Two New Isoflavones from the Bark of *Ficus microcarpa*

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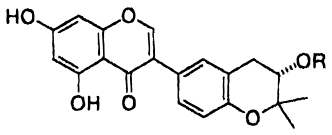
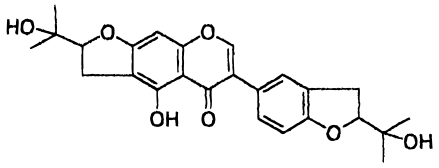
Two new isoflavones, ficuisoflavone (**1**) and isolupinisoflavone E (**2**), were isolated from the bark of *Ficus microcarpa* L.f.. Their structures were principally elucidated by spectral methods.

Fifty-two species of *Ficus* (Moraceace) grow in Taiwan. One of the species, *F. microcarpa* L.f., is a popular ornamental plant in the Orient. Only one chemical study of an EtOH extract from the leaves of *F. microcarpa* L.f. has been reported, and six triterpenoids were observed.[1] The crude MeOH extract from the bark of this plant showed antiplatelet activity, which caused us to determine the chemical components. After fractionation of this extract using repeated column chromatography on Si gel and HPLC, we isolated two new isoflavones, ficuisoflavone (**1**) and isolupinisoflavone E (**2**). In this paper, we report the structure of these isoflavones.



1 R=H

3 R=Ac



2

Ficuisoflavone (**1**), a yellow viscous liquid, gave a positive ferric chloride test and a negative MgHCl test. Compound **1** was formulated as $C_{20}H_{16}O_6$ on the basis of its HRMS. The IR spectrum of **1** showed bands attributable to hydroxy (3376 cm^{-1}) and conjugated carbonyl groups (1647 cm^{-1}) and a benzene ring (1610 and 1500 cm^{-1}). It was deduced to be an isoflavone due to a characteristic H-2 signal at δ 8.14 (s) and UV absorption bands at 263, 294 sh, and 340 sh nm.[2,3] The bathochromic shift with $AlCl_3$, which did not show any change on the addition of HCl, showed the presence of a chelated OH group at C-5. The addition of NaOAc caused a bathochromic shift suggesting that the C-7 phenolic hydroxy group is free. Two doublet signals at 6.27 (1H, d, J = 2.2 Hz) and 6.40 (1H, d, J = 2.2 Hz) were assigned to H-6 and H-8 in the A-ring, respectively. Signals due to three aromatic protons were discernible at δ 6.85 (1H, d, J = 8.2 Hz), 7.33 (1H, dd, J = 8.2, 2.1 Hz), and 7.38 (1H, d, J = 2.1 Hz), and these could be readily assigned to 1,3,4-trisubstituted ring B. The singlets at δ 9.00 and 13.03 were assigned to C-7 and C-5 phenolic protons, respectively. Both disappeared upon addition of D_2O. The presence of a 2,2-dimethyl chromane moiety with a secondary hydroxy attached on ring B was indicated by signals at δ 1.25 and 1.26 (3H each, s), 2.74 (1H, dd, J = 14.1, 9.9 Hz), 2.96 (1H, dd, J = 14.1, 1.9 Hz), and 3.67 (1H, dd, J = 9.9, 1.9 Hz). The chemical shifts of methylene protons (δ 2.74 and 2.96) indicated that this was a benzylic methylene. Compound **1** formed a triacetate **3** [viscous liquid, ν_{max} 1735, 1760 cm^{-1}; δ 1.93, 2.32, 2.37 (3H each, s) and 5.06 (1H, dd, J = 10.2, 3.0 Hz)] on reaction with Ac_2O in pyridine at 60 °C overnight. On the basis of the above evidence, ficuisoflavone (**1**) was assigned the structure as shown. This structure was fully supported by the ^{13}C-NMR spectrum, and additional proof for this structure **1** was obtained using NOESY, HMBC, and NOE techniques. The presence of NOEs between H-2 (2.5%) and H-5′, H-7, between H-5′ (5.8%) and H-4′β, between H-4′α (12.3%) and CH_3-2′α, and between H-3′ (4.4%) and CH_3-2′β was observed.

