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A MONOTERPENOID AND TWO SIMPLE PHENOLS FROM HEARTWOOD OF *FICUS MICROCARPA*

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Key Word Index—*Ficus microcarpa*; Moraceace; heartwood; ficusic acid; ficusol; ficuglucoside.

Abstract—A methanolic extract of the heartwood of *Ficus microcarpa* yielded three new compounds, (*Z*)-1,6,6-trimethyl-7-oxabicyclo[2,2,1]hexa-2(9)-en-10-oic acid, methyl (*S*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxypropanoate and 1β-(3-hydroxy-4,5-dimethoxyphenyl)-*O*-glucopyranoside were principally characterized by spectral techniques. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

More than fifty species of *Ficus* (Moraceace) grow in Taiwan, and one of the popular ornamental plants is *F. microcarpa* L. f.. The early chemical studies on the leaves of this plant were achieved in 1987 [1], only six terpenoids were isolated. Recently, we have carried out chemical studies on the bark of this plant, and found twenty-eight compounds including triterpenes, fatty alcohol, steroids, coumarin, flavane, 4-hydroxybenzoate, megastigmane [4,5-dihydroblumenol] [2] as well as two new isoflavones [3]. In connection with our interest in this plant, three new compounds were isolated from the methanolic extract of the heartwood, ficusic acid (**1**), ficusol (**2**) and ficuglucoside (**3a**). In this paper, we describe the structural elucidation of these new compounds.

RESULTS AND DISCUSSION

Ficusic acid (**1**) with molecular formula $C_{11}H_{16}O_3$ was deduced from the exact mass spectrum. The IR absorption bands at 3260–2500, 1686 and 1608 cm^{-1} and the significant UV absorption band at 224 nm were attributable to a conjugated carboxylic acid group. From the molecular formula $C_{11}H_{16}O_3$ of **1**, the index of hydrogen deficiency (IHD) of **1** is four; therefore, a bicyclic structure was suggested. The ^{13}C NMR spectrum (in Experimental) showed three signals at δ_C 180.7 (*s*), 171.5 (*s*), and 113.3 (*d*) owing to a carboxylic acid conjugated with a double bond. A signal at δ_H 5.69 (δ_C 113.3) was attributable to a vinyl proton situated at α-carbon of a conjugated acid. Two geminal methyl groups (δ_H 1.25 and 1.29) attached to a carbon (δ_C 35.0) and a singlet methyl group (δ_H 1.57) attached to an oxygenated carbon (δ_C 86.4) were revealed by HMBC (Fig. 1) and HMQC techniques. Examining the 2D NMR spectrum of **1**, two methylene groups signals appeared at δ_C 47.9 [δ_H 2.51 (H_β-3) and 1.49 (H_α-3)] and 49.8 [δ_H 2.00 (H_β-5) and 1.35 (H_α-5)] as well as H_β-3 and H_β-5 exhibited a W-type coupling (J = 2.2 Hz). It was suggested that the methine proton signal at δ_H 4.10 (*m*) (δ_C 65.1) was geminal to an ether linkage and situated between two methylene groups (H_2-3 and H_2-5) by analyzing HMBC and ^1H-^1H COSY spectrums. Based on the evidence above, ficusic acid is an oxa[2,2,1]bicyclic compound. Regarding the stereochemistry, H_β-5 and H_α-5 existed NOESY correlation with H_3-12 (δ_C 29.9) and H_3-11 (δ_C 25.1), respectively. In addition, H-9 and H_3-8 (δ_C 25.6) are on opposite sides of the double bond because of the absence of NOESY correlation between them. This novel homomonoterpene is in agreement with structure **1**.

Ficusol (**2**) was isolated as oil. It showed eleven ^{13}C NMR signals and the exact mass [M^+] at *m/z* 226.0844. Containing a hydroxy, an ester, and a benzene ring groups were inferred from the IR absorptions at 3419, 1731, 1602 and 1518 cm^{-1}. The UV spectrum of **2** showed significant absorptions at λ_{max} 230 and 278 nm. The bathochromic shift was

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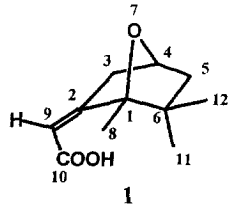

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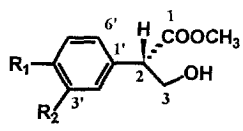
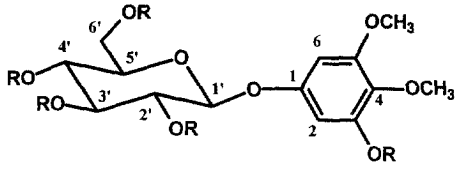


