Triterpenoids and Steroids from the Fruits of *Melia toosendan* and Their Cytotoxic Effects on Two Human Cancer Cell Lines

Shi-Biao Wu,[†] Jing-Jing Su,[†] Lian-Hui Sun,[‡] Wen-Xuan Wang,[†] Yun Zhao,[†] Hui Li,[§] Sheng-Ping Zhang,[‡] Guang-Hui Dai,[§] Chuan-Gui Wang,[‡] and Jin-Feng Hu*[,†]

Department of Natural Products for Chemical Genetic Research, Key Laboratory of Brain Functional Genomics, Ministry of Education & Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University, No. 3663 Zhongshan Road N, Shanghai 200062, People's Republic of China, Institute of Biomedical Sciences, School of Life Science, East China Normal University, No. 500 Dongchuan Road, Shanghai 200240, People's Republic of China, and Lab of Plant Health and Natural Product & Key Laboratory of Urban Agriculture, Ministry of Agriculture, School of Agriculture and Biology, Shanghai Jiao Tong University, No. 800 Dongchuan Road, Shanghai 200240, People's Republic of China

Received August 12, 2010

Ten new triterpenoids, named meliasenins I−R (**1**−**10**), one new steroid (**11**), and 11 related known compounds (**12**−**22**) were isolated from fruits of *Melia toosendan*. The structures of the new compounds were established on the basis of spectroscopic methods, including 2D NMR techniques and mass spectrometry. The relative configuration of **1**, (20*R**,23*E*)-25-hydroperoxyeupha-7,23-diene-3β,16β-diol (meliasenin I), was confirmed by single-crystal X-ray diffraction analysis. All isolated triterpenoids (**1**−**10**, **12**−**15**) and two steroids (**11**, **20**) were tested for their cytotoxicity against U20S human osteosarcoma and MCF-7 human breast cancer cells using the MTT assay, and some of them were significantly cytotoxic (IC_{50} <10 μg/mL). The insecticidal properties of compounds **1**−**15** and **20** were also briefly evaluated.

The genus *Melia* (Meliaceae) has proven to be a rich source of highly functionalized nortriterpenoids (e.g., limonoids) and triterpenoids (e.g., euphanes/tirucallanes) with promising biological activities.[1] The number of triterpenoids identified from different parts of the Chinaberry tree, *Melia toosendan* Sieb. et Zucc. (Meliaceae), cultivated in different regions of southwestern China (mainly Sichuan and Yunnan Provinces) continues to grow.[2] Investigation of fruits of *M. toosendan* ("Chuan-Lian-Zi" in Chinese) in continuation of our project toward the discovery of novel antitumor agents from natural products[3] yielded 11 euphane-type (**1**−**7**, **12**−**15**) and three tirucallane-type (**8**−**10**) triterpenoids and eight steroids (**11**,**16**−**22**). In this paper we report the isolation and structure elucidation of 11 new compounds (**1**−**11**), their cytotoxic effects on U20S and MCF-7 cancer cells, and their activity against aphids (*Lipaphis erysimi* Kaltenbach).

Results and Discussion

A methanol extract of fruits of *M. toosendan* was successively subjected to column chromatography (CC) over silica gel, MCI gel, Sephadex LH-20, and semipreparative HPLC to afford 22 compounds (**1**−**22**). Comparing their spectroscopic data and physicochemical properties with literature reports, or by direct comparison with authentic samples, the known compounds were identified as meliastatin 3 (**12**),[4] kulinone (**13**),[5] methyl kulonate (**14**),[5] meliastatin 5 (**15**),[4] β-sitosterol (**16**), daucosterine (**17**), 7α-hydroxysitosterol (**18**),[6] 7β-hydroxysitosterol (**19**),[6] (20*S*)-5-ergostene-3β,7α,16β,20-tetrol (**20**),[3d] (22*E*,24*S*)-5α,8α-epidioxy-24-methylcholesta-6,22-dien-3β-ol (**21**),[7] and (22*E*,24*S*)-5α,8α-epidioxy-24-methylcholesta-6,9,22-trien-3β-ol (**22**).[7]

The molecular weight of compound **1** and its chemical formula, $C_{30}H_{50}O_4$, were determined from HRESIMS, which gave a pseudomolecular ion at *m/z* 497.3615 $[M + Na]^+$. The ^1H NMR spectrum (Table 1) of **1** displayed signals for seven tertiary methyl groups with singlets at δ 0.75 (3H, Me-19), 0.84 (3H, Me-18), 0.86 (3H, Me-29), 0.96 (3H, Me-28), 1.21 (3H, Me-30), and 1.33 (6H, Me-26/Me-27), one secondary methyl doublet at δ 1.02 (3H, J = 6.2 Hz, Me-21), two oxymethines [δ 3.23 (1H, br d, J = 9.0 Hz, H-3α) and 4.05 (1H, br dd, J = 6.9, 6.7 Hz, H-16α)], and three olefinic protons resonating at δ 5.22 (1H, br s, H-7), 5.54 (1H, br d, J = 15.8 Hz, H-24), and 5.65 (1H, br dt, J = 15.8, 6.6 Hz, H-23). The ^{13}C and DEPT NMR spectra of **1** exhibited 30 signals classified as eight sp^3 methyl, seven sp^3 methylene, six sp^3 (two oxygenated at δ 77.6, 79.2) and three sp^2 (δ 118.1, 129.9, 135.2) methines, and five sp^3 (one oxygenated at δ 81.9) and one sp^2 (δ 144.8) quaternary carbons (Table 2). The NMR data demonstrated that **1** was a 25-hydroperoxyeuphane-type triterpenoid similar to the known compound **12** (meliastatin 3), previously isolated from the stem bark of *M. dubia*.[4] The carbonyl at C-3 and the methoxycarbonyl at C-21 in **12** were replaced by a secondary OH and a methyl in **1**, respectively. The secondary OH groups at C-3 and C-16 and the double bonds at C-7 and C-23 in **1** were reinforced by 2D NMR (COSY, HSQC, and HMBC) experiments. β-Orientation of the secondary OH at C-3 was determined according to the splitting pattern (br d, 9.0 Hz) of H-3 resonating at δ 3.23. The *E* geometry of the double bond at C-23 was based on the large coupling constant (15.8 Hz) between H-23 and H-24. In the NOESY spectrum of **1** (Figure 1), correlations were observed between Me-18 and H-16, as well as between Me-30 and H-17, indicating that H-16 was β-oriented. The configuration at C-20 was the same as that previously deduced in **12**.[4] The relative configuration of **1** was confirmed by single-crystal X-ray diffraction data (Figure 2).[8] Therefore, **1** was elucidated as (20*R**,23*E*)-25-hydroperoxyeupha-7,23-diene-3β,16β-diol (meliasenin I).[9]

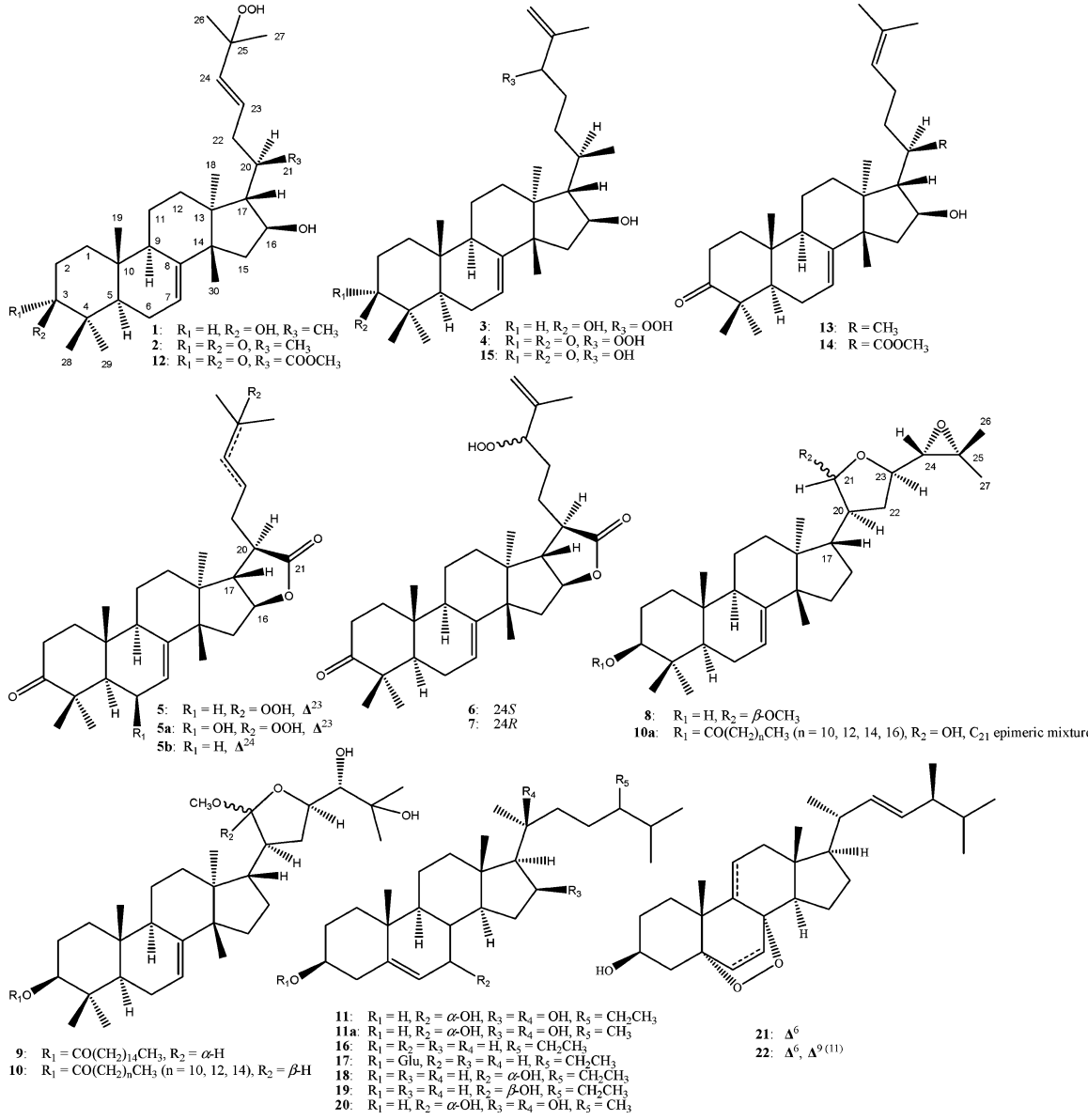
Compound **2**, meliasenin J, showed a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 495.3470 corresponding to the molecular formula $C_{30}H_{48}O_4$. The ^1H and ^{13}C NMR data (Tables 1 and 2) showed that **2** had features similar to those of **1**. The only difference between **2** and **1** was that the oxymethine group at C-3 in **1** was replaced by a carbonyl group (δ_C 216.9, IR: 1708 cm^{-1}) in **2**. Thus, compound **2** was deduced to be (20*R**,23*E*)-25-hydroperoxyeupha-7,23-dien-16β-ol-3-one, which was confirmed by 2D NMR (COSY, HMQC, HMBC, and NOESY) experiments.

