Research Article

A New Megastigmane Glucoside and Other Constituents from Desmodium gangeticum

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1.Introduction

Desmodium gangeticum (L.) DC. is a woody perennial herb plant, which distributed wildly in the mountainous area in Vietnam, or several regions in Asia and Africa [1, 2]. D. gangeticum has been used for the treatment of asthma, stomatitis, arthritis, eczema, or some other diseases [1]. Previous studies proved the contents of flavonoids, alkaloids, and sterols in D. gangeticum with hepatoprotective, anti-inflammatory, antileishmanial, immunomodulatory, cardioprotective, antinociceptive wound healing, antidiabetic, and antiulcer activities [3–6]. Nitric oxide (NO) has been known to involve in the regulation of various physiological processes in mammals and the overproduction of NO is responsible for the pathological development of inflammation, cancer, and diabetes [7, 8]. Therefore, inhibitors of NO production have potential therapeutic value as anti-inflammatory agents [9]. In this study, we reported the isolation of a new megastigmane glycoside 1 and three known compounds from D. gangeticum (Figure 1). The isolated compounds were evaluated for their inhibition of nitric oxide (NO) production in LPS-stimulated RAW264.7 cells.

2.Materials and Methods

2.1. Plant Materials. D. gangeticum was collected in Me Linh, Vinh Phuc Province, in June 2017, and identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources (IEBR). Voucher specimens (No TL-DG200617) have been deposited at the Pharmaceutical Chemistry Laboratory, Institute of Natural Product Chemistry, VAST.

2.2. General Experimental Procedures. NMR experiments were performed on a Bruker AM500 FT-NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The optical rotations were read on a JASCO P-2000 digital polarimeter. High-resolution mass spectra (ESI, positive mode) were obtained with a Thermo LTQ Orbitrap XL mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates (Merck, Germany), and spots were detected under UV illumination at 254 nm and spraying with H2SO4 10% reagent followed by heating. Column chromatography (CC) was carried out using D101 resin (0.3–1.5 mm, Extrepure, China), silica gel
60 (70-230 mesh, Merck, Germany), or YMC RP-C18 resin (150 μm, YMC, Japan). The sugar was analysed by an Agilent 1260 Series Single Quadrupole LC/MS Systems.

2.3. Extractions and Isolation. The air-dried powdered aerial parts of Desmodium gangeticum were extracted with methanol (6 L × 3 times) at room temperature in 24 hours. The combined extracts were concentrated to obtain a methanol (MeOH) crude residue (180 g), which was suspended in water and then successively partitioned with ethyl acetate. Evaporation of solvents under reduced pressure gave ethyl acetate residues (87.5 g). The water layer was adsorbed on a Diaion HP-20 column and then eluted with water, methanol 50%, and 100% to collect W, M50, and M100 fractions, respectively. Fraction M100 was subjected to a silica gel chromatography column with gradient mixtures of CH₂Cl₂–MeOH (1/0-0/1, v/v) to yield seven fractions, B1-B7. Fraction B2 was separated on a Sephadex LH-20 column eluted with methanol 50% to obtain two subfraction B2.1 and B2.2. The fraction B2.1 was purified on a YMC-C18 chromatography column eluted with methanol 67% to yield compound 1 (5.2 mg). The fraction B2.2 was isolated on a silica gel chromatography column with CH₂Cl₂–MeOH (6/1, v/v) elution to obtain compound 2 (10.3 mg). Compound 3 (6.1 mg) was separated from the fraction B4 using a YMC-C18 chromatography column eluted with methanol 50%. Fraction B6 was isolated on a Sephadex LH-20 column eluted with methanol 60%, followed by a YMC RP-C18 column eluted with methanol 50% to collect compound 4 (6.6 mg).

Gangeticoside (1): white solid; (α)D²⁴ = −8.5° (c 0.1, CH₃OH); ¹H NMR (500 MHz, CD₃OD): δ 0.94 (3H, br s, H-8'), 1.17 (3H, br s, H-7'), 2.02 (3H, br s, H-6), 1.83 (2H, m, H-2'a, 4'a), 1.98 (1H, dd, J = 14.0, 6.5 Hz, H-2''b), 2.20 (1H, dd, J = 13.5, 7.0 Hz, H-4'b), 3.16 (1H, dd, J = 9.0, 8.0 Hz, H-2'''), 3.35 (3H, m, H-3'', 4'', 5''), 3.68 (1H, dd, J = 11.5, 5.5 Hz, H-6''a), 3.77 (1H, d, J = 7.5 Hz, H-9'a), 3.82 (1H, d, J = 7.5 Hz, H-9''b), 3.88 (1H, d, J = 11.5 Hz, H-6''b), 4.27 (1H, m, H-3'), 4.38 (1H, d, J = 8.0 Hz, H-1'''), 5.85 (1H, s, H-2), 6.35 (1H, d, J = 15.5 Hz, H-5), 7.89 (1H, d, J = 15.5 Hz, H-4). ¹³C NMR (125 MHz, CD₃OD): δ 167.9 (C-1), 125.7 (C-3), 144.5 (C-3'), 132.7 (C-4), 131.7 (C-5), 20.7 (C-6), 49.8 (C-1'), 42.8 (C-2', 4'), 73.9 (C-3'), 87.6 (C-5'), 83.2 (C-6'), 19.7 (C-7'), 16.3 (C-8'), 77.9 (C-9'), 103.1 (C-1'''), 75.1 (C-2''''), 78.0 (C-3''), 71.6 (C-4'''), 77.9 (C-5'''), 62.7 (C-6'''). HR-ESI-MS: m/z 467.1896 [M + Na]+ (calcd. 467.1893 for C₂₁H₃₂NaO₁₀).

