New Cyclopropyl-Triterpenoids from the Aerial Roots of *Ficus microcarpa*

Yi-Ming CHIANG, Jen-Kuan SU, Yi-Hung LIU, and Yueh-Hsiung KUO*

Department of Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China.
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Four new cyclopropyl-triterpenes, 27-*nor*-3β-hydroxy-25-oxocycloartane (1), (22*E*)-25,26,27-*trinor*-3β-hydroxycycloart-22-en-24-al (2), 3β-acetoxy-15α-hydroxy-13,27-cyclours-11-ene (3), 3β-acetoxy-12α-formyloxy-13,27-cycloursan-11α-ol (4), together with (23*E*)-27-*nor*-3β-hydroxycycloart-23-en-25-one (5) were isolated from the aerial roots of *Ficus microcarpa*. Compounds 3 and 4 are rare 13,27-cycloursane-type triterpenes. Their structures were elucidated by spectroscopic and chemical methods.

Key words *Ficus microcarpa*; Moraceae; aerial roots; triterpene; 27-*nor*-3β-hydroxy-25-oxocycloartane; (22*E*)-25,26,27-*trinor*-3β-hydroxycycloart-22-en-24-al

Ficus microcarpa L. f. (Moraceae) is a popular ornamental plant in Taiwan. Antiplatelet activity as well as the strong vitality of this plant prompted us to research its chemical components. Phytochemical studies of the plant have identified six triterpenoids from the leaves.[1] Two isoflavones,[2] twenty-eight known components[3] and six new compounds were previously isolated from the bark and heartwood.[4,5] Recently, new triterpenes were isolated and identified from its aerial roots.[6—8] Reinvestigation of the aerial root extract has led to the isolation of four cycloartane and 13,27-cycloursane derivatives (**1**—**4**) together with a known compound (**5**). Compound **5** was identified as (23*E*)-27-*nor*-3β-hydroxycycloart-23-en-25-one by comparison with spectral data (^{1}H and ^{13}C) of those previously reported.[9,10] This paper deals with the isolation and structural determination of these metabolites.

Compound **1** was assigned to be the molecular formula $C_{29}H_{48}O_2$ by high resolution electron impact (HR-EI)-MS. The IR spectrum indicated absorption bands for a hydroxyl (3423 cm^{-1}) and carbonyl (1716 cm^{-1}). The ^{1}H-NMR spectrum showed similar peaks to the co-occurring (23*E*)-27-*nor*-3β-hydroxycycloart-23-en-25-one (**5**) including the cyclopropyl protons (δ 0.31, 0.52, d, J=4.0 Hz, each 1H), five singlet methyl groups (δ 0.78, 0.86, 0.93, 0.94, 2.11), one doublet methyl group (δ 0.85, J=5.2 Hz) and one carbinol methine proton (δ 3.26, dd, J=10.8, 4.0 Hz), but two olefinic proton signals in **5** had disappeared (Table 1). Comparison of the ^{13}C-NMR data (Table 2) of **1** with those of **5** suggested **1** had the identical skeleton as **5** but a different side chain due to the olefinic carbons at δ_C 147.5 and 132.5 instead of a saturation in **5**. The compound was therefore concluded to be the 27-*nor*-3β-hydroxy-25-oxocycloartane. The ^{1}H- and ^{13}C-NMR assignments were confirmed by distortionless enhancement by polarization transfer (DEPT), proton-detected heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiplebond correlation (HMBC) experiments. The side chain's structure was revealed by the HMBC correlations which were shown to be as follows: C-22 and H_3-21, H_2-24; C-23 and H_2-24; and C-25 and H_2-24, H_3-26. Furthermore, hydrogenation of **5** gave **1** exclusively.

Compound **2**, $C_{27}H_{42}O_2$ (from HR-EI-MS and ^{13}C-NMR), gave an IR spectrum which indicated the presence of a hydroxyl (3458 cm^{-1}), an α,β-unsaturated carbonyl (1695 cm^{-1}) and olefinic (1635 cm^{-1}) functional groups. The ^{1}H-NMR showed signals for a cyclopropane methylene (δ 0.33, 0.55, d, J=4.0 Hz, H_2-19), four singlet methyl groups (δ 0.79, 0.89, 0.95, 1.01), one doublet methyl group (δ 1.07, J=6.4 Hz), one carbinol methine proton (δ 3.27, dd, J=10.8, 4.4 Hz), and a *trans* α,β-unsaturated aldehyde (δ 6.70, dd, J=15.6, 8.8 Hz, H-22; 6.04, dd, J=15.6, 7.6 Hz, H-23; 9.46, d, J=7.6 Hz, H-24) (Table 1). In addition, the ^{13}C-NMR spectrum showed signals at δ_C 164.7 (C-22), 130.8 (C-23), and 194.6 (C-24) also confirming the presence of the above functionality. Comparison of the ^{13}C-NMR data of **2** with those of **1** (Table 2) suggested **2** had the identical ring structure but had a two carbon shorter side chain bearing an α,β-unsaturated aldehyde. The gross structure of compound **2** was established by detailed analyses of the homonuclear cor-