2 R_1=OH, R_2=OCH_3

4 R_1=R_2=H



3a R=H

3b R=Ac

attributable to a methoxy and a hydroxy auxochromes (attached on benzene ring) which showed resonances at δ_H 3.87 (*s*) and 5.50 (*br s*, OH, disappeared upon addition of D_2O), respectively. An ABX system [δ_H 6.72 (1H, *dd*, J = 8.6, 1.9 Hz, H-6'), 6.76 (1H, *d*, J = 1.9 Hz, H-2'), and 6.85 (1H, *d*, J = 8.6 Hz, H-5')] was assigned to phenyl protons. Another ABX system has signals occurring at δ_H 3.80, 4.09 and 3.72 (H-2) which exhibited the ^1H-^1H COSY correlation to one another. The appearance of HMBC correlation between δ_H 3.69 (3H, *s*) and ester carbonyl was assigned as an ester methoxy group, besides which, H-2 expressed the HMBC correlation with δ_C 173.8 (C-1), 127.3 (C-1'), and 64.7 (C-3). Thus, the structure **2** is a methyl tropate derivative (**4**) [4] with a hydroxy and a methoxy groups attached on benzene ring. MeO-3' exhibited nOe correlation with H-2', and H-2 showed nOe correlation with H-2' and H-6', that illustrated its relative position. The base peak of EI-MS of **2** at *m/z* 196 (100%) (as Fig. 2) can be explained *via* a McLafferty rearrangement. In addition, ficusol (**2**) has the (*S*)-form because of the negative specific rotation based upon Watson's confirmation on the *S*-configuration of (−)-methyl tropate (**4**) [4]. Therefore, **2** is methyl 4'-hydroxy-3'-methoxytropate.

Ficuglucoside (**3a**) was isolated as pentaacetate (**3b**), $[\alpha]_D^{25}$ −16.1° ($CHCl_3$), mass spectrum *m/z* 542 [M^+], whose composition was determined to be $C_{24}H_{30}O_{14}$ by measurement of the high-resolution MS. Compound **3b** exhibited IR absorption bands at 1755, 1615 and 1505 cm^{-1} due to ester and aromatic groups and the ^1H NMR spectrum signals at δ 2.01, 2.03, 2.04, 2.05 (alcoholic acetyl), and 2.28 (phenolic acetyl). The ^1H and ^{13}C NMR signals (H- and C-1' to 6') were attributable to a glucoside moiety. The observation of two nonequivalent methoxyl groups (δ 3.75 and 3.81) and aromatic protons [δ 6.30 (1H, *d*, J = 2.4 Hz, H-2) and 6.47 (1H, *d*, J = 2.4 Hz, H-6)], indicated that the aglycone moiety possesses an unsymmetrical substitution system. Furthermore, it indicated a phenolic β-*O*-glucoside because of the coupling constant 7.6 Hz for H-1' which exhibited nOe correlation with H-2 and H-6. Thus, the -*O*-glucoside moiety linked to the benzene

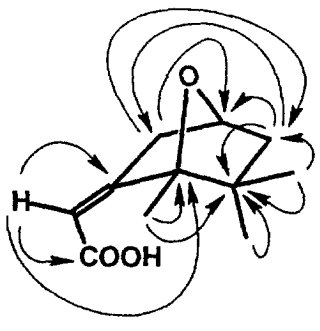


Fig. 1. HMBC correlation for compound **1**, indicated by arrows from ^1H to ^{13}C.

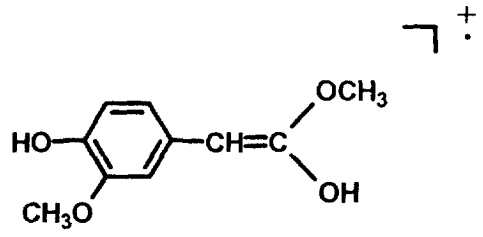


Fig. 2. *m/z* 196 (100%).

ring carbon between two free positions. Both of H-6 and MeO-4 (δ 3.75) correlated to MeO-5 (δ 3.81) in NOESY spectrum, that established the relative situation of aromatic ring moiety. The assignment was also supported by HMQC and HMBC experiments. Accordingly, **3a** is 1β-(3-hydroxy-4,5-dimethoxyphenyl)-*O*-glucopyranoside.

EXPERIMENTAL

General experimental procedures

Extracts were chromatographed on Silica gel (Merck 3374, 70–230 mesh).

