Two Novel Triterpenes from the Leaves of *Ficus microcarpa*

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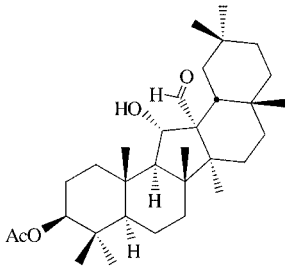
Two novel triterpenes, 29(20 → 19)abeolupane-3,20-dione (**4**) and 19,20-secoursane-3,19,20-trione (**5**), besides (3β)-3-hydroxy-29(20 → 19)abeolupan-20-one (**2**), lupenone, and α-amyrone (**6**), were isolated from the leaves of *Ficus microcarpa* and were characterized by spectroscopic means, including 2D-NMR techniques and chemical methods. Compound **4** is the second derivative having the 29(20 → 19)abeolupane skeleton, and **5** is a novel skeleton. A biosynthetic pathway to **5** is proposed (*Scheme*).

1. Introduction. – About 850 species of *Ficus* have been found in tropic and subtropic areas. In Taiwan, twenty-one endemic and twenty transplanted species have been reported. *Ficus microcarpa* L. f. (Moraceae) is a popular ornamental plant in the orient. The strong vitality of this plant with its unique aerial roots as well as antiplatelet activity prompted us to study the chemical compounds present in different parts of the plant. From the bark, two new isoflavones besides 28 components [1][2] were discovered, and from the heartwood, nine new compounds containing lignans, lactones, and phenols were isolated [3–5]. Aerial roots in the plant kingdom is a very unique event. More than 20-years-old aerial roots were collected on the campus of National Taiwan University, and 24 new triterpenes (including twelve taraxastanes, seven ursanes, three oleananes, and two lupanes) [6–10] and three novel triterpenoides, *i.e.*, (3β,11α)-3-(acetyloxy)-11-hydroxy-11(12 → 13)abeooleanan-12-al (**1**), (3β)-3-hydroxy-29(20 → 19)abeolupan-20-one (**2**), and (3β)-3-acetoxy-29,30-dinor-18,19-secolupane-18,19-dione (**3**) [11], besides two novel spirotocopheroids [12], were isolated and elucidated.

Previously, *Higa et al.* [13] have investigated the leaves of *F. microcarpa:* only six known compounds (three friedelanes, one lupane, one taraxerane, and one oleanane) were purified. This result encouraged us to reinvestigated the components of the leaves. In the present study, the two novel triterpenes **4** and **5** were isolated from the leaves, besides **2**, lupenone [14], and α-amyrone (**6**) [15]. Compound **4** has the same novel skeleton as **2**, with which it is chemically correlated. Compound **5** has a uniquely novel skeleton and was elucidated as 19,20-secoursane-3,19,20-trione. A biogenetic pathway for the formation of **5** is proposed.

2. Results and Discussion. – Compound **4**, isolated as colorless crystals, analyzed for $C_{30}H_{48}O_2$, which requires indices of hydrogen deficiency (IHD) seven on the basis of its HR-EI-MS and ^{13}C-NMR spectrum (*Table*). Its IR spectrum showed only a ketone (1707 cm^{-1}) functionality. Lupenone, isolated from the same source, had similar ^{1}H- and ^{13}C-NMR data (*Table*) for the A, B, and C rings. Thus, the structure of **4** may be a



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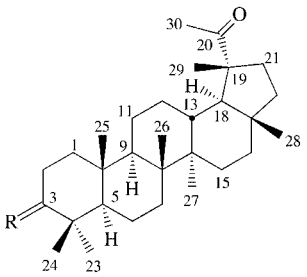
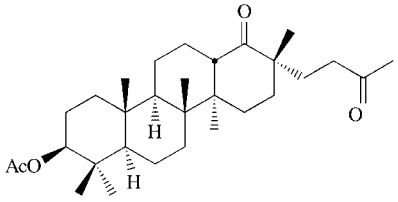


2 R = α-H, β-OH
4 R = O



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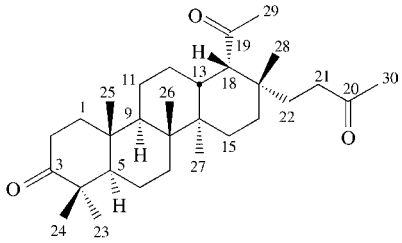