Table 1. ^{1}H-NMR Data for **1**, **2**, **3**, and **4** in $CDCl_3$ (400 MHz, J in Hz)

H	1	2	3	4
3	3.26 dd (10.8, 4.0)	3.27 dd (10.8, 4.4)	4.44 dd (10.4, 6.4)	4.45 dd (11.2, 4.8)
5	1.28[a]	1.28[a]	0.84[a]	0.90[a]
8	1.48[a]	1.52[a]	—	—
9	—	—	1.62[a]	1.22[a]
11	1.95,[a] 1.20[a]	2.02 m, 1.14[a]	5.12 dd (10.4, 3.2)	3.52 br d (5.6)
12	1.60[a]	1.64[a]	5.69 dd (10.4, 3.2)	5.13 s
15	1.30[a]	1.31[a]	4.41 dd (11.2, 7.2)	1.75 td (12.8, 6.4); 1.48[a]
17	1.55[a]	1.74[a]	—	—
18	0.93 s	1.01 s	1.00[a]	0.90[a]
19	0.31 d (4.0) 0.52 d (4.0)	0.33 d (4.0) 0.55 d (4.0)	0.88[a]	0.92[a]
20	1.20—1.40[a]	2.40 m	1.45[a]	1.30[a]
21	0.85 d (5.2)	1.07 d (6.4)	1.27[a]	1.31[a]
22	1.20—1.40[a]	6.70 dd (15.6, 8.8)	1.35,[a] 1.18[a]	1.34,[a] 1.23[a]
23	1.58[a]	6.04 dd (15.6, 7.6)	0.82 s	0.82 s
24	2.37 m	9.46 d (7.6)	0.82 s	0.83 s
25	—	—	0.88 s	0.91 s
26	2.11 s	—	0.98 s	1.19 s
27	—	—	1.32[a]; 0.48 d (5.6)	1.34[a] 0.22 d (6.4)
28	0.94 s	0.95 s	0.82 s	0.87 s
29	0.78 s	0.79 s	0.95 d (6.0)	0.96[a]
30	0.86 s	0.89 s	0.86 d (5.6)	0.85[a]
CH_3CO	—	—	2.01 s	2.01 s
HCO	—	—	—	8.18 s
OH	—	—	—	1.99 br s

a) Overlapped with other signals.

* To whom correspondence should be addressed. e-mail: yhkuo@ccms.ntu.edu.tw

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

C	1	2	3	4
1	32.0	31.9	38.7[a)]	38.6
2	30.4	30.3	23.3	23.5
3	78.8	78.8	80.7	80.6
4	40.5	40.5	37.7	37.7
5	47.1	47.0	54.9	55.6
6	21.1	21.0	18.2	18.1
7	26.0	26.0[a)]	38.3[a)]	38.3
8	48.0	47.9	34.6	37.1
9	20.0	19.8	52.6	58.1
10	26.1	26.1	36.3	37.9
11	26.5	26.3[a)]	120.5	70.3
12	32.9	32.8	138.5	83.8
13	45.3	45.8	31.9	26.5
14	48.8	48.8	33.5	32.3
15	35.5	35.6	65.9	21.3
16	28.1	28.2	38.2	27.1
17	52.1	51.3	40.6	31.5
18	18.0	18.3	51.6	46.0
19	29.9	29.9	40.9	41.3
20	35.9	40.7	38.1	38.0
21	18.2	18.6	30.8	31.1
22	35.7	164.7	41.7	42.0
23	20.7	130.8	27.7	27.8
24	44.3	194.6	16.2	16.3
25	209.4	—	17.3	17.9
26	29.9	—	17.9	18.5
27	—	—	12.8	10.1
28	25.4	25.4	28.7	28.0
29	14.0	14.0	17.7	17.4
30	19.3	19.3	20.5	20.6
CH_3CO	—	—	170.9	170.9
CH_3CO	—	—	21.3	21.3
HCO	—	—	—	161.5

a) Values may be interchanged.

relation spectroscopy (COSY), HMQC, HMBC, and Nuclear overhauser and exchange spectroscopy (NOESY) spectral data. The signal at δ 2.40 (m, H-20) had ^{1}H–^{1}H correlations with H-17 (δ 1.74), H_3-21 (δ 1.07, d), and H-22 (δ 6.70, dd). The long-range ^{13}C–^{1}H correlations (HMBC) were observed as follows: C-20/H_3-21, H-23; C-21/H-22; C-22/H_3-21; C-23/H-24; and C-24/H-22. This result confirmed the structure of the side chain's moiety. Thus, the structure of compound **2** was deduced to be (22*E*)-25,26,27-*trinor*-3β-hydroxycycloart-22-en-24-al.

