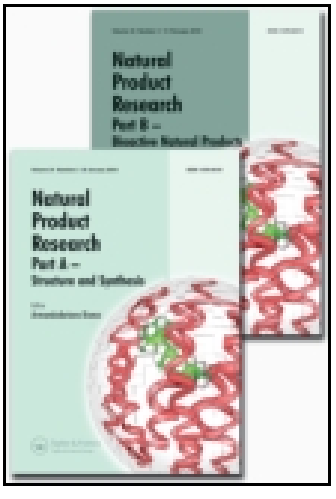

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New diterpenoids from the roots of Euphorbia ebracteolata Hayata

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New diterpenoids from the roots of *Euphorbia ebracteolata* Hayata

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Three new diterpenoids, ingenol-5β,20-*O*,*O*-isopropylidene-3β-palmitate (**1**), ingenol-5β,20-*O*,*O*-isopropylidene-3β-myristinate (**2**) and 3β,19-dihydroxy-1(10),15-rosadien-2-one (**3**), were isolated from the roots of *Euphorbia ebracteolata* Hayata. Their structures were deduced by spectroscopic means and analytic techniques.

Keywords: *Euphorbia*; *Euphorbia ebracteolata* Hayata; diterpenoids

1. Introduction

Euphorbia ebracteolata Hayata is a type of perennial herb widely distributed in China, Korea and Japan (Fu, Yu, & Zhu, 2006). The root of *E. ebracteolata* is a traditional Chinese medicine to treat pulmonary tuberculosis, psoriasis, neuropathic dermatitis and chronic bronchitis (Xie, 1996). Previous phytochemical studies have demonstrated the presence of some terpenoids, acetophenone derivatives and flavonoids in this plant (Yan & Zhao, 2008). Our further research has led to the isolation of seven diterpenoids from the roots of *E. ebracteolata* (Deng, Mu, Huang, Song, & Hao, 2009). As a continuation of our investigations into the chemical constituents of this plant, three new diterpenoids, ingenol-5β,20-*O*,*O*-isopropylidene-3β-palmitate (**1**), ingenol-5β,20-*O*,*O*-isopropylidene-3β-myristinate (**2**) and 3β,19-dihydroxy-1(10),15-rosadien-2-one (**3**), were obtained from this plant. In this article, we report the isolation and structural elucidation of three new diterpenoids (**1**–**3**, Figure 1).

2. Results and discussion

Compound **1** was obtained as yellow oil. Its molecular formula was determined as $C_{39}H_{62}O_6$ by (+)-HR-ESI-MS (*m*/*z* 649.4455, $[M+Na]^+$, Calcd 649.4444). IR absorptions at 3525, 3435 and 1728 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. All of the 39 carbons observed in the ^{13}C-NMR and DEPT spectra could be classified into two trisubstituted olefins, two carbonyls, four sp^3 quaternaries, six sp^3 methines and 16 sp^3 methylenes, together with seven methyls, as shown in Table 1. Among them, two carbonyls (δ_C 207.5 and 174.4) were assigned as

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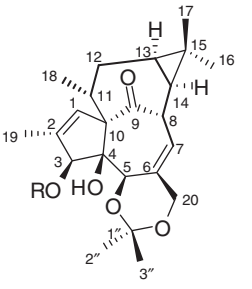
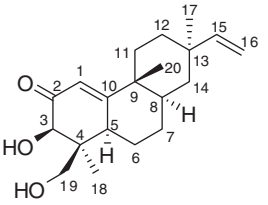


1 R=CO(CH$_2$)$_{14}$CH$_3$
2 R=CO(CH$_2$)$_{12}$CH$_3$



3

Figure 1. Structures of compounds **1–3**.

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compounds **1–2** in $CDCl_3$.

