Five New Taraxastane-Type Triterpenes from the Aerial Roots of *Ficus microcarpa*

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Five new taraxastane-type triterpenes, 22-oxo-20-taraxasten-3 β -ol (1), 20(30)-taraxastene-3 β ,21 α -diol (2), 20 α ,21 α -epoxytaraxastan-3 β -ol (3), 20-taraxastene-3 β ,22 β -diol (4), and 3 β -acetoxy-20-taraxasten-22-one (5), together with 20-taraxasten-3 β -ol (6) and ptiloepoxide (7) were isolated from the aerial roots of *Ficus microcarpa*. Their structures were elucidated by spectroscopic and chemical methods.

Key words *Ficus microcarpa*; Moraceae; aerial root; triterpene; 22-oxo-20-taraxasten-3 β -ol; 20(30)-taraxastene-3 β ,21 α -diol; 20 α ,21 α -epoxytaraxastan-3 β -ol; 20-taraxastene-3 β ,22 β -diol; 3 β -acetoxy-20-taraxasten-22-one

Ficus microcarpa L. f. (Moraceae) is a popular ornamental plant in Taiwan. Previous phytochemical studies by Higa on the leaves of this plant have identified six triterpenoids.¹⁾ The strong vitality of this plant, as well as its antiplatelet activity, led us to study its chemical components. In previous reports, we investigated its chemical components and found two new isoflavones together with twenty-eight components²⁾ from the bark and three new compounds from the heartwood.³⁾ In connection with our interest in this plant, chemical studies on the aerial part of this plant were undertaken in our laboratory.

The methanol extract of the aerial roots of *F. microcarpa* was subjected to partition with ethyl acetate and water. The organic layer was repeatedly purified by silica gel chromatography using a hexane–ethyl acetate solvent system. Detailed purification by HPLC resulted in the isolation of five new taraxastane-type triterpenes, 22-oxo-21-taraxasten-3 β -ol (1), 20(30)-taraxastene-3 β ,21 α -diol (2), 20 α ,20 α -epoxytaraxastan-3 β -ol (3), 20-taraxastene-3 β ,22 β -diol (4), and 3 β -acetoxy-20-taraxasten-22-one (5) as well as 20-taraxasten-3 β -ol (6)⁴⁾ and ptiloepoxide (7).⁵⁾ In this paper, we report the structure of these triterpenes.

Compound 1 had high resolution mass spectroscopy (HR-MS) and ¹³C-NMR data consistent with the molecular formula C₃₀H₄₈O₂, indicating seven index of hydrogen deficiency (IHD). Analysis of the IR spectrum of 1 suggested that it contained a hydroxyl group (3447 cm⁻¹) and a conju-

gated ketone (1670 and 1647 cm⁻¹). The UV absorption at $\lambda_{\max}$ 232 nm was consistent with the presence of a conjugated ketone. The ¹H-NMR spectrum of 1 exhibited signals for six singlet methyl groups (δ 0.75, 0.84, 0.91, 0.95, 0.96, and 1.04), a doublet methyl group [δ 1.10 (d, J =6.6 Hz)], a vinyl methyl group [δ 1.87 (br s)], a carbinol methine proton [δ 3.19 (dd, J =10.7, 5.3 Hz)], and an olefinic proton vicinal to carbonyl (δ 5.69 br s). All these data suggested that compound 1 is a taraxastane triterpene with one hydroxyl group and a conjugated carbonyl group with dialkyl substituents. Comparison of the ¹³C-NMR data (Table 1) of 1 with those of the known 20-taraxasten-3 β -ol (6)⁴⁾ suggested that 1 possesses the same skeletal structure with an additional oxo group located at C-22. These ¹H- and ¹³C-NMR data were resolved by distortionless enhancement by polarization transfer (DEPT) and by proton detected heteronuclear multiple-quantum coherence (HMOC) experiments. The structures were confirmed by the proton detected heteronuclear multiple-bond correlation (HMBC) technique. Thus, the structure of compound 1 was deduced to be 22-oxo-20-taraxasten-3 β -ol.