Compound **3**, a colorless solid, had a molecular formula of $C_{32}H_{50}O_3$ on the basis of its HR-EI-MS and ^{13}C-NMR (Table 2) data. It contained hydroxyl and acetoxyl groups due to the IR absorption bands at 3449, 1734, and 1248 cm^{-1}. The ^{1}H-NMR data of **3** showed six singlet groups (δ 0.82, 0.82, 0.82, 0.88, 0.98, 2.01), two doublet methyl groups [δ 0.86 (J=5.6 Hz), 0.95 (J=6.0 Hz)], two methine protons [δ 4.41 (dd, J=11.2, 7.2 Hz, H-15), 4.44 (dd, J=10.4, 6.4 Hz, H-3)], two olefinic protons [δ 5.12 (dd, J=10.4, 3.2 Hz, H-11), 5.69 (dd, J=10.4, 3.2 Hz, H-12)], and a high field signal of a cyclopropyl proton [δ 0.48 (d, J=5.6 Hz, H-27)] (Table 1). The ^{13}C-NMR and DEPT data showed an ester carbonyl (δ_C 170.9), a disubstituted double bond (δ_C 138.5, 120.5), and two oxygenated carbons (δ_C 80.7, 65.9). On account of the molecular formula $C_{32}H_{50}O_3$, the index of hydrogen deficiency (IHD) of **3** was eight including one ester carbonyl and one olefinic functionality. Thus, the number of rings of **3** should be six. 13,27-Cycloursane, a rare skeleton of triterpene in nature, possessed six cyclic rings including one cyclopropyl ring. HMQC and HMBC experiments showed two cyclopropyl protons at δ 1.32 and 0.48 had long-range correlations with one of the olefinic carbons (δ_C 138.5, C-12) (Table 2), one of the carbinol methine carbons at δ_C 65.9 (C-15), three quaternary carbons [δ_C 34.6 (C-8), 31.9 (C-13), 33.5 (C-14)], and one tertiary carbon (δ_C 51.6, C-18). All these data suggested that compound **3** may be a 13,27-cycloursane-type triterpene with an acetoxyl group at C-3, a hydroxyl group at C-15 and a double bond at C-11, 12. The proposed structure was also confirmed by HMQC, HMBC and COSY experiments. H-15 was assigned to have β-orientation as H_3-26 ascribed to NOESY correlation with H_3-26. Based on the above evidence, the structure of **3** could be assigned as 3β-acetoxy-15α-hydroxy-13,27-cyclours-11-ene. This unique structure was further proved by a single-crystal X-ray diffraction study (Fig. 1). To our knowledge, the only derivative of the 13,27-cycloursane skeleton is phyllanthol (13,27-cycloursan-3β-ol), which was first isolated from *Phyllanthus engleri* (Euphorbiaceae) in the 1951.[11)]

