SHORT REPORT

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Rec. Nat. Prod. 7:2 (2013) 157-160

records of natural products

The First Occurrence of A *Mallotus* 3,4-*Seco*-Taraxerane Triterpenoid from *Mallotus barbatus*

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(Received November 21, 2012; Revised December 29, 2012; Accepted January 31, 2013)

Abstract: The first occurrence of a 3,4-*seco*-taraxerane triterpenoid in *Mallotus* species (Euphorbiaceae) is reported. The triterpenoid was isolated from the leaves of the Vietnamese medicinal plant *Mallotus barbatus* (Wall.) Muell.-Arg. and its structure was determined to be 3,4-*seco*-taraxer-14-en-3-oic acid on the basis of HR-MS and NMR spectroscopic methods. For the first time, the ^{1}H and ^{13}C NMR data and stereochemistry of this compound were fully established on the basis of the ^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC spectroscopic data.

Keywords: *Mallotus barbatus*; Euphorbiaceae; triterpenoid; 3,4-*seco*-taraxerane.

1. Plant Source

The genus *Mallotus* of Euphorbiaceae family in Vietnam is of study interest because of the occurrence of particular groups of natural compounds which are significant for several biological activities including antioxidant, antiviral, anti-inflammatory, and cytotoxic [1]. *M. barbatus* (Wall.) Muell.-Arg. is recorded as a Vietnamese traditional medicinal plant [2] and was studied first by us aiming at the isolation of antioxidant phenolic compounds [3]. For the systematic comparison of chemical constituents of *Mallotus* species, the search for minor compounds of *M. barbatus* was carried out by us which resulted in the isolation of a 3,4-*seco*-taraxerane triterpenoid (**1**), possessing the less common saturated C-4(23) bond of *seco*-ring-A triterpenoids, together with a dimeric chalcone, kamalachalcone A (**2**).

The fresh leaves of *M. barbatus* were collected in Lao Cai province, Vietnam in June 2008 and the plant (voucher number VN 517) was identified by Mr. Nguyen Quoc Binh, a botanist of the Institute of Biological Resources and Ecology, Vietnam Academy of Science and Technology, Hanoi, Vietnam.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 3/15/2013 EISSN: 1307-6167

2. Previous Studies

Previous studies of the chemical constituents of *M. barbatus* showed the presence of several groups of natural products which can be chemotaxonomically meaningful such as chromene (8-cinnamoyl-5,7-dihydroxy-2,2,6-trimethylchromene) and 2-pyridone (*N*-methyl-5-carboxamide-2-pyridone); the other constituents are taraxerane triterpenoids (taraxerone and taraxerol), phenolic acids (methyl gallate, gallic acid, protocatechuic acid, cinnamic acid, and methyl *p*-hydroxybenzoate), quercetin and kaempferol and their glycosides (kaempferol 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside, and kaempferol 3,7-di-*O*-β-D-glucopyranoside), phytosterols (β-sitosterol and β-sitosterol 3-*O*-β-D-glucopyranoside), and fatty acids (palmitic acid and pentadecanoic acid) [3-5].

3. Present Study

The leaves were air-dried and then oven-dried at 50 ^{o}C. The dried leaves (6.0 kg) was powdered and then extracted with MeOH at room temperature. The aqueous MeOH extract was partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc, and 1-BuOH. Removal of the extraction solvents gave *n*-hexane- (146.7 g), CH_2Cl_2- (81 g), EtOAc- (48.3 g), and 1-BuOH- (262.4 g) soluble fractions. A part of the CH_2Cl_2-soluble fraction (44.8 g) was separated by CC on silica gel (*n*-hexane-acetone 49:1, 9:1, 6:1, 3:1, 2:1, and 1:1) to give 13 fractions. Fraction 8 (1.1 g) was divided into 6 subfractions by C-18 RP CC (MeOH-H_2O 7:3, 9:1, MeOH, and acetone). Subfraction 6 was further purified by repeated CC and FC using the following solvent systems: *n*-hexane-EtOAc 30:1, 19:1, 9:1, 6:1, and 3:1; *n*-hexane-acetone 25:1; *n*-hexane-EtOAc 12:1; and *n*-hexane-EtOAc 20:1 to give **1** (2.0 mg). A part of the *n*-hexane-soluble fraction (43.4 g) was subjected to CC on silica gel (*n*-hexane-acetone 19:1, 9:1, 6:1, 3:1, 2:1, and 1:1) to give 14 fractions. Fraction 8 (1.71 g) was separated by CC on silica gel (*n*-hexane-EtOAc 19:1, 9:1, 6:1, and 3:1) to give **2** (1.0 mg).