5

lupane-type triterpene. Comparison of the ^{1}H- and ^{13}C-NMR data of **4** with those of **2** suggests that **4** has the same skeleton as **2**, the sole difference being the 3-oxo group in **4** instead of the 3-hydroxy group in **2**. Oxidation of **2** with pyridinium chlorochromate (PCC) in CH_2Cl_2 yielded a product identical to **4**. The configuration of **2** was determined previously as (3*S*,5*R*) by the modified *Mosher* method. Therefore, **4** has (5*R*) configuration, and the structure of **4** can be assigned as 29(20 → 19)abeolupane-3,20-dione. It is the second derivative of the novel 29(20 → 19)abeolupane skeleton.

The ^{1}H-NMR spectrum (*Table*) of **4** exhibited seven *s* for Me groups (δ 0.88 (Me(25)), 0.96 (Me(28)), 0.99 (Me(27)), 1.01 (Me(24)), 1.05 (Me(23)), 1.06 (Me(26)), and 1.21 (Me(29))) and one *s* for an acetyl group (δ 2.13 (Me(30))). The ^{13}C-NMR spectrum exhibited 30 signals (eight Me, ten CH_2, four CH, and eight C) including an acetyl C=O (δ 213.9, C(20)) and a cyclohexanone C=O (δ 218.1, C(3)). Since the IHD of **4** was seven including two C=O functionalities, and since no sp^2 C-atom was observed, the number of rings in **4** should be five. In the HMBC spectrum, the long-range ^{1}H,^{13}C correlations Me(29)/C(18) and C(20), H–C(18)/C(19) and C(20), Me(30)/C(20), and H_β–C(21)/C(18), C(19), C(22), and C(29) established the partial structure of the E ring (*Fig. 1,a*). NOESY Correlations for Me(27)/H–C(18), Me(29)/Me(28) and H–C(13), and Me(30)/H–C(18), H_α–C(21), and Me(29) suggested that Me(29) should be on the same face as H–C(13) and Me(28) (*Fig. 1,b*). The signals of the axial and equatorial H-atoms were assigned by NOESY experiments.

Compound **5** was isolated as a colorless solid. The molecular formula $C_{30}H_{48}O_3$ was established by its ^{13}C-NMR and HR-EI-MS data, representing IHD seven. The IR spectrum of **5** showed only a ketone functionality at 1712 cm^{-1}. Comparison of the ^{1}H- and ^{13}C-NMR data of **5** with those of **2** revealed that the signals of the A, B, and C rings

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$ 400 and 100 MHz) *of Compounds* **4** and **5**. δ in ppm, J in Hz.

