Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 69 (2008) 812–819
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Ingenane diterpenoids from *Euphorbia esula*

Zhi-Qiang Lu, Min Yang, Jin-Qiang Zhang, Guang-Tong Chen, Hui-Lian Huang, Shu-Hong Guan, Chao Ma, Xuan Liu, De-An Guo *

Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Guo Shoujing Road 199, Zhangjiang, Shanghai 201203, PR China

Received 15 January 2007; received in revised form 11 June 2007
Available online 31 October 2007

Abstract

An extensive study of metabolites present in *Euphorbia esula* led to isolation of 16 ingenane diterpenoids **1**–**16** together with the known ingenane derivative **17** and four known cycloartane triterpenoids. Their structures were elucidated on the basis of spectroscopic studies and comparison with known related compounds. All the compounds were assayed for their inhibitory activity against human HeLa cervical cancer cell line.

Keywords: *Euphorbia esula*; Euphorbiaceae; Diterpenoids; Ingenane

1. Introduction

The genus *Euphorbia*, belonging to the Euphorbiaceae family, comprises about 2000 species ranging from annuals to trees (Shi and Jia, 1997a). About 80 species are distributed in China, many of which have series of applications in either traditional Chinese medicine (TCM) or folklore herbs (Shi et al., 1998). To our knowledge, over 400 diterpenoids, incorporating more than 23 skeletal types, have been isolated from *Euphorbia* plants, and some showed biological activities such as skin irritating, anti-tumor and tumor promoting activities (Amir, 2006; Singla and Kamala, 1990).

Euphorbia esula L. or leafy spurge (Euphorbiaceae) is distributed worldwide and contains a skin-irritant, toxic, milky latex. Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings and warts (Hohmann et al., 1997). Previous phytochemical investigations on this plant yielded a number of cytotoxic diterpenoid ingenol esters (Halaweish et al., 2002, 2003), jatrophane diterpenoids (Günther et al., 1999; Hohmann et al., 1997; Liu et al., 2002) and lathyrane diterpenoid esters (Onwukaeme and Rowan, 1992). In the course of our search for bioactive metabolites from plants, we became interested in the *Euphorbia* plants due to their folk use to treat cancers and began to study their chemical constituents to clarify their anticancer principles. As a part of this program, we systematically investigated the chemical constituents of the petroleum-soluble extract of *E. esula*, which resulted in the isolation of 16 new ingenane diterpenoids (**1**–**16**) together with five known compounds. Herein, we describe the structure elucidation and cytotoxic assay of these new compounds.

2. Results and discussion

The dried and powdered whole plant of *E. esula* was extracted with 95% EtOH at room temperature. The combined extracts were concentrated to dryness to afford a crude extract which was suspended in water and partitioned with petroleum ether and EtOAc. The petroleum ether layer after evaporation of the solvent was fractionated by repeated silica gel and Sephadex LH-20 column chromatography and finally by semipreparative HPLC to

* Corresponding author. Tel.: +86 21 50271516; fax: +86 21 50272789.
E-mail address: gda5958@163.com (D.-A. Guo).

0031-9422/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2007.09.013

give 16 new diterpenoids **1**–**16**, as well as the known ingenane derivative **17** and four known triterpenoids. The structures of the known compounds were identified by spectroscopic data measurement and by comparing the data obtained with published values, i.e. as 3-*O*-benzoyl-13,17-dibenzoyloxy-20-deoxyingenol (**17**) (Gotta et al., 1984), 25-hydroperoxycycloart-23E-en-3β-ol (Cabrera et al., 1996), cycloart-23E-en-3β, 25-diol (Shi and Jia, 1997b), cycloart-23Z-en-3β, 25-diol (Greca et al., 1994) and 23-methylenecycloart-3β, 24-diol (Ferreira et al., 2001).

Compound **1** was obtained as a colorless gum and its molecular formula, $C_{33}H_{42}O_7$, was deduced from the pseudomolecular ion peak at *m/z* 573.2863 (calcd. for $C_{33}H_{42}O_7Na$, 573.2828) in the HRESIMS, indicating the presence of thirteen degrees of unsaturation. The characteristic features of the ^1H and ^{13}C NMR spectra suggested that **1** was an ingenane diterpenoid derivative (Mbwambo et al., 1996). Further analysis of 2D NMR spectroscopic data allowed us to determine the gross structure of the diterpenoid part. Besides the NMR signals of the diterpenoid unit, the resonances of 2,3-dimethylbutanoyl and benzoyl moieties were found in the ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2). The location of the 2,3-dimethylbutanoyl and benzoyl units were deduced to be at C-3 and C-17, respectively, through ester linkage by the HMBC correlation peaks of H-3 with the carbon signal at δ_C 177.4 (2, 3-dimethylbutanoyl carbonyl), and H_2-17 with the carbon resonance at δ_C 167.0 (benzoyl carbonyl). The occurrence of esters of 17-hydroxyingenol, as opposed to 16-hydroxyingenol, had been established previously by a combination of 1D- and 2D-dimensional NMR methods (Jakupovic et al., 1998) and had been confirmed by NOE measurements. The stereochemistry of the ingenane diterpenoids had been confirmed by the single X-ray crystallography of ingenol-3, 5, 20-triacetate (Zechmeister et al., 1970). In conclusion, the structure of **1** was established as 17-benzoyloxy-3-*O*-(2,3-dimethylbutanoyl)-20-deoxyingenol.

Compounds **2**–**16** were analogues of 17-benzoyloxy-3-*O*-(2,3-dimethylbutanoyl)-20-deoxyingenol (**1**). Complete NMR spectroscopic studies on each new compound were performed in order to unambiguously determine the structures of the isolated compounds and to assign all the proton and carbon resonances. 1D and 2D NMR spectra showed that compounds **2**–**16** contained the same ingenane skeleton and similar substituent groups. Careful analysis of the ^1H and ^{13}C NMR spectroscopic data, and interpretation of the HMBC spectrum also allowed us to locate the substituent groups. Their stereostructures were determined by comparison of their spectroscopic data with those of **1** and some known ingenane diterpenoids. Some key points for structure determination of compounds **2**–**16** were described below.

Compound **2**, a colorless gum, showed a molecular formula of $C_{39}H_{52}O_9$ as determined by the HRESIMS. Overall spectroscopic analysis indicated that the structure of **2** was closely related to that of **1**. The only difference was the presence of one more 2,3-dimethylbutanoyl ester moiety substituent in **2**, on the basis of MS and NMR spectroscopic data (Tables 1 and 2). Inspection of the ^{13}C NMR data of **2** indicated that carbon signals of C-12 (δ_C 35.6), C-13 (δ_C 68.3) and C-14 (δ_C 28.7) were shifted to a more downfield position, when compared to the chemical shifts of C-12 (δ_C 30.5), C-13 (δ_C 23.8) and C-14 (δ_C 23.4) in **1**. This was consistent with analogous changes in the ^1H NMR signals of H_2-12 (δ_H 2.34 and 2.80), H-13 (disappeared) and H-14 (δ_H 1.42, $J = 12.0$ Hz, H-14), similar to those in the spectra of 13-hydroxyingenol derivatives (Wang et al., 2003). These observations implied that the additional 2,3-dimethylbutanoyl group was attached to the tertiary OH at C-13. Therefore, compound **2** was elucidated as 17-benzoyloxy-3-*O*-(2,3-dimethylbutanoyl)-13-(2,3-dimethylbutanoyloxy)-20-deoxyingenol.

