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Anti-inflammatory constituents from *Psychotria prainii* H. Lév

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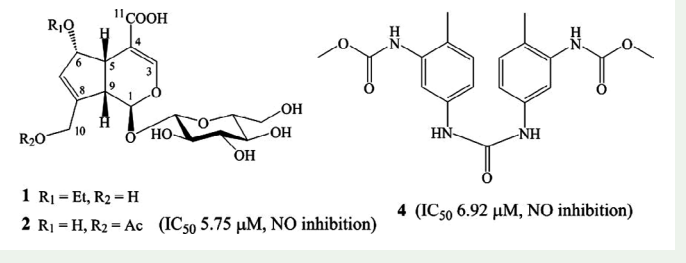
ABSTRACT

One new and three known compounds were isolated from the ethanol extract of *Psychotria prainii* aerial parts. By means of spectroscopic methods, their structures were elucidated to be deacetylasperulosidic acid 6-ethyl ether (**1**), asperulosidic acid (**2**), asperuloside (**3**) and obtucarbamates C (**4**). The isolated compounds were evaluated for their inhibitory effect on NO production in LPS-stimulated RAW264.7 cells. Among them, compounds **2** and **4** exhibited strong effect with the IC_{50} values of 5.75 ± 0.85 and 6.92 ± 0.43 μM, respectively. This is the first report for the chemical composition and biological activity of *P. prainii*.



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1. Introduction

The genus *Psychotria* is the largest genus within the Rubiaceae family, distributed throughout tropical and subtropical regions of the world, with approximately 1500 species (Yang et al. 2016). To date, 41 species of the *Psychotria* genus have been chemically investigated, including alkaloids of indole, quinoline and benzoquinolizidine, terpenoids, steroids, phenolics and aliphatic compounds (Yang et al. 2016). Those compounds exhibit several biological properties, such as antioxidant (Matsuura and Fett-Neto 2013), cytotoxicity (Adjibadé et al. 1989;

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Mahmud et al. 1993), antimicrobial (Khan et al. 2001; Jayasinghe et al. 2002), antiviral (Khan et al. 2001; Kuo et al. 2001) and antiparasitic activities (Kato et al. 2012). In Vietnamese traditional medicine, *P. prainii* is often used for the treatment of a variety of diseases as an antiflatulent or antitussive agent. It exhibits antifungal, appetite-inducing and counter-irritant properties and has been used to treat digestive and intestinal disorders. However, the studies of chemical constituents and biological effects of *P. prainii* are still unknown.

In the course of the screening programme for biologically active natural products, *P. prainii* has been a subject of extensive investigation due to its biological activities. This paper describes the isolation and structural elucidation of four compounds (**1**–**4**), including one new iridoid derivative (**1**) and three known compounds (**2**–**4**) from the aerial parts of *P. prainii* (Figure 1). Their anti-inflammatory effects were also examined via the inhibition of nitric oxide (NO) production.

2. Results and discussion

One new iridoid derivative (**1**) and three known compounds (**2**–**4**) were isolated from the water layer of an EtOH extract using combined chromatographic methods. Detailed analysis of the spectroscopic data (1D, 2D NMR and MS) and comparison with previously reported values led to the elucidation of the known as asperulosidic acid (**2**) (Tzakoua et al. 2007), asperuloside (**3**) (Otsuka et al. 1991) and obtucarbamate C (**4**) (Huang et al. 2012).

Compound **1** was obtained as a white solid. A $C_{18}H_{26}O_{11}$ molecular formula was deduced from the ion peak at *m/z* 441.1401 $[M + Na]^+$ observed in the HR-ESI-MS. The ^1H NMR spectrum of **1** showed two olefinic signals [δ_H 7.57 and 6.11 (each singlet, H-3 and H-7)], along with one hemiacetal signal [δ_H 5.04 (d, J = 8.5 Hz, H-1)], one oxymethylene [δ_H 4.24 (d, J = 15.5 Hz, H-10a) and 4.49 (d, J = 15.5 Hz, H-10b), one oxymethine [δ_H 4.51 (br s, H-6)]

