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Eupatorione A, an Unusual Sesquiterpenoid from the Aerial Parts of *Eupatorium adenophorum*

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Abstract: Herein, the chemical analysis of petroleum ether extract of *Eupatorium adenophorum* was conducted which led to the identification of a previously unreported sesquiterpenoid, named eupatorione A (**1**). The structure of eupatorione A (**1**) was elucidated through comprehensive spectroscopic analysis, including 1D, 2D-NMR techniques, as well as HRMS. Additionally, X-ray diffraction was employed to further confirm the structure. The anti-inflammatory activity of compound 1 was assessed by measuring its inhibitory effects on the production of nitric oxide (NO), induced by lipopolysaccharide (LPS) in RAW264.7 LPS-activated macrophages.

Keywords: Compositae; *Eupatorium adenophorum*; sesquiterpenoid; chemical structure.

1. Plant Source

The aerial parts of *Eupatorium adenophorum* Spreng. (Compositae) were procured from Xichang, located at GPS coordinates of 30º52′N/104º44′E, within Sichuan province, during the wet season of July 2021. The plant was identified by Prof. Qing-Shan Yang from Anhui University of Chinese Medicine. An authentic sample with the designation EA20210711 was preserved at Anhui University of Traditional Chinese Medicine for reference.

2. Previous Studies

Plants can produce a multitude of natural products, many of which play a significant role in developing lead compounds in the treatment of diseases and the production of plant-derived

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insecticides. The fascinating structures and diverse biological activities of these compounds have garnered substantial interest from researchers in both the fields of natural products and medicinal chemistry [1–2]. Terpenoids, characterized by an extensive array of chemical structures and biological functions, are the most abundant compounds in the family of natural products. To date, over 95000 terpenoids have been documented [3–4]. *Eupatorium adenophorum* Spreng., a member of the Compositae family (also known as Asteraceae), is a significant invasive plant [5]. However, this plant exhibits a rich content of terpenoids. A previous phytochemical study revealed that the aerial parts of this plant possessed an abundance of sesquiterpenoids. These sesquiterpenoids demonstrated noteworthy activities such as antibacterial and antiviral activities [6–9]. In our effort to discover bioactive sesquiterpenoids from this plant, we have identified a sesquiterpenoid possessing an unprecedented 5/5/6 tricyclic rare skeleton as depicted in Figure 1.

3. Present Study

The air-dried and powdered aerial parts of *E. adenophorum* (12 kg) were finely ground and immersed in petroleum ether (25 L) at room temperature for 48 h. This extraction was conducted thrice under identical conditions. The combined extract was evaporated under reduced pressure to obtain an oily residue (162 g), which was subjected to silica-gel column chromatography (CC). The column was eluted using a stepwise gradient of petroleum ether (PE) and ethyl acetate (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 0:10, v/v). The elution led to the separation into four distinct fractions labeled as Frs. I–IV). Fr. II (48 g) was fractionated on silica-gel CC with PE/acetone (from 10:1 to 2:1, v/v) as the eluent to give three sub-fractions (Frs. IIA–IIC). Fr. IIB (6.4 g) was passed through a Sephadex LH-20 column, eluted with acetone, resulting in additional sub-fractions (Fr. IIB1–Fr. IIB2). Subsequently, Fr. IIB2 (700 mg) was isolated through silica-gel CC, employing a mixture of PE and acetone (8:1, *v/v*) as the eluent. Further purification was achieved by subjecting it to a Sephadex LH-20 column eluted with acetone, followed by the separation using semi-preparative RP -HPLC using MeOH-H_2O (70:30, *v/v*) as the mobile phase. This process yielded compound 1 (t_R 28.9 min, 4.6 mg).

Equipment: Preparative HPLC was performed on an Ruihe with a Zorbax SB-C18 column (5 μm, 9.4 × 150 mm, Agilent, America) with a flow rate of 3.5 mL/min. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were recorded on Bruker AVANCE III 600 MHz. UV spectrum was obtained on a Shimadzu 2700 or 2401 PC double-beam spectrophotometer. IR spectra were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. Mass spectra were obtained on Agilent G6230 spectrometer. Optical rotation was measured with a Horiba SEPA-300 polarimeter. X-ray analysis was conducted on a Bruker SMART APEX CCD crystallography system.

Measurement of Nitric Oxide (NO) Production: NO production was assayed in supernatants of cultured RAW264.7 cells by using an NO assays kit (Beyotime Institute of Biotechnology, Haimen, China). Cells were seeded in 96 well culture plates and pretreated with tested compounds (50 μM) and stimulated with LPS (1 lg/mL) for 24 h. The supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2.5% phosphoric acid) and incubated at room temperature for 24 h [10. The concentration of nitrite was measured by reading the absorbance at 570 nm. L-NMMA was used as a positive control [11-12].