Compounds **3** and **4** gave the same molecular formula of $C_{39}H_{52}O_{10}$ as determined by HRESIMS, which were 16 mass units higher than that of compound **2**. Both compounds displayed similar NMR spectroscopic features and the same fragmentation pattern in the EIMS. These similarities suggested that **3** and **4** were isomers. Detailed analysis of the NMR spectra of **2** and **3** indicated that these two compounds were similar except for the presence of a hydroxyl group at C-20 in **3**. Thus, the structure of compound **3** was established to be 17-benzoyloxy-3-*O*-(2, 3-dimethylbutanoyl)-13-(2, 3-dimethylbutanoyloxy)ingenol. Comparing the ^1H NMR spectrum of **4** with that of **3**, it was found that the chemical shifts of H-3 and H_2-20 were drastically shifted upfield by 1.09 ppm and downfield by about 0.5 ppm, respectively. These observations implied that one of 2,3-dimethylbutanoyl groups was located at C-20 through an ester linkage rather than at C-3, which was supported from the HMBC correlation observed between H_2-20 (δ_H 4.41 and 4.63) and carbon signal at δ_C 176.3. Compound **4** was thus elucidated as 17-benzoyloxy-20-*O*-(2,3-dimethylbutanoyl)-13-(2,3-dimethylbutanoyloxy)ingenol.

Compound **5** showed a molecular formula of $C_{35}H_{46}O_9$ as assigned by HRESIMS. The ^1H NMR spectrum of **5** had a broad singlet at δ 1.27 indicative of the presence of a long-chain fatty acid moiety. The EIMS suggested the presence of benzoyl (*m/z* 105, $C_7H_5O^+$) and octanoyloxy (*m/z* 466, $M^+-C_7H_{15}COOH$) units in the structure. Detailed analysis of the NMR spectroscopic data of **5** (Tables 1 and 2) and HMBC spectra suggested that these two ester moieties were located at C-17 and C-13 in **5**, respectively. Thus compound **5** was identified as 17-benzoyloxy-13-octanoyloxyingenol.

Compound **6** was isolated as a colorless gum and exhibited a quasimolecular ion peak at *m/z* 737.3280 $[M + Na]^+$ in the HRESIMS, appropriate for a molecular formula of $C_{42}H_{50}O_{10}$. The EIMS exhibited a base peak at *m/z* 105 ($C_7H_5O^+$) and four significant fragment peaks at *m/z* 592 (M^+-C_6H_5COOH), 570 ($M^+-C_7H_{15}COOH$), 470 ($M^+-2\times C_6H_5COOH$) and 326 ($M^+-C_7H_{15}COOH$-$2\times C_6H_5$-COOH). These data suggested the presence of one octa-

Table 1
^{1}H NMR spectroscopic data for compounds **1–16** [$CDCl_3$, δ (ppm) and mult][a,b,c]