The molecular formula $C_{30}H_{50}O_4$ of meliasenin K (**3**) was identical to that of **1**, and they had similar absorption bands in their IR spectra and similar features in their ^1H and ^{13}C NMR spectra

* To whom correspondence should be addressed. Tel: +86 21 62607510. Fax: +86 21 62606791. E-mail: jfhu@brain.ecnu.edu.cn.
[†] Key Laboratory of Brain Functional Genomics, East China Normal University.
[‡] Institute of Biomedical Sciences, East China Normal University.
[§] Shanghai Jiao Tong University.

10.1021/np100566v
Published on Web 10/20/2010

Chart 1



(Tables 1 and 2). The difference between **3** and **1** was in the positions of the double bond and the hydroperoxy group. In the ^{1}H NMR spectrum of **3**, the signals for an exomethylene [δ 5.00 (1H, br s, H-26)/5.02 (1H, br s, H-26′)], a hydroperoxymethine [δ 4.27 (1H, t, J = 6.7 Hz, H-24)], and a vinylic methyl [δ 1.73 (3H, br s, Me-27)] were observed, which were very similar to those for H-24, H-26, and H-27 of known compound **15** (meliastatin 5).[4] The secondary OH group at C-24 in **15** was replaced by a hydroperoxy group in **3**, and hence both H-24 and C-24 were shifted downfield [**3**: δ_H 4.27 (1H, t, J = 6.7 Hz), δ_C (89.7/90.0) (Tables 1 and 2); **15**: δ_H 4.03 (1H, br t, J = 6.0 Hz), δ_C (76.4)[4]]. Unlike **15**, the carbons around C-24 in the side chain of **3** displayed a doubling of signals (Table 2), indicating that **3** was a mixture of C-24 epimers (24*R*/24*S*). Therefore, **3** was established as (20*R*,24*RS*)-hydroperoxyeupha-7,25-diene-3β,16β-diol, which was supported by 2D NMR (COSY, HSQC, and HMBC) experiments.

Meliasenin L (**4**) was also obtained as a mixture of C-24 epimers, as indicated by the duplicated signals of the side chain in the ^{13}C NMR spectrum of **4** (Table 2). Its molecular formula $C_{30}H_{48}O_4$ was determined by HRESIMS, with an $[M + Na]^+$ peak at *m*/*z* 495.3454 ($C_{30}H_{48}O_4Na$). The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) closely resembled those of compound **3**. As with compounds **2** and **1**, the only difference between **4** and **3** was that the oxymethine group at C-3 in **3** was replaced by a carbonyl group (δ_C 217.0, IR: 1703 cm^{-1}) in **4**. Thus, compound **4** was established as (20*R**,24*RS*)-hydroperoxyeupha-7,25-dien-16β-ol-3-one.

The molecular formula of meliasenin M (**5**) was deduced to be $C_{30}H_{44}O_5$, and the ^{1}H and ^{13}C NMR data (in $CDCl_3$, Tables 1 and 2) showed that **5** had general features similar to those of **2**. The difference between **5** and **2** was that the secondary C-21 methyl group in **2** was carboxylated, and a γ-lactone was formed between C-21 and C-16 in **5**. This was supported by the following: H-16 in **5** was shifted downfield to δ 4.17 (1H, ddd, J = 11.9, 10.6, 7.7 Hz) compared to that in **2** [δ 4.06 (1H, br dd, J = 6.5, 6.0 Hz)] (Table 1). In the ^{13}C NMR and DEPT spectra of **5**, one more carbonyl carbon at δ 179.8 was found instead of one of the methyl carbons in **2** (Table 2). Taking into account the molecular formula and nine degrees of unsaturation, an additional ring should be present in **5**. A strong absorption band at 1778 cm^{-1} attributable to a γ-lactone was observed in the IR spectrum of **5**. Finally, the 21,16 γ-lactone in **5** was confirmed by an HMBC NMR experiment. Key HMBC correlations were observed between H-16 at δ 4.17 and C-21 at δ 179.8 and between H-20 at δ 2.50 (1H, m) and C-16 (δ 82.6), C-17 (δ 56.9), C-21, C-22 (δ 31.6), and C-23 (δ 127.4), respectively. The relative configuration of **5** was determined by a combination of the coupling patterns (Table 1) and a NOESY NMR

Table 1. ^{1}H NMR Data of Compounds **1**−**7** (298 K, 500 MHz, *J* in Hz)[a]