Compound 2 has the molecular formula C₃₀H₅₀O₂ on the basis of HR-MS. The IR spectrum showed the presence of a hydroxyl group (3323 cm⁻¹) and a terminal double bond (3065, 1646, and 906 cm⁻¹). The ¹H-NMR spectrum exhibits that 2 has six singlet methyl groups (δ 0.75, 0.75, 0.83, 0.93, 0.95, and 1.00), one doublet methyl group [δ 1.19 (d, J =

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7.1 Hz)], a terminal double bond [δ 4.87, 4.96 (brs, each 1H)], two carbinol methine protons [δ 3.18 (dd, 1H, $J=10.9$, 5.3 Hz) and 4.38 (dd, 1H, $J=9.1$, 5.2 Hz)]. The above evidence as well as ^{13}C -NMR data (Table 1) indicate that **2** is a taraxastane triterpene with one terminal double bond instead of a methyl group and two additional hydroxyl groups. Comparison of the ^1H - and ^{13}C -NMR data of **2** with those of the known 20-taraxastene-3 β -ol (**6**)⁴ and ptiloepoxide (**7**)⁵ suggested that **2** is a taraxastane triterpene with two hydroxyl groups located at C-3 and C-21 and a terminal double bond positioned at C-20(30). Stereochemistries for H-3 and H-21 were also assigned as having an axial orientation due to their large coupling constants (>9 Hz). HMQC, HMBC, and nuclear Overhauser enhancement and exchange spectroscopy (NOESY) [H_3 -28 and H-21 have nuclear Overhauser effect (NOE) correlation] methods also confirmed the assigned structure. From the above evidence, compound **2** was deduced to be 20(30)-taraxastene-3 β ,21 α -diol.

Compound **3** showed a molecular ion peak at m/z 442 and its HR-MS indicated a molecular formation of $\text{C}_{30}\text{H}_{50}\text{O}_2$. The IR absorption band of **3** at 3442 cm^{-1} indicated the presence of a hydroxyl group. On account of the molecular formula $\text{C}_{30}\text{H}_{50}\text{O}_2$ of **3**, the IHD of **3** is six, similarly to compound **2**. The presence of eight methyl groups could be attributed to eight NMR signals at δ 0.73, 0.77, 0.81, 0.85, 0.94, 0.99, 1.29 (s, each 3H), and 1.09 (d, 3H, $J=6.4$ Hz). Comparison of ^1H - and ^{13}C -NMR data (Table 1) of **3** with those of **6** suggested that **3** is a derivative of taraxastane. The ^{13}C -NMR signal at δ 78.9 and corresponding proton signal at δ 3.17 (dd, 1H, $J=10.8$, 5.4 Hz) were assigned as C-3 and H_{α} -3, respectively. No olefinic or carbonyl signals (lower field than δ 100) were observed. There remained only two oxygenated carbons at δ 61.3 and 60.6 (corresponding ^1H signal at δ 3.03), which predicted an epoxide functionality which was located at C-20 and C-21 due to the H_3 -30 shifting downfield to δ 1.29. Its α -oriented epoxide could be reasonably explained by the quasi-axial H_{β} -21 having coupling constants ($J=6.6$, 1.2 Hz) and weak NOE correlation with H_3 -28.

Compound **4** was considered to be an isomer of **2** on the basis of HR-MS and ^{13}C -NMR data, together with the molecular formula $\text{C}_{30}\text{H}_{50}\text{O}_2$ having six IHD. The IR spectrum showed the presence of a hydroxyl group and a trisubstituted double bond ascribed to the absorptions at 3390, 1655, 1040, 1026 and 840 cm^{-1} . The ^1H -NMR spectrum of **4** showed signals for six singlet methyl groups (δ 0.65, 0.74, 0.83, 0.93, 0.95, 1.02), one vinyl methyl group [δ 1.64 (brs, 3H)], a doublet methyl group [δ 0.96 (d, 3H, $J=6.8$ Hz)], one carbinol methine proton [δ 3.19 (dd, 1H, $J=11.2$, 5.2 Hz)], an allylic carbinol methine proton [δ 3.75 (brs, 1H, H-22)], and a vinyl proton [δ 5.11 (brs, 1H, H-21)]. The proton at δ 3.75 was assigned to H-22 due to having a NOESY correlation with H-18. H-22 was assigned as having an α -axial orientation owing to no NOE with H_3 -28 and a small coupling constant to H-21. The structure for **4** was thus assigned as 20-taraxastene-3 β ,22 β -diol.

