Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 66 (2005) 495–501
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cytotoxic triterpenes from the aerial roots of *Ficus microcarpa*

Yi-Ming Chiang [a], Jang-Yang Chang [b], Ching-Chuan Kuo [b], Chi-Yen Chang [b], Yueh-Hsiung Kuo [c,*]

[a] *Institute of BioAgricultural Sciences, Academia Sinica, Taipei 115, Taiwan, ROC*
[b] *Division of Cancer Research, National Health Research Institutes, Taipei 115, Taiwan, ROC*
[c] *Department of Chemistry, National Taiwan University, Taipei 106, Taiwan, ROC*

Received 27 July 2004; received in revised form 8 November 2004
Available online 29 January 2005

Abstract

Six triterpenes, 3β-acetoxy-12,19-dioxo-13(18)-oleanene (**1**), 3β-acetoxy-19(29)-taraxasten-20α-ol (**2**), 3β-acetoxy-21α,22α-epoxytaraxastan-20α-ol (**3**), 3,22-dioxo-20-taraxastene (**4**), 3β-acetoxy-11α,12α-epoxy-16-oxo-14-taraxerene (**5**), 3β-acetoxy-25-methoxylanosta-8,23-diene (**6**) along with nine known triterpenes, 3β-acetoxy-11α,12α-epoxy-14-taraxerene (**7**), 3β-acetoxy-25-hydroxylanosta-8,23-diene (**8**), oleanonic acid (**9**), acetylbetulinic acid (**10**), betulonic acid (**11**), acetylursolic acid (**12**), ursonic acid (**13**), ursolic acid (**14**), and 3-oxofriedelan-28-oic acid (**15**) were isolated from the aerial roots of *Ficus microcarpa*, and their structures elucidated by spectroscopic methods. The in vitro cytotoxic efficacy of these triterpenes was investigated using three human cancer cell lines, namely, HONE-1 nasopharyngeal carcinoma, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells. Compound **8** and pentacyclic triterpenes **9**–**15** possessing a carboxylic acid functionality at C-28 showed significant cytotoxic activities against the aforementioned cell lines and gave IC_{50} values in the range 4.0–9.4 μM.

Keywords: *Ficus microcarpa*; Moraceae; Triterpenoid; Cytotoxic activity

1. Introduction

Ficus microcarpa L. f. (Moraceae) is a popular ornamental plant in Taiwan. The plant's anti-platelet activity prompted us to investigate its phytochemical constituents. Phytochemical studies of this species promptly led to identification of two isoflavones together with twenty-eight components from the bark (Kuo and Li, 1997; Li and Kuo, 1997); a monoterpenoid, phenoids, lignans, and a γ-lactone from the heartwood (Li and Kuo, 1998; Li and Kuo, 2000); many types of triterpenoids including cycloartanoid, lupanoid, oleanoid, ursanoid, taraxastane from the leaves and the aerial roots (Chiang and Kuo, 2002, and references cited therein); and two novel α-tocopheroids from the aerial roots (Chiang and Kuo, 2003). Triterpenes bearing a carboxylic acid at C-28 have been shown to exhibit cytotoxicity to tumor cells (Baglin et al., 2003; Lee et al., 2003; Sakai et al., 2004; Chang et al., 2004a). As part of our research into anti-tumor effects of plant products, we identified fifteen triterpenes from the EtOAc-soluble fraction of the aerial roots of *F. microcarpa* MeOH extract. Herein, we describe the structural elucidation of six new triterpenes (**1**–**6**). In addition, the isolated compounds **1**, **4**, **6**, and **8**–**15** were also evaluated for their in vitro cytotoxic efficacy against a small panel of cancer cell lines.

2. Results and discussion

Compound **1** was isolated as a colorless solid. The molecular formula was found to be $C_{32}H_{48}O_4$ by

* Corresponding author. Tel.: +886 2 23638146; fax: +886 2 23636359.
E-mail address: yhkuo@ccms.ntu.edu.tw (Y.-H. Kuo).

0031-9422/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.12.026

HREIMS. The IR spectrum of **1** showed absorptions for acetoxyl (1734, 1248 cm^{-1}) and conjugated carbonyl (1697, 1684 cm^{-1}) functionalities. The ^1H NMR (Table 1) spectrum in $CDCl_3$ exhibited signals for eight singlet methyl groups (δ_H 0.84, 0.85, 0.92, 0.95, 0.97, 1.05, 1.07, 1.23), one acetoxyl group [δ_H 2.02 (*s*)], a methylene flanked by a ketone and a tertiary carbon [δ_H 2.35 (*dd*, $J = 15.6$, 5.6 Hz, H_α-11), 2.41 (*dd*, $J = 15.6$, 12.4 Hz, H_β-11)], and one methine proton attached to an acetoxyl group [δ_H 4.46 (*dd*, $J = 10.8$, 5.2 Hz, H-3)]. The ^{13}C NMR (Table 2) and DEPT spectra of **1** exhibited one acetoxy (δ_C 21.2,

Table 1
^1H-NMR spectroscopic data for compounds **1**–**6**[a] (400 MHz in $CDCl_3$)