no.	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**5**[c]	**6**[b]	**7**[b]
1	1.12, 1H, m	1.44, 1H, br dt (14.5, 4.1)	1.12, 1H, m	1.45, 1H, br dt (14.0, 4.2)	1.43, 1H, br dt (14.5, 3.7)	1.44, 1H, br dt (14.6, 4.1)	1.46, 1H, br dt (14.5, 3.0)	1.46, 1H, br dt (14.5, 3.0)
	1.64, 1H, m	1.98, 1H, ddd (14.5, 5.0, 3.2)	1.66, 1H, m	1.98, 1H, ddd (14.0, 5.1, 3.0)	1.92, 1H, ddd (13.1, 5.1, 2.8)	1.99, 1H, ddd (13.2, 5.4, 2.4)	1.98, 1H, ddd (14.5, 5.5, 2.5)	1.98, 1H, ddd (14.5, 5.5, 2.5)
2	1.60, 1H, m	2.25, 1H, ddd (14.5, 4.1, 3.2)	1.58, 1H, m, overlapped	2.25, 1H, ddd (14.0, 4.2, 3.0)	2.22, 1H, ddd (14.5, 3.7, 2.8)	2.19, 1H, ddd, overlapped	2.26, 1H, ddd (14.5, 3.0, 2.5)	2.26, 1H, ddd (14.5, 3.0, 2.5)
	1.66, 1H, m	2.75, 1H, dt (14.5, 5.0)	1.65, 1H, m	2.76, 1H, dt (14.0, 5.1)	2.71, 1H, dt (14.5, 5.1)	2.85, 1H, dt (14.6, 5.6)	2.75, 1H, dt (14.5, 5.5)	2.75, 1H, dt (14.5, 5.5)
3	3.23, 1H, br d (9.0)		3.23, 1H, dd (11.6, 3.0)					
5	1.31, 1H, dd, overlapped	1.72, 1H, dd (10.3, 7.2)	1.28, 1H, dd (12.1, 5.7)	1.70, 1H, dd (10.2, 7.0)	1.68, 1H, dd, overlapped	1.68, 1H, dd, overlapped	1.72, 1H, dd, overlapped	1.72, 1H, dd, overlapped
6	1.97, 1H, m	2.10, 1H, m, overlapped	1.98, 1H, m	2.09, 1H, m	2.10, 1H, m	2.16, 1H, m	2.11, 1H, m	2.11, 1H, m
	2.11, 1H, m, overlapped	2.11, 1H, m, overlapped	2.16, 1H, m	2.11, 1H, m	2.11, 1H, m	2.18, 1H, m	2.12, 1H, m	2.12, 1H, m
7	5.22, 1H, br s	5.28, br d (3.0)	5.23, 1H, br d (2.5)	5.28, 1H, br d (3.0)	5.31, 1H, br d (3.5)	5.37, 1H, br d (3.1)	5.34, 1H, br d (3.0)	5.33, 1H, br d (3.0)
9	2.20, 1H, m	2.29, 1H, m	2.19, 1H, m	2.29, 1H, m	2.44, 1H, m	2.54, 1H, m, overlapped	2.49, 1H, m	2.48, 1H, m
11	1.55, 1H, m, overlapped	1.61, 1H, m	1.54, 1H, m, overlapped	1.60, 1H, m, overlapped	1.61, 2H, m, overlapped	1.60, 2H, m, overlapped	1.64, 1H, m	1.63, 1H, m
	1.57, 1H, m, overlapped	1.63, 1H, m	1.55, 1H, m	1.61, 1H, m			1.69, 1H, m	1.68, 1H, m
12	1.54, 1H, m, overlapped	1.65, 1H, m	1.58, 1H, m, overlapped	1.58, 1H, m	1.58, 1H, m	1.58, 1H, m	1.68, 1H, m	1.67, 1H, m
	1.85, 1H, m	1.89, 1H, m	1.87, 1H, m	1.88, 1H, m	1.67, 1H, m	1.67, 1H, m	1.70, 1H, m	1.71, 1H, m
15	1.54, 1H, dd, overlapped	1.58, 1H, dd, overlapped	1.53, 1H, dd, overlapped	1.55, 1H, m	1.68, 1H, dd, overlapped	1.68, 1H, overlapped	1.73, 1H, dd (10.1, 7.5)	1.73, 1H, dd (10.0, 7.5)
	2.10, 1H, dd, overlapped	2.10, 1H, dd, overlapped	2.07, 1H, dd (13.4, 6.9)	2.09, 1H, m	2.24, 1H, dd, overlapped	2.31, 1H, m	2.29, 1H, dd (11.8, 10.0)	2.28, 1H, dd (11.9, 10.0)
16	4.05, 1H, br dd (6.9, 6.7)	4.06, 1H, br dd (6.5, 6.0)	4.02, 1H, br dd (6.9, 6.5)	4.04, 1H, br dd (7.3, 5.7)	4.17, 1H, ddd (11.9, 10.6, 7.7)	4.27, 1H, ddd (11.8, 10.5, 7.5)	4.18, 1H, ddd (12.2, 10.5, 8.0)	4.17, 1H, ddd (11.9, 10.7, 7.5)
17	1.58, 1H, dd, overlapped	1.60, 1H, dd (10.1, 6.0)	1.52, 1H, dd, overlapped	1.54, 1H, dd (10.5, 6.4)	2.17, 1H, dd (13.0, 10.6)	2.25, 1H, dd (13.1, 10.6)	2.13, 1H, dd (13.5, 10.5)	2.13, 1H, dd (13.2, 10.7)
18	0.84, 3H, s	0.83, 3H, s	0.81, 3H, s	0.81, 3H, s	0.92, 3H, s	0.98, 3H, s	0.96, 3H, s	0.95, 3H, s
19	0.75, 3H, s	1.00, 3H, s	0.75, 3H, s	1.02, 3H, s	0.99, 3H, s	1.02, 3H, s	1.02, 3H, s	1.02, 3H, s
20	1.71, 1H, m	1.71, 1H, m	1.59, 1H, m, overlapped	1.60, 1H, m, overlapped	2.50, 1H, m	2.56, 1H, ddd, overlapped	2.45, 1H, ddd, overlapped	2.40, 1H, ddd (13.2, 6.5, 6.0)
21	1.02, 3H, d (6.2)	1.02, 3H, d (6.9)	1.02, 3H, d (6.4)	1.03, 3H, d (6.8)				
22	1.80, 1H, m	1.80, 1H, m	1.11, 1H, m	1.10, 1H, m	2.34, 1H, m	2.34, 1H, m	1.49, 1H, m	1.59, 1H, m
	2.35, 1H, br d (12.4)	2.34, 1H, br d (14.0)	1.57, 1H, m, overlapped	1.59, 1H, m	2.48, 1H, m	2.69, 1H, m	1.97, 1H, m	1.89, 1H, m
23	5.65, 1H, br dt (15.8, 6.6)	5.67, 1H, br dt (15.5, 6.5)	1.36, 1H, m	1.36, 1H, m	5.63, 1H, overlapped	5.59, 1H, br dt (15.8, 7.4)	1.62, 1H, m	1.65, 1H, m
			1.78, 1H, m	1.77, 1H, m			1.69, 1H, m	1.70, 1H, m
24	5.54, 1H, br d (15.8)	5.53, 1H, br d (15.5)	4.27, 1H, t (6.7)	4.27, 1H, t (6.8)	5.64, 1H, overlapped	5.69, 1H, br d (15.8)	4.35, 1H, br t (6.5)	4.33, 1H, br t (6.5)
26	1.33, 3H, s	1.33, 3H, s	5.00, 1H, br s	5.01, 1H, br s	1.30, 3H, s	1.28, 3H, s	5.03, 1H, br s	5.03, 1H, br s
			5.02, 1H, br s	5.03, 1H, br s			5.05, 1H, br s	5.04, 1H, br s
27	1.33, 3H, s	1.33, 3H, s	1.73, 3H, br s	1.73, 3H, br s	1.31, 3H, s	1.29, 3H, s	1.74, 3H, br s	1.75, 3H, br s
28	0.96, 3H, s	1.10, 3H, s	0.96, 3H, s	1.12, 3H, s	1.02, 3H, s	1.06, 3H, s	1.05, 3H, s	1.05, 3H, s
29	0.86, 3H, s	1.03, 3H, s	0.85, 3H, s	1.04, 3H, s	1.09, 3H, s	1.13, 3H, s	1.12, 3H, s	1.12, 3H, s
30	1.21, 3H, s	1.24, 3H, s	1.20, 3H, s	1.25, 3H, s	1.20, 3H, s	1.25, 3H, s	1.24, 3H, s	1.24, 3H, s
−OOH	8.25, 1H, br s	NA	8.29, 1H, br s	NA	7.81, 1H, br s	NA	8.01, 1H, br s	8.02, 1H, br s

[a] Assignments were made by a combination of 1D and 2D NMR (COSY, HSQC, HMBC) experiments. NA = not available. [b] Recorded in $CDCl_3$. [c] Recorded in CD_3OD.

Table 2. ^{13}C NMR Data of Compounds **1**−**7** (in $CDCl_3$, 298 K, 125 MHz)[a]

no.	1	2	3	4	5	6	7
1	37.0, CH_2	38.5, CH_2	37.1, CH_2	38.4, CH_2	38.3, CH_2	38.3, CH_2	38.3, CH_2
2	27.5, CH_2	34.8, CH_2	27.6, CH_2	34.9, CH_2	34.7, CH_2	34.7, CH_2	34.7, CH_2
3	79.2, CH	216.9, qC	79.2, CH	217.0, qC	216.4, qC	216.3, qC	216.3, qC
4	38.9, qC	47.9[c], qC	38.9, qC	47.9[f], qC	47.8[g], qC	47.9[i], qC	47.9[j], qC
5	50.7, CH	52.4, CH	50.7, CH	52.3, CH 52.4, CH	52.5, CH	52.6, CH	52.6, CH
6	23.9, CH_2	24.3, CH_2	23.9, CH_2	24.3, CH_2	24.3, CH_2	24.3, CH_2	24.3, CH_2
7	118.1, CH	118.1, CH	118.2, CH	118.1, CH	118.5, CH	118.6, CH	118.6, CH
8	144.8, qC	145.0, qC	144.9, qC	145.0, qC	143.3, qC	143.4, qC	143.4, qC
9	48.3, CH	47.9[c], CH	48.4, CH	47.9[f], CH	47.8[g], CH	47.9[i], CH	47.9[j], CH
10	34.9, qC	35.0, qC	35.0, qC	35.0, qC	35.4, qC	35.5, qC	35.5, qC
11	17.8, CH_2	18.0, CH_2	18.0, CH_2	18.0, CH_2 18.1, CH_2	16.7, CH_2	16.8, CH_2	16.8, CH_2
12	33.0, CH_2	33.0, CH_2	33.2, CH_2 33.3, CH_2	33.1, CH_2	29.2, CH_2	29.6, CH_2	29.6, CH_2
13	45.3, qC	45.7[d], qC	45.4, qC	45.3, qC	39.5, qC	39.6, qC	39.5, qC
14	49.7, qC	49.8, qC	49.9, qC	49.8, qC 49.9, qC	55.1, qC	55.2, qC	55.2, qC
15	45.7, CH_2	45.7[d], CH_2	45.8, CH_2	45.7, CH_2	35.6, CH_2	35.7, CH	35.6, CH
16	77.6, CH	77.6, CH	78.0, CH 78.1, CH	78.0, CH 78.1, CH	82.6, CH	82.6, CH	82.6, CH
17	61.5, CH	61.6, CH	62.2, CH 62.4, CH	62.1, CH 62.4, CH	56.9, CH	57.9, CH	58.0, CH
18	23.4, CH_3	23.5, CH_3	23.3, CH_3 23.4, CH_3	23.4, CH_3	21.6, CH_3	21.6, CH_3	21.6, CH_3
19	13.0, CH_3	12.7, CH_3	13.1, CH_3	12.7, CH_3	12.4, CH_3	12.4, CH_3	12.4, CH_3
20	33.8, CH	33.8, CH	33.9, CH 34.0, CH	34.0, CH	45.6, CH	45.6, CH	45.7, CH
21	18.6, CH_3	18.7, CH_3	18.4, CH_3 18.5, CH_3	18.4, CH_3 18.5, CH_3	179.8, qC	180.3, qC	180.3, qC
22	38.5, CH_2	38.4, CH_2	30.8, CH_2 31.1, CH_2	30.6, CH_2 30.9, CH_2	31.6, CH	25.1, CH_2	25.2, CH_2
23	129.9, CH	130.1, CH	27.6, CH_2 27.7, CH_2	27.7, CH_2 27.8, CH_2	127.4, CH_2	28.3, CH_2	28.7, CH_2
24	135.2, CH	135.1, CH	89.7, CH 90.0, CH	89.7, CH 90.0, CH	136.8, C H_2	89.23, CH	89.19, CH
25	81.9, qC	82.1, qC	143.8, qC 144.0, qC	143.7, qC 143.8, qC	81.8, qC	143.0, qC	143.1, qC
26	24.3[b], CH_3	24.3[e], CH_3	114.1, CH_2 114.3, CH_2	114.3, CH_2 114.5, CH_2	24.4[h], CH_3	114.6, CH_2	114.6, CH_2
27	24.4[b], CH_3	24.4[e], CH_3	17.0, CH_3 17.1, CH_3	17.0, CH_3 17.1, CH_3	24.4[h], CH_3	17.4, CH_3	17.3, CH_3
28	27.5, CH_3	24.5, CH_3	27.7, CH_3	24.5, CH_3	24.1, CH_3	24.5, CH_3	24.4, CH_3
29	14.7, CH_3	21.5, CH_3	14.7, CH_3	21.5, CH_3	21.4, CH_3	21.4, CH_3	21.4, CH_3
30	27.7, CH_3	27.8, CH_3	27.6, CH_3	27.8, CH_3	32.1, CH_3	32.2, CH_3	32.2, CH_3