Compound **5** had the molecular formula $\text{C}_{32}\text{H}_{50}\text{O}_3$ on the basis of HR-MS and ^{13}C -NMR data (Table 1). Its IR spectrum showed the presence of an acetoxy and a conjugated ketone group (1732, 1671 and 1635 cm^{-1}). The UV spectrum indicated the presence of conjugated ketone with two alkyl substituents (λ_{max} 235.5 nm). Its ^1H -NMR data were similar

Table 1. ^{13}C -NMR Data for **1**, **2**, **3**, **4**, **5** and **6** (100 MHz in CDCl_3)

Position	1	2	3	4	5	6
1	38.7	38.7	38.6	38.7	38.4	38.8
2	27.3	27.4	27.3	27.3	23.6	27.4
3	78.9	79.0	78.9	79.0	80.9	79.0
4	38.8	38.9	38.8	38.8	37.8	38.9
5	55.2	55.3	55.1	55.3	55.3	55.3
6	18.2	18.3	18.2	18.3	18.1	18.3
7	34.2	34.0	34.2	34.2	34.2	34.3
8	41.1	40.9	40.9	41.0	41.1	41.1
9	50.2	50.4	50.0	50.3	50.2	50.5
10	37.1	37.1	37.0	37.1	37.0	37.1
11	21.6	21.4	21.4	21.6	21.6	21.6
12	27.6	26.2	27.7	28.0	27.6	27.7
13	38.4	38.9	39.4	38.2	38.4	39.2
14	41.9	42.2	42.3	42.1	41.9	42.4
15	26.3	26.4	26.4	26.6	26.3	27.1
16	28.5	37.7	36.3	32.2	28.4	36.7
17	44.8	33.9	34.0	39.8	44.8	34.4
18	45.2	48.4	45.7	46.8	45.3	48.7
19	36.8	38.1	34.1	36.4	36.8	36.3
20	162.6	156.6	61.3	140.8	162.5	139.9
21	122.9	71.3	60.6	124.1	122.9	118.9
22	206.1	48.8	42.5	77.2	206.0	42.2
23	28.0	28.0	27.9	28.0	27.9	28.0
24	15.4	15.4	15.4	15.4	16.5	15.4
25	16.3	16.2	16.3	16.3	16.4	16.3
26	16.1	15.9	16.0	16.0	16.1	16.1
27	14.5	14.8	14.4	14.7	14.5	14.8
28	18.7	18.2	18.3	11.2	18.7	17.7
29	22.6	28.4	19.0	22.5	22.6	22.6
30	22.1	113.6	23.2	21.1	22.1	21.6
CH_3CO					171.0	
CH_3CO					21.3	

to those of compound **1** except for the presence of an acetoxy group [δ 2.02 (s, 3H)] instead of a hydroxyl group in **1**. H-3 exhibited a downfield shift at δ 4.46 (dd, 1H, $J=10.8$, 5.6 Hz) compared with the corresponding proton in **1**. Comparison of ^1H - and ^{13}C -NMR spectral data (Table 1) of **5** with those of **1** indicated that compound **5** is 3 β -acetoxy-20-taraxastene-22-one.

The chemical correlation of the five new taraxastane derivatives listed above was employed as follows. Wolff-Kishner reduction of **1** afforded **6**, and the treatment of **3** with saturated HCl in CHCl_3 solution yielded **2** in good yield, thereby, the epoxide positioned at 20 α and 21 α in **3** was proved. *m*-Chloroperbenzoic acid (*m*-CPBA) oxidation of **6** provided the oxidative product **3**, because the α -face gives less hindrance.⁶ Sodium borohydride reduction of **1** gave compound **4**. Saponification of **5** produced a product which was identified with **1**.

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^1H - and ^{13}C -spectra were run on a Varian Unity Plus 400 spectrometer. EI-MS, UV, and specific rotations were taken on a JEOL JMS-HX 300 mass spectrometer, a Hitachi S-3200 spectrometer, and a JASCO DIP-1000 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 70—230 mesh, 230—400 mesh, ASTM).

