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20-EPIBRYONOLIC ACID, PHYTOSTEROLS AND ELLAGIC ACID FROM *CORIARIA INTERMEDIA*

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Key Word Index—*Coriaria intermedia*; Coriariaceae; phytosterols; ellagic acid 3,3′-dimethyl ether; coriamyrtin; β-tutin; naringenin; ursolic acid; 20-epibryonolic acid.

Abstract—*Coriaria intermedia* was shown to contain phytosterols, ellagic acid 3,3′-dimethyl ether, coriamyrtin, β-tutin, naringenin, ursolic acid and a new triterpenoic acid, 20-epibryonolic acid. The compounds were characterized by spectroscopic analysis and/or comparison with authentic samples.

INTRODUCTION

Coriaria intermedia Matsum has been used as a folk remedy in Taiwan for gastrointestinal disturbance, rheumatism and uterus cancer. This plant is widely distribution on riverbanks and mountainsides [1]. In this study, we have identified some of the chemical constituents of *C. intermedia*, which have not been reported previously.

RESULTS AND DISCUSSION

The air-dried and powdered leaves and roots of *C. intermedia* were extracted with methanol. The crude extracts were partitioned with *n*-hexane, chloroform, *n*-butanol and water followed by chromatographic separation on silical gel. Coriamyrtin, β-tutin, [2–4] and naringenin [5] were identified from the chloroform soluble fraction of the leaves. Ellagic acid 3,3′-dimethyl ether [6, 7] and a mixture of phytosterols (campesterol, stigmasterol and sitosterol) [2] were found in the *n*-hexane soluble fraction. From the chloroform fraction of the roots, ursolic acid [8] and a triterpenoic acid, 20-epibryonolic acid (**1**), were isolated. The known compounds were readily identified from their spectral data and/or comparison with authentic samples as well as GC-MS analysis. Compound **1**, a triterpenoic acid, recrystallized from chloroform–methanol as white needles and it gave a molecular ion peak at *m/z* 456 as the base peak. The mass fragmentation pattern (*m/z* 441, 259, 247, 229 and 203) showed this compound to be a triterpenoic acid. No olefinic proton signal was found in the ^{1}H NMR spectrum. A carboxylic proton signal (δ 11.99), a hydroxyl proton signal (δ 4.28; $J = 4.8$ Hz), a hydroxylated proton signal (δ 2.99 *m*, H-3) and a doublet proton signal at δ 2.28 (H-18; $J = 15$ Hz) were also present. The downfield shift of H-18 was attributed to its position *cis* to the C-20 carboxylic acid moiety. This is proved by HMBC experiments (Fig. 1) that gave cross signals between H-18 and C-30 ($^4J_{CH}$). Cross peaks were also found between H-29 and C-30 as well as from H-23 and H-24 to C-3, respectively. The remaining four methyl groups were determined with the support of HMBC, ^{13}C NMR, DEPT and HETCOR measurements. Carbon signals of a secondary hydroxylated carbon (δ 76.86, C-3), a carbonyl carbon (δ 179.80) and two olefinic carbons (δ 133.89 and 133.41) were also present, which indicated that this compound might be a pentacyclic triterpenoic acid with a tetra-substituted double bond situated between C-8 and C-9. By comparison of the $[\alpha]_D$, melting-point and ^{13}C NMR

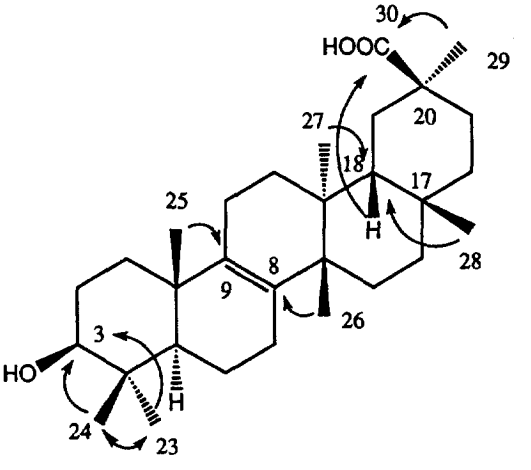

Fig. 1. Selected HMBC correlations of compound **1**.

