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Research Article

A New Megastigmane Glucoside and Other Constituents from *Desmodium gangeticum*

Hau Viet Dang,[1] Giang Hoang Do,[1] Phuong Thi Ngo,[2] Tien Dat Nguyen ,[1] and Ha Minh Le [2]

[1]*Center for Research and Technology Transfer, Vietnam Academy of Science and Technology (VAST), 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*
[2]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam*

Correspondence should be addressed to Tien Dat Nguyen; ngtiend@imbc.vast.vn and Ha Minh Le; leminhha.inpc@gmail.com

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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*. Their structures were determined by 1D and 2D NMR spectra. The isolated compounds were evaluated for their inhibitory effect on NO production in LPS-stimulated RAW264.7 cells. Among them, compounds **1**, **2,** and **3** exhibited strong effect with the IC_{50} values of 22.3, 15.6, 7.3 μM, respectively.

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 H_2SO_4 10% reagent followed by heating. Column chromatography (CC) was carried out using D101 resin (0.3–1.5 mm, Extrepure, China), silica gel

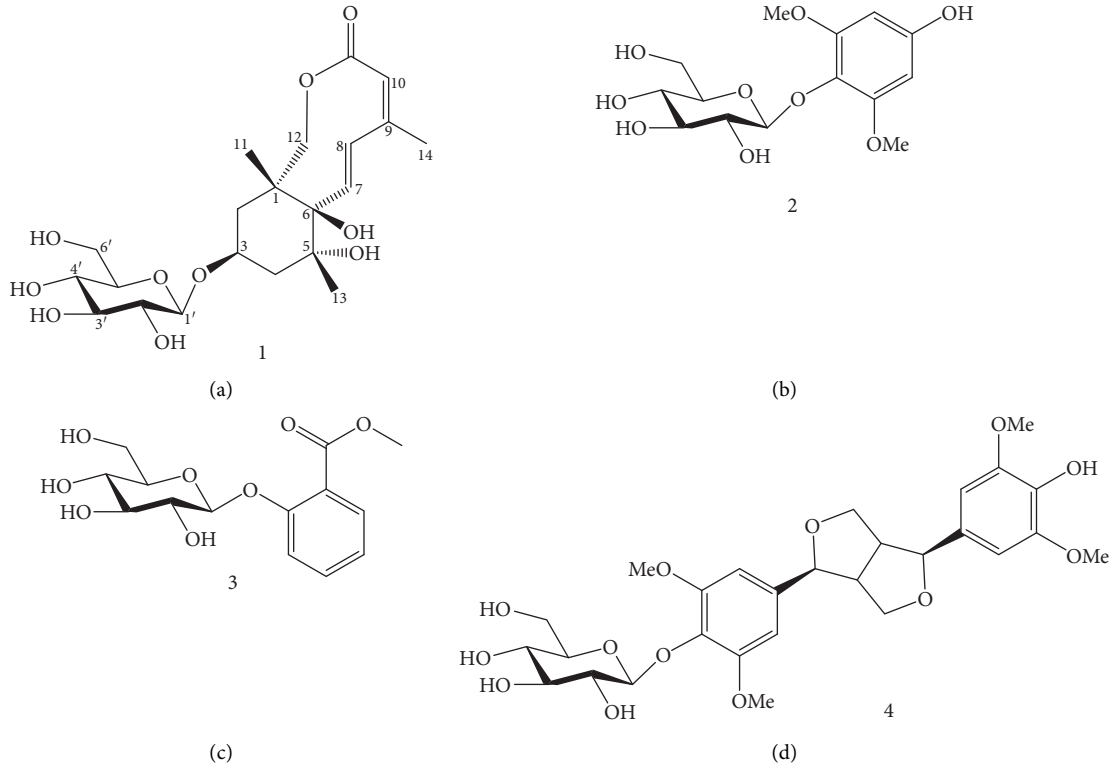

Figure 1: The structure of compounds isolated from *Desmodium gangeticum*.

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 CH_2Cl_2–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 CH_2Cl_2–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; $(\alpha)_D^{24} = -8.5°$ (c 0.1, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): 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). ^{13}C NMR (125 MHz, CD_3OD): 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 $C_{21}H_{32}NaO_{10}$).

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 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 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 (t_R 9.05 min).

