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CONSTITUENTS FROM STEM BARKS OF *ANACOLOSA POILANEI* GAGNEP. (OLACACEAE)

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Abstract

Four compounds were isolated from the stem barks of *Anacolosa poilanei* Gagnep. Theirs structures were established by spectroscopic analysis including MS and NMR. Accordingly, the isolates were identified as trichadenic acid B (**1**), trichadonic acid (**2**), amentoflavone (**3**) and *β*-sitosterol (**4**).

Keywords. *Anacolosa poilanei,* Olacaceae, trichadenic acid B, trichadonic acid, amentoflavone.

1. INTRODUCTION

Anacolosa genus comprises about 22 species and belongs to the Olacaceae family. The plants of this genus are distributed in the tropical regions [1]. An overview in the literature revealed that only two species have been examined for their chemical contents [2, 3]. These phytochemical studies led to the isolation and characterization of terpenoids and unsaturated faty acids containing triple bonds which were relatively rare in nature. In our screening program, a stem bark extract of A. *poilanei* Gagnep. (Olacaceae) showed cytotoxicity against KB cells (> 50 % inhibition at 1 μg/mL). In this paper, we report the isolation and structural determination of four compounds **1-4**.

2. EXPERIMENTAL

2.1. General experimental procedures

Optical rotations were measured on a Polax-2 L polarimeter. Melting points were determined using a Buchi B-545 instrument. ESI-MS were obtained on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on Bruker 500.13 MHz spectrometer, operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C NMR, respectively.

2.2. Plant material

Stem barks of *A. poilanei* were collected in Sapa, Lao Cai province, Vietnam in June 2003. A voucher specimen (VN-1124) was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy Science and Technology.

2.3. Extraction and isolation

Dry powdered stem barks of *A. poilanei* (1.18 kg) was extracted with EtOAc (3×2.5 L). The EtOAc solution was concentrated under reduced pressure to dryness. The EtOAc extract (55.3 g) was subjected to column chromatography (CC) on silica gel eluting with *n*-hexane/EtOAc gradient to yield 13 fractions.

Fraction 6 (1.2 g) was separated by column chromatography (CC) on silica gel, eluting with *n*-CH_2Cl_2 gradient to afford **1** (15 mg). Fraction 8 (1.3 g) was subjected to a Sephadex LH-20 CC to give 3 subfractions. Subfraction 2 was purified by CC on silica gel (CH_2Cl_2/MeOH gradient), followed by recrystallization in EtOAc to afford **2** (16 mg). Fraction 9 was recrystallized in EtOH to yield **3** (15 mg). Fraction 12 was purified on a silica gel CC (CH_2Cl_2/MeOH gradient), furnishing **4** (15 mg).

Trichadenic acid B (1): white powder, m.p. 302-304 ^{o}C, $[\alpha]_D$ +30 (*c*, 0.14, $CHCl_3$) (literature: 292-294 ^{o}C), $[\alpha]_D$ +32.2 (c, 0.69, pyridine). ^{1}H-NMR

(500 MHz, $CDCl_3$+CD_3OD), δ (ppm): 3.26 (1H, td, H-3), 0.83 (3H, d, *J* = 7.0 Hz, H-23) 0.72 (3H, s, H-24), 0.81 (3H, s, H-25), 0.90 (3H, s, H-26), 1.12 (3H, s, H-28), 1.10 (3H, s, H-26), 0.94 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$+CD_3OD): δ (ppm): 19.5 (C-1), 35.6 (C-2), 72.0 (C-3), 52.8 (C-4), 37.1 (C-5), 41.1 (C-6), 18.0 (C-7), 52.8 (C-8), 37.7 (C-9), 59.9 (C-10), 37.8 (C-11), 27.8 (C-12), 54.5 (C-13), 39.1 (C-14), 32.9 (C-15), 35.8 (C-16), 30.5 (C-17), 43.1 (C-18), 36.3 (C-19), 28.3 (C-20), 32.5 (C-21), 38.0 (C-22), 9.7 (C-23), 14.0 (C-24), 18.0 (C-25), 19.5 (C-26), 179.7 (C-27), 31.0 (C-28), 35.1 (C-29), 30.6 (C-30). ESI-MS (negative): 457 $[M-H]^-$.