	Compound **1**	Compound **2**	Compound **3**	Compound **4**	Compound **5**	Compound **6**
1	1.57[b] (H_β), 1.02[b] (H_α)	1.65[b] (H_β), 1.00[b] (H_α)	1.64[b] (H_β), 1.00[b] (H_α)	1.92[b], 1.40[b]	1.88 *dt* (13.6, 3.6 H_β), 1.35[b] (H_α)	1.68[b], 1.26[b]
2	1.64[b]	1.60[b]	1.60[b]	2.49 *ddd* (16.0, 9.2, 7.2, H_β), 2.42 *ddd* (16.0, 8.0, 4.8, H_α)	1.74[b] (H_α), 1.64[b] (H_β)	1.66[b], 1.56[b]
3	4.46 *dd* (10.8, 5.2)	4.45 *dd* (10.8, 5.6)	4.45 *dd* (10.4, 4.4)	–	4.50 *dd* (10.8, 5.2)	4.46 *dd* (11.2, 4.7)
4	–	–	–	–	–	–
5	0.84[b]	0.80[b]	0.77 *br d* (11.2)	1.35[b]	0.84[b]	1.10[b]
6	1.62[b], 1.30[b]	1.51[b], 1.36[b]	1.50[b], 1.36[b]	1.46[b]	1.16[b]	1.64[b], 1.46[b]
7	1.46[b]	1.41[b], 1.30[b]	1.37[b]	1.46[b]	2.03[b] (H_α), 1.35[b] (H_β)	2.00[b]
8	–	–	–	–	–	–
9	1.68[b]	1.40[b]	1.35[b]	1.37[b]	1.03[b]	–
10	–	–	–	–	–	–
11	2.41 *dd* (15.6, 12.4, H_β), 2.35 *dd* (15.6, 5.6, H_α)	1.44[b]	1.45[b]	1.57[b], 1.37[b]	3.14 *t* (4.4)	2.01[b]
12	–	1.83 *br d* (10.8, H_β), 0.79[b] (H_α)	2.03[b] (H_β), 1.10[b] (H_α)	1.68[b]	2.88 *d* (4.4)	1.60[b]
13	–	1.71[b]	1.31[a]	1.93 *m*	–	–
14	–	–	–	–	–	–
15	1.14[b]	1.02[b]	1.67[b] (H_β), 1.08[b] (H_α)	1.20[b], 1.14[b]	5.82 *s*	1.13[b]
16	1.57[b]	1.47[b], 1.23[b]	1.78 *td* (13.6, 4.0, H_α), 1.26[b] (H_β)	1.28[b]	–	1.90[b], 1.30[b]
17	–	–	–	–	–	1.44[b]
18	–	2.42 *d* (11.6)	1.74[b]	1.44[b]	1.66[b]	0.66 *s*
19	–	–	1.45[b]	2.01 *m*	1.48[b]	0.97 *s*
20	–	–	–	–	–	1.43[b]
21	1.77[b]	1.75–1.50[b], 1.15–1.00[b]	3.09 *d* (4.0)	5.70 *br s*	1.35[b]	0.86 *d* (6.3)
22	1.46[b]	1.75–1.50[b], 1.15–1.00[b]	2.89 *d* (4.0)	–	1.63[b], 1.23[b]	2.15 *dd* (13.6, 5.4), 1.74[b]
23	0.85 *s*	0.82 *s*	0.82 *s*	1.07 *s*	0.86 *s*	5.49 *ddd* (15.8, 8.1, 5.4)
24	0.84 *s*	0.82 *s*	0.81 *s*	1.02 *s*	0.88 *s*	5.35 *d* (15.8)
25	0.92 *s*	0.86 *s*	0.83 *s*	0.94 *s*	1.10 *s*	–
26	1.07 *s*	1.03 *s*	0.99 *s*	1.08 *s*	1.14 *s*	1.21 *s*
27	0.97 *s*	0.99 *s*	0.94 *s*	0.92 *s*	0.78 *s*	1.21 *s*
28	0.95 *s*	0.64 *s*	0.93 *s*	0.96 *s*	1.14 *s*	0.84 *s*
29	1.23 *s*	5.05 *s* (H_a), 4.48 *s* (H_b)	1.01 *d* (7.2)	1.10 *d* (6.4)	1.00 *s*	0.84 *s*
30	1.05 *s*	1.35 *s*	1.27 *s*	1.88 *br s*	1.02 *s*	0.82 *s*
CH_3CO	2.02 *s*	2.02 *s*	2.02 *s*		2.03 *s*	2.01 *s*
OMe						3.11 *s*
OH			2.12 *s*			

[a] Figures in parentheses are coupling constants (*J*) in Hz.
[b] Data obtained from HMQC spectrum.

Table 2
^{13}C-NMR spectroscopic data for compounds **1**–**6** (100 MHz in $CDCl_3$)

