Cytotoxic Triterpenoids from the Leaves of *Microtropis fokienensis*

I-Hsiao Chen,[†] Fang-Rong Chang,*[,†] Chin-Chung Wu,[†] Shu-Li Chen,[†] Pei-Wen Hsieh,[†] Hsin-Fu Yen,[‡] Ying-Chi Du,[†] and Yang-Chang Wu*[,†]

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, Republic of China, and National Museum of Natural Science, Taichung 404, Taiwan, Republic of China

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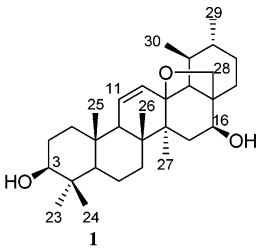
Five new triterpenoids, microfokienoxanes A−D (**1**−**4**) and 3β,28-dihydroxy-11α-methoxyurs-12-ene (**5**), were isolated and identified from the leaves of *Microtropis fokienensis*, along with nine known compounds. The structures of the new compounds were elucidated by spectroscopic methods. The compounds obtained in this investigation were evaluated against a small panel of human cancer cell lines for cytotoxicity. Only compounds **3** and **5** exhibited cytotoxicity (IC_{50} $\leq$ 5 μg/mL) for one or more cell lines.

About 70 species of *Microtropis* belonging to the family Celastraceae are distributed in India, Malaysia, Mainland China, Japan, Central America, and Mexico. Two species, *M. fokienensis* Dunn. and *M. japonica* (Fr. & Sav.) Hall. f., have been found in Taiwan.[1] Recently, Chen et al. reported several cytotoxic dihydroagarofuran sesquiterpenes from the stems of *M. fokienensis*.[2]

In a continuing search for bioactive compounds from Celastraceous plants,[3−6] a MeOH−H_2O extract of the leaves of *M. fokienensis* was found to be cytotoxic and selected for fractionation. We report herein the isolation and structural elucidation of five new compounds, microfokienoxanes A−D (**1**−**4**) and 3β,28-dihydroxy-11α-methoxyurs-12-ene (**5**), along with nine known compounds, 3β,28-dihydroxyurs-12-ene,[7,8] 13β,28-epoxy-3β-hydroxyolean-11-ene,[7] 13β,28-epoxy-3β-hydroxyurs-11-ene,[8,9] 3β-hydroxy-11α-methoxyurs-12-ene,[9] 30-hydroxylupeol,[10] 30-hydroxybetulin,[10,11] 1-methoxy-4((*E*)-2-methoxyvinyl)benzene,[12] *epi*-catechin,[13,14] and kaempferol.[4] This is the first report of triterpenoids from this species. The cytotoxicity of the isolated compounds against a small panel of human cancer cells was also investigated.



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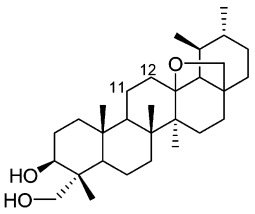
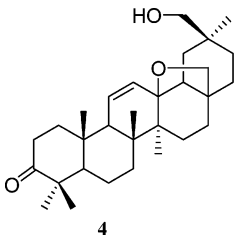


2 $\triangle^{11,12}$

3 C=O (C-12)



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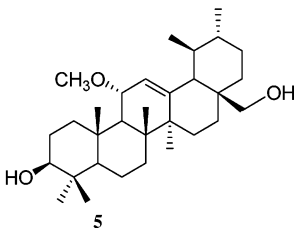