[a] Assignments were made by a combination of 1D and 2D NMR (COSY, HSQC, HMBC) experiments. [b,e] Assignments may be interchangeable within the same superscript in the same column. [c,d,f−j] Signals were overlapped within the same superscript in the same column.

experiment. In the ^1H NMR spectrum of **5** (in $CDCl_3$), the olefinic protons H-23 and H-24 were overlapping at δ 5.63 and 5.64. However, the geometry of this double bond was assigned as *E* on the basis of the coupling constant (15.8 Hz) between H-23 and H-24 recorded in CD_3OD (Table 1). The large coupling constants ($J_{16,17}$ = 10.6 Hz, $J_{17,20}$ = 13.0 Hz) observed for H-17 (δ 2.17) indicated that H-17 was in a pseudo-axial position (β-orientation), whereas the vicinal protons H-16 and H-20 both took opposite positions (α-orientation). Moreover, clear NOE correlations (Figure 1) were found between H-16 and Me-18, between H-16 and H-20, and between H-17 and Me-30. Therefore, **5** was defined as (23*E*)-25-hydroperoxyeupha-7,23-dien-3-oxo-16β,21β-olide, which is closely related to meliasenin A (**5a**), recently isolated from the stem bark of *M. toosendan*.[2a]

Meliasenins N (**6**) and O (**7**) possessed the same molecular formula ($C_{30}H_{44}O_5$). The ^1H and ^{13}C NMR data (Tables 1 and 2) and IR spectra showed that **6** and **7** both had general features similar to those of **5**, indicating they shared the same skeleton with a γ-lactone at C-21 and C-16. The difference between **6** and **5** was the positions of the double bond and the hydroperoxy group in the side chain. In the ^1H NMR spectrum of **6**, signals for an exomethylene at δ 5.03 (1H, br s, H-26)/5.05 (1H, br s, H-26′), a hydroperoxymethine at δ 4.35 (1H, br t, J = 6.5 Hz, H-24), and a vinylic methyl at δ 1.74 (3H, br s, Me-27) were observed, which were very similar to the corresponding resonances of compounds **3** and **4** (Table 1). Unlike **3** and **4**, the carbons around C-24 in the side chain of **6** did not show any duplicated signals in the ^{13}C NMR spectrum (Table 2), indicating that **6** was not a mixture of C-24 epimers. The ^1H and ^{13}C NMR spectra of **7**, particularly in the side chain, were very similar to those of **6** (Tables 1 and 2), implying that **7** was the C-24 epimer of **6**. The proposed structures of **6** and **7** were investigated by detailed 2D NMR (COSY, HSQC, and HMBC) analysis. Using a strategy similar to that used by Pettit et al. regarding the configuration at C-24 in the known compounds meliastatins 4 and 5,[4] examination of the NOESY spectra of **6** and **7** led to the 24*S* configuration at C-24 in **6** and 24*R* in **7**. As for **6**, NOE correlations were observed between H-16α and Me-18, between H-16α and H-20α, between H-20α and H-22α, between H-22α and H-23α, between H-23α and the exchangeable proton of −OOH resonating at δ 8.01, and between H-22β and H-24β (Figure 1). Meanwhile, key NOE correlations of **7** were found between H-17β and Me-30, between H-16α and Me-18, between H-16α and H-20α, between H-20α and H-22α, between H-22α and H-23α, and between H-23α and H-24α (Figure 1). The data revealed that **6** and **7** were (24*S*)- and (24*R*)-hydroperoxyeupha-7,25-dien-3-oxo-16β,21β-olide, respectively. Interestingly, the chemi-

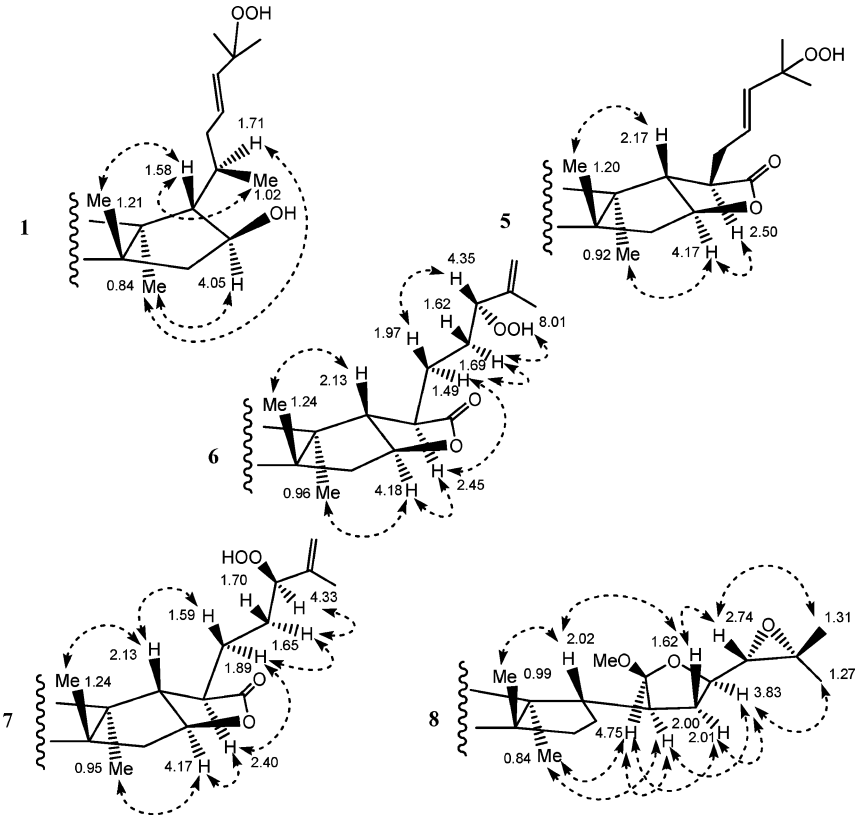


Figure 1. Observed key NOE correlations for partial structures of compounds **1** and **5**−**8**.

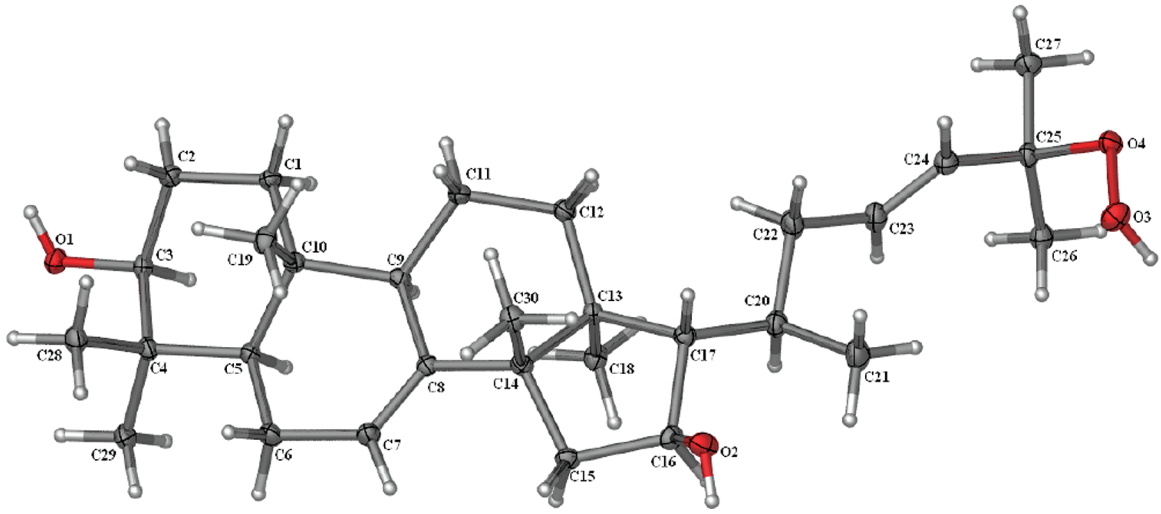


Figure 2. Single-crystal X-ray structure of **1**.