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	5.99 (*d*)	6.02 (*d*)	5.96 (*d*)	5.88 (*d*)	5.89 (*d*)	6.10 (*s*)	5.90 (*d*)	6.13 (*d*)	5.97 (*d*)	5.90 (*d*)	6.12 (*d*)	6.04 (*d*)	6.09 (*s*)	6.07 (*d*)	6.10 (*d*)	6.01 (*s*)
3	5.46 (*s*)	5.40 (*s*)	5.48 (*s*)	4.39 (*s*)	4.42 (*s*)	5.79 (*s*)	4.45 (*s*)	5.69 (*s*)	5.48 (*s*)	4.41 (*s*)	5.84 (*s*)	5.51 (*s*)	5.78 (*s*)	5.41 (*s*)	5.74 (*s*)	5.44 (*s*)
5	3.60 (*s*)	3.65 (*s*)	3.95 (*s*)	3.67 (*s*)	3.82 (*s*)	4.09 (*s*)	3.74 (*s*)	3.74 (*d*)	3.98 (*s*)	3.67 (*s*)	4.14 (*s*)	4.04 (*s*)	4.09 (*s*)	3.69 (*s*)	4.14 (*s*)	4.05 (*s*)
7	5.66 (*d*)	5.64 (*d*)	5.89 (*d*)	6.05 (*d*)	5.96 (*d*)	5.94 (*d*)	6.08 (*d*)	5.68 (*d*)	5.90 (*d*)	6.05 (*d*)	6.00 (*d*)	5.98 (*d*)	5.93 (*d*)	5.71 (*d*)	6.04 (*d*)	6.02 (*s*)
8	4.25 (*m*)	4.13 (*dd*)	4.24 (*dd*)	4.21 (*dd*)	4.24 (*dd*)	4.29 (*dd*)	4.26 (*dd*)	4.16 (*dd*)	4.26 (*dd*)	4.23 (*dd*)	4.42 (*dd*)	4.36 (*dd*)	4.26 (*dd*)	4.23 (*dd*)	4.10 (*dd*)	4.09 (*dd*)
11	2.44 (*m*)	2.55 (*m*)	2.61 (*m*)	2.48 (*m*)	2.49 (*m*)	2.76 (*m*)	2.49 (*m*)	2.67 (*m*)	2.70 (*m*)	2.46 (*m*)	2.79 (*m*)	2.66 (*m*)	2.77 (*m*)	2.58 (*m*)[c]	2.71 (*m*)	2.60 (*m*)
12a	2.38 (*m*)	2.80 (*dd*)	2.77 (*dd*)	2.82 (*dd*)	2.81 (*dd*)	2.84 (*dd*)	2.82 (*dd*)	2.82 (*dd*)	2.75 (*dd*)	2.80 (*dd*)	2.95 (*dd*)	2.94 (*dd*)	2.86 (*dd*)	2.92 (*dd*)	2.74 (*dd*)	2.71 (*dd*)
12b	1.85 (*m*)	2.34 (*dd*)	2.32 (*dd*)	2.32 (*dd*)	2.36 (*dd*)	2.42 (*dd*)	2.36 (*dd*)	2.44 (*dd*)	2.36 (*dd*)	2.37 (*dd*)	2.61 (*dd*)	2.57 (*dd*)	2.32 (*dd*)	2.58 (*m*)[c]	2.23 (*dd*)	2.21 (*dd*)
13	0.91 (*m*)															
14	1.12 (*m*)	1.42 (*d*)	1.41 (*d*)	1.49 (*d*)	1.46 (*d*)	1.45 (*d*)	1.48 (*d*)	1.32 (*d*)	1.40 (*d*)	1.48 (*d*)	1.62 (*d*)	1.61 (*d*)	1.46 (*d*)	1.60 (*d*)	1.23 (*d*)	1.22 (*d*)
16	1.17 (*s*)	1.20 (*s*)	1.19 (*s*)	1.25 (*s*)	1.26 (*s*)	1.24 (*s*)	1.25 (*s*)	1.24 (*s*)	1.11 (*s*)	1.25 (*s*)	1.28 (*s*)	1.29 (*s*)	1.24 (*s*)	1.28 (*s*)	1.04 (*s*)	1.05 (*s*)
17a	4.50 (*d*)	4.46 (*d*)	4.52 (*d*)	4.55 (*d*)	4.50 (*d*)	4.62 (*d*)	4.58 (*d*)	4.56 (*d*)	4.51 (*d*)	4.54 (*d*)	4.65 (*d*)	4.65 (*d*)	4.62 (*d*)	4.62 (*d*)	1.15 (*s*)	1.18 (*s*)
17b	4.35 (*d*)	4.42 (*d*)	4.37 (*d*)	4.48 (*d*)	4.46 (*d*)	4.33 (*d*)	4.45 (*d*)	4.35 (*d*)	4.45 (*d*)	4.46 (*d*)	4.39 (*d*)	4.46 (*d*)	4.34 (*d*)	4.49 (*d*)		
18	0.95 (*d*)	0.99 (*d*)	0.97 (*d*)	1.00 (*d*)	0.98 (*d*)	1.08 (*d*)	1.00 (*d*)	1.08 (*d*)	0.96 (*d*)	0.99 (*d*)	1.08 (*d*)	1.00 (*d*)	1.08 (*d*)	1.01 (*d*)	1.05 (*d*)	0.96 (*d*)
19	1.71 (*d*)	1.74 (*d*)	1.72 (*d*)	1.84 (*d*)	1.84 (*d*)	1.85 (*s*)	1.84 (*d*)	1.84 (*d*)	1.73 (*d*)	1.84 (*d*)	1.83 (*d*)	1.78 (*d*)	1.84 (*s*)	1.78 (*d*)	1.85 (*d*)	1.77 (*s*)
20	1.65 (*s*)	1.64 (*s*)	3.96	4.63 (*d*)	4.04	4.02	4.82 (*d*)	1.70 (*s*)	3.98	4.65 (*d*)	4.07	4.04	4.02	1.70 (*s*)	4.19	4.14
			(br, *s*)	4.41 (*d*)	(br, *s*)	(br, *s*)	4.72 (*d*)		(br, *s*)	4.43 (*d*)	(br, *s*)	(br, *s*)	(br, *s*)		(br, *s*)	(br, *s*)
3-OR																
2′	2.26 (*m*)	2.28 (*m*)	2.25 (*m*)			8.02 (*dd*)		8.01 (*dd*)	2.24 (*m*)		8.01 (*dd*)	2.30 (*m*)	8.01 (*dd*)	2.33 (*m*)	8.02 (*dd*)	2.31 (*m*)
3′	1.85 (*m*)	1.87 (*m*)	1.85 (*m*)			7.46 (*m*)[c]		7.46 (*t*)	1.85 (*m*)		7.44 (*m*)[c]	1.90 (*m*)	7.46 (*m*)[c]	1.91 (*m*)	7.48 (*t*)	1.91 (*m*)
4′	0.88 (*d*)	0.90 (*d*)	0.88 (*d*)			7.56 (*m*)[c]		7.59 (*t*)	0.88 (*d*)		7.56 (*m*)[c]	0.93 (*d*)	7.57 (*m*)[c]	0.94 (*d*)	7.61 (*t*)	0.95 (*d*)
5′	1.07 (*d*)	1.08 (*d*)	1.07 (*d*)						1.07 (*d*)			1.13 (*d*)		1.14 (*d*)		1.14 (*d*)
6′	0.85 (*d*)	0.87 (*d*)	0.84 (*d*)						0.84 (*d*)			0.89 (*d*)		0.90 (*d*)		0.92 (*d*)
13-OR																
2′		2.17 m)	2.15 (*m*)	2.19 (*m*)	2.44 (*t*)	2.26 (*t*)	2.25 (*t*)	2.26 (*t*)	2.23 (*t*)	2.24 (*t*)	8.02 (*dd*)	8.01 (*dd*)	2.20 (*m*)	8.02 (*d*)	2.19 (*t*)	2.20 (*t*)
3′		1.95 (*m*)	1.92 (*m*)	1.97 (*m*)	1.59 (*m*)	1.59 (*m*)	1.59 (*m*)	1.58 (*m*)	1.56 (*m*)	1.60 (*m*)	7.44 (*m*)[c]	7.44 (*dd*)	1.98 (*m*)	7.48 (*m*)[c]	1.56 (*m*)	1.55 (*m*)
4′		0.92 (*d*)	0.90 (*d*)	0.93 (*d*)	1.27 (*m*)	1.29 (*m*)	1.27 (*m*)	1.28 (*m*)	1.26 (*m*)	1.27 (*m*)	7.56 (*m*)[c]	7.58 (*m*)[c]	0.96 (*d*)	7.58 (*m*)[c]	1.26 (*m*)	1.25 (*m*)
5′		1.04 (*d*)	1.02 (*d*)	1.06 (*d*)	1.27 (*m*)	1.29 (*m*)	1.27 (*m*)	1.28 (*m*)	1.26 (*m*)	1.27 (*m*)			1.07 (*d*)		1.26 (*m*)	1.25 (*m*)
6′		0.85 (*d*)	0.85 (*d*)	0.88 (*d*)	1.27 (*m*)	1.29 (*m*)	1.27 (*m*)	1.28 (*m*)	1.26 (*m*)	1.27 (*m*)			0.89 (*d*)		1.26 (*m*)	1.25 (*m*)
7′					1.27 (*m*)	1.29 (*m*)	1.27 (*m*)	1.28 (*m*)	1.26 (*m*)	1.27 (*m*)					1.26 (*m*)	1.25 (*m*)
8′					0.86 (*t*)	0.88 (*t*)	0.88 (*t*)	0.88 (*t*)	0.86 (*t*)	0.88 (*t*)					0.87 (*t*)	0.86 (*t*)
17-OBz																
2′, 6′	7.99 (*dd*)	8.07 (*dd*)	8.05 (*dd*)	8.10 (*dd*)	8.12 (*dd*)	8.08 (*dd*)	8.06 (*dd*)	8.09 (*dd*)	8.06 (*dd*)	8.11 (*dd*)	8.11 (*dd*)	8.14 (*dd*)	8.07 (*dd*)	8.16 (*dd*)		
3′, 5′	7.40 (*t*)	7.42 (*t*)	7.41 (*t*)	7.46 (*t*)	7.47 (*t*)	7.46 (*m*)[c]	7.40 (*m*)	7.44 (*t*)	7.42 (*t*)	7.46 (*t*)	7.44 (*m*)[c]	7.49 (*t*)	7.46 (*m*)[c]	7.48 (*m*)[c]		
4′	7.51 (*t*)	7.53 (*t*)	7.52 (*t*)	7.57 (*t*)	7.58 (*t*)	7.56 (*m*)[c]	7.49 (*t*)[c]	7.55 (*t*)	7.53 (*t*)	7.57 (*t*)	7.56 (*m*)[c]	7.58 (*m*)[b]	7.57 (*m*)[c]	7.58 (*m*)[c]		