Trichadonic acid (2): white powder, m.p. 249-252 ^oC, $[\alpha]_D$ +5.3 (c, 1.5, $CHCl_3$) (literature: 248-249 ^oC), $[\alpha]_D$ +5 (c, 0.3, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 0.87 (3H, d, *J* = 7.0 Hz, H-23), 0.72 (3H, s, H-24), 0.91 (3H, s, H-25), 1.14 (3H, s, H-26), 1.22 (3H, s, H-28), 1.00 (3H, s, H-29), 0.96 (3H, s, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$, δ (ppm): 22.7 (C-1), 41,3 (C-2), 213.0 (C-3), 58.1 (C-4), 42.1 (C-5), 41.3 (C-6), 18.5 (C-7), 53.0 (C-8), 37.6 (C-9), 59.4 (C-10), 37.8 (C-11), 27.8 (C-12), 54.8 (C-13), 39.2 (C-14), 33.0 (C-15), 41.0 (C-16), 30.7 (C-17), 43.3 (C-18), 35.7 (C-19), 28.4 (C-20), 32.4 (C-21), 36.0 (C-22), 16.8 (C-23), 14.7 (C-24), 18.4 (C-25), 22.7 (C-26), 181.0 (C-27), 31.0 (C-28), 30.5 (C-29), 35.4 (C-30). ESI-MS (negative): 455 $[M-H]^-$.

Amentoflavone (3): yellow powder, m.p. 260-261 ^oC (literature: 254-256 ^oC). ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm): 6.55 (1H, s, H-3), 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 7.97 (1H, d, *J* = 2.5 Hz, H-2'), 7.08 (1H, d, *J* = 8.5 Hz, H-5'), 7.83 (1H, dd, *J* = 2.0 Hz, 9.0 Hz, H-6'), 6.56 (1H, s, H-3''), 6.34 (1H, s, H-6"), 7.50 (2H, d, *J* = 8.7 Hz, H-2''', 6'''), 6.71 (2H, d, *J* = 8.7 Hz, H-3''', 5'''). ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm): 166.2 (C-2), 103.9 (C-3), 183.8 (C-4), 163.1 (C-5), 100.2 (C-6), 163.1 (C-7), 95.2 (C-8), 159.3 (C-9), 105.3 (C-10), 123.2 (C-1'), 132.8 (C-2'), 121.8 (C-3'), 161.2 (C-4'), 117.7 (C-5'), 129.3 (C-6'), 165.9 (C-2''), 103.3 (C-3''), 184.2 (C-4''), 162.5 (C-5''), 100.2 (C-6''), 164.0 (C-7''), 100.3 (C-8''), 156.5 (C-9''), 105.6 (C-10''), 123.1 (C-1'''), 129.3 (C-2'''), 115.8 (C-3'''), 162.5 (C-4'''), 115.8 (C-5'''), 129.3 (C-6''').

***β*-Sitosterol (4):** white powder, m.p. 140-142 ^oC, ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 3.52 (1H, m, H-3), 5.35 (1H, dd, J = 3.0 Hz, H-6), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.92 (3H, d, *J* = 7.0 Hz, H-21), 0.82 (3H, d, *J* = 7.0 Hz, H-26), 0.83 (3H, d, *J* = 7.0 Hz, H-27), 0.85 (3H, t, *J* = 7.0 Hz, H-29). ^{13}C-NMR (125 MHz, $CDCl_3$, δ (ppm): 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 12.0 (C-18), 19.8 (C-19), 36.2 (C-20), 18.8 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.1 (C-26), 19.4 (C-27), 23.1 (C-28), 12.0 (C-29).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as white powder and optically active, $[\alpha]_D$ +30 (c, 0.14, $CHCl_3$). Its ESI-MS (negative) showed pseudo-molecular ion at m/z 455 $[M-H]^-$. The ^{1}H-NMR spectrum of **1** indicated signals of a doublet methyl at δ_H 0.83 (*J* = 7.0 Hz, CH_3-23), six singlet meyhyls at δ_H 0.72 (CH_3-24), 0.81 (CH_3-25), 0.90 (CH_3-26), 1.12 (CH_3-28), 1.10 (CH_3-29), 0.94 (CH_3-30), an oxymethine proton at δ_H 3.26, and a complex set of overlapping signals at aliphatic region. Analyses of the ^{13}C-NMR and DEPT spectra with the aid of HSQC spectrum of **1** revealed the signals of 30 carbons, including a carboxylic group, seven methyls, eleven methylenes, five methines and six quaternary carbons. This observation strongly suggested that compound **1** should be a triterpene belonging to friedelane skeleton. The observation of only seven methyl groups and the presence of a carboxylic carbon suggested one methyl group being oxidized into a carboxylic functionality. Analyses of 2D NMR spectra of **1** confirmed the structure of **1** in which the methyl group CH_3-27 was oxidized into a carboxylic function as indicated by the cross-peak of C-27 (δ_C 179.7) with the protons of methyl group CH_3-26 at δ_H 0.90. The proton H-3 had two anti coupling constants which indicated a *trans*-diaxial relationship between H-3/H-4. The NMR data of **1** was in agreement with that reported for trichadenic acid B [4].