cal shifts of H-24/C-24 in an *S*-configuration (**6**: δ 4.35/δ 89.23) were slightly lower field compared to those of the corresponding *R*-configuration (**7**: δ 4.33/δ 89.19). These findings were in good agreement with those of 24*S*/24*R*-hydroxylated cycloartane-type triterpenoids possessing a side chain similar to **6** and **7**, in which the configuration at C-24 was successfully defined by application of the modified Mosher's method.[10]

Compounds **1**−**7** are euphane-type triterpenoids, each bearing a hydroperoxy group in the side chain, and hydroperoxylated triterpenoids are often found in *Melia* spp.[2a,4] Naturally occurring epimeric mixtures of 24-hydroperoxylated triterpenoids have also been isolated from other plants (e.g., *Xanthosoma robustum*[11] and *Tillandsia recurvata*[12]). According to a hypothesis raised by Cabrera and Seldes,[12] compounds **1**−**4** might be biosynthesized from the known compound **13** (kulinone) through a naturally sensitized photooxygenation in the plant. The combination of molecular oxygen with olefins is a common phenomenon, which often involves the formation of an allylic hydroperoxide from an olefin by a process involving abstraction of an allylic proton along with migration of the carbon−carbon double bond. As there are two different types of allylic protons in **13** and as photooxygenation is nonstereoselective, different products could be obtained.[12] Similarly, kulactone (**5b**) could be considered as the biosynthetic precursor of compounds **5**−**7**. However, the above photooxygenation process might be restricted because of steric (spatial) strain between the hydroperoxy group and the bulky γ-lactone formed at C-21 and C-16, and hence both **6** and **7** were separable (Experimental Section). Kulactone (**5b**) was previously isolated from the bark of both *M. dubia*[4] and *M. azedarach*,[5] but unfortunately it was not obtained from the fruits of *M. toosendan* this time.

Table 3. ^{1}H (500 MHz, *J* in Hz) and ^{13}C (125 MHz) NMR Data of Compounds **8**−**10** (in $CDCl_3$, 298 K)[a]

	8		**9**		**10**	
no.	δ_H (*J* in Hz)	δ_C, mult.	δ_H	.	δ_H	δ_C, mult.
1	1.15, m	37.2, CH_2	1.21, m	36.8, CH_2	1.22, m	36.7, CH_2
	1.68, m		1.67, m		1.67, m	
2	1.58, m	27.7, CH_2	1.65, m	24.2, CH_2	1.65, m	24.2, CH_2
	1.62, m					
3	3.24, dd (11.3, 3.9)	79.2, CH	4.52, dd (11.1, 3.7)	80.7, CH	4.52, dd (11.3, 3.9)	80.7, CH
4		39.0, qC		37.9, qC		37.9, qC
5	1.31, overlapped	50.7, CH	1.41, dd (12.0, 5.6)	50.7[b], CH	1.41, dd (12.0, 5.6)	50.7, CH
6	1.97, m	24.0, CH_2	1.97, m	23.8, CH_2	1.97, m	23.7, CH_2
	2.13, m		2.10, m		2.10, m	
7	5.26, br s	118.1, CH	5.26, br d (2.7)	118.0, CH	5.26, br d (3.1)	118.0, CH
8		145.6, qC		145.5, qC		145.5, qC
9	2.24, m	48.8, CH	2.23, m	48.7, CH	2.28, m	48.6, CH
10		35.0, qC		34.9, qC		34.9[c], qC
11	1.48, m	17.7, CH_2	1.50, m	17.7, CH_2	1.50, m, overlapped	17.6, CH_2
	1.56, m		1.58, m		1.57, m	
12	1.32, m	31.1, CH_2	1.31, m	31.1, CH_2	1.51, m	31.6, CH_2
	1.88, m		1.86, m, overlapped		1.72, m	
13		43.6, qC		43.5, qC		43.7, qC
14		50.8, qC		50.7[b], qC		50.9, qC
15	1.49, m	34.2, CH_2	1.49, m, overlapped	34.2, CH_2	1.71, m	34.4, CH_2
	1.51, m		1.51, m, overlapped		1.92, m	
16	1.31, m	27.4, CH_2	1.22, m	27.3, CH_2	1.24, m	27.4, CH_2
	1.87, m		1.86, m, overlapped		1.88, m	
17	2.02, m, overlapped	45.1, CH	2.01, m	45.0, CH	1.76, m	50.3, CH
18	0.84, s	23.2, CH_3	0.84, s	23.2, CH_3	0.84, s	22.5, CH_3
19	0.75, s	13.1, CH_3	0.78, s	13.1, CH_3	0.78, s	13.1, CH_3
20	2.00, m, overlapped	46.8, CH	1.99, m	46.3, CH	2.10, m	47.7, CH
21	4.75, br s	104.6, CH	4.70, d (3.5)	104.9, CH	4.78, d (3.5)	109.0, CH
22	1.62, m 2.01, m, overlapped	31.9, CH_2	1.88, m	31.6, CH_2	1.50, m, overlapped	33.7, CH_2
23	3.83, ddd (9.8, 7.9, 7.7)	78.6, CH	4.43, br dt (9.1, 1.9)	78.8, CH	4.22, br dt (9.2, 1.7)	76.7, CH
24	2.74, d (7.9)	67.8, CH	3.17, br s	76.6, CH	3.24, br d (8.4)	75.5, CH
25		57.2, qC		72.9, qC		73.1, qC
26	1.31, s	24.9, CH_3	1.26, s	26.3, CH_3	1.26, s	26.3, CH_3
27	1.27, s	19.3, CH_3	1.27, s	26.4, CH_3	1.28, s	26.4, CH_3
28	0.86, s	14.7, CH_3	0.85, s	15.9, CH_3	0.86, s	15.9, CH_3
29	0.97, s	27.6, CH_3	0.94, s	27.6, CH_3	0.92, s	27.6, CH_3
30	0.99, s	27.3, CH_3	0.98, s	27.2, CH_3	0.96, s	27.1, CH_3
21-OMe	3.36, s	54.1, CH_3	3.36, s	55.2, CH_3	3.35, s	55.6, CH_3
$CH_3(CH_2)_{n-1}CH_2\underline{C}OO-$				173.7, qC		173.7, qC
$CH_3(CH_2)_{n-1}\underline{CH_2}COO-$			2.29, t (7.4)	34.8, CH_2	2.29, t (7.5)	34.9[c], CH_2
$CH_3(\underline{CH_2})_{n-1}CH_2COO-$			1.25−1.28	22.7, CH_2	1.25−1.29	22.5, CH_2
				29.1−29.6, CH_2		29.1−29.6, CH_2
				31.9, CH_2		31.9, CH_2
$\underline{CH_3}(CH_2)_{n-1}CH_2COO-$			0.88, t (6.8)	14.1, CH_2	0.87, t (6.8)	14.1, CH_2

[a] Assignments were made by a combination of 1D and 2D (COSY, HSQC, HMBC) NMR experiments. [b,c] Signals were overlapped within the same superscript in the same column.

Meliasenin P (**8**) had the molecular formula $C_{31}H_{50}O_4$ (HRESIMS), which corresponded to nine unsaturation degrees in the structure. The IR spectrum indicated the presence of OH (3433 cm^{-1}), double-bond (1629 cm^{-1}), and epoxide (1247 and 1078 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 3) showed seven tertiary methyl groups (δ 0.75, 0.84, 0.86, 0.97, 0.99, 1.27, and 1.31), one OCH_3 (δ 3.36), and one olefinic proton (δ 5.26). The ^{13}C and DEPT NMR spectra of **1** indicated that the molecule contained seven sp^3 methyl, eight sp^3 methylene, eight sp^3 (four oxygenated at δ 67.8, 78.6, 79.2, and 104.6) and one sp^2 (δ 118.1) methine, and five sp^3 (one oxygenated at δ 57.2) and one sp^2 (δ 145.6) quaternary carbon, in addition to an OCH_3 carbon (δ 54.1). These data suggested that **8** was a tirucallane-type triterpenoid,[13,14] with a tetrahydrofuran ring at C-21 and C-23, an OCH_3 bound to the hemiacetalic C-21, and a 24,25-epoxy group in the side chain. In the HMBC spectrum, key correlations were observed between H-21 and C-23, between H-21 and OMe, between H-23 and C-24, and between Me-26/Me-27 and C-24. The relative configuration of the chiral centers in the tetrahydrofuran ring and the orientation of the epoxide ring in the side chain were indicated by a combination of the coupling patterns and a NOESY NMR experiment. H-21 was a broad singlet at δ 4.75, and hence both H-20 and H-21 were *cis*. NOE correlations were observed between Me-18 and H-20, between Me-18 and H-21, between H-20 and H-21, between H-21 and H-22α, between H-20 and H-23, H-22α, and H-23, between H-23 and Me-27, between Me-30 and H-17, between H-17 and H-22β, between H-22β and H-24, and between H-24 and Me-26 (Figure 1), indicating that H-20, H-21, H-23, and the 24,25-epoxide ring were all in α-orientation. It is noteworthy that the chemical shift (δ 104.6) of C-21 bound with a β-OMe was in agreement with a ^{13}C NMR-based general rule for assignment of the C-21 configuration provided by Yue et al. in 2007.[13]