[a] J_{H-H} (values in Hz) for **1–17**: 1–19 = 1.2; 7–8 = 3.6; 8–14 = 12.0, 11–12a = 3.2; 11–12b = 5.2; 12a–12b = 16.8; 17a–17b = 12.0; 18–11 = 7.2. Bz: 2–3 = 7.6, 2–4 = 1.2, 3–4 = 7.6; Octanoyloxy: 2–3 = 7.2, 7–8 = 6.8; 2,3-dimethylbutanoyl: 2–5 = 3–4 = 3–6 = 6.8.

[b] Other signals. For **4**: 20-2,3-dimethylbutanoyl 2.19 (*m*), 1.83 (*m*), 0.85 (*d*), 1.03 (*d*), 0.84 (*d*). For **7**: 20-benzoyl 7.94 (*dd*), 7.40 (*t*), 7.54 (*t*). For **10**: 20-2,3-dimethylbutanoyl: 2.18 (*m*), 1.83 (*m*), 0.85 (*d*), 1.04 (*d*), 0.84 (*d*).

[c] Overlapped signals.

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**16**[a,b]

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	132.1	131.5	131.0	128.6	128.6	131.5	128.4	132.0	131.1	128.8	131.5	131.2	131.4	131.8	131.8	131.5
2	136.2	136.3	136.8	139.7	139.8	136.8	139.7	136.3	136.7	139.6	136.8	136.8	136.8	136.4	136.3	136.2
3	82.0	82.7	82.1	80.4	80.2	83.0	80.1	83.6	82.0	80.4	83.0	82.2	83.0	82.9	83.3	82.6
4	85.2	84.9	84.8	84.3	84.3	84.9	84.3	85.1	84.8	84.3	85.1	84.9	84.9	85.0	84.6	84.5
5	76.7	77.0	76.0	73.8	75.2	77.2	74.0	77.2	76.0	73.8	76.3	76.5	77.3	77.4	76.9	76.9
6	138.4	138.2	140.2	137.7	141.2	139.8	137.5	138.2	140.1	137.8	140.2	140.1	139.8	138.3	139.4	139.6
7	122.8	122.0	125.6	126.8	125.2	126.4	125.5	122.3	125.7	126.7	126.0	126.1	126.4	122.2	127.6	127.3
8	42.8	42.6	42.7	43.2	43.3	42.9	43.3	42.8	42.6	43.3	43.0	43.0	42.9	42.9	42.7	42.7
9	206.7	205.1	205.2	204.9	205.4	204.9	205.3	205.1	205.1	204.8	205.1	204.9	205.0	205.0	205.8	205.8
10	71.5	71.9	71.9	72.9	72.7	72.0	72.8	72.1	71.7	72.7	72.0	71.9	72.2	72.0	72.0	71.9
11	38.5	38.1	37.8	38.8	38.8	38.1	38.8	38.5	37.8	38.9	38.2	38.0	38.0	38.4	37.6	37.5
12	30.5	35.6	35.6	35.9	35.4	35.4	35.6	35.4	35.0	35.4	35.3	35.3	35.9	35.3	35.2	34.4
13	23.8	68.3	68.3	68.1	68.4	68.6	68.3	68.6	68.5	68.3	69.3	69.3	69.4	69.3	69.0	69.0
14	23.4	28.7	28.5	28.7	28.7	28.6	28.6	28.8	28.4	28.7	28.6	28.6	28.6	29.0	28.3	28.3
15	27.5	33.9	34.0	33.8	33.9	34.1	33.9	34.1	34.0	33.9	34.4	34.5	34.0	34.4	30.3	30.3
16	24.2	18.7	18.5	18.8	18.7	18.7	18.6	18.7	18.5	18.7	18.6	18.7	18.9	18.7	22.5	22.5
17	66.2	65.5	65.5	65.4	65.7	65.5	65.5	65.6	65.5	65.5	65.6	65.6	65.5	65.7	16.7	16.7
18	16.4	18.0	18.1	18.5	18.2	18.1	18.2	18.1	17.9	18.2	18.1	18.0	18.4	17.9	18.4	18.2
19	15.3	15.5	15.4	15.2	15.4	15.6	15.3	15.6	15.5	15.3	15.6	15.6	15.6	15.6	15.6	15.4
20	21.6	21.7	66.6	65.7	66.6	67.1	66.4	21.8	66.7	65.7	66.9	67.0	67.1	21.8	67.4	67.2
3-OR	177.4	177.2	177.2			130.2[b]		129.5	177.3		130.1[b]	177.3	129.7	177.2	129.6	177.6
2′	46.0	46.2	46.3			129.8		129.8	46.3		129.7[b]	46.4	129.8	46.3	129.8	46.5
3′	30.7	30.9	30.8			128.6[b]		128.6	30.9		128.4[b]	31.0	128.5[b]	31.1	128.6	31.0
4′	20.3	20.6	20.6			133.4		133.6	20.6		133.3	20.7	133.4	20.6	133.5	20.7
5′	13.6	13.8	13.9			128.6[b]		128.6	13.9		128.4[b]	14.0	128.5[b]	13.9	128.6	14.06
6′	18.8	19.0	19.1			129.8		129.8	19.0		129.7[b]	19.1	129.8	19.1	129.8	19.2
7′						167.2		167.2			167.2		167.2		167.4	
13-OR		176.2	176.2	176.3	173.5	173.6	173.6	173.6	173.5	173.5	129.96[b]	129.98	176.3	130.3[b]	174.0	174.0
2′		45.8	45.8	45.8	35.4	34.5	34.4	34.5	34.4	34.5	129.8[b]	129.7	45.9	129.7	34.4	34.4
3′		29.9	30.0	30.1	24.8	24.8	24.8	24.8	24.7	24.8	128.4[b]	128.4	30.1	128.4	24.8	24.8
4′		21.2	21.1	21.2	29.2	29.2	29.1	29.2	29.1	29.2	133.2	133.2[b]	21.3	133.2	29.2	29.2
5′		13.2	13.3	13.3	28.9	28.9	28.9	28.9	28.8	28.9	128.4[b]	128.4	13.4	128.4	28.9	28.9
6′		18.5	18.7	18.6	31.7	31.7	31.6	31.6	31.6	31.6	129.8[b]	129.7	18.6	129.7	31.6	31.6
7′					22.6	22.6	22.6	22.6	22.5	22.6	166.2	166.2		166.2	22.5	22.6
8′					14.0	14.0	14.0	14.0	14.0	14.0					14.0	14.0
17-OBz	130.3	130.1	130.1	133.2	130.1	129.7[b]	129.88	130.2	130.0	130.1	129.8[b]	130.2	130.2	130.1[b]		
2′, 6′	129.5	129.6	129.7	129.8	129.8	129.7	129.6	129.76	129.7	129.8	129.8[b]	129.8	129.74	129.8		
3′, 5′	128.3	128.3	128.4	128.4	128.5	128.5[b]	128.3	128.4	128.4	128.5	128.5[b]	128.5	128.6[b]	128.4		
4′	133.0	132.8	133.0	133.0	133.2	133.1	133.0	132.9	133.0	133.1	133.1	133.1[b]	133.1	133.0		
7′	167.0	166.6	166.6	166.8	166.9	166.5	166.8	166.7	166.6	166.8	166.6	166.6	166.5	166.8		