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Results and Discussion

Compound **1** was found to have the molecular formula $C_{30}H_{48}O_3$ on the basis of the HRESIMS molecular ion at *m*/*z* 479.3501 ([M + Na]$^+$, calcd for 479.3501), accounting for seven degrees of unsaturation. The IR data showed the presence of a hydroxyl group (3326 cm^{-1}). In the ^{1}H NMR spectrum, signals were observed for two olefinic protons at δ_H 5.53 (1H, dd, J = 10.4, 3.2 Hz) and 5.83 (1H, d, J = 10.4 Hz), an AB system of methylene protons on a carbon bonded to an oxygen at δ_H 3.00 (1H, d, J = 6.8 Hz) and 3.87 (1H, d, J = 6.8 Hz), two carbinol protons at δ_H 3.15 (1H, dd, J = 11.6, 4.4 Hz) and 4.20 (1H, dd, J = 9.4, 6.4 Hz), five tertiary methyl groups at δ_H 0.78, 0.92, 0.97, 1.08, and 1.13, and two secondary methyl groups at δ_H 0.96 and 1.00. On the basis of the molecular formula and the ^{1}H NMR spectroscopic data analysis, it was concluded that compound **1** is a triterpene based on the ursane skeleton,[15] with a double bond and six rings, one of which is an epoxide bridge between a CH_2 at δ_C 73.1 and a quaternary carbon at δ_C 85.8, as clearly shown by the ^{13}C NMR spectrum (Table 2). In the HMBC spectrum of **1** (Figure 1), the correlations of the signals due to a carbon doublet bearing a hydroxyl group at δ_C 79.6 with those of H-23 (δ_H 0.97) and H-24 (δ_H 0.78) of the *gem*-dimethyl moiety were used to place the hydroxyl group at C-3. The coupling constant of H-3 (J = 11.6, 4.4 Hz) indicated a β-orientation of the hydroxyl group at C-3.[11] In addition, the proton signals at δ_H 3.00 and 3.87 (H_2-28) correlated with the carbon signal at δ_C 66.4 (J_3), and the proton signals at δ_H 1.45 (H-15) with the carbon signals at δ_C 46.9 (J_2), 66.4 (J_2), and 85.8 (J_3). This clearly indicated that the hydroxyl group was attributed to C-16 and assigned with a β-configuration due to the coupling constant of H-16α (dd, J = 9.4, 6.4 Hz).[7,15] Moreover, the oxygenated methylene signals showed cross-peaks to the oxygen-bearing quaternary carbon signal at δ_C 85.8, which in turn correlated to the olefinic proton signal at δ_H 5.83. The disubstituted double bond was placed at the $\Delta^{11,12}$ position on the C ring of the ursane skeleton, with the sixth ring formed via an ether linkage between C-28 and C-13. Thus, compound **1** was elucidated as 13β,28-epoxy-3β,16β-dihydroxyurs-11-ene and named microfokienoxane A.

Compound **2** showed the molecular formula $C_{30}H_{48}O_3$, as confirmed by the HRESIMS molecular ion at *m*/*z* 479.3501 ([M + Na]$^+$, calcd for 479.3500) to be the same as **1**. The ^{1}H NMR spectrum (Table 1) suggested that compound **2** also possesses the ursane skeleton. Comparison of the ^{13}C NMR and HMBC spectral data of **2** with **1** showed that a hydroxyl group could be accommodated at the C-23 in **2**.[16] In the HMBC spectrum (Figure S1, Supporting Information), the proton signal at δ_H 3.76 (H-23) correlated with the carbon signal at δ_C 73.1 (J_3). This clearly indicated that the hydroxyl group was attached to C-3. Furthermore, a clear 1,3-diaxial correlation was observed between δ_H 4.25 (H-3) and 1.56 (H-5α) in the NOESY spectrum (Figure 2). These facts showed that the relative configuration of the hydroxyl group was β at C-3. From the aforementioned data, the structure of **2** was

* Corresponding authors. Tel: +886-7-312-1101, ext. 2197. Fax: +886-7-311-4773. E-mail: yachwu@kmu.edu.tw; aaronfrc@kmu.edu.tw.
[†] Kaohsiung Medical University.
[‡] National Museum of Natural Science.

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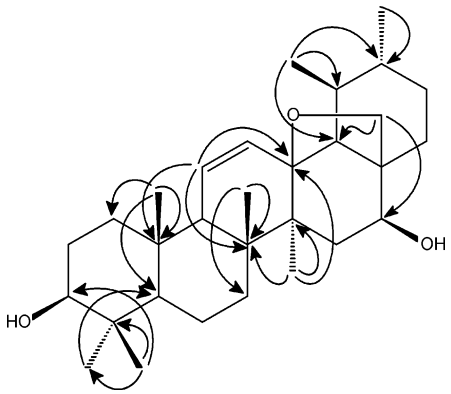

Figure 1. Key HMBC correlations of **1**.

determined as 13β,28-epoxy-3β,23-dihydroxyurs-11-ene, and this compound has been named microfokienoxane B.