Meliasenin Q (**9**) had the molecular formula $C_{47}H_{82}O_6$, and the ^{1}H NMR spectrum (Table 3) showed seven tertiary methyl groups (δ 0.78, 0.84, 0.85, 0.94, 0.98, 1.26, and 1.27), one OCH_3 (δ 3.36), and one olefinic proton (δ 5.26), which closely resembled those of **8**. Differing from **8**, **9** contained an ester moiety. This was supported by a typical ester absorption band (1731 cm^{-1}) in its IR spectrum and a carbonyl carbon at δ 173.7 in the ^{13}C NMR spectrum (Table 3). The 24,25-epoxide ring in the side chain of **8** was opened in **9** on the basis of both C-24 and C-25 having shifted downfield to δ 76.6 and 72.9, respectively (Table 3). Taking the molecular formula into account, the ester moiety was determined to be a palmitoyl group, which was linked at C-3 by an HMBC correlation between H-3 at δ 4.52 (1H, dd, J = 11.1, 3.7 Hz) and the ester carbonyl carbon. On the basis of the similarity of the NOEs the configurations of **9** and **8** were considered to be the same. In the side chain of **9**, H-20 and H-21 were on the same side of the tetrahydrofuran ring, as indicated by the small coupling constant ($J_{20,21}$ = 3.5 Hz) between H-21 at δ 4.70 and H-20 at δ 1.99. NOE correlations between Me-

18 and H-20, between H-20 and H-21, and between H-20 and H-23 indicated that H-20, H-21, and H-23 were all α-oriented. The H-24 signal at δ 3.17 was a broad singlet, requiring a dihedral angle of almost 90° between H-23α and H-24β. The chemical shift (δ 104.9) of C-21 in **9** was also in good agreement with the presence of a 21β-OMe group in the structure.[13,15]

The general features in the ^{1}H and ^{13}C NMR spectra (Table 3) of meliasenin R (**10**) were very similar to those of **9**. The HRESIMS of **10** exhibited three sets of data corresponding to three molecular formulas: $C_{43}H_{74}O_6$, $C_{45}H_{78}O_6$, and $C_{47}H_{82}O_6$. These data indicated that **10** was a mixture consisting of triterpene alcohol esters of fatty acids (lauric, myristic, and palmitic acids) similar to lipomelianol (**10a**), a tirucallane-type triterpenoid derivative that was previously isolated from the fruits of *M. toosendan*.[14] Lipomelianol (**10a**) was a mixture (in solution) of two epimers with respect to the hemiacetalic C-21; however, **10** was an optically pure compound with an α-OMe group anchored at C-21 on the basis of its chemical shift (δ 109.0).[13,15] The structure of **10** was verified by extensive 2D NMR (COSY, HMQC, HMBC, and NOESY) experiments.

The molecular formula, $C_{29}H_{50}O_4$, of compound **11** was deduced by HRESIMS, from an $[M + Na]^+$ peak at *m/z* 485.3613. The IR spectrum of **11** showed strong absorption bands at 3385 cm^{-1} (OH groups) and 1640 cm^{-1} (double bond). ^{1}H NMR signals for three oxymethines at δ 3.57 (1H, m, H-3α), 3.85 (1H, br s, H-7β), and 4.65 (1H, dt, J = 7.4, 3.6 Hz, H-16α) and one olefinic proton resonating at δ 5.61 (1H, br d, J = 4.0 Hz, H-6) were observed. The ^{13}C NMR and DEPT NMR spectra of **11** exhibited 29 peaks, classified as six methyl, nine sp^3 methylene, one sp^2 (δ 123.7) and nine sp^3 (three oxygenated at δ 74.2, 71.3, 65.2) methine and one sp^2 (δ 146.5) and three sp^3 (one oxygenated at δ 76.8) quaternary carbons. The above data were very similar to those of (20*S*)-5-ergostene-3β,7α,16β,20-tetrol (**11a**), a C_{28}-steroid previously isolated from *M. azedarach*.[3d] The only difference between these two steroids was that the methyl group at C-24 in **11a** was replaced by a terminal ethyl group [1.33 (1H, m, H-28)/1.28 (1H, m, H-28′) and 0.88 (3H, t, J = 7.4 Hz, Me-29)] in **11**. Therefore, **11** was determined to be (20*S*)-5-stigmastene-3β,7α,16β,20-tetrol.

Except for the common plant constituents **16** and **17**, the known isolates (**12**−**15** and **18**−**22**) were obtained from *M. toosendan* for the first time. Compounds **12**−**15** were previously found to be cytotoxic against the P388 cancer cell line, with ED_{50} values of 1.7−5.1 μg/mL.[4] Compounds **21** and **22** previously exhibited antiproliferative activity against the MCF-7 cell line at 10 μM.[7] In this study, the isolated triterpenoids (**1**−**10**, **12**−**15**) and two selected steroids (**11**, **20**) were tested for cytotoxicity against U20S human osteosarcoma cells and MCF-7 human breast cancer cells using the MTT assay method. The results (Table 4) showed that most compounds exhibited significant cytotoxic effects against these two cell lines. Meliasenin Q (**9**) had the strongest cytotoxic effect against the U20S cell line, with an IC_{50} value of 3.9 μg/mL; however, meliasenin R (**10**), with a different configuration at C-21, was not active (IC_{50} >50 μg/mL) against U20S cells. Methyl kulonate (**14**) showed the most significant cytotoxic effect against the MCF-7 cell line, with an IC_{50} value of 0.41 μg/mL, whereas its C-21 methoxycarbonylated derivative (**13**) was about 100 times less active (IC_{50} 42.5 μg/mL) on MCF-7 cells. Selected isolates (**1**−**15** and **20**) were also tested against aphids (*Lipaphis erysimi* Kaltenbach) for their insecticidal properties, but they were all inactive.

Table 4. Cytotoxicity of Compounds **1**−**15** and **20** against Human Cancer Cell Lines

	IC_{50} (μg/mL, mean ± SEM)[b]	
compound[a]	U20S	MCF-7
1	9.5 ± 0.90	11.2 ± 0.04
2	14.3 ± 0.74	1.1 ± 0.59
3	7.2 ± 0.63	2.4 ± 0.11
4	9.5 ± 0.70	9.8 ± 0.38
5	29.2 ± 0.75	16.4 ± 0.12
6	8.8 ± 0.59	3.0 ± 0.08
7	23.9 ± 0.93	2.3 ± 0.12
8	48.7 ± 0.71	6.4 ± 0.11
9	3.9 ± 0.80	>50
10	>50	28.1 ± 0.12
11	23.2 ± 0.52	>50
12	31.1 ± 0.64	48.6 ± 0.04
13	6.9 ± 0.91	42.5 ± 0.08
14	14.6 ± 0.49	0.41 ± 0.02
15	9.5 ± 0.90	16.0 ± 0.05
20	9.4 ± 0.74	47.4 ± 0.51
5-FU[c]	7.2 ± 0.97	34.0 ± 0.05

[a] Purity of tested compounds and the positive control ranged from 96.2% to 99.5% as determined by analytical HPLC with ELSD detection. [b] IC_{50} values refer to the 50% inhibition concentration and were calculated from regression using six different concentrations of at least three independent assays. [c] 5-FU, 5-fluorouracil (Sigma-Aldrich, catalog no. F6627), was used as a positive control.

Experimental Section

General Experimental Procedures. Melting points were measured on a WRS-1A digital melting-point apparatus (Shanghai YICE Apparatus & Equipments Co., Ltd.), but are uncorrected. Optical rotations were determined by using a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet NEXUS-670 FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer. Chemical shifts are expressed in δ (ppm) and are referenced to the residual solvent signals. Electrospray ionization mass spectra (ESIMS) were measured on a Bruker Daltonics micrOTOF-QII mass spectrometer. Semipreparative HPLC was performed on a Beckman system consisting of a Beckman Coulter System Gold 508 autosampler, Gold 126 gradient HPLC pumps with a Beckman System Gold 168 UV detector, a Sedex 80 (SEDERE, France) evaporative light-scattering detector (ELSD), a YMC-Pack ODS-A column (250 × 10 mm, dp 5 μm) (reverse phase), and a Kromasil-Si column (250 × 10 mm, dp 5 μm) (normal phase). Column chromatography (CC) was performed using silica gel (200−300 mesh, Ji-Yi-Da Silysia Chemical Ltd., Qingdao, China), MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Industries, Tokyo, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Silica gel-precoated plates (GF_{254}, 0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd., Yantai, China) were used for TLC detection. Spots were visualized using UV light (254 and/or 366 nm) and by spraying with 5% (v/v) H_2SO_4−EtOH followed by heating to 120 °C.

Plant Material. Dried fruits of *M. toosendan* were purchased from Shanghai Jiu-Zhou-Tong Medicine Co. Ltd. and were originally collected in October 2007 from Wanyuan County, Sichuan Province of China. The plant was identified by Prof. Jian-Wei Chen (College of Pharmacy, Nanjing University of Traditional Chinese Medicine). A voucher specimen (No. 100310) was deposited at the Herbarium of the Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University.