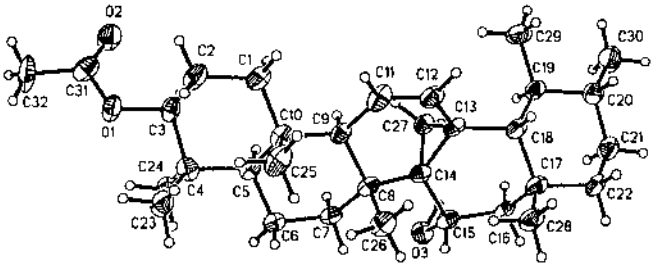


Fig. 1. ORTEP Drawing of **3**

Compound **4**, $C_{33}H_{52}O_5$ (from HR-EI-MS and ^{13}C-NMR), also had a high field signal of a cyclopropyl proton at δ 0.22 (d, J=6.4 Hz). IR absorption bands at 3491, 1734, 1248 cm^{-1} indicated it contained hydroxyl and ester carbonyl groups. ^{1}H-NMR spectrum of **4** exhibited signals for seven methyl groups (δ 0.82, 0.83, 0.85, 0.87, 0.91, 0.96, 1.19) (Table 1), one acetoxyl and one formyloxyl group [δ 2.01 (3H, s), 8.18 (1H, s)] and three oxygenated methine protons [δ 3.52 (br d, J=5.6 Hz, H-11), 4.45 (dd, J=11.2, 4.8 Hz, H-3), 5.13 (s, H-12)]. The signal at δ 8.18 corresponded to a carbon signal at δ_C 161.5 ppm as well as the MS spectrum [base peak at *m*/*z* 482 (M^+−HCOOH)] typically exhibited for a formyloxyl group. ^{13}C-NMR and DEPT spectra of **4** indicated eight CH_3, nine CH_2, nine CH, and seven C, including three carbons to which oxygen was attached (C-3, C-11, C-12), and two carbonyl carbons. Because of the molecular formula $C_{33}H_{52}O_5$, the IHD of **4** was eight including one acetoxyl and one formyloxyl functionality. Thus, the number of rings in **4** should be six. All these data suggested that **4** was a 13,27-cycloursane-type triterpene with one hydroxyl, one acetoxyl, and one formyloxyl group. Comparison of the ^{13}C-NMR data (Table 2) of **4** with those of **3** as well as two dimensional (2D) NMR (HMQC, HMBC, COSY) experiments (see Fig. 2) suggested that **4** was a 13,27-cycloursane-type triterpene with an acetoxyl group at C-3, a hydroxyl group at C-11, and a formyloxyl group at C-12. The β-orientation of H-11 and H-12 was elucidated by a NOESY experiment (Fig. 3). H-11 had NOESY correlations with H-12, H_3-25, and H_3-26; and

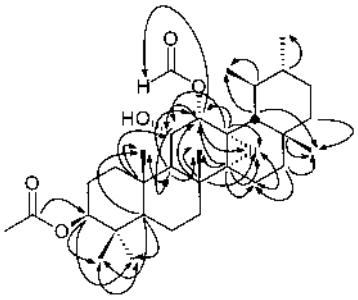


Fig. 2. HMBC Correlations of **4**

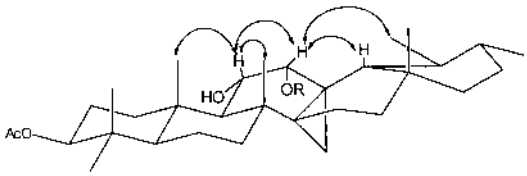
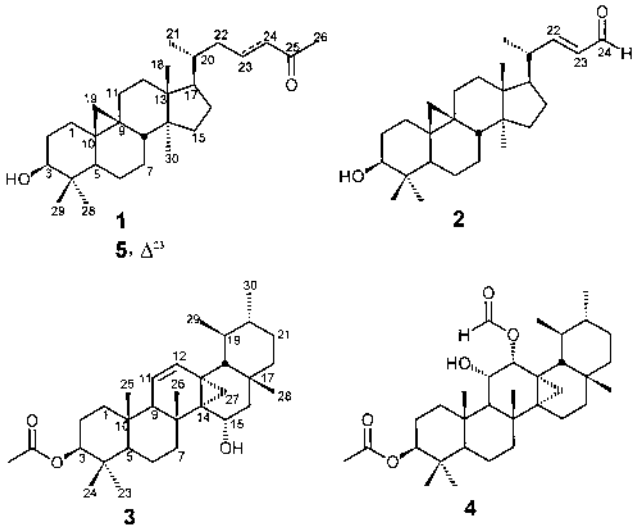


Fig. 3. Key NOSEY Correlations of **4** (R=formyl)



H-12 with H-11, H-18, and H_3-29. Therefore, the structure of **4** was found to be 3β-acetoxy-12α-formyloxy-13,27-cycloursan-11α-ol.

Experimental

Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 983G spectrophotometer. ^{1}H- 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 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_2O (1 l), and this phase was then partitioned with ethyl acetate (1 l×3). The combined 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 **3** and **4** were eluted with 5% EtOAc in hexane. Further purification by HPLC [Merck LichroCART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μm)] gave **3** (19 mg) and **4** (6 mg) using 20% EtOAc/hexane and 25% EtOAc/hexane, respectively. Crude compounds **1**, **2**, and **5** were eluted with 20% EtOAc in hexane. Further purification by HPLC [Merck LichroCART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μm)] gave **1** (5 mg), **2** (4 mg), **5** (9 mg) using 25% EtOAc/hexane and 30% EtOAc/hexane and 30% EtOAc/hexane, respectively.