Compound **3**, obtained as a powder, showed a molecular ion peak at *m*/*z* 495.3450 [M + Na]$^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3453) in the HRESIMS. Its IR spectrum contained absorption bands due to hydroxyl (3424 cm^{-1}) and carbonyl (1706 cm^{-1}) groups. According to the ^{1}H and ^{13}C NMR spectra (Table 1 and 2), **3** was similar to **2** expect for the presence of a carbonyl group and the lack of any olefinic proton in **3**. In the HMBC spectrum of **3** (Figure S2, Supporting Information), the proton signals at δ_H 2.54 and 2.71 (H-11) showed correlations with carbon signals at δ_C 42.1 (C-8), 48.9 (C-9), and 209.8 (C-12), while the proton signals at δ_H 3.74 and 4.24 (H-23) showed correlations with carbon signals at δ_C 72.8 (C-3) and 48.1 (C-5). Finally, the key NOESY correlations of **3** and its relative stereochemistry were determined as shown in Figure S3 (Supporting Information). According to the data obtained, the structure of **3** (microfokienoxane C) was determined as 13β,28-epoxy-3β,23-dihydroxyursan-12-one.

Compound **4** was assigned the molecular formula $C_{30}H_{46}O_3$ (HRESIMS, *m*/*z* 477.3344 [M + Na]$^+$, calcd for 477.3342). Its IR spectrum showed the presence of a hydroxyl group at 3447 cm^{-1} and a carbonyl group at 1701 cm^{-1}, which was also suggested by the ^{13}C NMR data at δ_C 215.9. The NMR spectra (Table 1 and 2) indicated that **4** is a 13β,28-epoxy-substituted triterpene possessing an oleanane skeleton with a hydroxymethylene, a carbonyl group, and a double bond.[17] In the HMBC data (Figure S4, Supporting Information), the carbonyl signal at δ_C 215.9 showed distinct correlations with the δ_H 1.16 (H-23) and 1.04 (H-24) signals and suggested the carbonyl group is attached to C-3. On the basis of the 2D NMR analysis, the disubstituted double bond was placed at the $\Delta^{11,12}$ positions on the C ring of the oleanane framework. Furthermore, the proton signals (δ_H 3.70 and 3.84) of oxygenated methylene (δ_C 65.0) were correlated to the carbon signals at 28.9 (C-29) and 32.4 (C-19). According to a previous study,[17,18] the chemical shifts of C-29 (equatorial) hydroxymethyl groups resonate around 75 ppm, while the values of the C-30 (axial) methyl groups are found around 20 ppm in the ^{13}C NMR spectrum. In contrast, the chemical shifts of the C-30 hydroxymethyl groups appear around 67 ppm, while the C-29 methyl groups appear around 28 ppm. Therefore, the hydroxyl group was placed at C-30 in **4**. Finally, the relative stereochemistry was determined from the NOESY spectrum, as shown in Figure S5 (Supporting Information). Thus, the structure of **4** was determined as 13β,28-epoxy-30-hydroxy-olean-11-en-3-one (microfokienoxane D).

Compound **5** was obtained as an amorphous powder. Its IR spectrum indicated the presence of one or more hydroxyl groups (3396 cm^{-1}). The HRESIMS showed a sodiated molecular ion at *m*/*z* 495.3814 ([M + Na]$^+$, calcd for 495.3815, $C_{31}H_{52}O_3Na$). The NMR spectra (Table 1 and 2) and molecular formula suggested that compound **5** belongs to the urs-12-ene type of triterpenes.[9] The ^{1}H NMR spectrum of compound **5** further showed a singlet at δ_H 3.31 and a doublet of doublets at δ_H 3.76 (1H, dd, J = 8.8, 3.2 Hz, H-11β), which indicated the presence of a methoxyl group at C-11. The position and the stereochemistry of H-11 were determined by the NMR data.[9] The larger coupling constant (J = 8.8 Hz) could be rationalized as a result of *trans*-diaxial coupling with the α-axial proton at C-9 (δ_H 1.87) and the smaller one (J = 3.2 Hz) as an interaction of the same proton with the vinylic hydrogen atom at C-12. Accordingly, the methoxyl proton H-11 is β-axial.[9] The EIMS displayed ions at *m*/*z* 441 [M − CH_2OH]$^+$, 318, 264, and 207. The characteristic retro-Diels−Alder fragment peaks at *m*/*z* 207 (A/B ring) and 264 (D/E ring) (Figure S6, Supporting Information) confirmed a double bond located at C-12 and C-13, a hydroxyl at the A/B ring, and hydroxyl and methoxyl groups at the C/D/E ring.[19,20] In addition, the configuration of the 11-methoxy group was further confirmed from the NOESY spectrum (Figure S7, Supporting Information), in which a correlation was seen between H-11 and CH_3-25, which confirmed the orientation of H-11 in the β-position. In the HMBC spectrum of **5** (Figure S8, Supporting Information), the proton signals at δ_H 1.66 (H-18) and 2.01 (H-22β) showed correlations with the oxygenated methylene carbon signal δ_C 69.1; the proton signals at δ_H 1.92 (H-2α), 1.27 (H-23), and 1.09 (H-24) showed a correlation with the carbon signal at δ_C 77.9 (C-3). Thus, the hydroxyls were located at C-28 and C-3. On the basis of the aforementioned data, **5** is formulated as 3β,28-dihydroxy-11α-methoxyurs-12-ene.