	δ(C)		δ(H)	
	4	**5**	**4**	**5**
CH_2(1)	39.5 (*t*)	39.5 (*t*)	1.37 (*m*, H_α), 1.86 (*m*, H_β)	1.40 (*m* H_α), 1.88 (*m*, H_β)
CH_2(2)	34.1 (*t*)	34.0 (*t*)	2.40 (*ddd*, *J* = 17.6, 7.6, 4.4, H_α), 2.46 (*ddd*, *J* = 17.6, 9.0, 7.7, H_β)	2.41 (*m*, H_α), 2.44 (*m*, H_β)
C(3)	218.1 (*s*)	218.1 (*s*)	–	–
C(4)	47.3 (*s*)	47.3 (*s*)	–	–
H–C(5)	54.9 (*d*)	54.6 (*d*)	1.31 (*dd*, *J* = 11.9, 3.0)	1.32[a])
CH_2(6)	19.7 (*t*)	19.6 (*t*)	1.45[a])	1.45[a])
CH_2(7)	33.4 (*t*)	32.3 (*t*)	1.36 (*m*, H_α), 1.45 (*m*, H_β)	1.33 (*m*, H_α), 1.45 (*m*, H_β)
C(8)	40.9 (*s*)	41.8 (*s*)	–	–
H–C(9)	49.9 (*d*)	49.5 (*d*)	1.43[a])	1.40[a])
C(10)	36.9 (*s*)	36.8 (*s*)	–	–
CH_2(11)	21.3 (*t*)	22.0 (*t*)	1.50 (*m*, H_α), 1.40(*m*, H_β)	1.56 (*m*, H_α), 1.40 (*m*, H_β)
CH_2(12)	25.4 (*t*)	28.7 (*t*)	1.26 (*m*, H_α), 1.45 (*m*, H_β)	1.40 (*m*, H_α), 1.63 (*m*, H_β)
H–C(13)	34.8 (*d*)	35.2 (*d*)	1.74 (*td*, *J* = 12.0, 2.6)	2.24 (*m*)
C(14)	43.2 (*s*)	41.5 (*s*)	–	–
CH_2(15)	27.5 (*t*)	26.3 (*t*)	1.04 (*m*, H_α), 1.66 (*m*, H_β)	1.08 (*m*, H_α), 1.60 (*m*, H_β)
CH_2(16)	37.8 (*t*)	29.8 (*t*)	1.50 (*m*, H_α), 1.40 (*m*, H_β)	2.05 (*m*, H_α), 1.12 (*m*, H_β)
C(17)	43.4 (*s*)	35.5 (*s*)	–	–
H–C(18)	50.6 (*d*)	59.6 (*d*)	1.98 (*d*, *J* = 12.0)	2.46 (*d*, *J* = 5.7)
C(19)	54.9 (*s*)	213.2 (*s*)	–	–
C(20)	213.9 (*s*)	209.1 (*s*)	–	–
CH_2(21)	37.7 (*t*)	38.3 (*t*)	1.88 (*m*, H_α), 1.58 (*m*, H_β)	2.16 (*m*), 2.36 (*m*)
CH_2(22)	40.5 (*t*)	34.8 (*t*)	1.30 (*m*, H_α), 1.58 (*m*, H_β)	1.47 (*m*), 1.55 (*m*)
Me(23)	26.6 (*q*)	26.7 (*q*)	1.05 (*s*)	1.05 (*s*)
Me(24)	21.6 (*q*)	21.0 (*q*)	1.01 (*s*)	1.00 (*s*)
Me(25)	15.9 (*q*)	16.0 (*q*)	0.88 (*s*)	0.90 (*s*)
Me(26)	15.7 (*q*)	15.7 (*q*)	1.06 (*s*)	1.03 (*s*)
Me(27)	15.2 (*q*)	13.7 (*q*)	0.99 (*s*)	0.94 (*s*)
Me(28)	20.0 (*q*)	24.0 (*q*)	0.96 (*s*)	0.92 (*s*)
Me(29)	20.2 (*q*)	36.3 (*q*)	1.21 (*s*)	2.12 (*s*)
Me(30)	25.4 (*q*)	29.7 (*q*)	2.13 (*s*)	2.09 (*s*)

[a]) Overlapping.

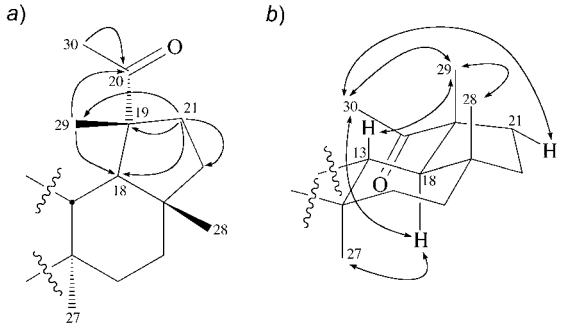

Fig. 1. a) *Selected HMBC correlations and* b) *selected NOESY correlations of* **4**

were almost identical. A HMBC experiment confirmed the structure of **5**, suggesting that oxidative cleavage between C(19) and C(20) of an ursane-type compound produced two functionalities, *i.e.*, the acetyl and 3-oxobutyl groups. Therefore, the structure of compound **5** can be elucidated as 19,20-secoursane-3,19,20-trione. The absolute configuration of **5** is proposed to be the same as that of **4**, except for the configuration at C(18). The other stereogenic centers of **5** were confirmed by NOESY correlations.