[a] Other signals for **4**: 20-2,3-dimethylbutanoyl 176.2, 46.1, 30.9, 20.6, 13.6, 19.1. For **7** 20-benzoyl 129.95, 129.7, 128.4, 133.0, 166.4. For **10** 20-2,3-dimethylbutanoyl 176.3, 46.1, 31.0, 20.6, 13.6, 19.1.
[b] Assignments within a column may be interchangeable.

noyloxy and two benzoyl units in **6**. Comparison of NMR spectroscopic data of **6** with those of **5** (Tables 1 and 2) led to the deduction that the only difference was the presence of a benzoyl group at C-3 in **6**. This was further supported by the HMBC correlation of H-3 (δ_H 5.79) with benzoyl carbonyl (δ_C 167.2). Therefore, the structure of compound **6** was established as 3-*O*-benzoyl-17-benzoyloxy-13-octanoyloxyingenol.

Compound **7** was found by HRESIMS to possess the same molecular formula of $C_{42}H_{50}O_{10}$ and the same fragmentation pattern in the EIMS as **6**. It was found that the NMR spectroscopic data of **7** were very similar to those of **6** (Tables 1 and 2), suggesting that **7** might be an isomer of **6**. However, the significant downfield shift at H-20 ($\Delta\delta_H$ +0.75 ppm) and the upfield shift at H-3 ($\Delta\delta_H$ −1.34 ppm) relative to those of **6** (Table 1 and 2) suggested that one of the benzoyloxy units was located at C-20 in **7** rather than at C-3. The HMBC correlation of H_2-20 (δ_H 4.72 and 4.82) with benzoyl carbonyl (δ_C 166.4) also corroborated this inference. Thus, compound **7** was identified as 20-*O*-benzoyl-17-benzoyloxy-13-octanoyloxyingenol.

Compound **8** exhibited a molecular formula of $C_{42}H_{50}O_9$ as established by HRESIMS, which was 16 mass units less than that of compound **6**. Detailed analysis of the NMR spectra of **6** and **8** made it clear that these two compounds were very similar except for the absence of the hydroxyl group at C-20 in **8**. Therefore, the structure of compound **8** was established as 3-*O*-benzoyl-17-benzoyloxy-13-octanoyloxy-20-deoxyingenol.

Compounds **9** and **10** gave the same molecular formula of $C_{41}H_{56}O_{10}$ as determined by HRESIMS. Both compounds displayed similar NMR spectroscopic features

and the same EIMS fragmentation patterns. The EIMS of **9** and **10** suggested the presence of benzoyl (*m/z* 105, $C_7H_5O^+$), 2,3-dimethylbutanoyloxy (*m/z* 592, $M^+-C_5H_{11}$-COOH) and octanoyloxy (*m/z* 564 $M^+-C_7H_{15}COOH$) units in the structures. The position of each ester group was deduced by careful analysis of ^1H and ^{13}C NMR data and the HMBC spectra. Hence, compound **9** was assigned as 17-benzoyloxy-3-*O*-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol. Similarly, compound **10** was elucidated to be 17-benzoyloxy-20-*O*-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol.

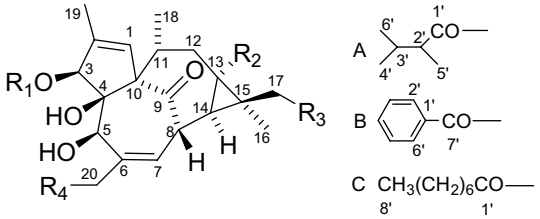
Compound **11** exhibited a molecular formula of $C_{41}H_{40}O_{10}$ as established by HRESIMS, which was 16 mass units higher than that of compound **17**. The NMR spectroscopic data (Tables 1 and 2) of **11** was identical to those of **17**, except for the presence of hydroxyl group at C-20 in **11**. The structure of **11** was thus established as 3-*O*-benzoyl-13,17-dibenzoyloxyingenol and the assignments of the proton and carbon signals were also confirmed by HSQC, HMBC and ^1H–^1H COSY spectra.

Compounds **12** and **13** displayed the same molecular formula of $C_{40}H_{46}O_{10}$ as determined by HRESIMS, similar NMR spectroscopic features and the same EIMS fragmentation patterns. The EIMS of **12** and **13** suggested the presence of two benzoyls (*m/z* 442, $M^+-2\times C_6H_5COOH$) and one 2,3-dimethylbutanoyloxy (*m/z* 592, $M^+-C_5H_{11}$-COOH) units in the structures. Precise locations of each ester group were deduced from further analysis of their HMBC spectra. Therefore, compounds **12** and **13** were identified as 13,17-dibenzoyloxy-3-*O*-(2,3-dimethylbutanoyl)ingenol and 3-*O*-benzoyl-17-benzoyloxy-13-(2,3-dimethylbutanoyloxy)ingenol, respectively.

Compound **14** exhibited a molecular formula of $C_{40}H_{46}O_9$ as determined by HRESIMS, which was 16 mass units less than that of **12**. From the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2), it was evident that compounds **14** and **12** were very similar and the only difference was the absence of hydroxyl group at C-20 in **14**. Thus the structure of compound **14** was elucidated as 13,17-dibenzoyloxy-3-*O*-(2,3-dimethylbutanoyl)-20-deoxyingenol (see Fig. 1).

Compound **15** had a molecular formula of $C_{35}H_{46}O_8$ as established by HRESIMS. The EIMS suggested the presence of a benzoyl unit (*m/z* 105, $C_7H_5O^+$) and an octanoyloxy (*m/z* 450, $M^+-C_7H_{15}COOH$) unit in the structure. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **15**, closely related to those of **6**, showed one methyl signal [δ_H 1.15 (*s*), δ_C 16.7] instead of the oxymethene signal in **6**. The HMBC correlations of the methyl group with C-13 (δ_C 69.0), C-14 (δ_C 28.3) and C-15 (δ_C 30.3) confirmed the additional methyl group at C-15. Precise locations of individual moieties were deduced from further analysis of the HMBC spectra. Therefore, compound **15** was established as 3-*O*-benzoyl-13-octanoyloxyingenol.