	1	2	3	4	5	6
1	37.9 *t*	38.4 *t*	38.2 *t*	39.6 *t*	37.7 *t*	35.2 *t*
2	23.4 *t*	23.7 *t*	23.6 *t*	34.0 *t*	23.1 *t*	24.1 *t*
3	80.3 *d*	81.0 *d*	80.9 *d*	218.1 *s*	80.4 *d*	80.8 *d*
4	37.7 *s*	37.8 *s*	37.7 *s*	47.3 *s*	37.7 *s*	37.7 *s*
5	55.3 *d*	55.5 *d*	55.2 *d*	54.7 *d*	54.4 *d*	50.5 *d*
6	18.0 *t*	18.2 *t*	18.2 *t*	19.6 *t*	18.6 *t*	18.1 *t*
7	34.6 *t*	33.6 *t*	34.0 *t*	33.5 *t*	39.5 *t*	26.3 *t*
8	41.3 *s*	41.1 *s*	41.2 *s*	41.0 *s*	40.0 *s*	134.4 *s*
9	49.8 *d*	50.9 *d*	49.8 *d*	49.6 *d*	52.6 *d*	134.3 *s*
10	37.3 *s*	37.1 *s*	36.9 *s*	36.7 *s*	36.6 *s*	36.9 *s*
11	39.3 *t*	21.0 *t*	21.3 *t*	22.2 *t*	51.4 *d*	20.9 *t*
12	205.2 *s*	24.7 *t*	27.2 *t*	27.6 *t*	55.4 *d*	30.9 *t*
13	145.3 *s*	33.0 *d*	37.3 *d*	38.4 *d*	37.6 *s*	44.4 *s*
14	45.1 *s*	42.1 *s*	43.2 *s*	42.0 *s*	173.9 *s*	49.8 *s*
15	24.8 *t*	26.5 *t*	26.1 *t*	26.3 *t*	120.4 *d*	30.8 *t*
16	36.6 *t*	36.6 *t*	34.0 *t*	28.5 *t*	206.5 *s*	28.1 *t*
17	40.1 *s*	36.2 *s*	35.9 *s*	44.7 *s*	45.1 *s*	50.0 *d*
18	148.0 *s*	44.5 *d*	37.0 *d*	45.2 *d*	45.1 *d*	15.8 *q*
19	211.4 *s*	151.5 *s*	40.2 *d*	36.7 *d*	32.2 *t*	19.1 *q*
20	46.3 *s*	72.8 *s*	70.5 *s*	162.5 *s*	28.1 *s*	36.6 *d*
21	36.3 *t*	37.8[a] *t*	60.7 *d*	122.9 *d*	36.3 *t*	18.7 *q*
22	33.7 *t*	37.6[a] *t*	65.6 *d*	205.9 *s*	27.2 *t*	39.3 *t*
23	27.9 *q*	27.9 *q*	27.9 *q*	26.8 *q*	27.8 *q*	128.6 *d*
24	16.5 *q*	16.4 *q*	16.4 *q*	21.0 *q*	16.5 *q*	136.7 *d*
25	15.9 *q*	16.4 *q*	16.2 *q*	16.2 *q*	17.1 *q*	74.8 *s*
26	16.9 *q*	15.8 *q*	15.9 *q*	15.9 *q*	26.0 *q*	25.7 *q*
27	20.6 *q*	15.7 *q*	15.5 *q*	14.5 *q*	26.0 *q*	26.1 *q*
28	23.1 *q*	16.8 *q*	17.9 *q*	18.7 *q*	30.9 *q*	27.9 *q*
29	24.7 *q*	107.1 *t*	17.0 *q*	22.6 *q*	33.9 *q*	16.5 *q*
30	24.5 *q*	28.1 *q*	27.1 *q*	22.1 *q*	30.6 *q*	24.2 *q*
$CH_3\underline{C}O$	170.9 *s*	171.0 *s*	171.0 *s*		170.8 *s*	170.9 *s*
$\underline{C}H_3CO$	21.2 *q*	21.3 *q*	21.3 *q*		21.2 *q*	21.2 *q*
OMe						50.2 *q*

[a] Values may be interchanged.

170.9), one carbon attached to an acetoxyl group [δ_C 80.3 (C-3)], two quaternary olefinic carbons [δ_C 145.3 (C-13), 148.0 (C-18)], and two carbonyl carbons [δ_C 205.2 (C-12), 211.4 (C-19)]. β-Amyrin acetate, an oleanane derivative, has similar ^{13}C NMR spectroscopic data for its A and B rings (Knight, 1974; Mahato and Kundu, 1994). This suggested that compound **1** and β-amyrin acetate possessed a similar structure on A and B rings. On the basis of the above evidence, the structure of compound **1** was proposed as an oleanane skeleton containing an acetoxy, a tetra-substituted olefin, and two ketone functionalities. The double bond was deduced to be located at C-13 and C-18 because it was the only possible structure to have a tetra-substituted olefin. In the HMBC spectrum, the long-range ^{13}C–^{1}H correlations C-12/H_2-11 and C-19/H_2-21, H_3-29, H_3-30 established partial structure of the C, D, and E rings. The UV absorption maximum at 252.0 nm was consistent with this partial structure. The 2D-NMR (HMQC, HMBC, NOESY, and COSY) analyses also confirmed the assigned structure. Therefore, compound **1** was assigned as 3β-acetoxy-12,19-dioxo-13(18)-oleanene.

Compound **2**, a colorless solid, analyzed for $C_{32}H_{52}O_3$ on the basis of HREIMS data. Its IR spectrum showed hydroxyl (3464 cm^{-1}), a terminal double bond (3091, 1642, and 900 cm^{-1}), and acetoxyl (1734 and 1249 cm^{-1}) functionalities. The ^{1}H NMR spectrum (Table 1) of **2** exhibited signals for seven singlet methyl groups (δ_H 0.64, 0.82, 0.82, 0.86, 0.99, 1.03, 1.35), one acetoxyl group [δ_H 2.02 (s)], an allylic proton [δ_H 2.42 (*d*, J = 11.6 Hz, H-18)], one methine proton attached with the acetoxyl group [δ_H 4.45 (*dd*, J = 10.8, 5.6 Hz, H-3)], and a terminal double bond [δ_H 4.48 (1H, *s*, H_b-29), 5.05 (1H, *s*, H_a-29)]. The ^{13}C NMR spectrum exhibited 32 signals (eight CH_3, eleven CH_2, five CH, and eight C) including one acetoxyl group (δ_C 21.3 and 171.0), two carbons to which oxygen was attached [δ_C 72.8 (C-20) and 81.0 (C-3)], and a terminal double bond [δ_C 107.1 (C-29) and 151.5 (C-19)]. Comparison of the ^{13}C NMR spectra between **2** and 3β-acetoxy-20β-*H*-19(29)-taraxastene (Bhutani et al., 1992) revealed a difference in the ring E of both the compounds. In the HMBC spectrum, long-range ^{13}C–^{1}H correlations for C-19/H-18, H_2-29, H_3-30 and C-20/H-18, H_2-29, H_3-30 established a partial structure of the E ring. NOESY

