.6	2.35–2.30 (*m*, H_a), 1.30–1.37 (*m*, H_b)	30.6
8	1.79–1.73 (*m*)	36.3	1.73–1.66 (*m*)	37.7
9	–	36.4	–	36.6
10	–	140.2	–	147.9
11	1.95–1.92 (*m*)	33.9	1.66–1.59 (*m*, H_a), 1.49–1.41 (*m*, H_b)	36.5
12	1.41–1.34 (*m*)	32.8	1.25, 1.50 (2*m*)	32.6
13	–	36.3	–	36.3
14	1.49–1.42 (*m*, H_a), 1.23–1.15 (*m*, H_b)	39.5	1.73–1.66 (*m*, H_a), 1.10–1.20 (*m*, H_b)	33.9
15	5.90 (*dd*, $J = 10.4, 17.2$)	151.1	5.90 (*dd*, $J = 10.8, 17.6$)	151.0
16	4.90 (*dd*, $J = 1.2, 10.4$, H_a), 4.98 (*dd*, $J = 1.2, 17.2$, H_b)	108.8	4.91 (*dd*, $J = 1.2, 10.8$, H_a), 5.00 (*dd*, $J = 1.2, 17.6$, H_b)	109.1
17	1.02 (*s*)	22.7	1.00 (*s*)	22.0
18		–	1.04 (*s*)	23.7
19	2.11 (*s*)	11.4	0.70 (*s*)	12.8
20	1.00 (*s*)	21.3	1.10 (*s*)	23.8

Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of the known compound 19-norrosa-1,3,5(10),15-tetraene-2,18-diol indicated that both compounds were very similar except for C(3) and C(19), implying they share the same basic skeleton of a rosane-type diterpeniod [4]. Detailed analysis of 1D-NMR spectra of **1** revealed the following differences of chemical shifts: one OH group and the Me group were indicated in place of the CH and CH_2OH groups, respectively, in the known compound 19-norrosa-1,3,5(10),15-tetraene-2,18-diol. On the basis of the HMBCs features of H–C(1) to C(3), and of Me(19) to C(4), C(3), and C(5), the OH group and the Me group were assigned to C(3) (δ(C) 139.9) and at C(19) (δ(C) 11.4, δ(H) 2.11),

respectively. The gross structure of **1** was finally established from its 2D-NMR spectra as shown in *Fig. 2*. The ^{1}H,^{1}H-COSY spectrum of **1** evidenced the presence of three partial structures, **a** – **c**. The connectivity of each partial structure was clarified by the HMBC spectrum as shown in *Fig. 2*.

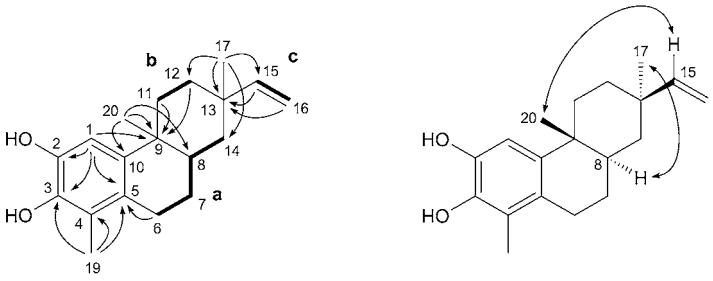

Fig. 2. *Key ^{1}H,^{1}H-COSY* (—), *HMB* (H → C), *and ROESY* (H ↔ H) *correlations of* **1**

The relative configuration of **1** was consistent with that of the known compound 19-norrosa-1,3,5(10),15-tetraene-2,18-diol as deduced from ROESY spectrum of **1** (*Fig. 2*). The correlation between Me(17) and H–C(8) indicated that Me(17) was α-oriented, while Me(20) had the β-orientation on the basis of the correlation between Me(20) and H–C(15). Thus, the structure of **1** was established as 2,3-dihydroxy-18-norrosa-1(10),2,4,15-tetraene, named euphebracteolatin A.

Compound **2** was obtained as optically active white amorphous powder. The molecular formula was determined as $C_{20}H_{32}O_2$ by HR-EI-MS at *m/z* 304.2401 (M^+; calc. 304.2402), with five degrees of unsaturation. The IR spectrum of **2** implied the presence of OH (3345 cm^{-1}) groups. The ^{13}C-NMR (*Table*) and DEPT data of **2** exhibited 20 signals due to one monosubstituted C=C bond (δ(C) 151.0 and 109.1), one trisubstituted C=C bond (δ(C) 116.2 and 147.9), three sp^3 quaternary C-atoms, and four sp^3-CH, five sp^3-CH$_2$, and four tertiary sp^3-Me groups. Of these, two CH groups (δ(C) 74.8 and 70.6) were ascribed as being attached to OH groups.