Isolupinisoflavone E (**2**), pale yellow prisms, mp 182−184 °C, was formulated as $C_{25}H_{26}O_7$ on the basis of its HRMS. The IR absorption bands showed that it contains a hydroxy group (3441 cm^{-1}), a conjugated carbonyl group (1654 cm^{-1}), and a benzene ring (1615 and 1492 cm^{-1}). The ^{1}H-NMR signal at δ 8.18 (s) and the UV absorption bands at 267 and 294 nm were all characteristic of an isoflavone. A chelated OH group at C-5 could be attributed to a lower field signal at δ 13.23 (s) and a bathochromic shift upon addition of $AlCl_3$, which did not change with the addition of HCl. Twenty-five signals existing in the ^{13}C-NMR spectrum of compound **2** indicated that two isoprenyl groups were attached to this isoflavone. A signal at δ 6.20 (1H, s) and a signal at δ 94.4, in addition to a positive Gibbs' test provided evidence that C-8 in the A-ring is free. An ABX system of three phenyl protons at δ 6.74 (d, J = 8.3 Hz), 7.28 (br d, J = 8.3 Hz), and 7.40 (br s) showed a B-ring with 1′,3′,4′-trisubstituted. Compound **2** afforded MS fragments at $[M - 59]^+$ and a m/z 59 indicative of the isoprenyl groups as hydroxyisopropyldihydrofuran substituents attached on the A- and B-rings. Four singlet methyl group signals at δ 1.22, 1.24, 1.27, and 1.29 provide additional proof for the structure of the isoprenyl group. The other ^{1}H-NMR and ^{13}C-NMR signals are also compatible with structure **2**. Additional proof for the structure **2** was obtained from HMBC and NOESY experiments. The ^{1}H-NMR data obtained for compound **2** was very similar to that

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of lupinisoflavone E,[4] but **2** had a different melting point and specific rotation. Lupinisoflavone E melted at 199−201 °C with $[\alpha]_D$ −111°. The specific rotation of isolupinisoflavone E is nearly zero. Therefore, the configurations of C-2″ and C-2‴ are *R* and *R* or *S* and *S* in lupinisoflavone E, and *R* and *S* or *S* and *R* in isolupinisoflavone E (**2**).

Experimental Section

General Experimental 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. 2D-NMR spectra were run on a Varian Unity 400 spectrometer. EIMS, FABMS, UV, and specific rotations were taken on a JEOL JMS-HX110, a Hitachi S-3200 spectrometer, and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on Si gel (Merck 3374, 70−230 mesh).

Plant Material. The bark of *F. 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. Air-dried bark of *Ficus microcarpa* L.f. was crushed into small pieces to give 3.0 kg of raw material, which was extracted with MeOH (30 L) three times (7 days each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue that was subsequently subjected to partition with *n*-BuOH and H_2O (each 2 L). The upper layer was purified by Si gel column chromatography with a gradient solvent system (hexane−EtOAc) to afford 22 fractions. Fraction 18 was purified again by Si gel column chromatography (hexane−EtOAc 4:1) to give crude **1** and **2**. Further purification by HPLC (hexane−EtOAc−*i*-PrOH 1:2:0.2) gave pure **1** (9 mg) and **2** (7 mg).

Ficuisoflavone (1): yellow viscous liquid; $[\alpha]^{25}_D$ + 3.2°(*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (4.12), 294 (sh, 3.60), 340 (sh, 3.07); +$AlCl_3$ 271, 310 (sh), 382; +NaOAc 271, 330 (sh); IR (dry film) ν_{max} 3376 (OH), 1647 (C═O, conjugated), 1610, 1500, 1262, 1202, 1065 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 13.04 (1H, s, OH), 8.14 (1H, s, H-2), 7.38 (1H, d, J = 2.1 Hz, H-5′), 7.33 (1H, dd, J = 8.2, 2.1 Hz, H-7′), 6.85 (1H, d, J = 8.2 Hz, H-8′), 6.40 (1H, d, J = 2.2 Hz, H-8), 6.27 (1H, d, J = 2.2 Hz, H-6), 3.67 (1H, dd, J = 9.9, 1.9 Hz, H-3′), 2.96 (1H, dd, J = 14.1, 1.9 Hz, H_a-4′), 2.74 (1H, dd, J = 14.1, 9.9 Hz, H_b-4′), 1.26, 1.25 (3H each, s, Me-2′); ^{13}C NMR (Me_2CO-d_6, 75 MHz) δ 181.6 (s, C-4), 165.0 (s, C-7), 163.9 (s, C-5), 159.0 (s, C-9), 157.1 (s, C-9′), 154.2 (d, C-2), 132.7 (d, C-5′), 129.2 (s, C-6′), 127.8 (d, C-7′), 124.1 (s, C-3), 123.2 (s, C-10′), 116.8 (d, C-8′), 106.1 (s, C-10), 99.8 (d, C-6), 94.4 (d, C-8), 80.9 (d, C-3′), 72.9 (s, C-2′), 34.5 (t, C-4′), 25.5, 25.4 (q, Me-2′); EIMS (70 eV) *m*/*z* $[M]^+$ 354 (100), 337 (24), 313 (30), 307 (48), 289 (55), 284 (66), 283 (68); HRMS calcd for $C_{20}H_{18}O_6$: 354.1103; found 354.1107.