	1		**2**	
Position	δ_C	δ_H [J (Hz)]	δ_C	δ_H [J (Hz)]
1	132.3	6.04 (s)	132.2	6.04 (s)
2	136.4		136.4	
3	81.7	5.54 (s)	81.7	5.54 (s)
4	84.1		84.1	
5	73.9	4.00 (s)	73.8	3.99 (s)
6	135.7		135.7	
7	122.2	5.78 (s)	122.2	5.78 (s)
8	43.6	4.14 (m)	43.6	4.14 (m)
9	207.5		207.5	
10	72.2		72.1	
11	37.7	2.56 (m)	37.7	2.56 (m)
12	31.1	2.18 (m), 1.76 (m)	31.0	2.18 (m), 1.79 (m)
13	23.4	0.72 (dd, 8.4, 14.8)	23.4	0.72 (dd, 8.4, 14.8)
14	22.9	0.86 (dd, 6.4, 13.2)	22.9	0.86 (dd, 6.4, 13.2)
15	24.1		24.2	
16	28.5	1.05 (s)	28.5	1.05 (s)
17	15.6	1.09 (s)	15.6	1.09 (s)
18	17.4	0.99 (d, 7.2)	17.3	0.99 (d, 6.8)
19	16.7	1.76 (s)	15.7	1.76 (s)
20	64.3	4.23 (m)	64.3	4.23 (m)
1′	174.4		174.8	
2′	34.7	2.37 (m)	34.7	2.39 (m)
3′	25.2	1.66 (m)	25.1	1.68 (m)
4′–11′	29.7–29.4	1.26 (s)	29.6–29.1	1.26 (s)
12′	29.3	1.26 (s)	31.9	1.24 (m)
13′	29.2	1.26 (s)	22.7	1.26 (m)
14′	31.9	1.24 (m)	14.1	0.86 (m)
15′	22.7	1.26 (m)		
16′	14.1	0.86 (m)		
1″	100.3		100.3	
2″	20.7	1.41 (s)	20.7	1.41 (s)
3″	26.8	1.46 (s)	26.8	1.46 (s)

a ketone carbonyl and an ester carbonyl, respectively. One methylene (δ_C 64.3, δ_H 4.23), one methine (δ_C 73.9, δ_H 4.00) and two quaternary carbons (δ_C 84.1 and 100.3) were ascribed to be bearing oxygen atoms. The ^{1}H-NMR spectrum of **1** revealed a broad singlet at δ_H 1.26 (20H, s), which indicated the presence of a long-chain fatty acid moiety. The chemical shifts at δ_C 29.3–29.7 and 174.4 further confirmed the above deduction.

Comparison of the NMR data of **1** with those of the known compound, ingenol-3-myristinate, from *Euphorbia wallichii* (Li & Suo, 2005) suggested that **1** possessed an ingenane-type skeleton. The main difference between them was the presence of three extra carbons at δ_C 100.3 (s), 26.8 (q), and 20.7 (q) in **1** and their molecular formulae. Further analysis of 1D-NMR, ^{1}H–^{1}H COSY and HMBC spectra, as shown in Figure 2, indicated that one acetonyl unit was connected to the C-5 and C-20 via two oxygen atoms, respectively, on the basis of HMBC correlations of H-20 (δ_H 4.23, 2H, m), H-2″ (δ_H 1.41, 3H, s) and H-3″ (δ_H 1.46, 3H, s) to C-1″, and of H-5 (δ_H 4.00, 1H, s) to C-20, C-2″ and C-3″ (Figure 2). The location of the long-chain fatty acid moiety at C-3 was determined by the HMBC cross-peaks from H-3 (δ_H 5.54, 1H, s) and C-1′ (δ_C 174.4). To further confirm the long-chain fatty acid moiety, the basic hydrolysis of compound **1** was carried to obtain the free long-chain fatty acid, which was determined as palmitic acid by a main peak in its GC-MS spectrum. Thus, the planar structure of **1** was deduced as ingenol-5,20-*O*,*O*-isopropylidene-3-palmitate.

The relative configuration of **1** was established by the analysis of its ROESY spectrum, as shown in Figure 2. Correlations of H-1–H-3, H_3-18–H_3-19, H-3–H-5 and H-5–H_3-2″ indicated that H-1, H-3, H-5 and H_3-2″ were in α-orientation, while correlations of H-8–H-7, H-7–H-11, H-11–H-17, H-17–H_2-20 and H_2-20–H_3-3″ indicated that H-7, H-8, H_3-17, H_2-20 and H_3-3″ were in β-orientation, which consisted of with the structural characters of ingenane-type diterpenoids. Thus, the structure of **1** was deduced as ingenol-5β, 20-*O*,*O*-isopropylidene-3β-palmitate.

Compound **2** was obtained as yellow oil. The ^{1}H-NMR, ^{13}C-NMR and DEPT data of **2** (Table 1) were almost the same as those of **1**, indicating that their structures should be very similar. However, the positive ESI-MS and negative ESI-MS spectra

