Three New Compounds, Ficusone, Ficuspirolide, and Ficusolide from the Heartwood of *Ficus microcarpa*

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One new apocarotenoid, ficusone (1), and two novel γ-lactone derivatives, ficuspirolide (2) and ficusolide (3) as well as 4,5-dihydroblumenol (4), were isolated from the heartwood of *Ficus microcarpa* L.f. Their structures were principally determined by spectral evidence.

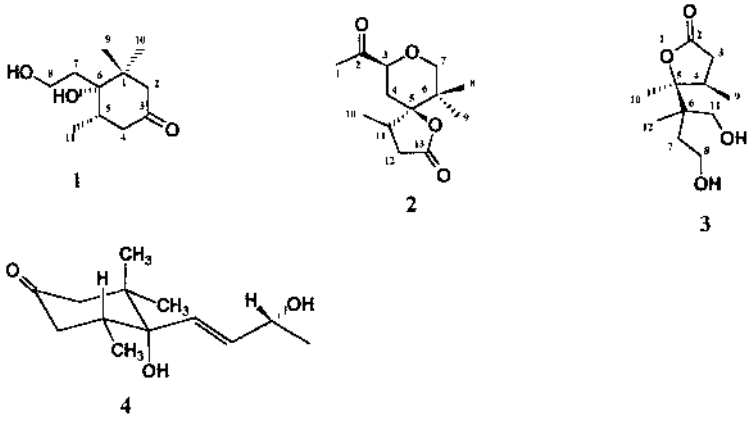
Key words *Ficus microcarpa* L.f.; Moraceace; homomonoterpene; ficusone; ficuspirolide; ficusolide

Ficus microcarpa L.f. (Moraceace) is a popular ornamental plant in the Orient. Chemical study reported that six terpenoids were observed in the leaves of the plant.[1)] The strong vitality of this plant as well as its antiplatelet activity from methanol extract of the bark caused us to determine the chemical components, and in our previous report, we reported the finding of twenty-eight compounds including triterpenes, a fatty alcohol, steroids, a coumarin, a flavane, 4-hydroxybenzoate, megastigmane [4,5-dihydroblumenol][2)] as well as two new isoflavones.[3)] In connection with our interest in components of the plant, chemical studies on the heartwood of the plant were undertaken in our laboratory.

The methanol extract of the heartwood of *F. microcarpa* was concentrated to give a residue which was suspended with water. The suspended aqueous solution was partitioned with *n*-hexane, ethyl acetate, and *n*-BuOH successively. The ethyl acetate extract was repeatedly purified by silica gel and HPLC, and we isolated one new compound, ficusone (**1**), two novel lactones, ficuspirolide (**2**) and ficusolide (**3**), as well as 4,5-dihydroblumenol (**4**).[2,4)] In this paper, we report the structure of these new compounds.

The molecular formula of ficusone (**1**) was established as $C_{11}H_{20}O_3$ by high resolution mass spectroscopy. The IR spectrum of **1** showed bands attributable to hydroxy (3418 cm^{-1}) and carbonyl (1696 cm^{-1}; 1690 cm^{-1} in **4**)[4)] groups. The ^{1}H-NMR spectrum of **1** showed two singlet methyl group signals at δ 0.85 (H_3-9) and 1.05 (H_3-10), an $ABXY_3$ system, corresponding to H_β-4 [δ 2.01 (ddd, J=14.4, 4.1, 2.3 Hz)], H_α-4 [δ 2.47 (dd, J=14.4, 14.4 Hz)], H_β-5 [δ 2.07 (dqd, J=14.4, 4.7, 4.1 Hz)], and H_3-11 [δ 1.02 (d, J=4.7 Hz)], an AX system corresponding to H_β-2 [δ 1.82 (dd, J=13.5, 2.3 Hz)] and H_α-2 [δ 2.83 (d, J=3.5 Hz)], and a hydroxyethyl group [δ 1.68, 2.00 (1H each, m, H-7), 3.95 (2H, m, H_2-8)]. H_β-2 (δ 1.82) and H_β-4 (δ 2.01) exhibited small coupling (J=2.3 Hz) due to W-form; this was also found in **4**.[2)] A comparison between the ^{1}H- and ^{13}C-NMR data (Table 1) of **1** and **4** showed a hydroxyethyl moiety in **1** instead of a 3-hydroxy-1-butenyl moiety in **4**. The ^{1}H- and ^{13}C-NMR data were resolved by distortionless enhancement by polarization transfer (DEPT) and proton detected heteronuclear multiple-quantum coherence (HMQC) experiments. The structure was confirmed by the proton detected heteronuclear mulitiple-bond correlation (HMBC) technique. The EI-MS fragment peaks at *m/z* 200 (M^+, 100%), 182 (M^+-H_2O, 22%), 167 ($M^+-H_2O-CH_3$, 58%), 155 ($M^+-CH_2CH_2OH$, 49%) also confirmed the assigned structure. The relative stereochemistry was elucidated by nuclear Overhauser effect (NOE) spectroscopy (NOESY) method; H_2-7 exhibited NOE correlation with three methyl groups, and H_3-9 expressed NOE correlation with H-5 (Fig. 1). This evidence caused us to conclude that C-5 methyl and C-6 hydroxyethyl groups are all in equatorial orientation. Therefore, the structure of ficusone can be assigned as 6α-hydroxy-6β-(2′-hydroxyethyl)-3,3,5α-trimethylcyclohexanone. Khare[5)] and his group synthesized the C-6 epimer of ficusone **1**, showing the two epimers with different physical data. The absolute configuration of **1** was determined from the circular dichroism (CD) spectra (Fig. 2). The CD spectrum of **1** showed negative Cotton effect at 191.5 nm ($\Delta\varepsilon$-181663), the 5-position was determined to have *S* configuration, and therefore 6-position is *S*-configuration.