Compound **16** was determined by HRESIMS to have a molecular formula of $C_{34}H_{52}O_8$. The EIMS of **16** suggested the presence of 2,3-dimethylbutanoyloxy (*m/z* 472, $M^+-C_5H_{11}COOH$) and octanoyloxy (*m/z* 444 $M^+-C_7H_{15}COOH$) units in the structure. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) clearly indicated that compounds **15** and **16** were similar, and the only difference was the ester moieties at C-3, which was defined as a 2,3-dimethylbutanoyloxy group on the basis of the MS and NMR data. Thus the structure of compound **16** was elucidated as 3-*O*-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol and the assignments of the proton and carbon signals were confirmed by HSQC and HMBC spectra.



	R_1	R_2	R_3	R_4
1	A	H	OB	H
2	A	OA	OB	H
3	A	OA	OB	OH
4	H	OA	OB	OA
5	H	OC	OB	OH
6	B	OC	OB	OH
7	H	OC	OB	OB
8	B	OC	OB	H
9	A	OC	OB	OH
10	H	OC	OB	OA
11	B	OB	OB	OH
12	A	OB	OB	OH
13	B	OA	OB	OH
14	A	OB	OB	H
15	B	OC	H	OH
16	A	OC	H	OH
17	B	OB	OB	H

Fig. 1. Chemical structures of **1**–**17**.

All the new compounds isolated in this study were evaluated for their cytotoxicity against the human hela cervical cancer cell line by using the MTT method. While the initial crude extract displayed moderate activity (IC_{50} 0.53 μg/mL), only 3-*O*-benzoyl-13-octanoyloxyingenol (**15**) and 3-*O*-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol (**16**) were found to have weak activity (IC_{50} 40.2 and 55.1 μM, respectively).

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, using a Bruker AM-400 spectrometer, with $CDCl_3$ (Aldrich, CA, USA) as solvent and TMS as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (*J*) in Hz. Optical rotations

were measured on a Perkin–Elmer 341 polarimeter. IR spectra were measured on a Permin–Elmer 577 spectrometer. EIMS (70 eV) was carried out on a Finnigan-MAT-95 mass spectrometer. ESI was carried on a Finnigan LC Q^{DECA} instrument. Silica gel H (200–300 mesh and 400–600 mesh), MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) and Sephadex LH-20 (Amersham Biosciences) were used for all CC separations, while silica gel 60 PF254 (Merck) was used for analytical TLC (0.50 mm). HPLC analyses were carried out using a Agilent 1100 with a Agilent DAD spectrophotometer set. Columns were RP18 (Phenomenex, C18, 4.6×250 mm for analysis and Alltima, C18, 10×250 mm for semi-preparative analysis).

3.2. Plant material

Whole plants of *E. esula* were collected in June 2004 in Shandong Province, P. R. China. The plant species was authenticated by Prof. Hu-Biao Chen. The voucher specimen (SC0062004) is available for inspection at the Institute of Materia Medica, SIBS, CAS.

3.3. Extraction and isolation

The whole plants of *E. esula* were collected and powdered by crusher. The powder of *E. esula* (5 kg) were extracted with 95% EtOH at room temperature (3×, each for 4 days). Removal of the solvent under reduced pressure gave rise to a crude extract (550 g), which was partitioned between petroleum ether (210 g), EtoAc (73 g) and H_2O (195 g). The petroleum ether fraction was applied to a silica gel CC (200–300 mesh, 2.0 kg) eluting with gradient mixtures of petroleum ether–acetone (from 1:0 to 0:1, 15.0 L) to give seven major fractions (F1–F7). F2 (55 g) was subjected to silica gel CC (200–300 mesh, 400 g) eluting with petroleum ether–EtOAc (10:1 to 3:1, 5 L) to afford four major subfractions, F2a–F2d. F2c (2.4 g) was further purified by silica gel CC (400–600 mesh, 50 g; petroleum ether–acetone, 20:1, 800 mL) and sephadex LH-20 column (2.0×80 cm, petroleum/$CHCl_3$/MeOH, 2:1:1) to obtain 25-hydroperoxycycloart-23E-en-3β-ol (25 mg), 25-hydroperoxycycloart-23E-en-3β-ol (17 mg), cycloart-23Z-en-3β,25-diol (100 mg) and 23-methylenecycloart-3β,24-diol (8 mg). F3 (35 g) was applied to a silica gel column (200–300 mesh, 300 g) eluted with petroleum ether–EtOAc (50:1 to 10:1, 4 L) to afford three major subfractions, F3a–F3c. F3a (1.8 g) was further purified by silica gel CC (400–600 mesh, 40 g; $CHCl_3$–EtOAc, 30:1, 800 mL) and sephadex LH-20 CC (2.0×80 cm; petroleum/$CHCl_3$/MeOH, 2:1:1) to obtain **1** (18 mg) and **2** (15 mg). F3c (0.8 g) was submitted to repeated chromatography by silica gel CC (petroleum–EtOAc, from 30:1 to 10:1; $CHCl_3$–EtOAc, 20:1) and sephadex LH-20 CC (petroleum/$CHCl_3$/MeOH, 2:1:1), followed by semipreparative HPLC to yield compounds **5** (10 mg) and **8** (4 mg). F4 (29 g) was subjected to silica gel CC (200–300 mesh, 400 g) eluting with gradient mixtures of petroleum ether–acetone (from 1:0 to 0:1; 4 L) to give three major fractions, F4a–F4c. F4a (3.5 g) was next purified further using MCI[1] gel CC (5.0×25 cm, 250 g; CH_3OH–H_2O; from 0:1 to 1:0; 6 L) and silica gel CC (400–600 mesh, 80 g ,$CHCl_3$–EtOAc, 20:1, 800 mL), followed by semipreparative HPLC to yield compounds **3** (32 mg), **4** (12 mg) and **6** (8 mg). F4b (1.2 g) was subjected to extensive silica gel CC (400–600 mesh, 50 g; $CHCl_3$–EtOAc, 20:1; 800 ml), followed by preparative TLC (developed with $CHCl_3$–EtOAc, 10:1) to yield compounds **11** (11 mg) and **12** (8 mg). F4c (0.8 g) was applied to a silica gel column (400–600 mesh, 30 g) eluted with $CHCl_3$–EtOAc (20:1 to 10:1, 1 L), followed by semipreparative HPLC to yield compounds **7** (4.5 mg), **9** (7 mg) and **10** (6 mg). F5 (15 g) was further purified using MCI gel column (5.0×25 cm, 250 g) eluting with MeOH–H_2O (1:1 to 1:0, 3 L) to obtain fractions F5a–F5c. In the same way, fraction F5a (1.3 g) afforded compounds **13** (9 mg), **14** (7 mg) and **15** (8 mg), whereas fraction F5b (0.9 g) gave compounds **16** (4 mg) and **17** (3 mg).