Extraction and Isolation. The dried fruits (10.0 kg) were extracted three times with 95% EtOH (3 × 10 L) at room temperature. The solvent was removed at reduced pressure to give a brown residue (675 g). The entire crude extract was suspended in H_2O (3 L) and extracted with EtOAc five times (5 × 3 L) to give an EtOAc extract (246 g), which was then subjected to CC over silica gel (column: 90 × 9 cm) with a petroleum ether (PE)−acetone gradient (15:1−1:1 to acetone neat, v/v) to yield 10 fractions (1−10). Fraction 2 (PE−acetone, 10:1, ca. 2.4 g) was chromatographed on silica gel (column: 70 × 4 cm) with a CH_2Cl_2−acetone gradient (10:1−8:1) to furnish compounds **16** (25.2 mg), **21** (8.9 mg), and **22** (9.5 mg). Compounds **2** (12.2 mg), **4** (25.8 mg), and **5** (17.5 mg) were isolated from fraction 3 (PE−acetone, 9:1, ca. 3.2 g) via silica gel CC (column: 70 × 3 cm) with a PE−EtOAc gradient (8:1−4:1). Fraction 6 (PE−acetone, 4:1, ca. 8.8 g) was chromatographed on silica gel (column: 70 × 6 cm) using a gradient of CH_2Cl_2−EtOAc (8:1−4:1) to afford five subfractions (6A−6F). Compounds **8** (19.1 mg), **13** (17.5 mg), and **14** (12.4 mg) were isolated from fraction 6C (CH_2Cl_2−EtOAc, 6:1, ca. 2.8 g) through silica gel CC (column: 70 × 3 cm) with PE−EtOAc (5:1) and were further purified by gel permeation chromatography on Sephadex LH-20 (column: 110 × 2.5 cm) in MeOH. Compounds **6** (10.5 mg) and **7** (15.1 mg) were separated from fraction 6D (CH_2Cl_2−EtOAc, 4:1, ca.

3.2 g) by using semipreparative normal-phase HPLC. The method employed consisted of a linear gradient of EtOAc in *n*-hexane from 30% to 70% over 10 min, then followed by an isocratic gradient of 70% EtOAc for 30 min and finally by 95% EtOAc for 5 min (flow rate: 3 mL/min; **6**: t_R = 30.0 min; **7**: t_R = 29.0 min). Fraction 6E (CH_2Cl_2−EtOAc, 3:1, ca. 2.1 g) was subjected to CC over MCI-gel using stepwise gradient elution with MeOH−H_2O (from 1:1 to 100% MeOH) to yield compounds **9** (8.2 mg), **10** (14.0 mg) **12** (12.8 mg), **15** (23.7 mg), **18** (15.2 mg), and **19** (14.9 mg). Compounds **1** (8.6 mg) and **3** (7.5 mg) were purified from fraction 6F (CH_2Cl_2−EtOAc, 2:1, ca. 2.0 g) by repeated silica gel CC (40 × 3 cm) using CH_2Cl_2−acetone (4:1, v/v) as the eluting solvent.

Compounds **11** (22.1 mg) and **20** (12.9 mg) were isolated from fraction 8 (PE−acetone, 1:1, ca. 4.8 g) by semipreparative reversed-phase HPLC. The method was a linear gradient of CH_3CN in H_2O from 40% to 80% over 10 min, followed by 80% CH_3CN for 30 min and then by 95% CH_3CN for 5 min (flow rate: 3 mL/min; **11**: t_R = 27.0 min; **20**: t_R = 31.0 min). Compound **17** (77.6 mg) was obtained from fraction 10 (acetone, 2.9 g) by crystallization from EtOAc.

Meliasenin I (1): monoclinic crystals (CH_3OH), mp 216.0−217 °C, $[\alpha]^{22}_D$ +49.2 (*c* 0.355, MeOH); IR (KBr) ν_{max} 3408−3226 (br), 2970, 2937, 1637, 1462, 1384, 1363, 1300, 1266, 1145, 1097, 1035, 969 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 497 $[M + Na]^+$, 971 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 497.3615 (calcd for $C_{30}H_{50}O_4Na$, 497.3601).

Meliasenin J (2): colorless, amorphous powder, $[\alpha]^{22}_D$ −9.0 (*c* 0.120, MeOH); IR (KBr) ν_{max} 3449 (br), 2946, 2856, 1708, 1623, 1455, 1382, 1014, 966 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 495 $[M + Na]^+$, 967 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 495.3470 (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

Meliasenin K (3): colorless, amorphous powder, $[\alpha]^{22}_D$ +3.4 (*c* 0.345, MeOH); IR (KBr) ν_{max} 3410 (br), 2970, 2933, 1621, 1460, 1384, 1300, 1266, 1145, 1097, 1035, 969 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 497 $[M + Na]^+$, 971 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 497.3610 (calcd for $C_{30}H_{50}O_4Na$, 497.3601).

Meliasenin L (4): colorless, amorphous powder, $[\alpha]^{22}_D$ −8.0 (*c* 0.150, MeOH); IR (KBr) ν_{max} 3422 (br), 2946, 2856, 1703, 1637, 1455, 1384, 1012, 966, 901 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 495 $[M + Na]^+$, 967 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 495.3454 (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

Meliasenin M (5): colorless, amorphous powder, $[\alpha]^{22}_D$ −47.0 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3423 (br), 2968, 2942, 2875, 1778, 1705, 1624, 1450, 1384, 1157, 1138, 1018, 953, 920, 835 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 507 $[M + Na]^+$, 991 $[2 M + Na]^+$; (−)-ESIMS *m/z* 519 $[M + Cl]^-$, 1003 $[2 M + Cl]^-$; (+)-HREIMS *m/z* 507.3101 $[M + Na]^+$ (calcd for $C_{30}H_{44}O_5Na$, 507.3081).

Meliasenin N (6): colorless, amorphous powder, $[\alpha]^{22}_D$ −39.0 (*c* 0.105, MeOH); IR (KBr) ν_{max} 3432 (br), 2945, 2872, 1780, 1703, 1639, 1448, 1385, 1161, 1056, 1028 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 507 $[M + Na]^+$, 991 $[2 M + Na]^+$; (+)-HRESI-MS *m/z* 507.3129 (calcd for $C_{30}H_{44}O_5Na$, 507.3081).

Meliasenin O (7): colorless, amorphous powder, $[\alpha]^{22}_D$ −72.0 (*c* 0.398, MeOH); IR (KBr) ν_{max} 3395 (br), 2927, 2855, 1779, 1706, 1636, 1454, 1384, 1162, 1088, 1058, 1027 cm^{-1}; ^1H and ^{13}C NMR data, Tables 1 and 2; (+)-ESIMS *m/z* 507 $[M + Na]^+$, 991 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 507.3108 (calcd for $C_{30}H_{44}O_5Na$, 507.3081).

Meliasenin P (8): colorless, amorphous powder, $[\alpha]^{22}_D$ +34.0 (*c* 0.180, MeOH); IR (KBr) ν_{max} 3433 (br), 2927, 2853, 1629, 1459, 1382, 1247, 1211, 1148, 1109, 1078, 1033, 979, 873, 819, 723 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; (+)-ESIMS *m/z* 509 $[M + Na]^+$, 995 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 509.3642 (calcd for $C_{31}H_{50}O_4Na$, 509.3601).

Meliasenin Q (9): colorless oil, $[\alpha]^{22}_D$ −14.0 (*c* 0.215, MeOH); IR (KBr) ν_{max} 3449 (br), 2925, 2854, 1731, 1635, 1462, 1374, 1247, 1207, 1146, 1107, 1093, 1077, 1035, 1013, 980 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; (+)-ESIMS *m/z* 765 $[M + Na]^+$, 1508 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 765.6021 (calcd for $C_{47}H_{82}O_6Na$, 765.6004).

Meliasenin R (10): colorless oil, $[\alpha]^{22}_D$ −31.0 (*c* 0.205, MeOH); IR (KBr) ν_{max} 3449 (br), 2925, 2854, 1731, 1638, 1463, 1383, 1245, 1201, 1151, 1109, 1097, 1075, 1034, 1011, 980 cm^{-1}; ^1H and ^{13}C NMR data, Table 3; (+)-ESIMS *m/z* 709 $[M^1 + Na]^+$, 737 $[M^2 + Na]^+$, 765 $[M^3 + Na]^+$, 1396 $[2M^1 + Na]^+$, 1452 $[2M^2 + Na]^+$, 1508 $[2M^3 + Na]^+$; (−)-ESIMS *m/z* 721 $[M^1 + Cl]^-$, 749 $[M^2 + Cl]^-$, 777 $[M^3 + Cl]^-$; (+)-HRESIMS *m/z* M_1 709.5371 (calcd for $C_{43}H_{74}O_6Na$, 709.5378), M_2 737.5672 (calcd for $C_{45}H_{78}O_6Na$, 737.5691), M_3 765.5980 (calcd for $C_{47}H_{82}O_6Na$, 765.6004).