Compound **2** was obtained as white solid, mp 249-252 ^oC and optically active, $[\alpha]_D^{25}$ +5.3 (*c*, 1.5, $CHCl_3$). The 1D NMR spectra of **2** were close to those of **1**, except for the presence of a ketone group for **2** instead of the oxymethine group of **1**. This data suggested that **2** was a derivative of **1** by oxidation of hydroxyl group at C-3 into ketone functionality. Complete analyses of 2D NMR spectra established the structure of **2** as trichadonic acid which was previously described [5].

Compound **3** was isolated as yellow powder. Its ^{1}H-NMR spectrum presented signals of twelve aromatic protons, including an A_2B_2 system [δ_H 6.71 (2H, d, *J* = 8.7 Hz, H-3''' and 5'''), 7.50 (2H, d, *J* = 8.7 Hz, H-2''' and 6''')], an ABX system [δ_H 7.08 (*J* = 8.5 Hz, H-5'), 7.82 (*J* = 2.0, 8.5 Hz, H-6'), 7.97 (*J* = 2.0 Hz, H-2')], three singlets at δ_H 6.34 (H-6'') and 6.55 (H-3) and 6.56 (H-3''), and two doublets

with meta-coupling at δ_H 6.17 (J = 2.0 Hz, H-6) and 6.39 (J = 2.0 Hz, H-8). Detailed analyses of ^{13}C-NMR and DEPT spectra with the aid of HSQC spectrum revealed the presence of two carbonyl groups at δ_C 183.8 (C-4) and 184.2 (C-4''), sixteen quaternary carbons and twelve aromatic methines. This NMR data suggested **3** was a biflavonoid. This was then confirmed by analyses of 2D NMR spectra, especially by HMBC spectrum. The linkage between the two flavonone units was established by the HMBC cross-peaks of C-8'' with H-6'' (δ_H 6.34) and H-2' (δ_H 7.97) of the ABX system. Intensive analysis of the 2D-NMR spectra defined the structure of **3** as amentoflavone which was previously reported [6].

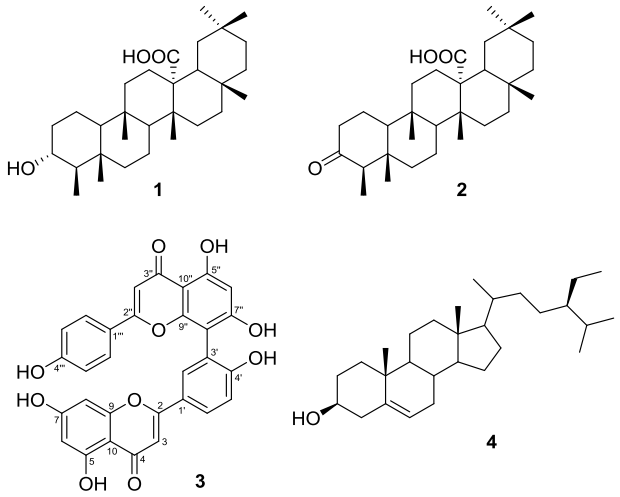

Figure 1: Compounds isolated from A. *poilanei*

Compound **4** was determined as *β*-sitosterol by comparison of its NMR data with the reported values [7], as well as by TLC analysis comparing with the authentic sample which was available in our laboratory.

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