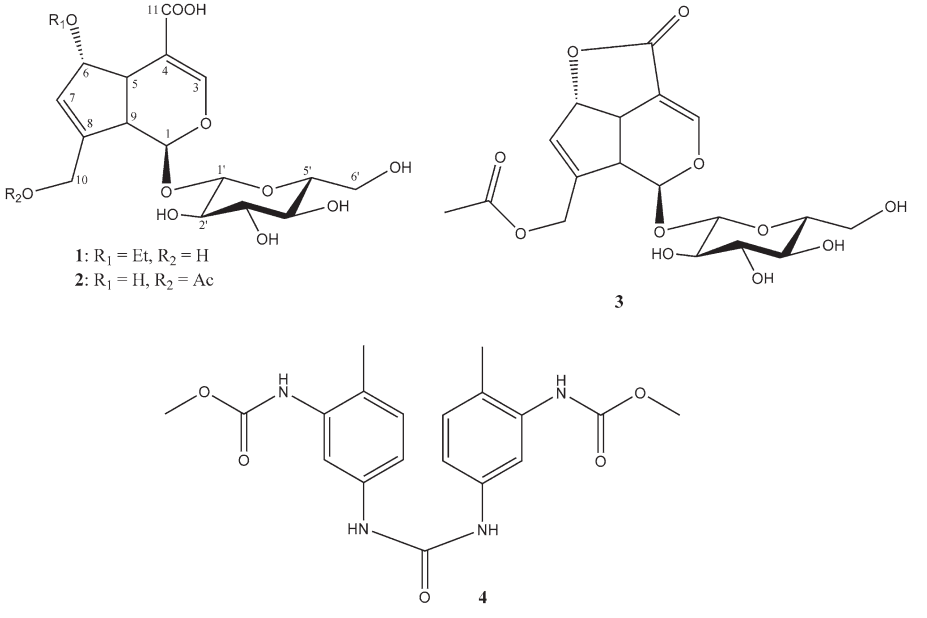

Figure 1. The structure of compounds **1**–**4**.

and two methines [δ_H 3.09 (m, H-5) and 2.52 (dd, J = 8.5, 7.5 Hz, H-9)]. Moreover, the ^{1}H NMR spectrum also displayed an anomeric signal at δ_H 4.75 (d, J = 8.0 Hz, H-1′) arising from a β-glucopyranose. These findings, taken together with the corresponding ^{13}C NMR data, revealed that **1** was an iridoid glycoside (Miyagoshi et al. 1987; Otsuka et al. 1991; Tzakoua et al. 2007). However, ^{1}H NMR spectrum of **1** exhibited additional signals of an ethyl group at δ_H 1.08 (t, J = 6.0 Hz, H-13) and 3.52 (m, H-12). The ^{13}C NMR spectrum of **1** contained 18 carbon signals, 12 of which were assigned to an iridoid aglycone and the remaining 6 carbons to a β-glucopyranosyl moiety. These NMR data suggested that **1** was an iridoid glycoside with a skeleton similar to that of deacetylasperulosidic acid (Tzakoua et al. 2007), except for the presence of an additional ethanolyl group at C-6. This suggestion was further confirmed by the HMBC correlations from H-12 (δ_H 3.52) to C-6 (δ_C 83.4) and from H-6 (δ_H 4.51) to C-12 (δ_C 66.2).

The relative configuration of **1** was deduced based on the NOESY correlations and other NMR data comparisons with a previous report. The relative configurations in **1** were established based on the NOESY experiment and ^{13}C chemical shifts. The NOE cross-peak between H-5 (δ_H 3.09) and H-9 (δ_H 2.52) indicated the *cis*-fused iridoid skeleton. The NOESY correlation between H-5 (δ_H 3.09) and H-6 (δ_H 4.51) suggested the α-orientation of the ethanolyl group. Therefore, compound **1** was determined to be deacetylasperulosidic acid 6-ethyl ether. Since the sample extraction by ethanol might produce ethyl ether adducts, the ethanol and methanol extracts of *P. prainii* aerial parts were prepared and analysed by HPLC. Compound **1** was detected in both extracts, which confirmed that compound **1** was not an artefact formed during the extraction process (see Supplemental).

Nitric oxide (NO) plays an important role in the regulation of various physiological processes in mammals. Overproduction of NO causes numerous human diseases including inflammation, arthritis, asthma and cancer. Therefore, an assay for inhibition of NO production is one of the possible ways to screen anti-inflammatory agents (Luiking et al. 2010). The inhibitory effect of the isolated compounds on the NO production was evaluated in LPS-stimulated RAW264.7 cells. Compounds **2** and **4** strongly inhibited NO production with the IC_{50} values of 5.75 ± 0.85 and 6.92 ± 0.43 μM, respectively. The MTT assay showed that those compounds had no significant toxicity to RAW264.7 cells up to 30 μM (data not shown) indicating that the inhibitory effect on NO production was not due to cytotoxicity. Compounds **1** and **3** were inactive at concentration up to 30 μM.

3. Experimental

3.1. General procedures

Optical rotations were read on a JASCO P-2000 digital polarimeter. NMR experiments were performed on a Bruker AM500 FT-NMR spectrometer. The HR-ESI-MS data were obtained from an API Q-STAR PULSAR I of Applied Biosystem. Thin layer chromatography (TLC) was performed using precoated Kiesel gel 60 F254 and visualised by UV light 254 nm and 10% H_2SO_4 reagent by heating. Column chromatography (CC) was performed using silica gel 60 (Merck, 70–230 mesh). HPLC analysis was carried out in an Agilent 1260 Series Single Quadrupole LC/MS Systems.

3.2. Plant materials

The aerial parts of *P. prainii* H. Lév were collected in Son La, Vietnam in July 2013, and identified by Dr. Pham Thanh Huyen, National Institute of Medicinal Materials and Assoc. Prof. Tran Van On, Hanoi University of Pharmacy. The voucher specimens (TB-9938 and HUP-1572013SL) were deposited at the Herbarium of the National Institute of Medicinal Materials and Hanoi University of Pharmacy.