Interestingly, in a previous study of the stems of *M. fokienensis*, agarofuran sesquiterpenes were reported.[2] However, careful examination of NMR spectra of all fractions of EtOAc-partitioned extracts from the leaves in this study revealed the absence of characteristic signals of agarofuran sesquiterpenes. On the basis of NMR spectra, isolation, and structural elucidation, the main components in EtOAc-partitioned extracts of the leaves are sugars, long-chain fatty acids, triglyceride fatty acids, triterpenoids, and flavonoids. Thus, the chemical composition of the leaves and stem of *M. fokienensis* is obviously different. In past studies,[21] intermediates with an 11-oxygenated-12,13-en-28β-hydroxymethylene function, such as **5**, may form 13β,28-epoxytriterpenoids under strong acid conditions. In the present study, such strong acids were not employed.

From the results of a cytotoxicity assay, compounds **1**−**5** and the nine known compounds obtained in this investigation were evaluated. Among these compounds, **3** was found to be the most active toward the HepG2 and Hep3B cancer cell lines, with IC_{50} values of 3.8 and 4.5 μg/mL, respectively; compound **5** was also active against the HepG2 cell line (IC_{50} 4.6 μg/mL). Compounds **3** and **5** were not active against any of the other cancer cell lines. All of the remaining compounds were inactive for all cell lines.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Mattson Genesis II spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Inova 500, Varian Unity Plus 400, or Varian Gemini 200 NMR spectrometers. Chemical shifts are reported in parts per million (δ), and coupling constants (J) are expressed in hertz. LREIMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer or Quattro GC-MS spectrometer having a direct inlet system. HRESIMS were measured on a Bruker Daltonics APEX II mass spectrometer. Silica gel 60 (Merck, 230−400 mesh) and Sephadex LH-20 were used for column chromatography, while TLC analysis was carried out on silica gel GF_{254} precoated plates with detection using 50% H_2SO_4 followed by heating on a hot plate. HPLC was performed with a Hitachi L-7100 pump and D-7000 interface equipped with a Bischoff RI detector using ODS (Lichrospher 60, 250 × 4 mm) column.

Plant Material. Leaves of *Microtropis fokienensis* were collected from Taichung County, Taiwan, in June 2004, and identified by a

Table 1. ^{1}H NMR Spectroscopic Data for Compounds **1**−**5** (in C_5D_5N, 400 MHz)