(20*S*)-5-Stigmastene-3*β*,7α,16*β*,20-tetrol (11): colorless, amorphous powder, $[\alpha]^{22}_D$ +126.0 (*c* 0.210, MeOH); IR (KBr) ν_{max} 3385 (br), 2934, 2869, 1640, 1462, 1380, 1057, 955 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.61 (1H, br d, *J* = 4.0 Hz, H-6), 4.65 (1H, dt, *J* = 7.4, 3.6 Hz, H-16α), 3.85, (1H, br s, H-7*β*), 3.57 (1H, m, H-3α), 2.41 (1H, m, H-15), 2.35 (1H, m, H-4), 2.34 (1H, m, H-4′), 2.15 (1H, m, H-12), 1.86 (1H, m, H-1), 1.85 (1H, m, H-2), 1.84 (1H, m, H-22), 1.60 (1H, m, H-8), 1.58 (1H, m, H-2′), 1.57 (2H, m, H-11), 1.49 (1H, m, H-22′), 1.42 (1H, m, H-25), 1.36 (1H, m, H-15′), 1.35 (1H, m, H-23), 1.35 (1H, m, H-14), 1.34 (1H, d, overlapped, H-17), 1.33 (1H, m, H-28), 1.31 (3H, s, Me-21), 1.28 (1H, m, H-28′), 1.24 (1H, m, H-9), 1.18 (1H, m, H-12′), 1.17 (3H, s, Me-18), 1.11 (1H, m, H-23′), 1.08 (1H, m, H-1′), 1.02 (3H, s, Me-19), 0.99 (1H, m, H-24), 0.88 (3H, t, *J* = 7.4 Hz, Me-29). 0.85 (3H, d, *J* = 6.6 Hz, H-26), 0.84 (3H, d, *J* = 6.6 Hz, H-27); ^{13}C NMR ($CDCl_3$) δ 146.5 (C-5), 123.7 (C-6), 76.8 (C-20), 74.2 (C-16), 71.3 (C-3), 65.2 (C-7), 59.4 (C-17), 47.7 (C-14), 46.1 (C-24), 42.6 (C-13), 42.5 (C-22), 42.2 (C-9), 41.9 (C-4), 39.7 (C-12), 37.4 (C-1), 37.4 (C-10), 36.9 (C-15), 36.5 (C-8), 31.3 (C-2), 29.1 (C-25), 27.0 (C-21), 24.9 (C-23), 23.1 (C-28), 20.3 (C-11), 19.6 (C-27), 19.2 (C-26), 18.3 (C-19), 14.7 (C-18), 12.1 (C-29); (+)-ESIMS *m/z* 485 $[M + Na]^+$, 947 $[2 M + Na]^+$; (+)-HRESIMS *m/z* 485.3613 (calcd for $C_{29}H_{50}O_4Na$, 485.3601).

X-ray Crystallographic Analysis of Meliasenin I (1). A colorless crystal was obtained from a solution of MeOH. The X-ray data were collected on a Bruker-AXS SMART APEX II CCD diffractometer, formula $C_{30}H_{50}O_4$, M_r = 474.70; triclinic crystal system; space group *P*1; *a* = 6.4322(3) Å, *b* = 13.3812(7) Å, *c* = 32.9546(17) Å, *V* = 2836.4(2) $Å^3$; *Z* = 4, *d* = 1.112 mg/m^3; crystal dimensions 0.34 × 0.14 × 0.11 mm^3; the final indices were R_1 = 0.0376, wR_2 = 0.0795.

Cytotoxicity Assay. The U20S human osteosarcoma and MCF-7 human breast cancer cell lines were purchased from the cell bank of Shanghai Institute of Cell Biology (Shanghai, China) and were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 units/mL streptomycin (Gibco BRL Gaithersburg, MD, USA), in a 37 °C incubator under an atmosphere of 5% CO_2.

Inhibition of cell proliferation was assessed by a standard MTT-based colorimetric assay.[3c] Briefly, the test cells in exponential growth were plated at a final concentration of 1 × 10^4 cells/well in 96-well tissue culture dishes for 12 h. The cells were then treated with compounds at varying concentrations. In order to exclude phototoxicity,[16] the process was kept away from bright light and the cells were incubated in a dark incubator. After 36 h, the cells were incubated in fresh cell culture medium without FBS. Then, MTT (U20S: 20 μL, 5 mg/mL; MCF-7: 10 μL, 5 mg/mL) was added. After incubating for an additional 4 h, the supernatant was discarded and 150 μL of DMSO was added. After 15 min, the optical density of each well was measured at 490 nm using an enzyme-immunoassay instrument. IC_{50} was calculated from the curves generated by plotting the percentage of the viable cells versus the test concentration on a logarithmic scale using SigmaPlot 10.0 software.

Acknowledgment. The authors thank Prof. Jian-Wei Chen (Nanjing University of Traditional Chinese Medicine) for the plant identification. We gratefully acknowledge Prof. Yi-Hua Yu from Shanghai Key Laboratory of Functional Magnetic Resonance Imaging (ECNU) for his assistance with NMR data acquisition. This work was supported by an NSFC grant (No. 90713040), STCSM grants (Nos. 06DZ19002, 07DZ22006), an overseas research grant from the Graduate School of East China Normal University, and a grant from the State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica (SIMM), Chinese Academy of Sciences (CAS).

Supporting Information Available: The 1D-/2D-NMR and HRESIMS spectra of compounds **1**−**11** and the cif file of X-ray data of compound **1** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Zhao, L.; Huo, C.-H.; Shen, L.-R.; Yang, Y.; Zhang, Q.; Shi, Q.-W. *Chem. Biodiversity* **2010**, *7*, 839–859. (b) Vishnukanta, A. C. R. *Pharmacogn. Rev.* **2008**, *2*, 173–179. (c) Mulholland, D. A.; Parel, B.; Coombes, P. H. *Curr. Org. Chem.* **2000**, *4*, 1011–1054.

(2) (a) Zhang, Y.; Tang, C.-P.; Ke, C.-Q.; Yao, S.; Ye, Y. *J. Nat. Prod.* **2010**, *73*, 664–668. (b) Sang, Y. S.; Zhou, C. Y.; Lu, A. J.; Yin, X. J.;

Min, Z. D.; Tan, R. X. *J. Nat. Prod.* **2009**, *72*, 917–920. (c) Zhou, C.-Y.; Tang, H.-Y.; Li, X.; Sang, Y.-S. *Helv. Chim. Acta* **2009**, *92*, 1191–1197.

(3) (a) Li, X.-W.; Guo, Z.-T.; Zhao, Y.; Zhao, Z.; Hu, J.-F. *Phytochemistry* **2010**, *71*, 682–687. (b) Fan, H.; Zang, Y.; Zhang, Y.; Zhang, H.-F.; Zhao, Z.; Hu, J.-F. *Helv. Chim. Acta* . doi:, 10.1002/hlca.201000095. (c) Wu, S.-B.; Pang, F.; Wen, Y.; Zhang, H.-F.; Zhao, Z.; Hu, J.-F. *Planta Med.* **2010**, *76*, 82–85. (d) Wu, S.-B.; Ji, Y.-P.; Zhu, J.-J.; Zhao, Y.; Xia, G.; Hu, Y.-H.; Hu, J.-F. *Steroids* **2009**, *74*, 761–765.

(4) Pettit, G. R.; Numata, A.; Iwamoto, C.; Morito, H.; Yamada, T.; Goswami, A.; Clewlow, P. J.; Cragg, G. M.; Schmidt, J. M. *J. Nat. Prod.* **2002**, *65*, 1886–1891.

(5) Chiang, C.-K.; Chang, F. C. *Tetrahedron* **1973**, *29*, 1911–1929.

(6) Chaurasia, N.; Wichtl, M. *J. Nat. Prod.* **1987**, *50*, 881–885.

(7) Ioannou, E.; Abdel-Razik, A. F.; Zervou, M.; Christofidis, D.; Alexi, X.; Vagias, C.; Alexis, M. N.; Roussis, V. *Steroids* **2009**, *74*, 73–80.

(8) Crystallographic data for compound **1** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-773391. Copies of the data can be obtained, free of charge, on application to the Director, 12 Union Road, Cambridge CB2 1EZ, UK [fax +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

(9) The new triterpenoids **1**−**10** were named meliasenins I−R, respectively. The trivial name was sequentially derived from the most recent work of Ye and co-workers (ref 2a herein).

(10) Luo, H.-F.; Li, Q. L.; Yu, S. G.; Badger, T. M.; Fang, N. B. *J. Nat. Prod.* **2005**, *68*, 94–97.

(11) Kato, T.; Frei, B.; Heinrich, M.; Sticher, O. *Phytochemistry* **1996**, *41*, 1191–1195.

(12) Cabrera, G. M.; Seldes, A. M. *J. Nat. Prod.* **1995**, *58*, 1920–1924.

(13) Xie, B.-J.; Yang, S.-P.; Chen, H.-D.; Yue, J.-M. *J. Nat. Prod.* **2007**, *70*, 1532–1535.

(14) Nakanishi, T.; Inada, A.; Lavie, D. *Chem. Pharm. Bull.* **1986**, *34*, 100–104.

(15) (a) Mitsui, K.; Maejima, M.; Saito, H.; Fukaya, H.; Hitotsuyanagi, Y.; Takeya, K. *Tetrahedron* **2005**, *61*, 10569–10582. (b) Biavatti, M. W.; Vieira, P. C.; da Silva, M. F. G. F.; Fernandes, J. B.; Albuquerque, S. *J. Nat. Prod.* **2002**, *65*, 562–565.

(16) Colombain, M.; Goll, V.; Muyard, F.; Girard, C.; Bévalot, F.; Richert, L. *Planta Med.* **2001**, *67*, 644–646.

NP100566V