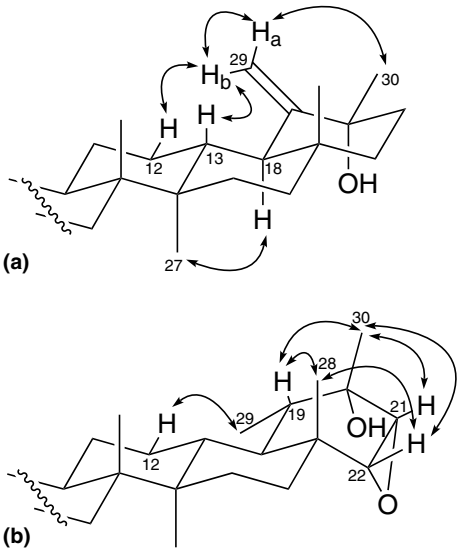

Fig. 1. Key NOESY correlations of compound **2** (a) and **3** (b).

correlations for H-18/H_3-27; H_a-29/H_b-29, H_3-30; and H_b-29/H_β-12, H-13, H_a-29 suggested that CH_3-30 should be on the β face (Fig. 1(a)). Therefore, compound **2** was assigned as 3β-acetoxy-19(29)-taraxasten-20α-ol.

HREIMS of compound **3** gave the molecular ion peak at m/z = 500.3845 corresponding to a molecular formula of $C_{32}H_{52}O_4$. IR absorption bands at 3513, 1728, and 1247 cm^{-1} indicated hydroxyl and acetoxyl groups. The ^1H NMR spectrum of **3** exhibited signals for seven singlet methyl groups (δ_H 0.81, 0.82, 0.83, 0.93, 0.94, 0.99, and 1.27), a doublet methyl group (δ_H 1.01), a methine proton associated with an acetoxyl group (δ_H 2.02 and 4.45), a D_2O exchangeable proton (δ_H 2.12), and two oxymethine protons coupled to each other (δ_H 2.89 and 3.09). Its ^1H and ^{13}C NMR data were similar to those of 3β-acetoxy-21α,22α-epoxide-20(30)-taraxastene (Menichini et al., 1996), except for the existence of one tertiary hydroxy group at C-20 in compound **3**, that was proposed from the hydration of 3β-acetoxy-21α,22α-epoxide-20(30)-taraxastene. NOESY correlations for H_3-28/H-19, H-22; H_3-29/H_β-12; and H_3-30/H-19, H-21, H-22 suggested that CH_3-30 should be on the same face as H-19, H-21, H-22, and CH_3-28 (Fig. 1(b)). Therefore, compound **3** was assigned as 3β-acetoxy-21α,22α-epoxytaraxastan-20α-ol.

Compound **4** had the molecular formula $C_{30}H_{46}O_2$ on the basis of its HREIMS data. Its IR spectrum indicated the presence of a carbonyl group (1707 cm^{-1}) and a conjugated carbonyl group (1677 and 1642 cm^{-1}). The UV absorption at λ_{max} 236.0 nm was consistent with the presence of a conjugated ketone. The ^1H and ^{13}C NMR spectroscopic data of compound **4** were similar to those of 22-oxo-20-taraxasten-3β-ol (Kuo and Chiang, 1999). Comparison of the NMR spectra between **4** and 22-oxo-20-taraxasten-3β-ol indicated a ketone group at C-3 in **4** instead of a hydroxy group at C-3 in 22-oxo-20-taraxasten-3β-ol. The ketone group caused H_2-2 in compound **4** to exhibit a downfield shift in NMR spectrum to δ_H 2.42 and 2.49. Thus, compound **4** was established as 3,22-dioxo-20-taraxastene.

Compound **5**, $C_{32}H_{48}O_4$ (form HREIMS), had IR absorption bands at 1734, 1247 cm^{-1} (acetoxyl group) and 3053, 1683 cm^{-1} (conjugated carbonyl group), respectively. The ^1H NMR data of compound **5** were similar to those of compound **7** (Tanaka and Matsunaga, 1988). The only difference was an addition of a carbonyl group at C-16 in **5**. The UV absorption band at λ_{max} 243.0 nm and an olefinic proton at δ_H 5.82 (s, H-16) confirmed the presence of a β,β-disubstituted cyclohexenone moiety. Thus, compound **5** was established as 3β-acetoxy-11α,12α-epoxy-16-oxo-14-taraxerene.

The ^1H and ^{13}C NMR spectroscopic data of compound **6** were similar to those of compound **8** (Fourrey et al., 1970) except for the presence of a methoxyl group [δ_H 3.11 (s)] in compound **6** instead of the hydroxyl group found in **8**. This evidence showed that the structure of **6** is 3β-acetoxy-25-methoxylanosta-8,23-diene.

