DOI 10.1007/s10600-020-03216-7
Chemistry of Natural Compounds, Vol. 56, No. 6, November, 2020

A NEW DIHYDROCOUMARIN FROM *Ficus sycomorus*

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A phytochemical study of the fruits and leaves of Ficus sycomorus *L. led to the isolation of a new dihydrocoumarin, 4-carboxylic-4-hydroxy-3,4-dihydrocoumarin (**1**), together with ten known compounds. Moreover, the different extracts* of F. sycomorus *were screened for cytotoxic activity using the brine shrimp lethality bioassay. The results revealed that all extracts were virtually nontoxic on the shrimps and showed LC_{50} values greater than 100 μg/mL.*

Keywords: *Ficus sycomorus*, dihydrocoumarin, isoquercitrin, cinaroside, brine shrimp lethality bioassay.

Ficus sycomorus L. is an evergreen spreading tree and grows in Egypt. The comparatively large fruit is edible and called gimmeiz [1, 2].

Our previous biological study of the different extracts of *F. sycomorus* L. showed significant hepatoprotective and hypotensive activities [3, 4].

Each of the ethanolic extract of *F. sycomorus* L. fruits and leaves was partitioned separately with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, successively. By several chromatographic techniques a new compound, 4-carboxylic-4-hydroxy-3,4-dihydrocoumarin (**1**), together with ten known compounds, was isolated. They were identified as ethyl-β-D-glucopyranoside (**2**) [5], ethyl-α-D-arabinofuranoside (**3**) [6, 7], vanillic acid (**4**) [8], quercetin-3-*O*-glucoside (isoquercitrin) (**5**), luteolin-7-*O*-glucoside (cinaroside) (**6**) [9–12], campesterol-3-*O*-glucoside (**7**) [13], and psoralen (**8**) [14, 15] by comparing their spectroscopic data with those described in the literature. In addition, β-amyrin (**9**), β-sitosterol (**10**), and β-sitosterol-3-*O*-glucoside (**11**) were identified by comparison with authentic samples (mp and co-TLC).

The UV (MeOH) absorption spectrum of compound **1** showed maxima at 208 and 253 and a shoulder at 285 nm, suggesting a dihydrocoumarin skeleton [16]. Its IR spectrum showed absorption bands at 1715 cm^{-1} due to a saturated δ-lactone, a strong broad absorption band at 3417 cm^{-1} due to OH and carboxylic OH groups, 1587 (CH_2) and 1024 (COC) [17]. The ^1H NMR spectrum revealed two signals at δ 1.97 and 2.33 (each 1H d, J = 15.0 Hz) assigned to C-3 pyran. Also, a pattern of 1,2-disubstituted benzene signals appeared at δ 6.76 (1H, d, J = 7.7 Hz), 6.89 (1H, br.t, J = 7.4 Hz), 7.14 (1H, td, J = 7.7, 0.8 Hz), and 7.33 (1H, d, J = 7.4 Hz) [18]. The ^{13}C NMR and DEPT spectra exhibited signals closely correlated with those expected for the 3,4-dihydrocoumarin skeleton at δ 42.2 (t), 73.8 (s), 109.3 (d), 121.3 (d), 123.9 (d), 128.3 (d), 134.1 (s), 141.1 (s), and 174.2 (s) [19] in addition to one carbonyl carbon of the carboxylic group at δ 178.8 (s). The disubstitution at C-4 was indicated by the appearance of the quaternary carbon at 73.8 (s). The structure was confirmed by ^1H–^1H COSY, HSQC, and HMBC experiments. The EI-MS spectrum did not show the molecular ion peak but it showed a peak at *m/z* 149 $[M - (CH_2 + CO + OH)]^+$ from the retro-Diels-Alder reaction characteristic for 4-substituted 3,4-dihydrocoumarin [20]. In addition, diagnostic fragments at *m/z* 107, 89, and 77 appeared. The positive FAB mass spectrum showed a prominent fragment ion peak at *m/z* 192 $[M - OH + H]^+$ $[C_{10}H_7O_4 + H]^+$. Based on the above-mentioned data, compound **1** was identified as 4-carboxylic-4-hydroxy-3,4-dihydrocoumarin and is considered as a new natural product.

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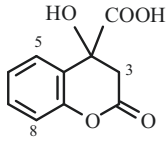
Published in *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2020, pp. 874–875. Original article submitted December 19, 2019.



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The brine shrimp lethality bioassay revealed that all the extracts were virtually nontoxic on the shrimps. The unripe fruits, stem bark, wood, and leaf extracts caused 17.54%, 14.04%, 11.67%, and 5.00% mortality, respectively, after 24 h and showed LC_{50} values greater than 100 μg/mL.

Consequently, these bioassay results support the use in traditional medicine of different organs of the plant in the treatment of diarrhea, skin and liver diseases, epilepsy, stomach and respiratory disorders, helminthiasis, mental disorders, infertility, and sterility [21, 22].