3.3. Extraction and isolation

The air-dried materials (10 kg) were refluxed with ethanol for 2 h (20 L × 3 times). The combined extracts were concentrated to give 339.3 g of crude extract, which was then resuspended in water (500 mL) and successively extracted with *n*-hexane and ethyl acetate (each 500 mL × 3 times). The water residue was filtered through a Diaion HP-20 column eluted by 0, 25 and 100% methanol in water. The 100% methanol-eluted fraction was concentrated and fractionated by a silica gel column using a gradient of 0–100% methanol in dichloromethane to afford five fractions (F-1–F-5). Fraction F-2 was passed through a silica gel column eluted by *n*-hexane–acetone (1.8:1 v/v) to obtain **4** (11.8 mg). Compound **3** (7.0 mg) were purified from fraction F-3 using RP-C_{18} column eluted with methanol–water (1:4 v/v) followed by a Sephadex LH-20 column (methanol–water, 1:3 v/v). Fraction F-4 was fractionated on a RP-C_{18} column eluted with methanol–water (1:3 v/v) to give seven subfractions, (F-4.1–F-4.7). A RP-C_{18} column chromatography using methanol–water (1:5 v/v) was applied for F-4.4 to give **1** (25.0 mg). Compound **2** (241.4 mg) was obtained from fractions F-4.4 and F-4.7 by a similar column with methanol–water (1:3 v/v).

3.3.1. Deacetylasperulosidic acid 6-ethyl ether (1)

White solid; $[\alpha]_D^{24}$ = +4.0 (*c* 0.1, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ 1.08 (3H, t, *J* = 6.0 Hz, H-13), 2.52 (1H, dd, *J* = 8.5, 7.5 Hz, H-9), 3.09 (1H, m, H-5), 3.27 (1H, m, H-2′), 3.30 (1H, m, H-5′), 3.35 (1H, m, H-4′), 3.43 (1H, dd, *J* = 9.0, 8.5 Hz, H-3′), 3.52 (2H, m, H-12), 3.70 (1H, dd, *J* = 12.0, 5.5 Hz, H-6′a), 3.85 (1H, dd, *J* = 12.0, 2.0 Hz, H-6′b), 4.24 (1H, d, *J* = 15.5 Hz, H-10a), 4.49 (1H, d, *J* = 15.5 Hz, H-10b), 4.51 (1H, br s, H-6), 4.75 (1H, d, *J* = 8.0 Hz, H-1′), 5.04 (1H, d, *J* = 8.5 Hz, H-1), 6.11 (1H, s, H-7), 7.57 (1H, s, H-3). ^{13}C NMR (125 MHz, CD_3OD): δ 101.6 (C-1), 154.8 (C-3), 108.9 (C-4), 42.2 (C-5), 83.4 (C-6), 128.4 (C-7), 152.1 (C-8), 45.9 (C-9), 61.7 (C-10), 171.2 (C-11), 66.2 (C-12), 15.9 (C-13), 100.6 (C-1′), 74.9 (C-2′), 77.8 (C-3′), 71.4 (C-4′), 78.2 (C-5′), 62.6 (C-6′); and HR-ESI-MS (positive): *m/z* 441.1401 $[M + Na]^+$ (calcd for 441.1373, $C_{18}H_{26}NaO_{11}$).

3.4. Acid hydrolysis of 1

Compound **1** (0.5 mg) was heated in 1 N HCl (400 µL) at 80 °C for 2 h, then the solution was extracted with ethyl acetate (1 mL × 3). The aqueous layer was neutralised with NH_4OH and then dried under reduced pressure. The obtained residue was redissolved in 150 µL pyridine containing 10 µmol of L-cysteine methyl ester and heated at 80 °C for 1 h. 6 µl *o*-tolyl isothiocyantate was added, and the solution was heated for another hour. The reaction solution was then analysed by HPLC using Cosmosil 5C18-MS-II column (4.6 × 150 mm), mobile phase of 20% acetonitrile in 0.2% TFA water, UV detection at 254 nm. The sugars were identified as D-glucose (t_R 9.02 min).

3.5. HPLC analysis of 1

The HPLC analysis was performed to confirm that compound **1** was not an artefact formed during the extraction process. Two parts of *P. prainii* materials (each 1 g) were individually extracted with 2 mL of ethanol and methanol in a sonic bath for 10 min. Each extracts were diluted by 8 mL of water and then mixed with 5 mL of hexane. The aqueous solutions were filtered through a 0.45-µm filter unit and injected to the HPLC system. The separation was done a Zorbax Eclipse XDB C_{18} column (250 × 4.6 mm, 5 µm) using a gradient of 10–90% acetonitrile in water for 25 min, flow rate: 0.5 mL/min. Compound **1** was detected in the tested solutions by retention time (16.76 min) and mass ion peak (*m/z* 419.1).

3.6. Assay for inhibition of NO production

Determination of NO production in LPS-induced RAW264.7 cells was performed as previously described (Dang et al. 2017). Cardamonin was used as a positive control (IC_{50}, 2.24 µM).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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