Ficuspirolide (**2**), $C_{13}H_{20}O_4$ (based on the exact mass of HR-EI-MS), a liquid, shows no significant absorption in its



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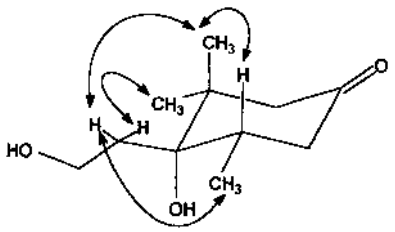

Fig. 1

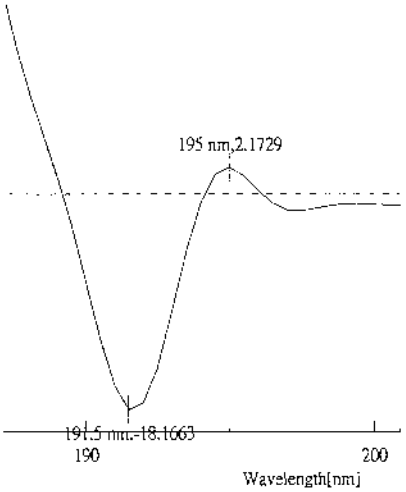

Fig. 2

UV spectrum. It has IR absorption bands at 1776 and 1716 cm^{-1}, suggesting that it contains a γ-lactone and an acetyl group. The presence of two functional groups is attribute to four NMR signals at δ 2.20 (3H, s), 31.3, 174.7 and 206.2. On account of molecular formula $C_{13}H_{20}O_4$ of **2**, the index of hydrogen deficiency (IHD) of **2** is four. Since no olefinic signal (δ 100—150) was observed, then **2** is a bicyclic compound. $ABXY_3$ system signals, corresponding to H_α-12 [δ 2.21 (dd, J=17.6, 9.6 Hz), H_β-12 [δ 2.75 (dd, J=17.6, 10.0 Hz)], H-11 [δ 2.65 (m)], and H_3-10 [(δ 1.18 (d, J=6.4 Hz)], were observed in the ^{1}H-NMR spectrum. Irradiating at H-11, the signals at 2.21, 2.75 and 1.18 collapsed to d, d, and s, respectively. Two H-12 signals had HMBC correlation with lactone carbonyl (δ 174.7) (Table 1). Therefore, the C-12–C-11–C-10 moiety is linked to lactone carbonyl. A quaternary C at δ 96.8 was believed to connect to lactone-*O* atom, meanwhile H-11 and H-10 have HMBC correlation to this carbon. The evidence suggests that this is a spiro-γ-lactone. Two ether carbons express at δ 78.5 (CH_2) and δ 76.6 (CH), and their corresponding signals at δ 3.59 and 3.76 (1H each, J=9.8 Hz, H-7), 4.74 (1H, dd, J=9.6, 2.0 Hz, H-3). The latter signal showed HMBC correlation to acetyl carbonyl carbon (δ 206.2). The acetyl group being connected to C-3 can reasonably show that the H-3 signal is at lower field. Two methylene protons at δ 2.28 (dd, J=16.0, 2.0 Hz, H_α-4) and 2.90 (dd, J=16.0, 9.6 Hz, H_β-4) have larger geminal coupling constants and also couple with signals at δ 4.74. They are discernible by the decoupling technique. The final signals of two singlet methyl groups were presented at δ 0.96 (H_3-8) and 1.12 (H_3-9). Two methyl groups at C-6 express HMBC correlation with each other and with C-5 (δ 96.8). Based on the above evidence together with HMBC method, the structure of ficuspirolide is identified as **2**. Regarding the relative stereochemistry, pronounced NOESY correlation (Fig. 3) between H-11 and H_3-9; H-3 and H_3-10; H_3-8 and H_β-7, H_β-4; H_3-9 and H_α-7 were observed.