Extraction and Isolation The dried aerial roots of *Ficus microcarpa* L. f. were crushed into pieces to give 18 kg of raw material, which was extracted with MeOH (150 l) at room temperature (7 d $\times$ 2). The extract was evaporated *in vacuo* to yield a residue which was suspended in H_2O (1 l), and this phase was then partitioned with ethyl acetate (1 l $\times$ 3). The com-

bined ethyl acetate layer afforded a black syrup (250 g) which was subsequently chromatographed over silica gel with a hexane/EtOAc gradient solvent system. Crude compounds, **5**, **6**, **7**, **3**, **1**, **4** and **2**, were all eluted with 20% EtOAc in hexane. Further purification by HPLC [Merck LichroCART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μ m)] gave **5** (17 mg), **6** (587 mg), **7** (53 mg), **3** (44 mg), **1** (18 mg), **4** (6 mg) and **2** (6 mg) using 10% EtOAc/hexane, 20% EtOAc/hexane, 20% EtOAc/hexane, 20% EtOAc/hexane, 20% EtOAc/hexane, 30% EtOAc/hexane, and 30% EtOAc/hexane, respectively. Two known compounds, **6** and **7**, were identified by comparing their physical data with those in the literature.

22-Oxo-20-taraxasten-3 β -ol (1): mp 264–266 °C, $[\alpha]_D^{26} + 33.1^\circ$ ($c=0.5$, CHCl₃). UV $\lambda_{\max}$ (log ϵ) nm: 232 (3.72). IR $\nu_{\max}$ cm⁻¹: 3447, 3045, 1670, 1647, 1380, 1361, 1042, 850. ¹H-NMR (400 MHz, CDCl₃) δ : 0.66 (br d, 1H, $J=10.6$ Hz, H-5), 0.75, 0.84, 0.91, 0.95, 0.96, 1.04 (s, each 3H), 1.10 (d, 3H, $J=6.6$ Hz, H-29), 1.87 (br s, 3H, H-30), 2.01 (m, 1H, H-19), 3.19 (dd, 1H, $J=10.7$, 5.3 Hz, H-3), 5.69 (br s, 1H, H-21). ¹³C-NMR data see Table 1. EI-MS (70 eV) m/z (rel. int. %): 440 (M⁺, 69), 422 (67), 379 (41), 299 (53), 207 (69), 189 (94), 135 (80), 121 (91), 95 (100). HR-EI-MS m/z M⁺ Calcd for C₃₀H₄₈O₂: 440.3656; Found 440.3657.

20(30)-Taraxasten-3 β ,21 α -diol (2): mp 246–248 °C, $[\alpha]_D^{26} + 22.1^\circ$ ($c=0.3$, CHCl₃). IR $\nu_{\max}$ cm⁻¹: 3323, 3065, 1646, 1384, 1373, 1043, 906. ¹H-NMR (400 MHz, CDCl₃) δ : 0.67 (br d, 1H, $J=9.1$ Hz, H-5), 0.75, 0.75, 0.83, 0.93, 0.95, 1.00 (s, each 3H), 1.19 (d, 3H, $J=7.1$ Hz, H-29), 1.32 (overlapped with other signals, 1H, H-22), 1.94 (dd, 1H, $J=13.9$, 9.2 Hz, H-22), 2.14 (quin, 1H, $J=7.4$ Hz, H-19), 3.18 (dd, 1H, $J=10.9$, 5.3 Hz, H-3), 4.38 (dd, 1H, $J=9.1$, 5.2 Hz, H-21), 4.87, 4.96 (brs, each 1H, H-30). ¹³C-NMR data see Table 1. EI-MS (70 eV) m/z (rel. int. %): 442 (M⁺, 41), 424 (39), 207 (100), 189 (96). HR-EI-MS m/z M⁺ Calcd for C₃₀H₅₀O₂: 442.3813; Found 442.3804.

20 α ,21 α -Epoxytaraxastan-3 β -ol (3): mp 234–236 °C, $[\alpha]_D^{31} + 15.7^\circ$ ($c=0.5$, CHCl₃). IR $\nu_{\max}$ cm⁻¹: 3442, 1383, 1372, 1045, 1025. ¹H-NMR (400 MHz, CDCl₃) δ : 0.65 (br d, 1H, $J=9.2$ Hz, H-5), 0.73, 0.77, 0.81, 0.85, 0.94, 0.99, 1.29 (s, each 3H), 1.09 (d, 3H, $J=6.4$ Hz, H-29), 3.03 (dd, 1H, $J=6.6$, 1.2 Hz, H-21), 3.17 (dd, 1H, $J=10.8$, 5.4 Hz, H-3). ¹³C-NMR data see Table 1. EI-MS (70 eV) m/z (rel. int. %): 442 (M⁺, 15), 424 (33), 207 (67), 189 (100), 133 (58), 121 (67). HR-EI-MS m/z M⁺ Calcd for C₃₀H₅₀O₂: 442.3813; Found 442.3816.