The ^{1}H-NMR (*Table*) of **2** confirmed the presence of three olefinic H-atoms (δ(H) 5.90 (*dd*, *J* = 10.8, 17.6, 1 H), 5.00 (*dd*, *J* = 1.2, 17.6, 1 H), and 4.91 (*dd*, *J* = 1.2, 17.6, 1 H)), four tertiary sp^3-Me groups (δ(H) 1.10 (*s*), 1.04 (*s*), 1.00 (*s*), and 0.70 (*s*)), and two O-bearing CH groups (δ(H) 4.05 – 3.99 (*m*) and 3.53 (*dd*, *J* = 6.0, 10.4). These data indicated that compound **2** was a rosane-type diterpeniod, similar to the known compound (3*R*)-*ent*-rosa-1(10),15-dien-3α-ol (**3**) [4]. Further comparison of the 1D-NMR data of **2** with those of **3** revealed that the main difference between the two compounds was the presence of one OH group in **2**. The OH group was located at C(6) evidenced by the HMBCs of H–C(6) to C(4), of CH$_2$(7) to C(6), and of H–C(8) to C(6). Detailed analysis of the 2D-NMR, including HMQC, ^{1}H,^{1}H-COSY, and HMBC as shown in *Fig. 3*, confirmed the above conclusion. The relative configuration of **1** was established from the ROESY spectrum. The correlations H–C(3)/Me(18), Me(18)/H–C(5), H–C(5)/H–C(6), and Me(20)/H–C(15) indicated that H–C(3), H–C(5), H–C(6), and Me(20) were α-oriented, and accordingl Me(17) and the OH at C(3) and C(6) were β-oriented. H–C(8) was β-oriented on the basis of the correlation Me(17)/

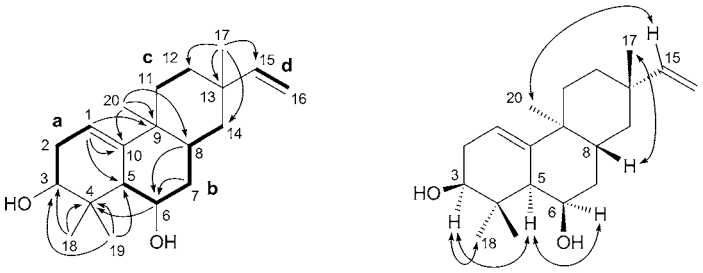

Fig. 3. *Key* $^1H,^1H$*-COSY* (—), *HMB* (H → C), *and ROESY* (H ↔ H) *correlations of* **2**

H–C(8). Thus, the structure of **2** was established as *ent*-rosa-1(10),15-dien-3β,6β-diol, named euphebracteolatin B.

By comparison with the NMR and MS data with those reported in the literature, compounds **3** and **4** were identified as (3*R*)-*ent*-rosa-1(10),15-dien-3α-ol [4] and yuexiandajisu D [5], respectively.

Compounds **1**–**4** were evaluated for their cytotoxic activities against human ovarian carcinoma cell line SK-OV-3. At the 1.00×10^{-5} M concentration, euphebracteolatin A (**1**) showed weak inhibition of tumor growth with a value of $14.90 \pm 13.46\%$, while compounds **2**–**4** were inactive.

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Experimental Part

General. All solvents used were distilled prior to use. Column chromatography (CC): silica gel (SiO_2; 200–300 and 300–400 mesh; *Qingdao Marine Chemical Company*, P. R. China), reversed-phase *RP-18* (40–63 μm; *Merck*), and *Sephadex LH-20* (40–70 μm; *Amersham Pharmacia Biotech AB*, Sweden). Optical rotations: *JASCO-P1020* digital polarimeter. UV: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra (KBr): *Bruker Tensor 27* FT-IR spectrophotometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *INOVA-400* MHz NMR spectrometer in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-EI-MS: *Waters AutoSpec Premier P776* spectrometer; in *m*/*z*.

Plant Material. The roots of *E. ebracteolata* were collected from Changchun, Jilin Province of China, in February 2006. The sample was identified by Prof. *Li Gao* from the Natural Drug Resources Laboratory of the Yunnan Institute of Materia Medica. A voucher specimen (GZCNP 08070724) was deposited with the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation. The air-dried roots (20 kg) of *E. ebracteolata* were powdered and extracted with 95% EtOH (3 × 30 l, each for 5 d) at r.t. and concentrated *in vacuo* to give a crude extract. The extract was suspended in H_2O and extracted successively with petroleum ether (PE) and $CHCl_3$. The $CHCl_3$ fraction (300 g) was subjected to CC (SiO_2 (200–300 mesh); PE/acetone 100:0 to 0:100) to afford five fractions, *Frs. 1*–*5*. *Frs. 1*, *3*, and *4* were purified by repeated CC (SiO_2; *RP-18*; and *Sephadex LH-20*; $CHCl_3$/MeOH 0:1–1:1) to afford **1** (83 mg), **2** (74 mg), **3** (61 mg), and **4** (16 mg).

Euphebracteolatin A (= rel-*(4b*R,*7*R,*8a*R*)-7-Ethenyl-4b,5,6,7,8,8a,9,10-octahydro-1,4b,7-trimethylphenanthrene-2,3-diol*; **1**). Light yellow oil. $[\alpha]_D^{23.1} = +97.6$ ($c = 0.42$, $CHCl_3$). UV ($CHCl_3$): 286 (3.1), 241 (2.9). IR (KBr): 3424, 2925, 1635, 1613, 1485, 1175, 1026. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-EI-MS: 286.1926 (M^+, $C_{19}H_{26}O_2^+$, calc. 286.1933).

Euphebracteolatin B (= rel-*(2*R*,4b*R*,7*R*,8a*S*,10*S*,10a*S*)-7-Ethenyl-1,2,3,4b,5,6,7,8,8a,9,10,10a-dodecahydro-1,1,4b,7-tetramethylphenanthrene-2,10-diol = rel-*(3β,6β,8β,9α,13α)-Rosa-1(10),15-diene-3,6-diol*; **2**). White amorphous powder. $[\alpha]_D^{23.0} = +6.2$ ($c = 1.00$, $CHCl_3$). IR (KBr): 3578, 3345, 2966, 2930, 1636, 1450, 1363, 1048. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-EI-MS: 304.2401 (M^+, $C_{20}H_{32}O_2^+$; calc. 304.2402).

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