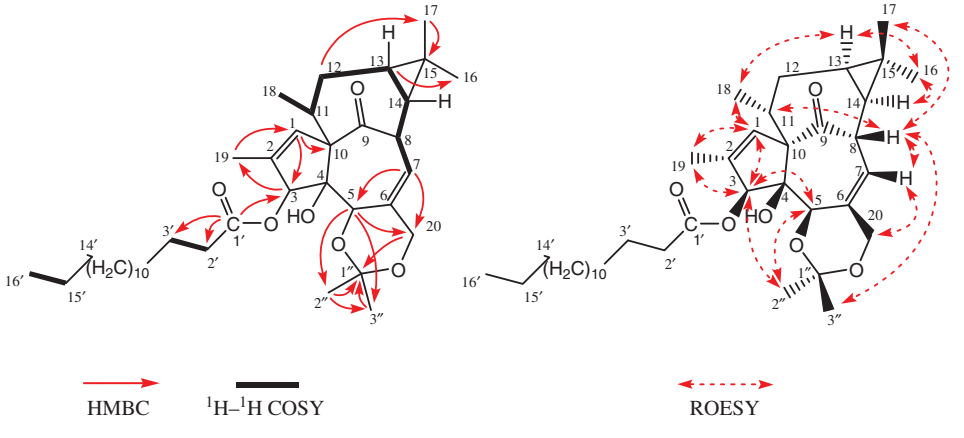


Figure 2. Key ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **1**.

Table 2. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data of compound **3** in $CDCl_3$.

Position	δ_C	δ_H [*J* (Hz)]	Position	δ_C	δ_H [*J* (Hz)]
1	119.8	6.13 (d, 2.4)	11	34.1	1.78 (m), 1.83 (m)
2	199.1		12	39.5	1.20 (m), 1.24 (m)
3	80.5	4.13 (s)	13	36.1	
4	46.9		14	32.3	1.35 (m), 1.38 (m)
5	44.0	2.69 (m)	15	150.3	5.79 (dd, 10.8, 17.6)
6	17.7	1.88 (m), 1.94 (m)	16	109.3	4.88 (m), 4.92 (m)
7	24.9	1.42 (m), 1.45 (m)	17	22.1	0.98 (s)
8	30.6	1.78 (m)	18	20.5	1.35 (s)
9	39.1		19	63.3	3.51 (d, 11.2)
					3.64 (d, 11.2)
10	177.3		20	19.1	1.04 (s)

of **2** showed ion peaks at *m/z* 621 $\{[M+Na]^+\}$ and *m/z* 597 $\{[M-H]^-\}$, respectively, and its molecular formula was determined to be $C_{37}H_{58}O_6$ by (+)-HR-ESI-MS (*m/z* 621.4120 $[M+Na]^+$, Calcd 621.4131), which is smaller than that of **1** by two CH_2 units. Moreover, further analysis using LC-MS spectra of **1** and **2** also showed their different retention times and molecular weights. Combining the above evidence, the difference between **1** and **2** should be a different fatty acid chain at C-3. Compared with **1**, the absence of two CH_2 units in **2** implied the presence of a myristic acid unit at C-3 of **2**, instead of the palmitic one in **1**. Thus, the structure of **2** was deduced to be ingenol-5β,20-*O*,*O*-isopropylidene-3β-myristinate.

Compound **3** was obtained as white solid powder with the molecular formula $C_{20}H_{30}O_3$, derived from its (+)-HR-ESI-MS at *m/z* 341.2094 $[M+Na]^+$ ($C_{20}H_{30}O_3Na^+$, Calcd 341.2092) and ^{13}C-NMR. The IR absorption bands at 3425 and 1673 cm^{-1} indicated the presence of hydroxyl and carbonyl groups. The ^{1}H- and ^{13}C-NMR data (Table 2) of **3** showed 20 carbon signals, including one carbonyl group (δ_C 199.1), one trisubstituted [δ_H 6.13 (1H, d, 2.4 Hz); δ_C 119.8 (d), 177.3 (s)] and one monosubstituted olefin [δ_H 5.79 (1H, dd, 10.8, 17.6 Hz), δ_H 4.88 (2H, m); δ_C 150.3 (d), 109.3 (t)], three tertiary methyls [δ_H 0.98, 1.35 and 1.04 (each CH_3, s); δ_C 22.1, 20.5 and 19.1], one-oxygened methylene [δ_H 3.51 (1H, d), 3.64 (1H, d); δ_C 63.3] and one oxygened methine [δ_H 4.13 (1H, s); δ_C 80.5].

Considering the structural characteristics of diterpenoids isolated from the genus *Euphorbia*, all of the spectral data of **3** implied that its structure was a rosane-type diterpenoid, similar to the known compound 18-hydroxyhugorosenone from *Hugonia casteneifolia* (Ladislaus, Reiner, Mayunga, Stephan, & Hans, 1998). Detailed analysis of the 2D-NMR (including HMQC, ^{1}H–^{1}H COSY and HMBC) of **3** suggested that **3** possessed the same planar structure as 18-hydroxyhugorosenone, but the differences of chemical shift values between **3** and 18-hydroxyhugorosenone in ring A (especially, C-3, C-4, C-5 and C-19) indicated that they should possess different stereochemistry, as shown in Figure 3.

