Research Article

Pogonatherumol, a Novel Highly Oxygenated Norsesquiterpene with Flavone C-Glycosides from *Pogonatherum crinitum*

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A novel highly oxygenated norsesquiterpene, pogonatherumol (**1**), with two known flavone C-glycosides (**2-3**), was isolated from *Pogonatherum crinitum*. The structure of the new compound was illuminated based on its spectroscopic data and X-ray analysis. Compounds **1** and **3** inhibited NO production in the mouse peritoneal macrophage ($64.5 \pm 7.2\%$ and $61.6 \pm 5.8\%$, respectively, at a concentration of $50 \mu\text{M}$). The three compounds were inactive when tested against two human cancer cell lines (IC_{50} values $> 50 \mu\text{M}$).

1. Introduction

Pogonatherum crinitum, is one of the species in the genus *Pogonatherum* of Gramineae family, which has been used as a natural medicine in Chinese folk [1], for treatment of diabetes, urinary tract infection, jaundice hepatitis, and nephritis [2–5]. Previous phytochemical studies on this plant have led to a series of flavones and lignins, some of which showed anti-inflammatory and antifungal activities [6, 7]. In order to discover structurally and biologically interesting compounds from *P. crinitum*, a novel highly oxygenated norsesquiterpene, pogonatherumol (**1**) with two known flavone C-glycosides was investigated (Figure 1). Herein, the isolation and structural characterization of the compounds and their biological activities are reported in this paper.

2. Experimental

2.1. General Experimental Procedures. X-ray diffractometry data of **1** were collected on a MAC DIP-2030K diffractometer with graphite-monochromated Mo K α

radiation and were corrected by Lorentz and polarization. An XT-5B micromelting point apparatus was used to determine the melting point. JEOL ECZ-400S, JASCO V-650, and Nicolet iS5N spectrophotometers manufactured by Thermo Scientific were used to record NMR, UV, and IR spectra, respectively. Thermo Scientific LTQ Orbitrap XL was used to collect HRESIMS spectra. Column chromatography (CC) was performed with silica gel (300–400 mesh), polyamide (60–100 mesh), Sephadex LH-20, D101 macroporous, and ODS ($50 \mu\text{m}$). Preparative HPLC made up for an LC-20AP instrument, an SPD-20A detector, and an ODS-A column (YMC, $250 \times 20 \text{ mm}$, $5 \mu\text{m}$) was used to purify the compounds.

2.2. Plant Material. *Pogonatherum crinitum* was collected in Jianyang, Fujian, China, in September 2015. Professor Xiaoxing Zou, from the Fujian Agriculture and Forestry University (FAFU), People's Republic of China (PRC), identified the plant. The plant specimen was deposited at the lab of the material basis and utilization of TCM, College of Plant Protection, FAFU, PRC.

FIGURE 1: The structure of compounds 1–3. *New compound.

2.3. Extraction and Isolation. Air-dried and powdered herba of *Pogonatherum crinitum* (10 kg) was heated under reflux two times with 75% aqueous EtOH (80 L $\times$ 2 h $\times$ 2) at 80°C. The extract was evaporated under reduced pressure, and the residue was suspended in H₂O and then extracted with EtOAc (8 L $\times$ 3). The ethyl acetate segment (113.2 g) was dissolved in water and subjected to passage over polyamide eluting with H₂O and 50% EtOH-H₂O (v/v), successively (segments A₁ and A₂). The 50% EtOH-water extract (segment A₂, 43.7 g) was passed over CC on silica gel eluting with CH₂Cl₂-MeOH (15:1–1:1) to afford 8 segments (B₁–B₈). Segment B₃ (1.032 g) was subjected to passage over Sephadex LH-20 eluting with MeOH and finally purified by preparative HPLC to give **1** (23 mg), using chromatographic condition as MeOH-H₂O (30:70, v/v), and detected at 210 nm, 8 mL/min. Segment B₅ (5.273 g) was subjected to passage over an RP-18 column with MeOH-H₂O (15%, 30%, and 45%, v/v, successively) to afford 3 segments (C₁–C₃). Finally, segment C₂ (1.518 g) was purified by preparative HPLC to give **2** (10 mg) and **3** (8 mg), under chromatographic condition as MeCN-H₂O (15:85, v/v), and detected at 210 nm, 8 mL/min.

2.3.1. Pogonatherumol (1). Colorless needles; mp 153°C–154°C; UV (MeOH) $\lambda_{\max}$ (log ϵ) 232 (3.53) nm; IR $\gamma_{\max}$ 3313, 2960, 2867, 1672, 1566, 1510, 1294, 1182, 922, 866 cm⁻¹; ¹H NMR (DMSO-*d*₆, 400 MHz) and ¹³C NMR (DMSO-*d*₆, 100 MHz) (Table 1); HRESIMS *m/z* 233.11728 [M + H]⁺ (calcd. for C₁₄H₁₆O₃, 233.12722).

2.3.2. Crystallographic Data of 1. Bond precision: C–C = 0.0059; wavelength = 0.71073; cell: *a* = 8.214 (4), *b* = 6.783 (3), *c* = 22.236 (11); α = 90; β = 90; γ = 90; temperature: 296 K; C₁₄H₁₆O₃, Mr 232.27; space group Pnma; hall group-p2ac2n; Dx = 1.245 g·cm⁻³; 1.092 g·cm⁻³, *F*(000) = 496; μ = 0.087 mm⁻¹; nref = 1607; *T*_{min} = 0.468, *T*_{max} = 0.746; data completeness = 0.982; θ (max) = 28.149; R(reflections) = 0.0888(946); WR2(reflections) = 0.2143(1607); *s* = 1.130; npar = 114.