Nine known compounds were identified as 3β-acetoxy-11α,12α-epoxy-14-taraxerene (**7**) (Tanaka and Matsunaga, 1988), 3β-acetoxy-25-hydroxylanosta-8,23-diene (**8**) (Fourrey et al., 1970), oleanonic acid (**9**) (Shirane et al., 1996), acetylbetulinic acid (**10**) (Nomura et al., 1981), betulonic acid (**11**) (González et al., 1983), acetylursolic acid (**12**) (Tkachev and Denisov, 1994), ursolic acid (**14**) (Fang et al., 1991), and 3-oxo-friedelan-28-oic acid (**15**) (Kumar et al., 1985), respectively, by comparing their NMR and MS data as well as optical rotations with those reported in the literature. Ursonic acid (**13**) was identified by direct comparison (^1H NMR) with an authentic sample, which was synthesized from ursolic acid (**14**) by Jones oxidation.

Eleven isolated compounds (**1**, **4**, **6**, and **8**–**15**) were evaluated for their cytotoxicity against HONE-1, KB, and HT29 cancer cell lines by using methylene blue dye assay and anti-cancer drugs, etoposide (Chang et al., 2004b) and cisplatin (Chang et al., 2002), as positive controls (Table 3). Among them, compounds **8**–**15** exhibited cytotoxicity against these cell lines and gave IC_{50} values in the range 4.0–9.4 μM. On the other hand, compounds **1**, **4**, and **6** displayed no cytotoxicity effects against these cancer cell lines (>10 μM). On the basis of structure and activity relationships, the pentacyclic triterpenes possessing a carboxylic acid at C-28, including compounds **9**–**15**, showed more potent cytotoxic efficacy than that of other tested triterpenes. Triterpenes with a C-28 carboxylic acid are reported to exhibit cytotoxic effects (Baglin et al., 2003; Sakai et al., 2004) and this was also observed in this study.

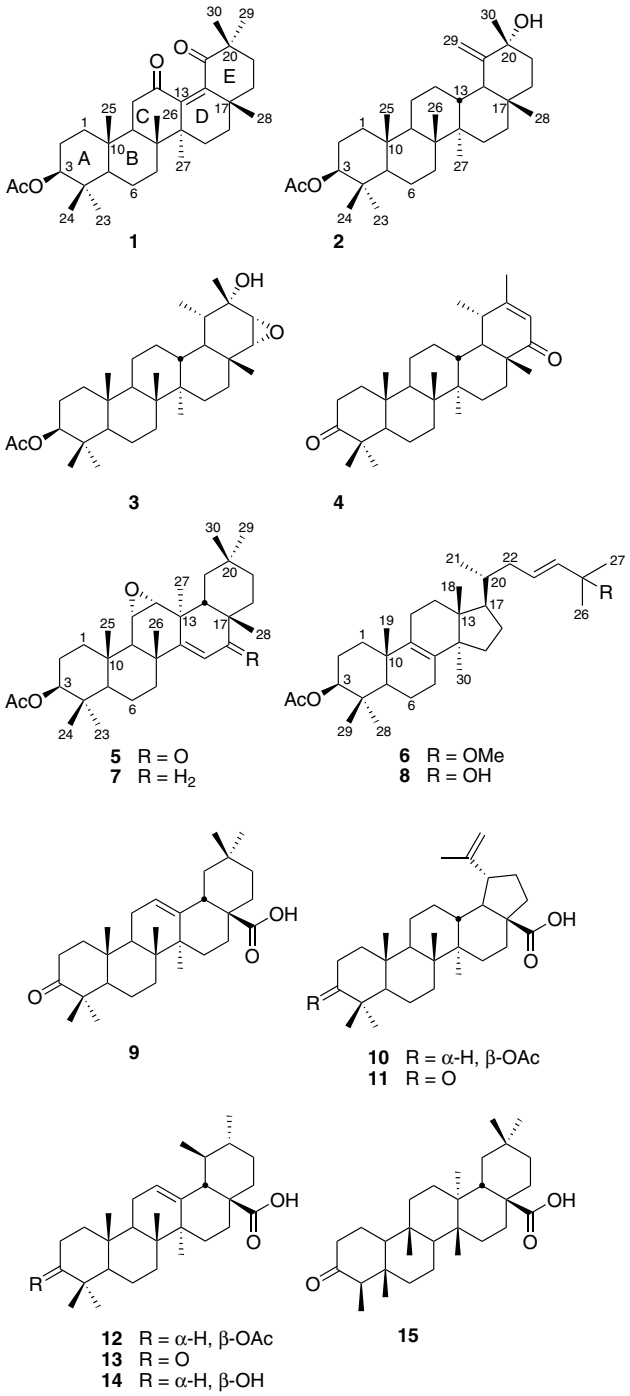

Table 3
Cytotoxicity of compounds **8**–**15** against cultured HONE-1, KB, and HT29 cancer cell lines

Compounds	Growth inhibition constant (IC_{50})[a] [μM]		
	HONE-1	KB	HT29
Etoposide[b]	0.5 ± 0.2	0.9 ± 0.3	2.4 ± 0.5
Cisplatin[b]	3.2 ± 0.5	4.4 ± 0.9	5.7 ± 1.1
8	>10	>10	9.3 ± 1.6
9	7.2 ± 1.9	6.3 ± 1.6	>10
10	4.7 ± 1.9	6.7 ± 2.6	>10
11	4.9 ± 2.1	8.2 ± 1.8	>10
12	>10	8.4 ± 2.9	>10
13	5.2 ± 0.7	4.0 ± 2.1	6.3 ± 1.8
14	8.8 ± 1.5	8.2 ± 2.7	4.7 ± 1.5
15	9.4 ± 2.8	8.3 ± 2.4	>10

[a] IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number and the results are means ± standard deviation of three independent replicates. The IC_{50} greater than 10 μM was considered to be no cytotoxicity.
[b] Positive control substance.