Isolupinisoflavone E (2): Mp 182−184 °C; $[\alpha]^{25}_D$ + 0.5°(*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.41), 267 (4.38), 294 (sh, 3.93); +$AlCl_3$ 230 (sh), 280, 320, 380; +NaOAc no shift; IR (dry film) ν_{max} 3441 (OH), 1654 (C═O, conjugated), 1615, 1492, 1256, 1164, 1060 cm^{-1}; ^{1}H NMR (Me_2CO-d_6, 300 MHz) δ 13.23 (1H, s, OH), 8.17 (1H, s, H-2), 7.40 (1H, br s, H-2′), 7.28 (1H, br d, J = 8.3 Hz, H-6′), 6.74 (1H, d, J = 8.3 Hz, H-5′), 6.20 (1H, s, H-8), 4.85 (1H, dd, J = 9.3, 8.1 Hz, H-2‴), 4.66 (1H, dd, J = 9.3, 8.1 Hz, H-2″), 3.34−3.26 (2H, m, H-1″), 3.25−3.18 (2H, m, H-1‴), 1.30, 1.28, 1.25, 1.23 (3H each, Me); ^{13}C NMR (Me_2CO-d_6, 75 MHz) δ 181.7 (s, C-4), 167.7 (s, C-5), 164.3 (s, C-7), 161.2 (s, C-4′), 153.9 (s, C-9), 153.9 (d, C-2), 129.6 (s, C-1′), 129.6 (d, C-6′), 126.7 (d, C-2′), 124.3 (s, C-3), 123.8 (s, C-3′), 109.3 (d, C-5′), 106.2 (s, C-10), 104.8 (s, C-6), 94.4 (d, C-8), 92.7 (d, C-2″), 90.6 (d, C-2‴), 71.5 (s, C-3‴), 71.4 (s, C-3″), 31.0 (t, C-1‴), 29.0 (t, C-1″), 27.2, 26.0, 25.6, 25.5 (q, Me-3″ and Me-3‴); EIMS (70 eV) *m*/*z* $[M]^+$ 438 (100), 423 (8), 405 (20), 379 (52), 361 (3); HRMS calcd for $C_{25}H_{26}O_7$: 438.1679; found 438.1687.

Lupinisoflavone E: ^{1}H NMR (Me_2CO-d_6, 400 MHz) δ 13.21 (1H, s, OH), 8.18 (1H, s, H-2), 7.42 (1H, d, J = 1.5 Hz, H-2′), 7.30 (1H, dd, J = 8.1, 1.5 Hz, H-6′), 6.75 (1H, d, J = 8.1 Hz, H-5′), 6.38 (1H, s, H-8), 4.85 (1H, dd, J = 9.5, 8.1 Hz, H-2‴), 4.67 (1H, dd, J = 9.5, 8.1 Hz, H-2″), 3.34−3.26 (2H, m, H-1″), 3.24−3.11 (2H, m, H-1‴), 1.30, 1.28, 1.25, 1.23 (3H each, Me).

Acetylation of 1. A mixture of **1** (2 mg) and Ac_2O (1 mL) in pyridine (1 mL) was heated at 60 °C for 12 h. The mixture solvent (15 mL each of EtOAc and hexane) was added to the reaction mixture and stirred at 25 °C for 0.5 h. The reaction mixture was then extracted with crushed ice to remove some AcOH and pyridine. The organic layer was washed with 3 N HCl, saturated aqueous $NaHCO_3$, and NaCl and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure produced **3** (2 mg): amorphous powder; IR (dry film) ν_{max} 1760, 1735, 1650, 1625, 1431, 1257, 1118; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.85 (1H, s), 7.38 (1H, d, J = 2.2 Hz), 7.29 (1H, dd, J = 8.2, 2.2 Hz), 7.09 (1H, d, J = 8.2 Hz), 7.22, 6.83 (1H each, d, J = 2.2 Hz), 5.06 (1H, dd, J = 10.5, 2.7 Hz), 2.95 (1H, dd, J = 14.2, 2.7 Hz), 2.76 (1H, dd, J = 14.2, 10.5 Hz), 2.37, 2.32, 1.94 (3H each, s), 1.26, 1.25 (3H each, s).

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