The crystallographic data of the structure of pogonatherumol (deposition number: CCDC 1842928) were deposited at the Cambridge Crystallographic Data Centre.

2.4. Biological Activities. Two cell lines, human ovarian neoplasm cell (A2780) and human colon cancer cell line (HCT-116), were used to test the tumor cytotoxic activities, and Taxol was used as a positive control. The method was

TABLE 1: ¹H and ¹³C NMR data of compound 1.

Number	δ_c^a	δ_H^b (mult. J/Hz)
1	191.5	—
2	138.7	—
3	26.1	3.43, <i>s</i>
4	139.1	—
5	151.0	—
6	112.1	6.76, <i>s</i>
7	142.1	—
8	126.7	—
9	137.7	—
10	129.5	6.56, <i>d</i> (7.1)
11	14.8	1.89, <i>d</i> (7.1)
12	26.2	4.06, <i>m</i>
13	23.2	1.12, <i>d</i> (6.8, overlap)
14	23.2	1.12, <i>d</i> (6.8, overlap)

^aIn DMSO-*d*₆ (100 MHz); ^bin DMSO-*d*₆ (400 MHz).

according to the previously described [8]. LPS-induced RAW264.7 macrophage was used to evaluate the NO production inhibition activity of the three compounds. Dexamethasone was used as a positive control, and the test method was according to the literature [9, 10].

3. Results and Discussion

Pogonatherumol (**1**) was obtained as colorless needles (MeOH). The IR spectrum of **1** showed 3313, 1672, and 1566 cm⁻¹ absorption bands, which were assigned to hydroxyl, carbonyl, and olefin functionalities, respectively. The molecular formula was determined to be C₁₄H₁₆O₃ on the basis of HRESIMS (an [M + H]⁺, *m/z* 233.11728), which corresponded to the molecular formula, indicating 7 degrees of unsaturation. Analysis of the ¹H NMR spectroscopic data of **1** (Table 1) revealed three methyls (δ_H 1.12, 1.12 (3H each, both *d*, *J* = 6.8 Hz, H₃-13, 14), and 1.89 (3H, *d*, *J* = 7.1 Hz, H₃-11)), a methylene (δ_H 3.43 (2H, *s*, H-3)), an aromatic proton δ_H 6.76 (1H, *s*, H-6), an olefin proton δ_H 6.56 (1H, *m*, H-10), and two hydroxyls (10.06 (1H, *s*) and 8.74 (1H, *s*)). And the ¹³C NMR (Table 1) and DEPT spectra showed fourteen carbons, including a carbonyl, a methene, three methines, three methyls, and six aromatic carbons, two of them bearing oxygen atoms (C-4 and C-5). HMBC correlations of H-6/C-4, C-5, and C-8; H-3/C-1, C-2, C-8, and C-9; 4-OH/C-5 and C-9; and 5-OH/C-4, C-5, and C-6 confirmed the presence of the indanone skeleton (Figure 2). An isopropyl (δ_H 1.12, 1.12 (3H each, both *d*, *J* = 6.8 Hz, H₃-13, 14) and 4.06 (1H, *m*, H-12)) was attached to C-7, which could be confirmed by the HMBC correlation from H-13 and H-14 to C-12 and C-7 and from H-12 to C-6, C-7, and C-8. Further HMBC correlations of H-11/C-10 and C-2 and of H-10/C-1 indicated that an ethylidene was fixed in C-2, and NOESY correlation from H₃-11 to H-3 suggested the *trans*-configuration of double bonds between C-2 and C-10 (Figure 2). Finally, the proposed structure of **1**, (*E*)-2-ethylidene-4,5-dihydroxy-7-isopropyl-2,3-dihydro-1H-inden-1-one, named “pogonatherumol” was confirmed by an X-ray crystallography analysis. The ORTEP drawing, with the atom-number scheme indicated, is shown in Figure 3.

FIGURE 2: Key ^1H , ^1H COSY, HMBC, and NOESY correlations of **1**.

FIGURE 3: ORTEP drawing of compound **1**.

In addition to pogonatherumol, two known C-glycosides, schaftoside (**2**) [11] and isoschaftoside (**3**) [12], were identified by comparing their ESIMS and ^1H and ^{13}C NMR data with those reported in the literature.

Pogonatherumol (**1**) with a fused indanone skeleton has not been previously reported as a natural product. Notably, it seems to be a novel rearrangement of norsesquiterpene.

Meanwhile, the inhibited NO production and the tumor cytotoxic activities of three compounds were evaluated. Compounds **1** and **3** inhibited NO production in the mouse peritoneal macrophage ($64.5 \pm 7.2\%$ and $61.6 \pm 5.8\%$, respectively, at a concentration of $50 \mu\text{M}$), while the positive control dexamethasone gave an inhibitory ratio of $59.4.1 \pm 6.2\%$ at a concentration of $10 \mu\text{M}$. All compounds were also evaluated *in vitro* for cytotoxicity against A2780 and HCT-116 cell lines employing an MTT assay with Taxol as the positive control. The three compounds were inactive when tested against the two human tumor cell lines (IC_{50} values $\geq 50 \mu\text{M}$).

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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Supplementary Materials

The supplementary materials contain HRESIMS, IR, and NMR spectra of compound **1** and NMR data of compounds **2** and **3**. (*Supplementary Materials*)

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