20-Taraxastene-3 β ,22 β -diol (4): mp 208–210 °C, $[\alpha]_D^{25} + 20.3^\circ$ ($c=0.2$, CHCl₃). IR $\nu_{\max}$ cm⁻¹: 3390, 1655, 1382, 1369, 1040, 1026, 1008, 840. ¹H-NMR (400 MHz, CDCl₃) δ : 0.68 (overlapped with other signals, 1H, H-5), 0.65, 0.74, 0.83, 0.93, 0.95, 1.02 (s, each 3H), 0.96 (d, 3H, $J=6.8$ Hz, H-29), 1.64 (br s, 3H, H-30), 3.19 (dd, 1H, $J=11.2$, 5.2 Hz, H-3), 3.75 (br s, 1H, H-22), 5.11 (br s, 1H, H-21). ¹³C-NMR data see Table 1. EI-MS (70 eV) m/z (rel. int. %): 442 (M⁺, 1), 189 (30), 175 (32), 119 (40), 105 (100), 91 (68). HR-EI-MS m/z M⁺ Calcd for C₃₀H₅₀O₂: 442.3813; Found 442.3809.

3 β -Acetoxy-20-taraxasten-22-one (5): mp 290–292 °C, $[\alpha]_D^{27} + 46.9^\circ$ ($c=1.1$, CHCl₃). UV $\lambda_{\max}$ (log ϵ) nm: 235.5 (3.97). IR $\nu_{\max}$ cm⁻¹: 1732,

1671, 1635, 1379, 1368, 1246, 1031, 981. ¹H-NMR (400 MHz, CDCl₃) δ : 0.78 (br d, 1H, $J=11.2$ Hz, H-5), 0.82, 0.83, 0.86, 0.90, 0.94, 1.04 (s, each 3H), 1.10 (d, 3H, $J=6.8$ Hz, H-29), 1.87 (br s, 3H, H-30), 2.00 (overlapped with other signals, 1H, H-19), 2.02 (s, 3H, CH₃CO), 4.46 (dd, 1H, $J=10.8$, 5.6 Hz, H-3), 5.68 (br s, 1H, H-21). ¹³C-NMR data see Table 1. EI-MS (70 eV) m/z (rel. int. %): 482 (M⁺, 16), 422 (100), 407 (49), 379 (99), 189 (80), 135 (55), 121 (63). HR-EI-MS m/z M⁺ Calcd for C₃₂H₅₀O₃: 482.3762; Found 482.3768.

Wolff–Kishner Reduction of 1 To a solution of compound **1** (10 mg) in 3 ml triethylene glycol, 35 mg of NaOH and 150 mg of hydrazine hydrate were added. The mixture was heated at 120 °C for 1 h, then the temperature was gradually raised to 170 °C for 3 h. At the end of the reaction, the solution was poured into ice-water (20 ml) and extracted with ether. The product (4 mg) was identified as compound **6**.

Treatment of 3 with HCl in CHCl₃ Compound **3** (17 mg) and one drop of conc. HCl were added to a solvent of CHCl₃ (10 ml). The mixture was stirred at room temperature for 18 h. After the usual treatment, the product was identified as compound **2** (13 mg).

Oxidation of 6 with *m*-CPBA Compound **6** (29 mg) and *m*-CPBA (17 mg) were dissolved in CH₂Cl₂ (2 ml), and the mixture was stirred at room temp. for 4 h. The mixture was diluted with CH₂Cl₂ (5 ml) and washed with aqueous NaHSO₃, aqueous NaHCO₃, and then brine. The usual purification gave **3** (25 mg).

Sodium Borohydride Reduction of 1 Excess NaBH₄ was added in a small portion to a solution of **1** (7 mg) in MeOH (2 ml), and the mixture was stirred for 1 h, then poured into H₂O (10 ml). The aqueous solution was extracted with EtOAc (10 ml $\times$ 2) and purified to yield **4** (4 mg).

Saponification of 5 in Methanolic NaOH Compound **5** (6 mg) was dissolved in 1 N methanolic NaOH solution (2 ml) for 5 h under stirring, and the solution was then quenched with 15 ml of H₂O. The product was extracted and purified to afford **1** (3 mg).

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