3. Experimental

3.1. General procedures

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter, whereas IR spectra were recorded on a Perkin–Elmer 983G spectrophotometer. ^1H and ^{13}C NMR spectra were run on a Varian Unity Plus 400 spectrometer, whereas EIMS and FABMS were obtained on a Finnigan TSQ-46C and JEOL JMS-HX 300 mass spectrometer respectively. Extracts were purified using Si gel chromatography (Merck 70–230 mesh, 230–400 mesh, ASTM).

3.2. Plant material

The aerial roots of *Ficus microcarpa* L. f. were collected on the campus of National Taiwan University, Taipei, Taiwan, in August 1996. The plant was identified by Mr. Muh-Tsuen Gun (retired), Department of Botany, National Taiwan University. A voucher specimen (No. 038671) has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

3.3. Extraction and isolation

The dried aerial roots of *F. microcarpa* were crushed to give 18 kg of raw material, which was extracted with MeOH (150 l) at room temperature (7 days × 2). The combined extracts were evaporated in vacuo to yield a residue, which was suspended in H_2O (1 l), and this was then partitioned with ethyl acetate (1 l × 3). The combined ethyl acetate layer afforded a black syrup (250 g), which was subsequently applied to a Si gel column (15 × 60 cm) eluted with hexane-EtOAc mixtures of increasing polarity (1:0, fr. 1, 23 l; 49:1, fr. 2, 4 l; 97:3, fr. 3, 3 l; 19:1, fr. 4, 13 l; 9:1, fr. 5, 6 l; 4:1, fr. 6, 23 l; 7:3, fr. 7, 19 l; 1:1, fr. 8, 15 l; 3:7, fr. 9 15 l; 0:1, fr. 10, 9 l). Fr. 2 was separated by preparative HPLC [Phenomenex Luna 5 μm Silica (2), 250 mm × 10 mm] eluted with 3% EtOAc

in hexane to afford compounds **1** (12 mg), **5** (7 mg), and **7** (11 mg). Fr. 6 was separated by preparative HPLC [Phenomenex Luna 5 μm Silica (2), 250 mm × 10 mm] eluted with 20% EtOAc in hexane to afford compounds **2** (11 mg), **4** (5 mg), **6** (33 mg), **8** (18 mg), and **10** (15 mg). Fr. 7 (13.3 g) was subjected to Si gel cc (5 × 30 cm) eluted with CH_2Cl_2–MeOH mixtures of increasing polarity (1:0, fr. 7-1, 500 ml; 49:1, fr. 7-2, 1500 ml; 19:1, fr. 7-3, 1000 ml; 9:1, fr. 7-4, 500 ml). Fr. 7-2 was separated by preparative HPLC [Phenomenex Luna 5 μm Silica (2), 250 mm × 10 mm] eluted with 35% EtOAc in hexane to afford compounds **3** (9 mg), **9** (6 mg), **11** (11 mg), **12** (20 mg), **13** (10 mg), and **15** (19 mg). Fr. 8 (7.1 g) was subjected to Si gel cc (3 × 20 cm) eluted with CH_2Cl_2–MeOH mixtures of increasing polarity (1:0, fr. 8-1, 250 ml; 49:1, fr. 8-2, 300 ml; 19:1, fr. 8-3, 1000 ml; 9:1, fr. 8-4, 500 ml; 4:1, fr. 8-5, 500 ml). Fr. 8-3 was separated by preparative HPLC [Phenomenex Luna 5 μm Silica (2), 250 mm × 10 mm] eluted with 5% acetone in CH_2Cl_2 to afford compound **14** (14 mg).

3.4. Compound identification

3.4.1. 3β-Acetoxy-12,19-dioxo-13(18)-oleanene (*1*)

Colorless solid, mp 244–247 °C; $[\alpha]_D^{24}$: −94.9° ($CHCl_3$; *c* 1.0); UV λ_{max}^{MeOH} nm (log ε): 252.0 (3.83); IR ν_{max}^{KBr} cm^{-1}: 1734, 1697, 1684, 1624, 1394, 1370, 1248, 1030, 1003, 971, 902, 737, 705. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 496 $[M]^+$ (30), 427 (100), 356 (16), 273 (14), 232 (62), 189 (30), 176 (28), 135 (25); HREIMS, *m/z*: found: 496.3557; calc. for $C_{32}H_{48}O_4$: 496.3554.

3.4.2. 3β-Acetoxy-19(29)-taraxasten-20α-ol (*2*)

Colorless solid, mp 245–248 °C; $[\alpha]_D^{24}$: +55.3° ($CHCl_3$; *c* 0.5); IR ν_{max}^{KBr} cm^{-1}: 3464, 3091, 1734, 1642, 1380, 1370, 1249, 1037, 982, 900, 739. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 484 $[M]^+$ (6), 466 (72), 451 (9), 426 (25), 406 (39), 391 (22), 363 (22), 337 (6), 303 (5), 295 (7), 269 (5), 255 (10), 229 (10), 217 (32), 202 (40), 189 (100), 173 (38), 133 (48), 121 (76), 107 (50); HREIMS, *m/z* found: 484.3927; calc. for $C_{32}H_{52}O_3$: 484.3919.