EXPERIMENTAL

Plant Material. The leaves and unripe fruits of *Ficus sycomorus* L. were collected in the period of February to April 2010 from the Experimental Station of Ornamental Plants, Faculty of Agriculture, Assiut University and kindly identified and authenticated by the late Prof. Dr. Naeem E. Keltawy, Professor of Ornamental Horticulture and Floriculture, Faculty of Agriculture, Assiut University. A voucher sample (No. 2010 FS) has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction and Isolation. The fresh unripe fruits (2.7 kg) were extracted by maceration with hot ethanol (3 × 10 L) until exhaustion. The ethanolic extract was concentrated under reduced pressure and left to dry, then weighed to give the corresponding viscous residue (92 g). The residue was suspended in distilled water (200 mL) and successively partitioned with *n*-hexane (4 × 500 mL), $CHCl_3$ (3 × 500 mL), EtOAc (3 × 500 mL), and *n*-BuOH saturated with H_2O (3 × 500 mL). Each phase was concentrated under reduced pressure to give the corresponding soluble fraction: *n*-hexane (20 g), $CHCl_3$ (6 g), EtOAc (9 g), *n*-BuOH (25 g), and H_2O extract (30 g).

About 9 g of EtOAc fraction was chromatographed over a silica gel column (270 g, 100 × 5 cm) using a gradient elution system of $CHCl_3$ and MeOH, and fractions of 200 mL each were collected. Five group fractions (Frs. E-1–E-V) were obtained: Fr. E-I (1.5 g, eluted with $CHCl_3$–MeOH, 90:10), Fr. E-II (1.5 g, eluted with $CHCl_3$–MeOH, 90:10), Fr. E-III (2.0 g, eluted with $CHCl_3$–MeOH, 85:15), Fr. E-IV (1.0 g, eluted with $CHCl_3$–MeOH, 80:20), and Fr. E-V (2.5 g, eluted with $CHCl_3$–MeOH, 70:30). About 1.0 g of group Fr. E-III was subjected to RP-18 column chromatography (70 × 3 cm) using MeOH–H_2O, and the fractions eluted with MeOH–H_2O (10:30) were collected and concentrated to afford a residue (100 mg). Further purification by PTLC using $CHCl_3$–MeOH–H_2O (75:23:2) afforded pure compound **1** (10 mg).

4-Carboxylic-4-hydroxy-3,4-dihydrocoumarin (1). This compound is purified by PTLC using $CHCl_3$–MeOH–H_2O, 75:23:2). It was obtained as a white solid, 10 mg; $[\alpha]_D^{25}$ –9.885° (*c* 0.244, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 208 (3.93). IR (film, ν, cm^{-1}): 3417 (br, OH, COOH), 1715 (C=O), 1587 (CH_2), 1024 (COC). ^1H NMR (600 MHz, DMSO-d_6, δ, ppm, J/Hz): 1.97 (1H, d, J = 15.0, H-3b), 2.33 (1H, d, J = 15.0, H-3a), 6.76 (1H, d, J = 7.7, H-8), 6.89 (1H, br.t, J = 7.4, H-6), 7.14 (1H, td, J = 7.7, 0.8, H-7), 7.33 (1H, d, J = 7.4, H-5), 10.13 (1H, s, 4-OH). ^{13}C NMR (150 MHz, DMSO-d_6, δ, ppm): 42.2 (CH_2, C-3), 73.8 (C, C-4), 109.3 (CH, C-8), 121.3 (CH, C-6), 123.9 (CH, C-5), 128.3 (CH, C-7), 134.1 (C, C-10), 141.1 (C, C-9), 174.2 (C, C-2), 178.8 (C, 4-COOH). FAB-MS *m/z* 192 $[M - OH + H]^+$; EI-MS *m/z* (I_{rel}, %): 149 $[M - (CH_2 + CO + OH)]^+$ (55), 107 (64), 89 (43), 77 (100), 44 (45); HR-ESI-MS *m/z* 208.0372 $[M]^+$ (calcd for $C_{10}H_8O_5$, 208.1690).

Brine Shrimp Lethality Bioassay. The dried extracts of the leaves, stem bark, wood, and fresh unripe fruits were prepared as mentioned previously [3, 4]. A specific weight (0.5 mg) of each extract was prepared by dissolving it in 20 μL DMSO and the volume made up to 5 mL in each tube with seawater; the concentration of the extract in this tube was 100 μg/mL. A vial containing 20 μL DMSO diluted to 5 mL with seawater was used as control.

The cytotoxicity assay was performed on brine shrimp nauplii using Meyer′s method [23, 24].

ACKNOWLEDGMENT

The authors gratefully acknowledge Prof. Dr. Samir A. Ross (National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA; BioMolecular Sciences, Division of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA) and Dr. Yoshiaki Takaya (Faculty of Pharmacy, Meijo University, Tempaku, Nagoya 468-8503, Japan) for the NMR measurements.

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