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spectral data with those of the very similar and only known Δ^8-unsaturated triterpenoic acid, bryonolic acid [9–11], and also because ursolic acid is present in this plant, we concluded that compound **1** was 20-epi-bryonolic acid. This is the first report of **1** from *C. intermedia* or any other natural source.

EXPERIMENTAL

Mps (Yanaco micromelting-point apparatus) are uncorr. NMR spectra were recorded on either a Bruker AC-300 or Varian Gemini-200 spectrometer; chemical shifts are reported in δ units relative to DMSO-d_6. Mass spectra were measured on a JOEL JMSD 300 mass spectrometer. CC was effected using E. Merck Kieselgel 60 (70–230 or 230–400 mesh).

Plant material. C. intermedia (*ca* 27 kg) was collected from Nantou county, Taiwan, in December 1992. It was identified by Mr Nien-Yung Chiu, Institute of Chinese Pharmaceutical Sciences, China Medical College, and a voucher specimen is deposited at the herbarium of this institute.

Extraction and isolation. The dried leaves (3.3 kg) and roots (3.2 kg) were extracted with MeOH. The extracts were concd and partitioned to give *n*-hexane, $CHCl_3$, *n*-BuOH and H_2O frs. After CC with silica gel, HP-20 dianion, polyamide and/or Sephadex LH-20, from the $CHCl_3$ fr of leaf we obtained coriamyrtin (500 mg), β-tutin (8 mg) and naringenin (20 mg). A mixt. of phytosterols (50 mg) and ellagic acid 3,3′-dimethyl ether (15 mg) were isolated from the *n*-hexane fr. of root by silica gel CC. After recrystallization from $CHCl_3$–MeOH of frs obtained from the sepn of the $CHCl_3$ layer of root by silica gel CC, eluting with a gradient of *n*-hexane to EtOAc, resulted in ursolic acid (25 mg) and **1** acid (30 mg), respectively.

20-*Epibryonolic acid* (**1**). Needles from $CHCl_3$–MeOH, mp 276–277°; $[\alpha]_D$ −48.6 (*c* 0.094, MeOH); TLC: R_f 0.6 (hexane–EtOAc, 1:1); EIMS *m/z* (rel. int.): 456 $[M]^+$ (100), 441 (75.3), 423 (41.8), 368 (17.7), 259 (87.9), 247 (68.1), 229 (51.5), 203 (30), 189 (37.7); HRMS *m/z* 456.3623 $[M]^+$, $C_{30}H_{48}O_3$ requires: 456.3603; IR ν_{max}^{KBr} cm^{-1}: 3465, 2950, 1725, 1680; ^1H NMR (DMSO-d_6): δ 4.28 (*br. d*, 1H, *J* = 4.8 Hz, –OH), 2.99 (*m*, 1H, H-3), 2.28 (*d*, 1H, *J* = 15 Hz, H-18), 1.10 (*s*, 3H, H-29), 0.99 (*s*, 3H, H-28), 0.92 (*s*, 3H, H-26), 0.89 (*s*, 3H, H-23), 0.88 (*s*, 3H, H-25), 0.85 (*s*, 3H, H-27), 0.68 (*s*, 3H, H-24). ^{13}C NMR (DMSO-d_6): δ 179.80 (C-30), 133.89 (C-9), 133.41 (C-8), 76.89 (C-3), 50.27 (C-5), 44.24 (C-18), 41.42 (C-14), 39.44 (C-20), 38.50 (C-4), 37.16 (C-10), 36.76 (C-16), 36.75 (C-13), 34.74 (C-1), 34.02 (C-22), 32.57 (C-29), 31.06 (C-17), 30.59 (C-28), 30.02 (C-19), 29.85 (C-21), 29.43 (C-12), 28.19 (C-23), 27.67 (C-7), 27.17 (C-2), 24.58 (C-15), 21.88 (C-26), 20.23 (C-11), 19.73 (C-25), 18.89 (C-6), 17.27 (C-27), 16.07 (C-24).

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