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, and a voucher specimen has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and isolation

Heartwood of *Ficus 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 days each time) at room temperature. The combined extracts were evaporated *in vacuo* to give a black residue (58.8 g). To this residue was added 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 on Silica gel column chromatography (hexane–EtOAc and EtOAc–MeOH solvent system) and give crude compounds **1** and **2**. The *n*-BuOH fraction (10.1 g) was acetylated with Ac_2O and pyridine in the usual way to give a complex acetate mixture, which was separated by Silica gel column chromatography (hexane–EtOAc and EtOAc–MeOH solvent system) to give a crude compound **3b**. Crude compounds **1** and **2** were eluted by hexane–EtOAc = 2:5 and 1:1 and crude **3b** eluted by hexane–EtOAc = 1:4. Further purification by HPLC gave pure **1** (2.5 mg), **2** (2.8 mg) and **3b** (3.0 mg) with the solvent systems hexane–EtOAc–*i*-PrOH = 1:1:0.2, hexane–EtOAc–*i*-PrOH = 2:1:0.2, and hexane–EtOAc–*i*-PrOH = 1:1:0.2, respectively.

Ficusic acid (**1**): amorphous; UV (MeOH) λ_{max} (log ϵ) 224 (4.16); IR (dry film) ν_{max}^{neat} 3260–2500 (COOH), 3072 (vinyl, C–H), 1686 (conjugated, C=O), 1608 (C=C), 1281, 1170 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.51 (1H, *ddd*, J = 11.6, 2.2, 2.2 Hz, H$_\beta$-3), 2.00 (1H, *ddd*, J = 12.8, 4.0, 2.2 Hz, H$_\beta$-5), 1.49 (1H, *dd*, J = 11.6, 4.2 Hz, H$_\alpha$-3), 1.35 (1H, *dd*, J = 12.8, 4.8 Hz, H$_\alpha$-5); EIMS (70 eV) *m/z* (rel. int.): 196 [M$^+$] (5), 178 (100), 163 (48), 153 (14), 140 (29), 111 (55); HRMS *m/z*: 196.1093, $C_{11}H_{16}O_3$ requires 196.1099.

Ficusol (**2**): oil; $[\alpha]_D^{25}$ −13.7° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (3.70), 278 (3.47); IR (dry film) ν_{max}^{neat} 3419 (OH), 1731 (C=O), 1602, 1518 (aromatic), 1376, 1275 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.09 (1H, *dd*, J = 12.9, 10.5 Hz, H$_a$-3), 3.80 (1H, *dd*, J = 12.9, 5.3 Hz, H$_b$-3), 3.72 (1H, *dd*, J = 10.5, 5.3 Hz, H-2); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 146.7 (*s*, C-4′), 145.3 (*s*, C-3′), 121.2 (*d*, C-6′), 114.7 (*d*, C-2′), 110.5 (*d*, C-5′), 56.0 (*q*, MeO-3′), 53.5 (*d*, C-2), 52.2 (*q*, MeO-1); EIMS (70 eV) *m/z* (rel. int.): 226 [M$^+$] (93), 196 (100), 181 (22), 167 (50), 149 (10), 137 (25), 107 (19); HRMS *m/z* 226.0844, $C_{11}H_{14}O_5$ requires 226.0841.

Ficuglucoside pentaacetate (**3b**): an amorphous solid; UV (MeOH) λ_{max} (log ϵ) 225 (3.82), 273 (3.20); IR (dry film) ν_{max}^{neat} 1755 (C=O), 1615, 1505 (aromatic), 1434, 1370, 1222, 1041 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz)_δ 5.26 (1H, *dd*, J = 9.2, 9.2 Hz, H-3′), 5.23 (1H, *dd*, J = 9.2, 7.6 Hz, H-2′), 5.12 (1H, *dd*, J = 9.2, 9.2 Hz, H-4′), 5.00 (1H, *d*, J = 7.6 Hz, H-1′), 4.23 (1H, *dd*, J = 12.4, 5.6 Hz, H$_a$-6′), 4.15 (1H, *dd*, J = 12.4, 2.4 Hz, H$_b$-6′), 3.84 (1H, *ddd*, J = 9.2, 5.6, 2.4 Hz, H-5′); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 170.5, 170.2, 169.4, 169.3, 168.9 (*s*, Ac), 154.0 (*s*, C-5), 152.5 (*s*, C-1), 144.1 (*s*, C-3), 137.1 (*s*, C-4), 102.7 (*d*, C-2), 100.9 (*d*, C-6), 99.1 (*s*, C-1′), 72.7 (*d*, C-3′), 72.0 (*d*, C-5′), 71.0 (*d*, C-2′), 68.2 (*d*, C-4′), 62.0 (*t*, C-6′), 60.8 (*q*, MeO-4), 56.1 (*q*, MeO-5), 20.7, 20.7, 20.6, 20.6, 20.6 (*q*, Ac); EIMS (70 eV) *m/z* (rel. int.) 542 [M$^+$] (10), 523 (5), 331 (67), 307 (5), 289 (6), 271 (8), 259 (9), 211 (18), 169 (100); HRMS *m/z* 542.1635, $C_{24}H_{30}O_{14}$ requires 542.1636.

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