The ^{1}H-NMR (*Table*) spectrum of **5** showed six *s* for Me groups (δ 0.90 (Me(25)), 0.92 (Me(28)), 0.94 (Me(27)), 1.00 (Me(24)), 1.03 (Me(26)), and 1.05 (Me(23))) and two *s* for two acetyl groups (δ 2.09 (Me(30)), 2.12 (Me(29))). ^{13}C-NMR (*Table*) and DEPT spectra suggested the presence of eight Me, ten CH_2, four CH, and eight C, including three C=O (δ 218.1 (C(3)), 213.2 (C(19)), 209.1 (C(20))), and no sp^2 C-atom was observed. Because the IHD of **5** was seven including three C=O groups, the number of rings in **5** should be four. Thus, **5** with 30 C-atoms including 8 Me groups could be an oleanane, ursane, or taraxastane-type triterpene. The location of the three C=O groups was determined as follows. One C=O arose from an acetyl group attached to the tertiary C(18) (δ 59.6) since Me(29) (δ 2.12) of this acetyl group exhibited an HMBC correlation to C(18). The second C=O was incorporated in a 3-oxobutyl group connected to the a quaternary C(17) (δ 35.5); the corresponding signals of Me(30) (δ(H) 2.09, δ(C) 29.7) and of C(20) (δ(C) 209.1) were typical of the terminal acetyl moiety of the 3-oxobutyl group, and the four mutually coupled methylene protons of CH_2(21) (δ 2.36, 2.16) and CH_2(22) (δ 1.55, 1.47) were flanked by a C=O and a quaternary C-atom (C(17)), respectively, the latter carrying a Me group, *i.e.*, Me(28) (δ 0.92). HMBC Correlations (*Fig. 2,a*) established that the acetyl and 3-oxobutyl groups were in vicinal position. NOESY Correlations for Me(28)/H–C(22), H–C(18), and H–C(13), Me(29)/H$_\alpha$–C(12), and Me(27)/H$_\alpha$–C(7), and the small coupling between H–C(18) and H–C(13) (J = 5.7 Hz) confirmed the α-axial position of the acetyl group and the α-equatorial position of the 3-oxobutyl group (*Fig. 2,b*). The signals of the axial and equatorial protons were assigned by NOESY experiments.

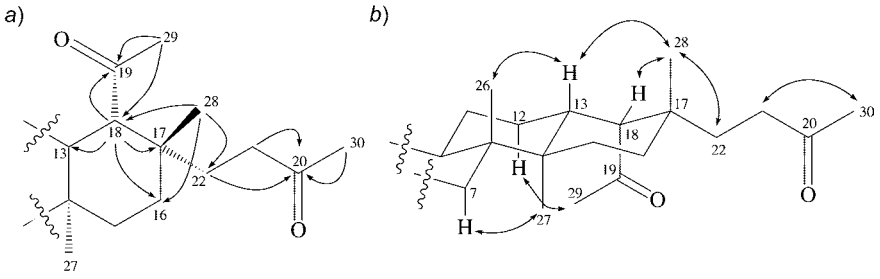

Fig. 2. a) *Selected HMBC correlations and* b) *selected NOESY correlations of* **5**

We suggest that the biosynthesis of **5** originates from α-amyrone (**6**) (*Scheme*). Reduction of **6** would produce (13β)-12,13-dihydro-α-amyrone (**7**), which is subsequently dehydrogenated (or hydroxylated and then dehydrated) to yield **8**. Dioxygenase would then oxidize **8** to afford **5**.

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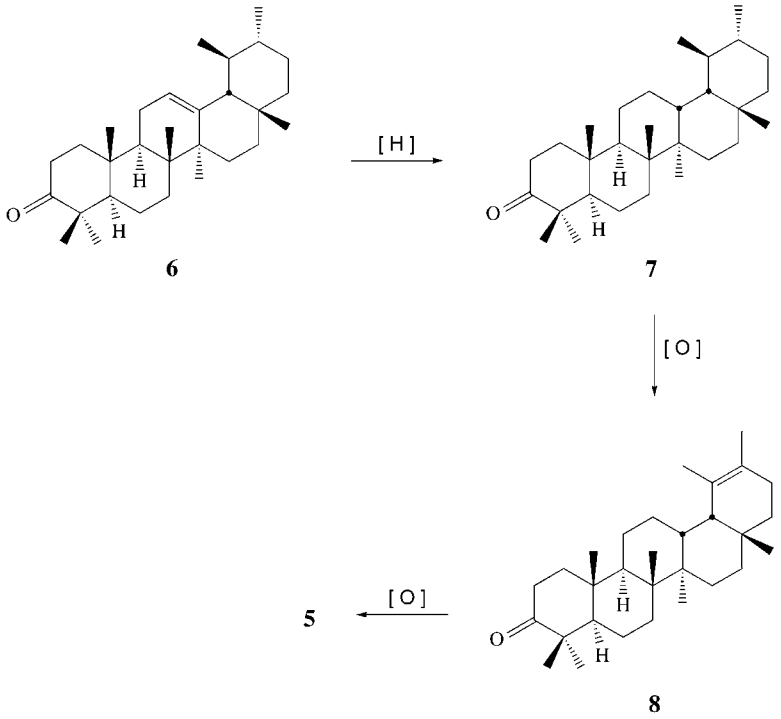
Scheme. *Proposed Mechanism for the Biosynthesis of* **5** *from α-Amyrone* (**6**)