Table 1. ^{13}C-NMR Spectral Data of Compounds **1**, **2**, and **3** (75 MHz, in $CDCl_3$)

No.	**1**	**2**	**3**
1	44.0	31.3	
2	51.6	206.2	175.8
3	212.0	76.6	37.1
4	45.9	44.5	33.6
5	39.3	96.8	93.1
6	75.3	46.2	43.4
7	34.3	78.5	33.3
8	61.1	17.4	59.0
9	23.5	23.4	16.7
10	24.4	17.3	20.6
11	16.0	30.4	68.9
12		37.4	20.4
13		174.7	

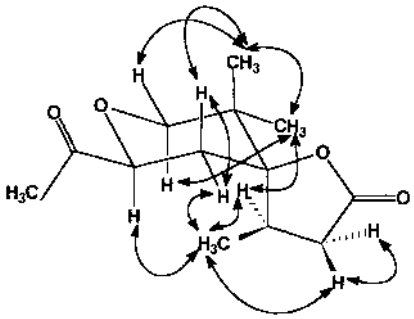

Fig. 3

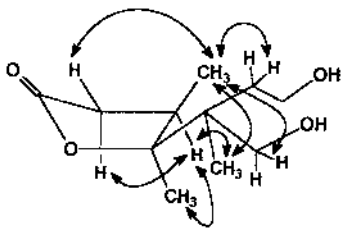

Fig. 4

The EI-MS of ficusolide (**3**) showed a molecular ion peak at *m/z* 216 and its HR-EI-MS indicated the molecular formula to be $C_{11}H_{20}O_4$. The IR spectrum of **3** exhibited absorption bands at 3419 and 1756 cm^{-1} which suggested the presence of hydroxy and γ-lactone groups. Based on the molecular formula $C_{11}H_{20}O_4$ of **3**, its IHD is two. Ficusolide (**3**) has $ABXY_3$ system signals, corresponding to H_β-3 [δ 2.29 (dd, J=18.0, 11.2 Hz)], H_α-3 [δ 2.64 (dd, J=18.0, 10.0 Hz)], H-4 [δ 2.88 (ddq, J=11.2, 10.0, 6.8 Hz)], and H_3-9 [δ 1.18 (d, J=6.8 Hz)]. With irradiation at δ 2.88, the signals at δ 2.29, 2.64 and 1.18 collapsed to d, d, and s, respectively. Carbonyl carbon at δ 175.8 (Table 1) showed HMBC correlation with H-3, so that the C-3–C-4–C-9 moiety was connected to carbonyl. The moiety (C-4–C-5–C-6) also connected to two singlet methyl groups [δ 0.96 (H_3-12), 1.03 (H_3-10)], a hydroxymethyl [δ 68.9; δ 3.47, 3.62 (1H each, d, J=11.2 Hz), and hydroxyethyl groups [δ 33.3, 59.0; δ 2.03, 2.22 (1H each, dt,