3.4. 17-Benzoyloxy-3-O-(2,3-dimethylbutanoyl)-20-deoxyingenol (*1*)

Colorless gum, $[\alpha]_D^{22} + 25$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.61) nm; IR γ_{max} (KBr) cm^{-1}: 3488, 2964, 1720, 1270, 756; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 550 [M^+] (2), 440 (5), 434 (1), 428 (3), 312 (8), 105 (100), 71 (38); HRESIMS (positive) *m/z*: 573.2863 (calcd. for $C_{33}H_{42}O_7Na$, 573.2828).

3.5. 17-Benzoyloxy-3-O-(2,3-dimethylbutanoyl)-13-(2,3-dimethylbutanoyloxy)-20-deoxyingenol (*2*)

Colorless gum, $[\alpha]_D^{22} + 35$ (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.15) nm; IR γ_{max} (KBr) cm^{-1}: 3504, 2968, 1724, 1270, 711; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 548 (0.4), 542 (0.3), 432 (3), 426 (2), 328 (15), 310 (10), 105 (52), 71 (100); HRESIMS (positive) *m/z*: 687.3483 (calcd. for $C_{39}H_{52}O_9Na$, 687.3509).

3.6. 17-Benzoyloxy-3-O-(2,3-dimethylbutanoyl)-13-(2,3-dimethylbutanoyloxy)ingenol (*3*)

Colorless gum, $[\alpha]_D^{22} + 22$ (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.12) nm; IR γ_{max} (KBr) cm^{-1}: 3446, 2966, 1724, 1270, 713; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 564 (0.8), 558 (0.2), 448 (1.2),

[1] MCI gel product is a kind of gel designed by Mitsubishi Chemical Industries Ltd based on resin, which can be used to separate many kinds of natural products.

442 (2), 326 (16), 105 (46), 71 (100); HRESIMS (positive) *m/z*: 703.3424 (calcd. for $C_{39}H_{52}O_{10}Na$, 703.3458).

3.7. 17-Benzoyloxy-20-O-(2,3-dimethylbutanoyl)-13-(2,3-dimethylbutanoyloxy)ingenol (**4**)

Colorless gum, $[\alpha]_D^{22}$ + 12 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.09) nm; IR γ_{max} (KBr) cm^{-1}: 3438, 2966, 1728, 1452, 1270, 714; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. nt): 546 (0.8), 448 (1.2), 442 (1.0), 326 (12), 105 (53), 71 (100); HRESIMS (positive) *m/z*: 703.3447 (calcd. for $C_{39}H_{52}O_{10}Na$, 703.3458).

3.8. 17-Benzoyloxy-13-octanoyloxyingenol (**5**)

Colorless gum, $[\alpha]_D^{22}$ + 4 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.22) nm; IR γ_{max} (KBr) cm^{-1}: 3425, 2927, 1722, 1452, 1270, 713; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 592 (0.2), 488 (0.5), 466 (0.2), 360 (13), 105 (100); HRESIMS (positive) *m/z*: 633.3032 (calcd. for $C_{35}H_{46}O_9Na$, 633.3040).

3.9. 3-O-benzoyl-17-benzoyloxy-13-octanoyloxyingenol (**6**)

Colorless gum, $[\alpha]_D^{22}$ + 82 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.41) nm; IR γ_{max} (KBr) cm^{-1}: 3434, 2927, 1720, 1452, 1274, 711. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 592 (0.2), 570 (0.3), 326 (10), 308 (5), 105 (100); HRESIMS (positive) *m/z*: 737.3280 (calcd. for $C_{42}H_{50}O_{10}Na$, 737.3302).

3.10. 20-O-benzoyl-17-benzoyloxy-13-octanoyloxyingenol (**7**)

Colorless gum, $[\alpha]_D^{22}$ + 70 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.34) nm; IR γ_{max} (KBr) cm^{-1}: 3450, 2928, 1720, 1269, 713. For ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 696 (0.1), 592 (0.9), 470 (0.6), 326 (5), 105 (100); HRESIMS (positive) *m/z*: 737.3285 (calcd. for $C_{42}H_{50}O_{10}Na$, 737.3302).

3.11. 3-O-benzoyl-17-benzoyloxy-13-octanoyloxy-20-deoxyingenol (**8**)

Colorless gum, $[\alpha]_D^{22}$ + 107 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.50) nm; IR γ_{max} (KBr) cm^{-1}: 3448, 2929, 1720, 1452, 1275, 712; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 680 $[M-H_2O]^+$ (0.1), 576 (1), 554 (0.4), 454 (0.8), 432 (3), 310 (5), 105 (100). HRESIMS (positive) *m/z*: 721.3328 (calcd. for $C_{42}H_{50}O_9Na$, 721.3353).

3.12. 17-Benzoyloxy-3-O-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol (**9**)

Colorless gum, $[\alpha]_D^{22}$ + 9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.98) nm; IR γ_{max} (KBr) cm^{-1}: 3462, 2929, 1724, 1271, 714; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 690 (0.1), 592 (0.8), 586 (0.4), 564 (0.8), 442 (0.2), 326 (24), 105 (100). HRESIMS (positive) *m/z*: 731.3735 (calcd. for $C_{41}H_{56}O_{10}Na$, 731.3771).

3.13. 17-Benzoyloxy-20-O-(2,3-dimethylbutanoyl)-13-octanoyloxyingenol (**10**)

Colorless gum, $[\alpha]_D^{22}$ + 26 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.88) nm; IR γ_{max} (KBr) cm^{-1}: 3458, 2970, 1720, 1270, 715; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 690 (0.1), 592 (0.6); 586 (0.5), 564 (0.3), 470 (4), 326 (10), 105 (100); HRESIMS (positive) *m/z*: 731.3742 (calcd. for $C_{41}H_{56}O_{10}Na$, 731.3771).

3.14. 3-O-benzoyl-13,17-dibenzoyloxyingenol (**11**)

Colorless gum, $[\alpha]_D^{22}$ + 51 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.54) nm; IR γ_{max} (KBr) cm^{-1}: 3435, 2933, 1724, 1272, 712; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 692 $[M^+]$ (0.1), 674 (0.5), 570 (0.8), 448 (1), 326 (4), 105 (100). HRESIMS (positive) *m/z*: 715.2510 (calcd. for $C_{41}H_{40}O_{10}Na$, 715.2519).

3.15. 13,17-Dibenzoyloxy-3-O-(2,3-dimethylbutanoyl)ingenol (**12**)

Colorless gum, $[\alpha]_D^{22}$ + 4 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.05) nm; IR γ_{max} (KBr) cm^{-1}: 3438, 2966, 1726, 1274, 714; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 686 $[M^+]$ (0.02), 668 (0.3), 570 (0.5), 442 (0.5), 326 (4), 105 (100); HRESIMS (positive) *m/z*: 709.2977 (calcd. for $C_{40}H_{46}O_{10}Na$, 709.2989).