proton	**1**[a]	**2**	**3**	**4**	**5**
H-1	1.00 (m, H-1β)	1.06 (m, H-1β)	1.53 (m, H-1β)	1.33 (m)	1.52 (m, H-1β)
	1.86 (dt, 12.8, 3.6, H-1α)	1.84 (brd, 13.0, H-1α)	1.89 (m, H-1α)	1.97 (m)	2.09 (dt, 13.6, 3.2; H-1α)
H-2	ca. 1.64	1.97 (m)	1.93 (m, H-2β)	2.44 (m)	1.96 (m, H-2β)
	ca. 1.67	ca. 2.00	1.88 (m, H-2α)	2.60 (m)	1.92 (m, H-2α)
H-3	3.15 (dd, 11.6, 4.4)	4.25 (dd, 11.2, 4.8)	4.22 (dd, 10.4, 5.2)		3.50 (brd, 10.4)
H-5	0.78 (m)	ca.1.56	1.56 (m)	1.35 (m)	0.92 (s)
H-6	1.63 (2H, m)	ca. 1.03	1.03 (m, H-6β)	1.43 (m)	1.40 (m)
		ca. 1.76	1.74 (m, H-6α)	1.62 (m)	1.58 (m)
H-7	ca. 1.24	ca. 1.26	1.56 (m, H-7β)	1.57 (m)	1.24 (m, H-7β)
	ca. 1.26	ca. 1.36	1.67 (m, H-7α)	1.67 (m)	1.52 (m, H-7α)
H-9	1.91 (brs)	2.11 (brs)	1.83 (dd, 13.2, 4.4)	2.00 (brs)	1.87 (d, 8.8)
H-11	5.53 (dd, 10.4, 3.2)	5.70 (dd, 10.4, 2.8)	2.71 (dd, 17.2, 13.2, H-11β)	5.60 (dd, 10.4, 2.8)	3.76 (dd, 8.8, 3.2)
			2.54 (dd, 17.2, 4.4, H-11α)		
H-12	5.83 (d, 10.4)	5.89 (d, 10.4)		5.85 (d, 10.4)	5.45 (d, 3.2)
H-15	ca. 1.21	0.93 (m)	0.98 (m, H-15β)	1.85 (m, H-15β)	1.05 (m, H-15β)
	ca. 1.45	1.82 (m)	1.91 (m, H-15α)	1.88 (m, H-15α)	2.00 (m, H-15α)
H-16	4.20 (dd, 9.4, 6.4)	1.03 (m)	0.98 (m)	1.82 (m, H-16β)	1.29 (m, H-16β)
		1.97 (m)	1.91 (m)	2.12 (td, 13.2, 4.8, H-16α)	1.60 (m, H-16α)
H-18	1.38 (d, 12.0)	1.22 (d, 12.4)	2.21 (d, 11.6)	1.94 (brd, 12.8)	1.66 (brd, 11.2)
H-19	1.78 (m)	1.71 (m)	1.66 (m)	1.83 (m)	0.96 (m)
				1.94 (m)	
H-20	ca. 0.96	1.23 (m)	1.22 (m)		1.50 (m)
H-21	1.29 (m)	ca. 1.24	ca. 1.35	1.25 (m)	1.37 (m)
	ca. 2.20	ca. 1.26	ca. 1.50	1.44 (m)	1.53 (m)
H-22	1.49 (m)	1.48 (m)	1.47 (m, H-22β)	1.29 (m)	2.01 (m, H-22β)
	1.52 (m)	1.50 (m)	1.92 (m, H-22α)	1.34 (m)	1.76 (td, 13.6, 4.0, H-22α)
H-23	0.97 (s)	3.76 (dd, 10.8, 4.4)	3.74 (dd, 10.4, 4.8)	1.16 (s)	1.27 (s)
		4.22 (dd, 10.8, 4.4)	4.24 (dd, 10.4, 4.8)		
H-24	0.78 (s)	1.08 (s)	1.06 (s)	1.04 (s)	1.09 (s)
H-25	0.92 (s)	1.05 (s)	0.95 (s)	0.96 (s)	1.10 (s)
H-26	1.08 (s)	1.36 (s)	1.35 (s)	1.33 (s)	1.05 (s)
H-27	1.13 (s)	1.08 (s)	1.03 (s)	1.08 (s)	1.29 (s)
H-28	3.00 (d, 6.8)	3.25 (d, 6.8)	3.29 (d, 6.8)	3.36 (d, 6.4)	3.52 (brd, 10.4)
	3.87 (d, 6.8)	3.67 (d, 6.8)	3.70 (d, 6.8)	3.76 (d, 6.4)	3.88 (brd, 10.4)
H-29	1.00 (d, 6.0)	1.05 (d, 5.6)	1.08 (d, 6.4)	1.23 (s)	1.03 (d, 6.4)
H-30	ca. 0.96	0.88 (d, 6.4)	ca. 0.88	3.70 (d, 10.4)	0.96 (brs)
				3.84 (d, 10.4)	

[a] ^{1}H NMR spectrum run in CD_3OD.