The relative stereochemistry of **3** was then elucidated by ROESY spectrum, as shown in Figure 3. Correlations of H-3–H-5, H-5–H-8 and H-5–H_3-18 indicated that H-5, H-8 and H_3-18 were in α-orientation, while the oxygened methylene group

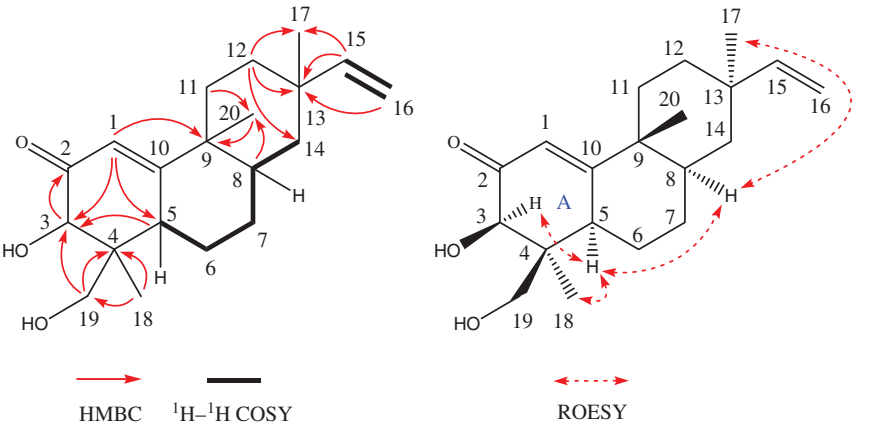


Figure 3. Key ^{1}H–^{1}H COSY, HMBC and ROESY correlations of **3**.

took β-configuration, which was opposite to 18-hydroxyhugorosenone. Thus, the structure of **3** was deduced to be 3β,19-dihydroxy-1(10),15-rosadien-2-one.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured on a Jasco-20C digital polarimeter. IR (KBr) spectra were recorded on a Bruker Tensor 27 FT-IR spectrophotometer. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. 1D- and 2D-NMR spectra were obtained on an INOVA-400 MHz NMR spectrometer in $CDCl_3$ using TMS as the internal standard. ESI-MS and LC-MS were carried out on a HP 1100MSD (ESI) apparatus. HR-ESI-MS was measured an API Qstar Pulsar 1 spectrometer. GC-MS was carried out on a HP 6890-5975C (EI) apparatus. Semi-preparative HPLC was performed on an Agilent 1100 apparatus. Column chromatography was performed on silica gel (200–300 mesh and H-60, Qingdao Marine Chemical Company, Qingdao, China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Sweden). Solvents used for extraction and isolation were distilled prior to use.

3.2. *Plant material*

The roots of *E. ebracteolata* were collected from Changchun, Jilin Province, China in February 2006. The sample was identified by Prof. L. Gao from the Natural Drug Resources Laboratory of the Yunnan Institute of Materia Medica.

3.3. *Extraction and isolation*

Air-dried and powdered roots of *E. ebracteolata* (20 kg) were extracted three times with 95% ethanol (3 × 30 L, each for 5 days) at room temperature and concentrated *in vacuo* to give a crude extract. The extract was then dissolved in H_2O and

partitioned successively with petroleum ether (PE) and $CHCl_3$. The PE fraction (735 g) was chromatographed on a silica gel column (200–300 mesh) eluted with a PE : acetone gradient (100 : 0–0 : 100) to obtain fractions 1–6. Fraction 2 (65 g) was submitted to a silica gel CC eluted with a PE : EtOAc gradient (1 : 0–0 : 1) to give four subfractions, 2.1–2.4. Fraction 2.2 (7 g) was submitted to repeated chromatography over silica gel H and preparative TLC to afford compound **3** (6 mg). Fraction 4 (18 g) was submitted to a silica gel CC eluted with a $CHCl_3$: acetone gradient (1 : 0–0 : 1) to give five subfractions, 4.1–4.5. Fraction 4.1 (8 g) was submitted to Sephadex LH-20 (MeOH) and semi-preparative HPLC using Kromail^{TM} 100-5-C18 ODS column (10 mm × 250 mm) with 95% MeOH at 2 mL min^{-1} under 30°C to afford compounds **1** (11 mg) and **2** (14 mg).