3,4-*Seco*-taraxer-14-en-3-oic acid (**1**): white amorphous powder; $[\alpha]_{,D}^{26}$ +39.2 (*c* 0.15, $CHCl_3$). IR (film) ν_{max} cm^{-1}: 3428, 1707, 1652, 1462, 1382, 1294. ^{1}H NMR (500 MHz, $CDCl_3$): δ0.82 (3H, s, CH_3-28), 0.83 (3H, d, *J* = 7.0 Hz, CH_3-24), 0.89 (3H, s, CH_3-27), 0.91 (3H, s, CH_3-30), 0.92 (3H, d, *J* = 7.0 Hz, CH_3-23), 0.92 (3H, s, CH_3-25), 0.96 (3H, s, CH_3-29), 0.96 (1H, m, H-19a), 0.97 (1H, m, H-18), 1.01 (1H, m, H-22a), 1.03 (1H, m, H-5), 1.09 (3H, s, CH_3-26), 1.24 (1H, m, H-21a), 1.26 (1H, m, H-7a), 1.31 (1H, m, H-19b), 1.34 (1H, m, H-21b), 1.36 (1H, m, H-22b), 1.47 (2H, m, 2H-6), 1.54 (3H, m, H-9, 2H-11), 1.56 (1H, m, H-12a), 1.62 (1H, m, H-12b), 1.65 (1H, m, H-16a), 1.67 (2H, m, 2H-1), 1.89 (1H, m, H-4), 1.92 (1H, dd, *J* = 14.5 Hz, 3.0 Hz, H-16b), 2.01 (1H, ddd, *J* = 12.5 Hz, 3.5 Hz, 3.0 Hz, H-7b), 2.09 (1H, td, *J* = 9.5 Hz, 7.5 Hz, H-2a), 2.21 (1H, ddd, *J* = 9.5 Hz, 9.0 Hz, 8.0 Hz, H-2b), 5.55 (1H, dd, *J* = 8.0 Hz, 3.0 Hz, H-15). ^{13}C NMR (125 MHz, $CDCl_3$): δ17.5 (C-11), 18.5 (C-6), 18.8 (C-24), 19.0 (C-25), 21.4 (C-27), 24.9 (C-23), 25.1 (C-4), 25.2 (C-26), 28.0 (C-2), 28.8 (C-20), 29.8 (C-28), 29.9 (C-30), 32.2 (C-1), 33.1 (C-21), 33.4 (C-29), 33.6 (C-12), 35.1 (C-22), 35.8 (C-17), 36.7 (C-19), 37.5 (C-13), 37.7 (C-16), 38.8 (C-10), 40.1 (C-7), 40.5 (C-9), 40.8 (C-8), 47.8 (C-5), 48.8 (C-18), 117.0 (C-15), 157.9 (C-14), 179.1 (C-3). Negative-ion HR-ESI-MS: *m/z* 441.3736 (calcd. for $C_{30}H_{49}O_2$, $[M–H]^–$, 441.3738).

Compound **1** was isolated from the CH_2Cl_2-soluble fraction in the form of a white amorphous powder. Compound **1** was assigned the molecular formula $C_{30}H_{50}O_2$ ($[M–H]^–$ *m/z* 441.3736; calcd. 441.3738) on the basis of high-resolution (HR)-ESI-MS. The IR spectrum showed the presence of hydroxy (3428 cm^{-1}), carboxyl (1707 cm^{-1}), and olefinic (1652 cm^{-1}) absorptions. The ^{1}H NMR and ^{13}C NMR spectroscopic data of **1** characterized a triterpenoid structure with 30 carbon signals, including those of eight methyl groups [δ_H 0.82 (s), 0.83 (d, *J* = 7.0 Hz), 0.89 (s), 0.91 (s), 0.92 (d, *J* = 7.0 Hz), 0.92 (s), 0.96 (s), and 1.09 (s); δ_C 18.8, 19.0, 21.4, 24.9, 25.2, 29.8, 29.9, and 33.4], ten methylenes, five methines, six carbons, and a carboxyl group (δ_C 179.1). A trisubstituted double bond was identified through an olefinic proton which appeared as a doublet of doublets at δ_H 5.55 (1H, dd, *J* = 8.0 Hz, 3.0 Hz) and two sp^2 ^{13}C NMR signals at δ_C 117.0 and 157.9. These NMR signals suggested a