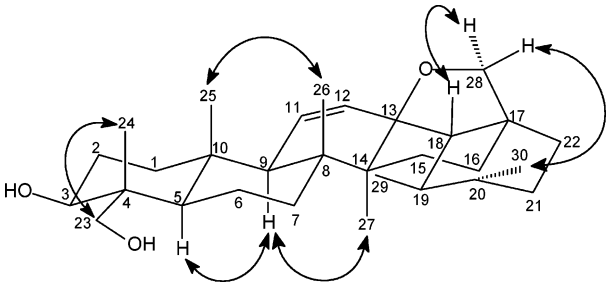

Figure 2. Key NOESY correlations of **2**.

botanist, Dr. Hsin-Fu Yen. A voucher specimen (Microtropis-01) was deposited at the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The dried leaves (1.2 kg) were extracted three times with MeOH overnight at room temperature to give 160 g of crude extract. The extract was partitioned between EtOAc and H_2O to produce an EtOAc-soluble fraction (fraction A, 29.5 g). Using *n*-hexane and MeOH−H_2O (4:1), fraction A was divided into an *n*-hexane and a MeOH−H_2O layer. The MeOH−H_2O layer exhibited cytotoxicity against the HepG2 (IC_{50} = 11.0 μg/mL) and A549 (IC_{50} = 19.5 μg/mL) cell lines. The MeOH−H_2O layer was chromatographed on silica gel and eluted with pure $CHCl_3$, $CHCl_3$−MeOH (20:1), $CHCl_3$−MeOH (10:1), and $CHCl_3$−MeOH (4:1) to give 10 fractions. Fraction 3 (1.0 g) was purified on silica gel eluting with *n*-hexane−$CHCl_3$ (1:1), $CHCl_3$, and $CHCl_3$−MeOH (4:1) to give 17 fractions. Fraction 3-8 (56.6 mg) was subjected to ODS HPLC (MeOH−H_2O, 90:10) to give **5** (6.3 mg, t_R 29 min, 1 mL/min). Fraction 3-(9+10) (69.0 mg) was chromatographed on Sephadex LH-20 with $CHCl_3$−MeOH (1:1) and further purified on silica gel eluting with *n*-hexane, *n*-hexane−EtOAc (1:1), and EtOAc−MeOH (5:1) to give **1** (9.5 mg, R_f 0.5, $CHCl_3$−MeOH, 12:1). Fraction 4 (1.6 g) was chromatographed on silica gel eluting with $CHCl_3$, $CHCl_3$−MeOH (20:1), $CHCl_3$−MeOH (10:1), and $CHCl_3$−MeOH (4:1) to give 11 fractions. Fraction 4-4 (561.9 mg) was chromatographed on Sephadex LH-20 with $CHCl_3$−MeOH (1:3) to give eight fractions. Fraction 4-4-3 (250.2 mg) was purified using ODS HPLC (MeOH−H_2O, 85:15) to give **2** (2.9 mg, t_R 29 min, 1 mL/min), **3** (1.1 mg, t_R 21 min, 1 mL/min), and **4** (1.3 mg, t_R 23 min, 1 mL/min). For the isolation procedure of known compounds, see the Supporting Information.

Microfokienoxane A (1) (13β,28-epoxy-3β,16β-dihydroxyurs-11-ene): yellow, amorphous powder; mp 250 °C; $[\alpha]^{24}_D$ +60.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.00) nm; IR (neat) ν_{max} 3326 (OH), 2916, 2856, 799, 744 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; EIMS *m*/*z* $[M]^+$ 456 (15), 290 (70), 257 (50); HRESIMS *m*/*z* 479.3501 ($[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501).

Microfokienoxane B (2) (13β,28-epoxy-3β,23-dihydroxyurs-11-ene): white, amorphous powder; mp 160 °C; $[\alpha]^{24}_D$ +24.0 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.64) nm; IR (neat) ν_{max} 3351 (OH), 2924, 2861, 1456, 1381, 803, 750 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 100 MHz), see Table 2; EIMS *m*/*z* $[M]^+$ 456 (20), 306 (11), 215 (16), 161 (54); HRESIMS *m*/*z* 479.3501 ($[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3500).