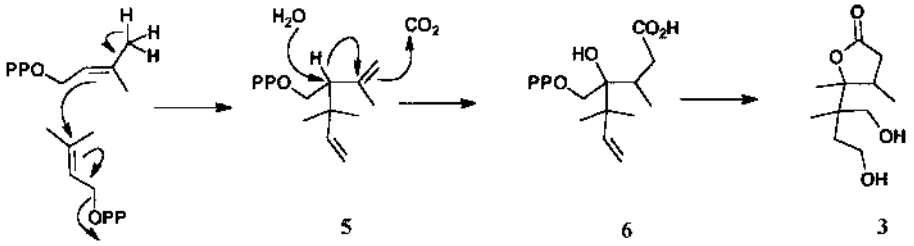

Chart 1

J=14.8, 7.0 Hz), 3.79 (2H, m)]. Six methylene protons in the hydroxymethyl and hydroxyethyl groups are all nonequivalent as well as expressing HMBC correlation with a quaternary C at δ 43.4. It is concluded that these two groups are linked to an asymmetric C-6 (δ 43.4). Three methyl group protons are also correlated to C-5 (δ 93.1) in the HMBC spectrum. The signal at δ 0.96 was assigned as H-12 owing to the C-11 correlated to δ 0.96 in the HMBC spectrum. According to the above evidence, the structure of ficusolide should be assigned as **3**. Its relative stereochemistry, can be shown by the NOESY technique (Fig. 4). The biosynthesis of **3** is proposed in Chart 1. Coupling of two moles 3,3-dimethylallypyrophosphate afforded **5** which followed incorporation of CO_2 and hydration to produce **6**. The compound **3** can be derived from **6** through the following four pathways: 1) oxidation of one of the methyl groups, 2) hydration of double bond, 3) reduction of pyrophosphate, and 4) cyclizing *via* dehydration.

Experimental

General Procedures Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AM-300 spectrometer. Two dimensional (2D) NMR spectra were run on a Varian Unity 400 spectrometer. EI-MS, HR-MS, FAB-MS were taken on a JEOL JMS-HX110. UV and specific rotations were taken on a Hitachi S-3200 spectrometer and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merck 3374, 70—230 mesh) and purified by a semi-preparative normal-phase HPLC column (250×10 mm, 7 μm, LiChrosorb Si 60).

Plant Material The heartwood of *Ficus microcarpa* L.f. was collected on the campus of the National Taiwan University and was identified by Prof. Shao-Shun Ying, Department of Forest, National Taiwan University; a voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation Heartwood of *F. microcarpa* was crushed into pieces to give 7.0 kg (air-dried) of raw material, which was extracted with MeOH (60 l) three times (7 d each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue (58.8 g) that was suspended in water (500 ml). Then, the aqueous solution was partitioned with hexane (500 ml×3), EtOAc (500 ml×4), and *n*-BuOH (500 ml×3), successively. The EtOAc fraction (13.3 g) was chromatographed by silica gel column chromatography (hexane–EtOAc to EtOAc–MeOH solvent system). Crude compounds **1**, **2** and **4** were eluted by hexane : EtOAc=1 : 1, and crude **3** eluted by CH_3OH : EtOAc=1 : 5. Further purification by HPLC gave **1** (3.1 mg), **2** (2.8 mg), **3** (3.0 mg), and **4** (7 mg) using acetone : CH_2Cl_2=1 : 10, hexane : EtOAc : iso-PrOH=7 : 1 : 0.2, and acetone : CH_2Cl_2 : iso-PrOH=1 : 3 : 0.2, respectively.

Ficusone (**1**): Amorphous, $[\alpha]_D^{15}$=+24.5° (c=0.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3418, 1696, 1380, 1370, 1288, 1151, 1057. ^{13}C-NMR data see Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 200 (M^+, 100), 182 (22), 167 (58), 155 (49), 152 (24). HR-EI-MS *m/z* [M^+] Calcd for $C_{11}H_{20}O_3$: 200.1413. Found: 200.1418.

Ficuspirolide (**2**): Liquid, $[\alpha]_D^{20}$=+70.8° (c=0.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1776, 1716, 1269, 1232, 1054. ^{13}C-NMR data see Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 240 (M^+, 8), 225 (44), 181 (27), 165 (27), 139 (100), 125 (22). HR-EI-MS *m/z* [M^+] Calcd for $C_{13}H_{20}O_4$: 240.1362. Found: 240.1359.

Ficusolide (**3**): Amorphous, $[\alpha]_D^{18}$=+3.4° (c=0.3, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3419, 1756, 1267, 1164, 1041, 957. ^{13}C-NMR data see Table 1. EI-MS (70 eV) *m/z* (rel. int. %): 216 (M^+, 65), 198 (25), 187 (90), 177 (65), 154 (100), 137 (95), 107 (42). HR-EI-MS *m/z* [M^+] Calcd for $C_{11}H_{20}O_4$: 216.1362. Found: 216.1353.

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