2.4. Acid Hydrolysis and Sugar Identification. Compound 1 (1 mg) was heated in 1N HCl (500 μL) at 80°C for 2 h, then the solution was extracted with ethyl acetate (1 mL × 3). The aqueous layer was neutralized with NH₄OH 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 isothiocyanate was added, and the solution was heated for another hour. The reaction solution was then analyzed 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 (tR 9.05 min).
2.5. Assay for Inhibition of NO Production. The anti-inflammatory effects of the isolated compounds were evaluated via the inhibition of NO production in LPS-stimulated RAW264.7 cells [10]. Briefly, RAW264.7 cells were seeded in 96-well plates at 2 × 105 cells/well and incubated for 24 h. The plate were pretreated with various concentrations of test samples for 30 min and then incubated for another 24 h with or without 1 μg/mL LPS. As a parameter of NO synthesis, nitrite concentration in the culture supernatant was measured by the Griess method. 100 μL of the culture supernatant was transferred to other 96-well plate and 100 μL of Griess reagent were added. The absorbance of the reaction solution was read at 570 nm with a microplate reader. 

*Table 1: Inhibitory activity of NO production of compounds 1-4.*

| Compounds                  | IC50 in μM |
|----------------------------|-----------|
| Gangeticoside (1)          | 22.3 ± 3.06 |
| Leonuriside A (2)          | 15.6 ± 2.45 |
| Methyl benzoate 2-O-β-D-glucopyranoside (3) | 7.3 ± 1.27 |
| Tortoside A (4)            | >30       |
| Cardamomín (positive control) | 2.72 ± 0.13 |

H-8 to C-6, C-7, C-9, and C-10. The position of glucose was elucidated by the correlation between H-1′ and C-3. The positions of three methyls were assigned by HMBC correlations between H-11 to C-1, H-13 to C-5, and H-14 to C-9 (Figure 2). The compound 1 showed similar structural properties to *dihydropaseic acid*, the other megastigmane in the previous study [11]. However, the presence of the quaternary carbon C-1 and the HMBC correlation between H-12 to the carboxyl group revealed the structure of a lactone, rather than a carboxylic acid. As can be seen from Figure 2, the NOE correlation between H-7/H-12 and no correlation between H-7/H-8 indicated E geometry of the double bond at C-7, whereas the correlation between H-10/H-14 suggested Z-geometry of C-9 double bond (Figure S6). Based on the above analysis, the structure of compound 1 was elucidated as a new megastigmane glycoside, which was named gangeticoside.

Three known compounds were elucidated by comparing their NMR data to the previous reports, including leonuriside A (2) [12], methyl benzoate 2-O-β-D-glucopyranoside (3) [13], and tortoside A (4) [14]. Compounds 1, 2, and 3 exhibited significant anti-inflammatory activity with NO inhibition IC50 at 22.3, 15.6, 7.3 μM, respectively, while compound 4 was inactive (Table 1). The MTT assay showed that those compounds had no significant toxicity to RAW264.7 cells up to 50 μM (data not shown), indicating that the inhibitory effect on NO production was not due to cytotoxicity.

It is reported that the extracts of *Desmodium gangeticum* exhibited significant anti-inflammatory effect in vitro as well as in animal models [15, 16]. Among phytochemical constituents isolated from *D. gangeticum*,...
(17Z,20Z)-hexacosa-17, 20-dien-9-one and geniandrogenone dose-dependently inhibited proinflammatory cytokines TNF-α and IL-6 in LPS-stimulated RAW264.7 cells [17]. The pterocarpenoid gangetin exhibited potent anti-inflammatory properties against the carrageenin-induced paw edema in rats [18]. Consistently, the present study identified other components in *Desmodium gangeticum* responsible for the antiinflammation effect of this plant.

4. Conclusions

In conclusion, a new megastigmane glycoside, gangeticoside (1), and three known compounds leonuriside A (2), methyl benzoate 2-O-β-D-glucopyranoside (3), and tortoside A (4) were isolated from the aerial part of *Desmodium gangeticum*. Compounds 1, 2, and 3 exhibited strong effect on NO production in LPS-stimulated RAW264.7 cells with the IC₅₀ values of 22.3, 15.6, and 7.3 μM, respectively.

Data Availability

The data used in the study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

HR-ESI-MS and NMR spectra of compound 1. Figure S1: HR-ESI-MS spectrum of 1. Figure S2: ¹H NMR spectrum of 1. Figure S3: ¹³C NMR spectrum of 1. Figure S4: HSQC spectrum of 1. Figure S5: HMBC spectrum of 1. Figure S6: NOESY spectrum of 1. (Supplementary Materials)

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