Microfokienoxane C (3) (13β,28-epoxy-3β,23-dihydroxyursan-12-one): white, amorphous powder; mp 185 °C; $[\alpha]^{24}_D$ +19.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.01) nm; IR (neat) ν_{max} 3424 (OH), 2921, 2862, 1706 (C=O), 1599, 1452, 1260, 1083, 1038, 800, 757 cm^{-1}; ^{1}H NMR (C_5D_5N, 400 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 100 MHz), see Table 2; EIMS *m*/*z* $[M]^+$ 472 (17), 440 (13), 318 (18),

Table 2. ^{13}C NMR Spectroscopic Data for Compounds **1**−**5** (in C_5D_5N, 100 MHz)

carbon	**1**[a]	**2**	**3**	**4**	**5**
1	39.5	38.6	38.5	39.0	40.6
2	27.7	27.6	27.6	34.2	28.5
3	79.6	73.1	72.8	215.9	77.9
4	40.0	43.1	43.1	47.6	38.5
5	56.2	48.2	48.1	54.5	55.8
6	18.8	18.0	17.9	19.2	18.8
7	32.7	31.6	33.0	30.9	33.6
8	43.1	42.1	42.1	41.7	43.2
9	53.7	53.5	48.9	52.8	53.1
10	37.5	36.6	37.0	36.3	39.7
11	132.9	133.8	37.4	132.3	76.7
12	131.5	129.3	209.8	131.3	124.9
13	85.8	84.9	89.4	84.9	143.1
14	46.9	44.6	45.9	44.2	42.2
15	36.0	25.8	26.3	25.7	26.7
16	66.4	27.3	26.4	26.0	23.7
17	48.4	42.5	42.7	41.9	38.6
18	63.3	61.6	55.0	51.1	53.8
19	39.3	37.9	38.0	32.4	39.8
20	42.1	41.0	40.7	36.7	39.4
21	30.5	31.7	31.6	30.9	31.1
22	31.8	35.2	34.9	30.6	36.1
23	28.4	67.4	67.2	26.2	28.8
24	15.7	12.5	12.9	21.0	16.6
25	18.4	18.5	16.1	17.3	17.4
26	20.1	19.8	18.7	19.4	18.3
27	19.0	17.4	17.7	19.6	22.7
28	73.1	76.8	77.2	77.1	69.1
29	18.9	18.4	19.1	28.9	17.6
30	19.8	19.5	19.8	65.0	21.6

[a] ^{13}C NMR spectrum run in CD_3OD.

264 (78); HRESIMS *m*/*z* 495.3450 ([M + Na]$^+$ (calcd for $C_{30}H_{48}O_4$-Na, 495.3453).

Microfokienoxane D (4) (13β,28-epoxy-30-hydroxyolean-11-en-3-one): white, amorphous powder; mp 210 °C; $[\alpha]^{24}_D$ +100.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.1) nm; IR (neat) ν_{max} 3347, 2924, 2858, 1701, 1458, 1383, 1260, 1087, 1022, 801, 756 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 100 MHz), see Table 2; EIMS *m*/*z* [M]$^+$ 454 (13), 288 (20), 273 (32), 193 (35); HRESIMS *m*/*z* 477.3344 ([M + Na]$^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3342).

3β,28-Dihydroxy-11α-methoxyurs-12-ene (5): white, amorphous powder; mp 90 °C; $[\alpha]^{24}_D$ −10.3 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (3.65) nm; IR (neat) ν_{max} 3396 (OH), 2924, 2865, 1453, 1384, 756 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz), see Table 1; ^{13}C NMR (C_5D_5N, 100 MHz), see Table 2; EIMS *m*/*z* [M]$^+$ 472 (25), 440 (25), 318 (20), 264 (70); HRESIMS *m*/*z* 495.3814 ([M + Na]$^+$ (calcd for $C_{31}H_{52}O_3Na$, 495.3815).

Bioassays.[22] Compounds **1**−**14** were assayed for cytotoxicity against the human hepatoma cell lines (HepG2 and Hep3B), breast cancer cell lines (MCF-7 and MDA-MB-231), and lung cancer cell line (A549) using the MTT method. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000−10 000 cells per well, and test compounds were added from DMSO stock solutions. After 3 days in culture, attached cells were incubated with MTT (0.5 μg/mL, 1 h) and subsequently solubilized in DMSO. The absorbance was measured at 550 nm using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions. Results represent the mean of two to three separate experiments, each performed in triplicate.