27-*nor*-3β-Hydroxy-25-oxocycloartane (**1**): mp 127—129 °C, $[\alpha]_D^{25}$ +38.0° (c=0.3, $CHCl_3$). IR ν_{max} cm^{-1}: 3423, 3039, 1716, 1377, 1163, 1099, 1026. ^{1}H-NMR data: see Table 1. ^{13}C-NMR data: see Table 2. EI-MS (70 eV) m/z (rel. int. %): 428 (M^+, 12), 413 (18), 410 (68), 395 (100), 367 (31), 341(24), 297 (45), 288 (26), 203 (46), 175 (60), 121 (56), 107 (60), 95 (68). HR-EI-MS m/z: 428.3650 (M^+ Calcd for $C_{29}H_{48}O_2$: 428.3656).

(22*E*)-25,26,27-*trinor*-3β-Hydroxycycloart-22-en-24-al (**2**): mp 110—113 °C, $[\alpha]_D^{25}$ +47.9° (c=0.2, $CHCl_3$). UV λ_{max} (log ε) nm: 224 (4.08). IR ν_{max} cm^{-1}: 3458, 3041, 1695, 1635, 1385, 1101, 1049, 738. ^{1}H-NMR data: see Table 1. ^{13}C-NMR data: see Table 2. EI-MS (70 eV) m/z (rel. int. %): 398 (M^+, 25), 380 (82), 365 (95), 337 (68), 311 (24), 297 (39), 258 (48), 227 (48), 187 (44), 175 (67), 147 (67), 133 (79), 119 (90), 107 (100), 95 (92). HR-EI-MS m/z: 398.3186 (M^+ Calcd for $C_{27}H_{42}O_2$: 398.3187).

3β-Acetoxy-15α-hydroxy-13,27-cyclours-11-ene (**3**): mp 130—135 °C, $[\alpha]_D^{25}$ +16.8° (c=1.6, $CHCl_3$). UV λ_{max} (log ε) nm: 252 (3.25). IR ν_{max} cm^{-1}: 3449, 3006, 1734, 1374, 1248, 1030, 757. ^{1}H-NMR data: see Table 1. ^{13}C-NMR data: see Table 2. EI-MS (70 eV) m/z (rel. int. %): 482 (M^+, 72), 464 (60), 451 (18), 422 (34), 391 (38), 353 (100), 293 (26), 269 (47), 253 (54), 189 (62), 135 (68), 123 (76). HR-EI-MS m/z: 482.3763 (M^+ Calcd for $C_{32}H_{50}O_3$: 482.3762).

3β-Acetoxy-12α-formyloxy-13,27-cyclours-11α-ol (**4**): mp 269—273 °C, $[\alpha]_D^{29}$ +43.9° (c=0.5, $CHCl_3$). IR ν_{max} cm^{-1}: 3491, 1734, 1458, 1369, 1248, 1182, 1090, 1030, 987, 739. ^{1}H-NMR data: see Table 1. ^{13}C-NMR data: see Table 2. EI-MS (70 eV) m/z (rel. int. %): 528 (M^+, 4), 510 (1), 482 (100), 464 (45), 422 (16), 381 (10), 369 (14), 355 (23), 329 (14), 317 (25), 277 (44), 230 (56), 189 (44), 147 (50), 135 (59), 95 (74). HR-EI-MS m/z: 528.3808 (M^+ Calcd for $C_{33}H_{52}O_5$: 528.3817).

Hydrogenation of 5 Compound **5** (9 mg) in ethyl acetate (2 ml) and 10% Pd/C (6 mg) were placed in a 25-ml flask under H_2 atmosphere for 2 h. The catalyst was filtered and washed with EtOAc. The solvent of combined filtrate was removed *in vacuo*, and the product was identified as compound **1** (7 mg).

X-Ray Crystal Structure Analysis of 3 A colorless crystal of **3** with dimensions 0.30×0.30×0.35 mm was selected for X-ray analysis. The X-ray crystal data were collected at room temperature using an Enraf-Nonius CAD4 diffractometer equipped with graphite-monochromated Mo-Kα radiation (λ=0.7107 Å), θ–θ scan mode. The structure was solved and refined by a full-matrix least-squares method using the NRCVAX[12] software package. The compound was crystallized in the monoclinic space group $P2_1$, a=7.9147(17) Å, b=16.326(6) Å, c=10.983(3) Å, β=90.676(20)°, V=1419.0(7) A^3, Z=2, D_{calc}=1.130 g/cm^3, F(000)=532, and T=298 K. A total of 3780 reflections yielded 3369 unique reflections. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using riding mode. The final indices were R=0.048, R_w=0.045 with goodness-of-fit=1.46. Scattering factors were taken from the International Tables for X-ray Crystallography.[13]

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