*Eupatorione A (**1**)*: Colorless needle crystal; $[\alpha]_D^{20}$ = – 6.7 (c = 0.01, MeOH); UV (MeOH): λ_{max} (log ε): 251 (3.78) ; IR ν_{max} (KBr): = 3449, 2958, 2924, 2852, 1716, 1645, 1454, 1383, 1150, 1075 cm-1; 1H NMR (600 MHz, acetone-d_6): δ (ppm) = 1.92 (3H, s, Me-1), 2.14 (3H, s, Me-3), 2.64 (1H, d, *J* = 5.0 Hz, H-6), 1.70 (1H, m, H-7), 1.89 (1H, m, H-8), 3.50 (1H, m, H-9), 2.27 (1H, m, H-10), 2.92 (1H, m, H-11a), 2.49 (1H, m, H-11b), 1.32 (3H, s, Me-14), 0.85 (3H, d, *J* = 7.1 Hz, Me-15), 4.19 (1H, s, OH); 13C NMR (150 MHz, acetone-d6): δ (ppm) =18.3 (CH_3, Me-15), 20.4 (CH_3, Me-3), 23.0 (CH_3, Me-1), 27.0 (CH_3, Me-14), 35.8 (CH, C-7), 45.2 (CH, C-8), 45.5 (CH_2, C-11), 48.1 (CH, C-9), 58.1 (CH, C-10), 59.1 (CH, C-6), 76.6 (C, C-13), 135.7 (C, C-4), 143.9 (C, C-2), 203.3 (C, C-5), 213.5 (C,

C-12); HRESIMS *m/z* 249.1487[M+H]$^+$ (calcd. 249.1485 for $C_{15}H_{21}O_3$). The crystal data (CCDC: 2271496) of **1** see Table S1.

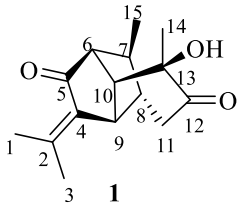

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Figure 1. Structure of eupatorione A (**1**) isolated from *E. adenophorum*

Compound **1** was obtained as a colorless needle-shaped crystal. The IR spectrum (Figure S8) of **1** exhibited absorption bands for hydroxyl groups (3449 cm-1) and α, β-unsaturated ketone ($\nu_{C=O}$ 1715, $\nu_{C=C}$ 1645, ν_{C-O} 1150 cm-1). HRESIMS recorded an ion peak at *m/z* 249.1487 [M+H]$^+$ (cal. 249.1485), its molecular formula was determined as $C_{15}H_{21}O_3$, signifying a total of six degrees of unsaturation. Further analysis of the ^{1}H and ^{13}C NMR data (Figure S1 and S2), along with the assistance of DEPT experiments, revealed the presence of four methyl groups (including one bimodal methyl), one methylene group, five methine groups, five quaternary carbons (corresponding to two olefinic carbons, two ketone carbonyls, and one oxygenated quaternary carbon, respectively). The comprehensive analysis of the NMR data indicated that compound **1** should be a tricyclic natural compound. The key HMBC interactions illustrated in Figure 2 provide valuable insights into the structure of compound **1**. The correlations are as follows: correlation of Me-1 (δ_H 1.92) with C-2/C-3/C-4, Me-3 (δ_H 2.14) with C-1/C-2/C-4, Me-14 (δ_H 1.32) with C-10/C-12/C-13, Me-15 (δ_H 0.85) with C-6/C-7/C-8, H-6 (δ_H 2.64) with C-4/C-8/C-9/C-15, H-7 (δ_H 1.70) with C-5/C-9/C-10/C-11, H-8 (δ_H 1.89) with C-4/C-6/C-10/C-12/C-15, H-9 (δ_H 3.50) with C-2/C-4/C-5/C-6/C-7/C-11, H-10 (δ_H 2.27) with C-4/C-5/C-7/C-8/C-14, H-11 (δ_H 2.92, 2.49) with C-7/C-9/C-12/C-13, 13-OH (δ_H 4.19) with C-10/C-12/C-13/C-14. These correlations conclusively establish the existence of a 5/5/6 tricyclic rare skeleton for compound 1. The apparent 1H-1H COSY correlations (Figure 2) of H-6 with H-7/H-10, H-7 with H-8/H-15, H-8 with H-9/H-11, H-10 with H-6/H-9 further confirmed the planar structure of the compound. While the ROESY spectrum only displayed limited correlations indicating the correlation of H-6 with H-9/Me-15, and Me-14 with H-9/H-10. Through the solvent volatilization, a needle-shaped crystal of **1** was obtained from a mixture of methanol and water (10:1, *v/v*). Subsequently, the structure, including the absolute configuration (6*S*, 7*S*, 8*S*, 9*R*, 10*S*, 13*R*), was unambiguously established through X-ray diffraction employing Cu Kα radiation (Figure 3). Consequently, compound **1** has been identified as shown in Figure 1, and designated as Eupatorione A.

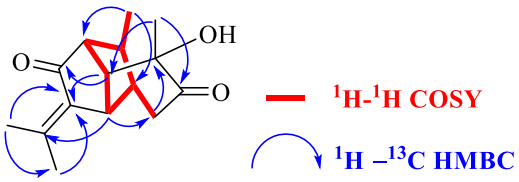

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Figure 2. Important ^{1}H-^{1}H-COSY and HMBC correlations for **1**

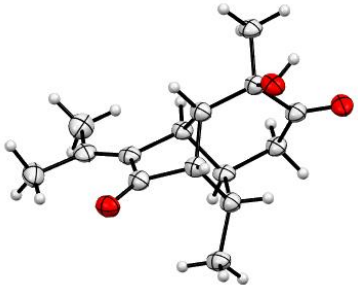

Figure 3. X-ray crystallographic structure of compound **1**

Eupatorione A (**1**) was tested for anti-inflammatory activity by measuring the inhibitory effects on LPS-induced NO production in RAW264.7 LPS-activated macrophages. At 50μM, compound **1** showed relatively weak anti-inflammatory activity with an inhibition rate of 30.56%. The results are summarized in Table 1.

Table 1. Inhibitory rate of LPS-induced NO production in RAW246.7 cells treated with compound **1**

Compound	concentration（μM）	NO Inhibitory rate（%）
1	50	30.56±0.48
L-NMMA	50	55.47±2.27

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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