3.3.1. *Ingenol-5β,20-O,O-isopropylidene-3β-palmitate (**1**)*

Yellow oil. $[\alpha]_D^{24} = +8.97$ (*c* 0.13, $CHCl_3$); UV λ_{max} ($CHCl_3$): 239 nm; IR ν_{max} (KBr): 3525, 3435, 2954, 2924, 1728, 1462, 1380, 1161 cm^{-1}; for ^{1}H-NMR and ^{13}C-NMR data, see Table 1; positive ESI-MS *m/z*: 649 $[M + Na]^+$; negative ESIMS *m/z*: 625 $[M - H]^-$; (+)-HR-ESI-MS *m/z* 649.4455 $[M + Na]^+$ ($C_{39}H_{62}O_6$ Na^+, Calcd 649.4444).

3.3.2. *Ingenol-5β,20-O,O-isopropylidene-3β-myristinate (**2**)*

Yellow oil. $[\alpha]_D^{16} = 0.00$ (*c* 0.47, $CHCl_3$); UV λ_{max} ($CHCl_3$): 239 nm; IR ν_{max} (KBr): 3428, 3068, 2924, 1726, 1463, 1380, 1079 cm^{-1}; for ^{1}H-NMR and ^{13}C-NMR data, see Table 1; positive ESIMS *m/z*: 621 $[M + Na]^+$; negative ESI-MS *m/z*: 597 $[M - H]^-$; (+)-HR-ESI-MS *m/z* 621.4120 $[M + Na]^+$ ($C_{37}H_{58}O_6$ Na^+, Calcd 621.4131).

3.3.3. *3β,19-dihydroxy-1(10),15-rosadien-2-one (**3**)*

White solid powder. $[\alpha]_D^{24} = +64.71$ (*c* 0.17, $CHCl_3$); UV λ_{max} ($CHCl_3$): 252 nm; IR ν_{max} (KBr): 3425, 2918, 2850, 1673, 1467, 1379, 1102, 1050 cm^{-1}; for ^{1}H-NMR and ^{13}C-NMR data, see Table 1; (+) ESI-MS *m/z*: 341 $[M + Na]^+$; negative ESI-MS *m/z*: 317 $[M - H]^-$; (+)-HR-ESI-MS *m/z* 341.2094 $[M + Na]^+$ ($C_{20}H_{30}O_3Na^+$, Calcd 341.2092).

3.4. *Basic hydrolysis and GC-MS analysis of 1*

Compound **1** (3 mg) was dissolved in 5 mL MeOH, and then 0.025 g NaOH was added. The mixture was stirred at room temperature for 3 h. After acidification with 5% HCl, the mixture was extracted with $CHCl_3$. The extraction of $CHCl_3$ was washed by H_2O, dried by Na_2SO_4 and evaporated under reduced pressure. The crude residue was subjected to a silica gel CC to obtain an ingenol derivative and fatty acid. Then the fatty acid was analysed by GC-MS.

3.5. *LC-MS analysis of the mixture of 1 and 2*

Compounds **1** (2 mg) and **2** (2 mg) were mixed together, and then analysed by an HP 1100MSD (ESI) using a Kromail^{TM} 100-5-C18 ODS column (4.6 mm × 250 mm) with 100% MeOH at 1 mL min^{-1} under 30°C.

Acknowledgements

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References

Deng, B., Mu, S.Z., Huang, L.J., Song, Z.Q., & Hao, X.J. (2009). Studies on diterpenoids from the roots of *Euphorbia ebracteolata* Hayata. *China Journal of Chinese Materia Medica, 34*(6), 789–791.

Fu, G.M., Yu, B.Y., & Zhu, D.N. (2006). Two novel phloroglucinol derivatives from *Euphorbia ebracteolata* Hayata. *Journal of Asian Natural Products Research, 8*(1–2), 149–153.

Ladislaus, K.M., Reiner, W., Mayunga, H.H.N., Stephan, A.J., & Hans, A. (1998). Rosane diterpenes and *bis*-dinorditerpenes from *Hugonia casteneifolia*. *Phytochemistry, 49*(4), 1107–1113.

Li, Y.L., & Suo, Y.R. (2005). Ingenane diterpene ester constituents from Tibetan medicine *Euphorbia wallichii*. *Chinese Traditional and Herbal Drugs, 36*(12), 1763–1767.

Xie, Z.W. (1996). *National herbal medicine collection* (2nd ed., pp. 305–306). Beijing: People's Medical Publishing House.

Yan, B.Q., & Zhao, Y.Q. (2008). Progress on *Euphorbia ebracteolata* Hayata. *Journal of Shandong University of Traditional Chinese Medicine, 32*(5), 234–237.