3.4.3. 3β-Acetoxy-21α,22α-epoxytaraxastan-20α-ol (*3*)

Colorless solid, mp >300 °C; $[\alpha]_D^{21}$: +5.6° ($CHCl_3$; *c* 0.6); IR ν_{max}^{KBr} cm^{-1}: 3513, 1728, 1386, 1375, 1247, 1014, 983, 936, 902, 869, 814. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 500 $[M]^+$ (4), 482 (6), 466 (10), 442 (8), 440 (12), 422 (16), 411 (12), 379 (10), 353 (6), 299 (8), 257 (10), 234 (16), 203 (100), 189 (84), 175 (38), 135 (48), 121 (52), 95 (57); HREIMS, *m/z* found: 500.3845; calc. for $C_{32}H_{52}O_4$: 500.3868.

3.4.4. 3,22-Dioxo-20-taraxastene (*4*)

Colorless solid, mp 245–248 °C; $[\alpha]_D^{24}$: +63.9° ($CHCl_3$; *c* 0.2); UV λ_{max}^{MeOH} nm (log ε): 236.0 (3.92); IR ν_{max}^{KBr} cm^{-1}: 3052, 1707, 1677, 1642, 1384, 738. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 438 $[M]^+$ (100), 423 (13), 395 (6), 368 (11), 353 (6), 243 (7), 236 (10), 219 (13), 205 (33), 189 (10), 163 (14), 152 (13), 107 (14), 97 (24); HREIMS, *m/z* found: 438.3503; calc. for $C_{30}H_{46}O_2$: 438.3500.

3.4.5. 3β-Acetoxy-11α,12α-epoxy-16-oxo-14-taraxerene (*5*)

Colorless solid, mp >300 °C; $[\alpha]_D^{24}$: −39.3° ($CHCl_3$; *c* 0.2,); UV λ_{max}^{MeOH} nm (log ε): 243.0 (3.77); IR ν_{max}^{KBr} cm^{-1}: 3053, 1734, 1683, 1375, 1247, 1031, 992, 738. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 496 $[M]^+$ (22), 481 (20), 478 (14), 463 (8), 436 (20), 403 (13), 372 (38), 356 (13), 312 (70), 294 (100), 279 (81), 175 (40), 148 (78), 135 (46); HREIMS, *m/z* found: 496.3546; calc. for $C_{32}H_{48}O_4$: 496.3554.

3.4.6. 3β-Acetoxy-25-methoxylanosta-8,23-diene (*6*)

Colorless solid, mp 148–150 °C; $[\alpha]_D^{26}$: +30.4° ($CHCl_3$; *c* 2.7); IR ν_{max}^{KBr} cm^{-1}: 1736, 1371, 1244, 1076, 1028, 979. For ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. EIMS 70 eV, *m/z* (rel. int.): 498 $[M]^+$ (5), 466 (14), 451 (32), 406 (24), 391 (100), 309 (16), 297 (18), 109 (84); HREIMS, *m/z* found: 498.4073; calc. for $C_{33}H_{54}O_3$: 498.4075.

3.4.7. Ursonic acid (*13*)

Colorless solid, mp 271–275 °C; $[\alpha]_D^{25}$: +56.7° ($CHCl_3$; *c* 0.9); IR ν_{max}^{KBr} cm^{-1}: 3400–2400, 3065, 1706, 1695, 1379, 1387, 1318, 1278, 1257, 1238, 978, 740; ^1H NMR (400 MHz, $CDCl_3$): δ 0.80 (*s*, H_3-26), 0.84 (*d*, *J* = 6.4 Hz, H_3-29), 0.93 (3H, *d*, *J* = 6.4 Hz, H_3-30), 1.00 (*s*, H_3-24), 1.03 (*s*, H_3-25), 1.06 (*s*, H_3-23 and H_3-27), 2.17 (*d*, *J* = 11.2 Hz, H-18), 2.36 (*ddd*, *J* = 16.0, 6.8, 3.6 Hz, H_α-2), 2.52 (*ddd*, *J* = 16.0, 10.8, 7.2 Hz, H_β-2), 5.24 (*t*, *J* = 3.2 Hz, H-12); FABMS *m/z* (rel. int.): 455 $[M + H]^+$ (10), 425 (14), 407 (14), 248 (19), 203 (35), 154 (100). Identified by direct comparison (^1H NMR) with an authentic sample, which was synthesized from ursolic acid (**14**) by Jones oxidation.

3.5. Antitumoral cytotoxic assay

Human nasopharyngeal carcinoma HONE-1, oral epidermoid carcinoma KB, and colorectal carcinoma HT29 cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cells in logarithmic phase were cultured at a density of 5000 cells/ml/well in a 24-well plate. The cells were exposed to various concentrations of the tested drugs for 72 h. The methylene

blue dye assay was used to evaluate the effects of the tested drugs on cell growth, as described previously (Finlay et al., 1984). The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control.

Acknowledgement

This research was supported by the National Science Council of the Republic of China (NSC 92-2323-B-002-003).