taraxer-14-ene structure of **1** [6]. The ^{13}C NMR spectroscopic data of **1** were compared with those of taraxer-14-ene [6] to reveal the intact B, C, D, and E rings of the taraxer-14-ene skeleton. The distinct difference was seen in the A ring and the observation of two doublet methyl groups at δ_H 0.83 and 0.92 and a carboxyl group at δ_C 179.1 indicated a cleavage of the C-3/C-4 bond leading to the structure of a 3,4-*seco*-taraxer-14-en-3-oic acid. Ring-A's NMR data of **1** were in agreement with those of 3,4-*seco*-olean-18-en-3,28-dioic acid [7]. In addition, ^{1}H-^{1}H COSY, HSQC, and HMBC spectra were recorded for **1** to confirm this structure (Figure 1). The location of the double bond was confirmed by HMBC correlations of H_2-16 (δ_H 1.65/1.92) to C-14 (δ_C 157.9) and C-18 (δ_C 48.8), and H_3-26 (δ_C 1.09) to C-14. The carboxyl group was located at C-3 by HMBC correlations of H_2-1 (δ_H 1.67) and H_2-2 (δ_H 2.09/2.21) to C-3 (δ_C 179.1), and H_3-25 (δ_H 0.92) to C-1 (δ_C 32.2). The isopropyl group was attached to C-5 (δ_C 47.8) by the observation of HMBC cross-peaks between H_3-23 (δ_H 0.92), H_3-24 (δ_H 0.83), and H-4 (δ_H 1.89) to C-5. The orientations of CH_3-25β and H-5α were determined by NOESY spectrum (Figure 1) which showed correlations between H_3-26 and H_3-25, and H-11β (δ_H 1.54), and H-5 (δ_H 1.03) and H-7α (δ_H 1.27). Therefore, the structure of **1** was unambiguously determined to be 3,4-*seco*-taraxer-14-en-3-oic acid.

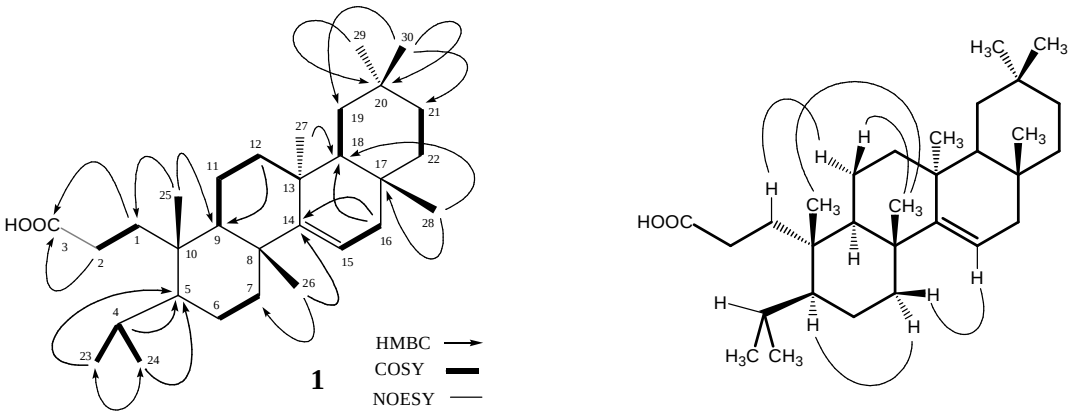

Figure 1. Structure of **1** and HMBC, ^{1}H-^{1}H COSY, and NOESY correlations of **1**.

Seco-taraxerane triterpenoids have not been found in the *Mallotus* species and 3,4-*seco*-taraxer-14-en-3-oic acid (**1**) may be biosynthesized from taraxerone *via* oxidative processes [8]. Setzer et al. reported this structure with very few spectroscopic evidences from *Alchornea latifolia* [9]. In our study the ^{1}H and ^{13}C NMR data and stereochemistry of this compound were fully established on the basis of the ^{1}H-^{1}H COSY, NOESY, HSQC, and HMBC spectroscopic data. Kamalachalcone A (**2**) has been isolated so far from *M. phillipensis* [10].

Acknowledgment

This work was supported by National Foundation for Science and Technology Development (NAFOSTED, Hanoi, Vietnam) (Project No. 104.01.137.09).

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