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-3β-ol (1), 20(30)-taraxastene-3β,21α-diol (2), 20α,21α-epoxytaraxastan-3β-ol (3), 20-taraxastene-3β,22β-diol (4), and 3β-acetoxy-20-taraxasten-22-one (5), together with 20-taraxasten-3β-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-3β-ol; 20(30)-taraxastene-3β,21α-diol; 20α,21α-epoxytaraxastan-3β-ol; 20-taraxastene-3β,22β-diol; 3β-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. 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 compounds that suggested the same skeletal structure with an additional oxo group located at C-22. These data suggested that compound 1 possessed the molecular formula C_{30}H_{50}O_{2} on the basis of HR-MS. The IR 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-3β-ol (6) 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 (HMQC) 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-3β-ol.

Compound 2 has the molecular formula C_{30}H_{50}O_{2} on the basis of HR-MS. The IR spectrum showed the presence of a hydroxyl group (3323 cm^{-1}) and a conjugated ketone (1670 and 1647 cm^{-1}). The UV absorption at λ_{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-3β-ol (6) 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 (HMQC) 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-3β-ol.

Compound 2 has the molecular formula C_{30}H_{50}O_{2} 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=
A terminal double bond \( [\delta 4.87, 4.96 \text{ (brs, each 1H)}] \), two carbonyl methine protons \( [\delta 3.18 \text{ (dd, 1H, } J=10.9, 5.3 \text{ Hz}) \text{ and 4.38 (dd, 1H, } J=9.1, 5.2 \text{ Hz})] \). The above evidence as well as \(^{13}\text{C}-\text{NMR} \) 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 \(^1\text{H}\)- and \(^{13}\text{C}-\text{NMR} \) data of 2 with those of the known 20-taraxasten-3 \( \beta \)-ol (6)\(^a\) and ptiloepoxide (7)\(^b\) 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). Stereochemistry 

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To those of compound 1 except for the presence of an acetoxyl group \( [\delta 2.02 \text{ (s, 3H)}] \) instead of a hydroxyl group in 1. H-3 exhibited a downfield shift at \( \delta 4.46 \text{ (dd, 1H, } J=10.8, 5.6 \text{ Hz}) \) compared with the corresponding proton in 1. Comparison of \(^1\text{H}\)- and \(^{13}\text{C}-\text{NMR} \) spectral data (Table 1) of 5 with those of 1 indicated that compound 5 is 3\( \beta \)-acetoxyl-20-taraxasten-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 CH\(_2\)Cl\(_2\) solution yielded 2 in good yield, thereby, the epoxide positioned at 20\( \alpha \) and 21\( \alpha \) in 3 was proved. m-Chloroperbenzoic acid (m-CPBA) oxidation of 6 provided the oxidative product 3, because the \( \alpha \)-face gives less hindrance.\(^5\) 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. \( ^1 \text{H} \) and \(^{13}\text{C}-\text{NMR} \) spectra were recorded on a Varian Unity Plus 400 spectrometer. EI-MS, UV, and specific rotations were taken on 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×2). The extract was evaporated in vacuo to yield a residue which was suspended in H\(_2\)O (1 l), and this phase was then partitioned with ethyl acetate (1×3). The com-
bined ethyl acetate layer afforded overall silica gel with 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 µm)] gave 5 (17 mg), 6 (587 mg), and 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, and 20% EtOAc/hexane, respectively. Two known compounds, 6 and 7, were identified by comparing their physical data with those in the literature.

22-Oxo-20-taraxast-3β-ol (1): mp 264—266 °C, [α]20 D +33.1° (c = 0.5, CHCl3). UV λmax (log ε) nm: 232 (3.72). IR νmax cm⁻¹: 3447, 3045, 1670, 1647, 1380, 1361, 1042, 850. 1H-NMR (400 MHz, CDCl3) δ: 0.64 (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 (brs, 3H, H-30), 2.01 (m, 1H, H-19), 3.19 (dd, 1H, J = 10.7, 5.3 Hz, H-3), 4.38 (brs, 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: 442.3816; Found 442.3813.

20(Taraxastene-3β,21β,22- triol (2): mp 246—248 °C, [α]20 D +22.1° (c = 0.3, CHCl3). UV νmax 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 = 9.2, 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 442.3814.

20β,21α-Epoxytaraxastene-3β-ol (3): mp 234—236 °C, [α]20 D +15.7° (c = 0.5, CHCl3). IR νmax 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.