References

Baglin, I., Mitaine-Offer, A.C., Nour, M., Tan, K., Cave, C., Lacaille-Dubois, M.A., 2003. A review of natural and modified betulinic, ursolic and echinocystic acid derivatives as potential antitumor and anti-HIV agents. Mini. Rev. Med. Chem. 3, 525–539.

Bhutani, K.K., Gupta, D.K., Kapil, R.S., 1992. Occurrence of D/E trans stereochemistry isomeric to ursane (cis) series in a new pentacyclic triterpene from *Calotropis procera*. Tetrahedron Lett. 33, 7593–7596.

Chang, J.Y., Liu, J.F., Juang, S.H., Liu, T.W., Chen, L.T., 2002. Novel mutation of topoisomerase I in rendering cells resistant to camptothecin. Cancer Res. 62, 3716–3721.

Chang, C.I., Kuo, C.C., Chang, J.Y., Kuo, Y.H., 2004a. Three new oleanane-type triterpenes from *Ludwigia octovalvis* with cytotoxic activity against two human cancer cell lines. J. Nat. Prod. 67, 91–93.

Chang, J.Y., Chang, C.Y., Kuo, C.C., Chen, L.T., Wein, Y.S., Kuo, Y.H., 2004b. Salvinal, a novel microtubule inhibitor isolated from *Salvia miltiorrhizae* Bunge (Danshen), with antimitotic activity in multidrug-sensitive and -resistant human tumor cells. Mol. Pharmacol. 65, 77–84.

Chiang, Y.M., Kuo, Y.H., 2002. Novel triterpenoids from the aerial roots of *Ficus microcarpa*. J. Org. Chem. 67, 7656–7661.

Chiang, Y.M., Kuo, Y.H., 2003. Two novel alpha-tocopheroids from the aerial roots of *Ficus microcarpa*. Tetrahedron Lett. 44, 5125–5128.

Fang, J.M., Wang, K.C., Cheng, Y.S., 1991. Steroids and triterpenoids from *Rosa laevigata*. Phytochemistry 30, 3383–3387.

Finlay, G.J., Baguley, B.C., Wilson, W.R., 1984. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. Anal. Biochem. 139, 272–277.

Fourrey, J.L., Rondest, J., Polonsky, J., 1970. Sur quelques oxydations de type biogénétique. Tetrahedron 26, 3839–3847.

González, A.G., Amaro, J., Fraga, B.M., Luis, J.G., 1983. 3-oxo-6β-hydroxyolean-18-en-28-oic acid from *Orthopterygium huancuy*. Phytochemistry 22, 1828–1830.

Knight, S.A., 1974. ^{13}C NMR spectra of some tetra- and pentacyclic triterpenoids. Org. Magn. Reson. 6, 603–611.

Kumar, N.S., Muthukuda, P.M., Balasubramaniam, S., 1985. Triterpenes from *Euonymus revolutus*. Phytochemistry 24, 2454–2455.

Kuo, Y.H., Chiang, Y.M., 1999. Five new taraxastane-type triterpenes from the aerial roots of *Ficus microcarpa*. Chem. Pharm. Bull. 47, 498–500.

Kuo, Y.H., Li, Y.C., 1997. Constituents of the bark of *Ficus microcarpa* L. f. J. Chin. Chem. Soc. 44, 321–325.

Lee, S.M., Min, B.S., Lee, C.G., Kim, K.S., Kho, Y.H., 2003. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. Planta Med. 69, 1051–1054.

Li, Y.C., Kuo, Y.H., 1997. Two new isoflavones from the bark of *Ficus microcarpa*. J. Nat. Prod. 60, 292–293.

Li, Y.C., Kuo, Y.H., 1998. A monoterpenoid and two simple phenols from heartwood of *Ficus microcarpa*. Phytochemistry 49, 2417–2419.

Li, Y.C., Kuo, Y.H., 2000. Four new compounds, ficusal, ficusesquilignan A, B, and ficusolide diacetate from the heartwood of *Ficus microcarpa*. Chem. Pharm. Bull. 48, 1862–1865.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids – a compilation and some salient features. Phytochemistry 37, 1517–1575.

Menichini, F., Di Benedetto, R., Delle Monache, F., 1996. A triterpene epoxide and a guaianolide from *Ptilostemmon gnaphaloides*. Phytochemistry 41, 1377–1379.

Nomura, M., Tokoroyama, T., Kubota, T., 1981. Biarylheptanoids and other constituents from wood of *Alnus japonica*. Phytochemistry 20, 1097–1104.

Shirane, N., Hashimoto, Y., Ueda, K., Takenaka, H., Katoh, K., 1996. Ring-A cleavage of 3-oxo-olean-12-en-28-oic acid by the fungus *Chaetomium longirostre*. Phytochemistry 43, 99–104.

Sakai, K., Fukuda, Y., Matsunaga, S., Tanaka, R., Yamori, T., 2004. New cytotoxic oleanane-type triterpenoids from the cones of *Liquidamber styraciflua*. J. Nat. Prod. 67, 1088–1093.

Tanaka, R., Matsunaga, S., 1988. Triterpene constituents from *Euphorbia supina*. Phytochemistry 27, 3579–3584.

Tkachev, A.V., Denisov, A.Y., 1994. Oxidative decarboxylation by hydrogen peroxide and a mercury (II) salt: a simple route to nor-derivatives of acetyloleanolic, acetylursolic and dehydroabietic acids. Tetrahedron 50, 2591–2598.