Experimental Part

General. Column chromatographed (CC): silica gel (*Merck* 70–230 mesh, 230–400 mesh, ASTM). HPLC: *LDC Analytical-III*; *LiChrosorb Si60* column (250 × 10 mm, 7 μm). M.p.: *Yanagimoto* micro-melting-point apparatus; uncorrected. Specific rotation: *Jasco DIP-180* digital polarimeter. IR Spectra: *Perkin-Elmer 983-G* spectrophotometer; in cm^{-1}. ^1H- and ^{13}C-NMR spectra: *Bruker DMX-400* spectrophotometer; δ in ppm, J in Hz. EI-MS: *Jeol JMS-HX-300* mass spectrometer; in m/z (rel. %).

Plant Material. The leaves of *F. microcarpa* were collected on the campus of National Taiwan University, Taipei, Taiwan, in 2000. The plant was identified by Mr. *Muh-Tsuen Gun*, formerly a technician of the 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.

Extraction and Isolation. The dried leaves of *F. microcarpa* (7.1 kg) were extracted with MeOH (80 l) at r.t. (3 × 10 d). After evaporation, the residue of the MeOH extract was mixed with H_2O to bring the total volume to 1 l. This phase was extracted with AcOEt (3 × 1 l), the combined AcOEt phase evaporated, and the obtained black syrup (345 g) repeatedly purified by CC (silica gel, hexane/AcOEt and HPLC (*LiChrosorb Si 60*, hexane/AcOEt). Compound **4** (6 mg), α-amyrone (**6**) (7 mg), lupenone (8 mg), **2** (9 mg), and **5** (8 mg) were eluted with 20%, 20%, 20%, 30%, and 50% AcOEt in hexane, respectively.

29(20→19)Abeolupane-3,20-dione (**4**). M.p. 167–168°. $[\alpha]_D^{23} = +17.7$ ($c = 0.17$, $CHCl_3$). IR (film): 1707, 1385, 1363, 1258, 1183, 759. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 440 (14, M^+), 397 (15), 294 (27), 236 (24), 221 (23), 205 (20), 178 (73), 161 (18), 147 (91), 97 (59), 57 (100). HR-EI-MS: 440.3646 ($C_{30}H_{48}O_2^+$; calc. 440.3649).

19,20-Secoursane-3,19,20-trione (**5**). M.p. 117–118°. $[\alpha]_D^{23} = +80.5$ ($c = 0.08$, $CHCl_3$). IR (film): 1712, 1386, 1355, 1168, 670. ^1H- and ^{13}C-NMR: *Table*. EI-MS: 456 (86, M^+), 438 (23), 413 (24), 395 (81), 249 (38), 175 (55), 95 (84), 69 (86), 55 (100). HR-EI-MS: 456.3612 ($C_{30}H_{48}O_3^+$; calc. 456.3598).

Oxidation of (+)-(3β)-3-Hydroxy-29(20→19)abeolupan-20-one (**2**). A mixture of **2** (3 mg), PCC (10 mg), and CH_2Cl_2 (5 ml) was stirred at r.t. for 1 h. Excess Et_2O (40 ml) was poured into the mixture, which

was then filtered over *Celite*. The filtrate was evaporated and the residue purified by HPLC: **4** (3 mg), which was identical to the isolated **4**.

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