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Supporting Information Available: This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Huang, T. C., Ed. In *Flora of Taiwan*, 2nd ed.; Lungwei Printing Co. Ltd Press: Taipei, 1993; Vol. 3, pp 640−658.
(2) Chen, J. J.; Chou, T. H.; Duh, C. Y.; Chen, I. S. *J. Nat. Prod.* **2006**, *69*, 685−688.
(3) Kuo, Y. H.; Kuo, L. M. Y. *Phytochemistry* **1997**, *44*, 1275−1281.
(4) Hatano, T.; Aga, Y.; Shintani, Y.; Ito, H.; Okuda, T.; Yoshida, T. *Phytochemistry* **2000**, *55*, 959−964.
(5) Spivey, A. C.; Weston, M.; Woodhead, S. *Chem. Soc. Rev.* **2002**, *31*, 43−59.
(6) Chang, F. R.; Hayashi, K. i.; Chen, I. H.; Liaw, C. C.; Bastow, K. F.; Nakanishi, Y.; Nozaki, H.; Cragg, G. M.; Wu, Y. C.; Lee, K. H. *J. Nat. Prod.* **2003**, *66*, 1416−1420.
(7) Rojas, R.; Caviedes, L.; Aponte, J. C.; Vaisberg, A. J.; Lewis, W. H.; Lamas, G.; Sarasara, C.; Gilman, R. H.; Hammond, G. B. *J. Nat. Prod.* **2006**, *69*, 845−846.
(8) González, A. G.; Andrés, L. S.; Ravelo, A. G.; Luis, J. G.; Bazzocchi, I. L.; West, J. *Phytochemistry* **1990**, *29*, 1691−1693.
(9) Ikuta, A.; Tomiyasu, H.; Morita, Y.; Yoshimura, K. *J. Nat. Prod.* **2003**, *66*, 1051−1054.
(10) Núñez, M. J.; Reyes, C. P.; Jiménez, I. A.; Moujir, L.; Bazzocchi, I. L. *J. Nat. Prod.* **2005**, *68*, 1018−1021.
(11) Fu, L.; Zhang, S.; Li, N.; Wang, J.; Zhao, M.; Sakai, J.; Hasegawa, T.; Mitsui, T.; Kataoka, T.; Oka, S.; Kiuchi, M.; Hirose, K.; Ando, M. *J. Nat. Prod.* **2005**, *68*, 198−206.
(12) Griesbaum, K.; Kim, W. S.; Nakamura, N.; Mori, M.; Nojima, M.; Kusabayashi, S. *J. Org. Chem.* **1990**, *55*, 6153−6161.
(13) Foo, L. Y.; Lua, Y.; Howellb, A. B.; Vorsa, N. *Phytochemistry* **2000**, *54*, 173−181.
(14) Baldé, A. M.; Pieters, L. A.; Gergely, A.; Kolodziej, H.; Claeys, M.; Vlietinck, A. J. *Phytochemistry* **1991**, *30*, 337−342.
(15) Fujioka, T.; Yoshida, K.; Fujii, H.; Nagao, T.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **2003**, *51*, 365−372.
(16) Zhang, A. L.; Ye, Q.; Li, B. G.; Qi, H. Y.; Zhang, G. L. *J. Nat. Prod.* **2005**, *68*, 1531−1535.
(17) González, A. G.; González, C. M.; Ravelo, A. G. *J. Nat. Prod.* **1986**, *49*, 148−150.
(18) Sanchez-Contreras, S.; Díaz-Lanza, A. M.; Bartolomé, C.; Bernabé, M. *Phytochemistry* **2000**, *54*, 783−789.
(19) Sukumar, E.; Balakrishna, K.; Rao, R. B.; Kundu, A. B. *Phytochemistry* **1995**, *38*, 275−276.
(20) Li, B. Z.; Wang, B. G.; Jia, Z. J. *Phytochemistry* **1998**, *49*, 2477−2481.
(21) Kitagawa, I.; Kitazawa, K.; Yosioka, I. *Tetrahedron* **1972**, *28*, 907−921.
(22) Sladowski, D.; Steer, S. J.; Clothier, R.; Balls, M. *J. Immunol. Methods* **1993**, *157*, 203−207.

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