3.16. 3-O-benzoyl-17-benzoyloxy-13-(2,3-dimethylbutanoyloxy)ingenol (**13**)

Colorless gum, $[\alpha]_D^{22}$ + 73.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.01) nm; IR γ_{max} (KBr) cm^{-1}: 3433, 2966, 1720, 1274, 712; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 570 (0.4), 564 (0.8), 448 (0.3), 326 (5), 105 (100); HRESIMS (positive) *m/z*: 709.2982 (calcd. for $C_{40}H_{46}O_{10}Na$, 709.2989).

3.17. 13,17-Dibenzoyloxy-3-O-(2,3-dimethylbutanoyl)-20-deoxyingenol (**14**)

Colorless gum, $[\alpha]_D^{22} + 29$ (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.11) nm; IR γ_{max} (KBr) cm^{-1}: 3446, 2966, 1726, 1274, 711; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 670 (0.1), 554 (0.4), 548 (0.8), 426 (0.9), 310 (10), 105 (100); HRESIMS (positive) *m/z*: 693.3066 (calcd. for $C_{40}H_{46}O_9Na$, 693.3040).

3.18. 3-O-benzoyl-13-octanoyloxyingenol (**15**)

Colorless gum, $[\alpha]_D^{22} + 40$ (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.14) nm; IR γ_{max} (KBr) cm^{-1}: 3442, 2956, 1720, 1274, 711; for ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 576 (0.2), 472 (0.8), 450 (0.4), 328 (12), 105 (100); HRESIMS (positive) *m/z*: 617.3068 (calcd. for $C_{35}H_{46}O_8Na$, 617.3090).

3.19. 3-O-(2, 3-dimethylbutanoyl)-13-octanoyloxyingenol (**16**)

Colorless gum, $[\alpha]_D^{22} + 7$ (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 229 (2.71) nm; IR γ_{max} (KBr) cm^{-1}: 3442, 2962, 1728, 1382; for ^1H NMR (CDCl3, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) spectra, see Tables 1 and 2; EIMS (70 eV) *m/z* (rel. int): 588 [M^+] (0.3), 570 (0.5), 472 (1.2), 444 (1.6), 328 (32), 310 (40), 57 (100). HRESIMS (positive) *m/z*: 611.3542 (calcd. for $C_{34}H_{52}O_8Na$, 611.3560).

Acknowledgements

We thank Shanghai Green Valley Pharmaceutical Co. Ltd. for financial support of this work. The authors are also grateful to Dr. Hu-Biao Chen for help with the identification of the plant.

References

Amir, R.J., 2006. Chemistry and biological activity of secondary metabolites in Euphorbia from Iran. Phytochemistry 67, 1977–1984.

Cabrera, G.M., Gallo, M., Seldes, .M., 1996. Cycloartane derivatives from *Tillandsia usneoides*. J. Nat. Prod. 59, 343–347.

Ferreira, M.J., Pinto, F.C., Ascenso, J.R., 2001. Cycloartane triterpenes from *Euphorbia tuckeyana*. Nat. Prod. Lett. 15, 363–369.

Gotta, H., Adolf, W., Opferkuch, H.J., Hecker, E., 1984. On the active principles of the Euphorbiaceae, IX Ingenane type diterpene esters from five *Euphorbia* species. Zeitschrift fuer Naturforschung, Teil B: Anorganische Chemie, Organische Chemie. 39, 683–694.

Greca, M.D., Fiorentino, A., Monaco, P., Previtera, L., 1994. Cycloartane triterpenes from *Juncus effuses*. Phytochemistry 35, 1017–1022.

Günther, G., Martinek, T., Dombi, G., Hohmann, J., Vasas, A., 1999. Structural characterization and dynamic NMR studies of a new peracylated macrocyclic diterpene. Magn. Reson. Chem. 37, 365–370.

Halaweish, F.T., Kronberg, S., Hubert, M.B., Rice, J.A., 2002. Toxic and aversive diterpenes of *Euphorbia esula*. J. Chem. Ecol. 28, 1599–1611.

Halaweish, F., Kronberg, S., Rice, J.A., 2003. Rodent and ruminant ingestive response to flavonoids in *Euphorbia esula*. J. Chem. Ecol. 29, 1073–1082.

Hohmann, J., Vasas, A., Günther, G., Máthé, I., Evanice, F., Dombi, G., Jerkovich, G., 1997. Macrocyclic diterpene polyesters of the jatrophane type from *Euphorbia esula*. J. Nat. Prod. 60, 331–335.

Jakupovic, J., Morgenstern, T., Macro, J.A., Berendsohn, W., 1998. Diterpenes from *Euphorbia paralias*. Phytochemistry 47, 1611–1619.

Liu, L.G., Meng, J.C., Wu, S.X., Li, X.Y., Zhao, X.C., Tan, R.X., 2002. New macrocyclic diterpenoids from *Euphorbia esula*. Planta Med. 68, 244–248.

Mbwambo, Z.H., Lee, S.K., Mshiu, E.N., Pezzuto, J.M., Kinghorn, A.D., 1996. Constituents from the stem wood of *Euphorbia quinquecostata* with phorbol dibutyrate receptor-binding inhibitory activity. J. Nat. Prod. 59, 1051–1055.

Onwukaeme, N.D., Rowan, M.G., 1992. Jatrophane and lathyrane diterpenoid esters from North American leafy spurge seed. Phytochemistry 31, 3479–3482.

Shi, Y.P., Jia, Z.J., 1997a. Recent studies on diterpene esters and their bioactivities from Euphorbia genus in China. Chem. J. Chin. Univ. 7, 1107–1112.

Shi, Y.P., Jia, Z.J., 1997b. Triterpenoids and other compounds from *Euphorbia esula* and *E. petiolata*. Indian J Chem. B 36, 1038–1043.

Shi, Y.P., He, Z.J., Jia, Z.J., 1998. Progress in the structures of diterpenoids and their bioactivities from *Euphorbia* genus (I). Nat. Prod. Res. Dev. 11, 85–89.

Singla, A.K., Kamala, P., 1990. Phytochemistry of *Euphorbia* species. Fitoterapia 61, 483–516.

Wang, L.Y., Wang, N.L., Yao, X.S., Miyata, S., Kitanaka, S., 2003. Diterpenes from the roots of *Euphorbia kansui* and their in vitro effects on the cell division of Xenopus (2). Chem. Pharm. Bull. 51, 935–941.

Zechmeister, K., Brandl, F., Hoppe, W., Hecker, E., Opferkuch, H.J., Adolf, W., 1970. Structure determination of the new tetracyclic diterpene ingenol triacetate with triple product methods. Tetrahedron Lett. 11, 4075–4078.