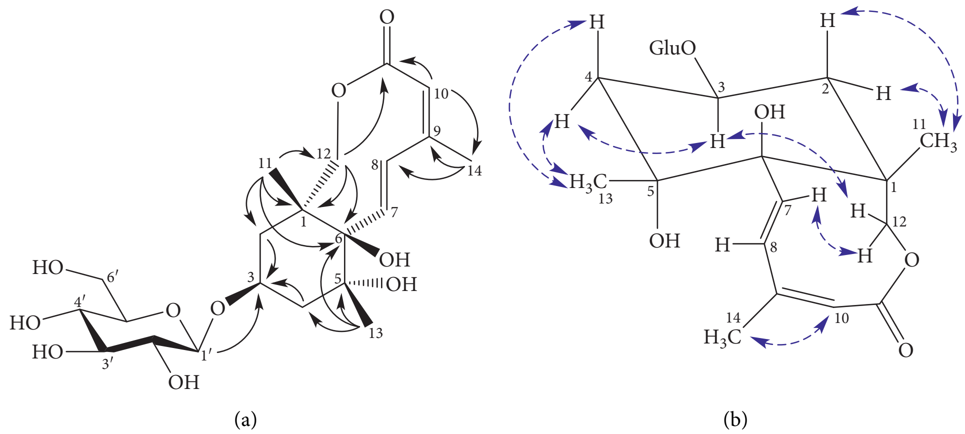

Figure 2: Key HMBC, and NOESY correlations of the new compound **1**.

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. The remaining cell solutions in cultured 96-well plates were used to evaluate cell viability by 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cardamonin was used as a positive control.

3. Results and Discussion

Compound **1** was obtained as a colorless solid. Its HRESIMS (Figure S1) showed the peak at *m/z* 467.1896 $[M + Na]^+$ corresponding to the molecular formula $C_{21}H_{32}O_{10}$. The ^1H NMR spectrum of **1** (Figure S2) showed signals for a *trans*-double-bond at δ_H 6.35 (1H, d, $J = 15.5$ Hz, H-5), 7.89 (1H, d, $J = 15.5$ Hz, H-4), an olefinic proton at δ_H 5.85 (1H, s, H-2), and three tertiary methyl groups at δ_H 0.94 (3H, br s, H-8′), 1.17 (3H, br s, H-7′), 2.02 (3H, br s, H-6). A β-anomeric proton of a sugar moiety was appeared at δ_H 4.38 (1H, d, $J = 8.0$ Hz, H-1″). The ^{13}C-NMR and HSQC spectra (Figures S3 and S4) of **1** indicated the presence of 21 signals including three methyl, four methylene, nine methine, and five quaternary carbon groups. A glucopyranose was recognized by six characteristic signals at δ_C 103.1 (C-1″), 75.1 (C-2″), 78.0 (C-3″), 71.6 (C-4″), 77.9 (C-5″), and 62.7 (C-6″). Acid hydrolysis followed by HPLC analysis allowed confirming the *D*-glucose. The remaining 15 carbon signals suggested the presence of a megastigmane structure. The positions of the two double bonds at C-7 and C-9 were assigned by HMBC (Figure S5), correlations of H-10 to C-8, C-9, and the carboxyl group, H-7 to C-6, C-8, and C-9, and 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 *dihydrophaseic 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.

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

Compounds	IC_{50} 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
Cardamonin (positive control)	2.72 ± 0.13

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 IC_{50} 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 gangenoid 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 carrageenininduced paw edema in rats [18]. Consistently, the present study identified other components in *D. 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 IC_{50} 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.

Acknowledgments

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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: ^{1}H NMR spectrum of **1**. Figure S3: ^{13}C NMR spectrum of **1**. Figure S4: HSQC spectrum of **1**. Figure S5: HMBC spectrum of **1**. Figure S6: NOESY spectrum of **1**. (*Supplementary Materials*)

References

[1] V. V. Chi, *Dictionary of Vietnamese Medicinal Plants*, Medical Publisher, Hanoi, Vietnam, 2012.

[2] S. Suman, P. Neha, and P. Bhupesh, "A review on Shalparni (*Desmodium gangeticum* DC.) and *Desmodium species* (*Desmodium triflorum* DC. & *Desmodium laxiflorum* DC.) Ethnomedicinal perspectives," *Journal of Medicinal Plants Studies*, vol. 3, no. 4, pp. 38–43, 2015.

[3] L. M. Ha, N. T. Luyen, N. T. Phuong et al., "Isoflavonoids from *Desmodium heterophyllum* aerial parts," *Natural Product Communications*, vol. 13, no. 6, pp. 699-700, 2018.

[4] R. Govindarajan, H. Asare-Anane, S. Persaud, P. Jones, and P. Houghton, "Effect of *Desmodium gangeticum* extract on blood glucose in rats and on insulin secretion in vitro," *Planta Medica*, vol. 73, no. 5, pp. 427–432, 2007.

[5] X. Ma, C. Zheng, C. Hu, K. Rahman, and L. Qin, "The genus Desmodium (Fabaceae)-traditional uses in Chinese medicine, phytochemistry and pharmacology," *Journal of Ethnopharmacology*, vol. 138, no. 2, pp. 314–332, 2011.

[6] S. Rastogi, M. M. Pandey, and A. K. S. Rawat, "An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC," *Journal of Ethnopharmacology*, vol. 136, no. 2, pp. 283–296, 2011.

[7] R. Korhonen, A. Lahti, H. Kankaanranta, and E. Moilanen, "Nitric oxide production and signaling in inflammation," *Current Drug Target -Inflammation & Allergy*, vol. 4, no. 4, pp. 471–479, 2005.

[8] Y. C. Luiking, M. P. Engelen, and N. E. Deutz, "Regulation of nitric oxide production in health and disease," *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 13, no. 1, pp. 97–104, 2010.

[9] W.-J. Jiang, K. i. Ishiuchi, M. Furukawa, T. Takamiya, S. Kitanaka, and H. Iijima, "Stereospecific inhibition of nitric oxide production in macrophage cells by flavanonols: synthesis and the structure-activity relationship," *Bioorganic & Medicinal Chemistry*, vol. 23, no. 21, pp. 6922–6929, 2015.

[10] N. M. Chau, H. M. Tuan, T. M. Hung, N. T. Dat, and N. H. Dang, "Anti-inflammatory constituents from *Eurycoma longifolia* roots," *Letters in Organic Chemistry*, vol. 17, no. 6, pp. 455–458, 2020.

[11] N. T. T. Ngan, T. H. Quang, S. B. Song, and Y. H. Kim, "Anti-inflammatory and PPAR transactivational effects of components from *Ginkgo biloba* seeds," *Bulletin of the Korean Chemical Society*, vol. 35, no. 9, pp. 2843–2846, 2014.

[12] H. Otsuka, M. Takeuchi, S. Inoshiri, T. Sato, and K. Yamasaki, "Phenolic compounds from *Coix lachryma-jobi* var. Mayuen," *Phytochemistry*, vol. 28, no. 3, pp. 883–886, 1989.

[13] M. Ushiyama and T. Furuya, "Glycosylation of phenolic compounds by root culture of *Panax ginseng*," *Phytochemistry*, vol. 28, no. 11, pp. 3009–3013, 1989.

[14] S.-Y. Lyu and W.-B. Park, "Modulation of IL-12 and IFN-γ secretions by eleutheroside E, tortoside A, and syringaresinol from acanthopanax koreanum nakai," *Biomolecules and Therapeutics*, vol. 18, no. 2, pp. 211–218, 2010.

[15] R. Govindarajan, M. Vijayakumar, C. V. Rao et al., "Anti-inflammatory and antioxidant activities ofDesmodium gangeticum fractions in carrageenan-induced inflamed rats," *Phytotherapy Research*, vol. 21, no. 10, pp. 975–979, 2007.

[16] A. Rathi, C. V. Rao, B. Ravishankar, S. De, and S. Mehrotra, "Anti-inflammatory and anti-nociceptive activity of the water decoction *Desmodium gangeticum*," *Journal of Ethnopharmacology*, vol. 95, no. 2-3, pp. 259–263, 2004.

[17] A. K. Yadav, J. Agrawal, A. Pal, and M. M. Gupta, "Novel anti-inflammatory phytoconstituents from *Desmodium gangeticum*," *Natural Product Research*, vol. 27, no. 18, pp. 1639–1645, 2013.

[18] D. Ghosh and A. Anandakumar, "Anti-inflammation and analgesic activity of Gangetin-a pterocarpenoid from *Desmodium gangeticum* DC," *Indian Journal of Pharmacology*, vol. 15, pp. 391–402, 1983.