SOME GLYCOSIDES ISOLATED FROM DESMODIUM GANGETICUM (L.) DC. OF VIET NAM

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ABSTRACT

From the methanol extract of the leaves of Desmodium gangeticum collected in Me Linh, Ha Noi, we isolated 5 compounds. In which, there are four glycosides including (6S,9R)-roseoside (1), kaempferol-3-O-rutinoside (nicotiflorin) (2), quercetin-3-O-rutinoside (rutin) (3), β-sitosterol-3-O-β-D-glucopyranoside (4), and the other is protocatechuic acid (5). Kaempferol-3-O-rutinoside (2) was isolated from Desmodium gangeticum for the first time while (6S,9R)-roseoside (1) was isolated from the genus Desmodium for the first time. Their structures were determined by 1D and 2D NMR spectra.

Keywords: Desmodium gangeticum, (6S,9R)-roseoside, kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside, β-sitosterol-3-O-β-D-glucopyranoside, protocatechuic acid.

1. INTRODUCTION

Desmodium gangeticum (L.) DC. (Fabaceae) is a slightly woody perennial herb and widely distributed in South East Asia, India and Africa. In Vietnam and other countries, D. gangeticum has been used for various purposes such as wound ulcers, snake bites, diuretic, edema, asthma, stomatitis, arthritis, eczema, hair loss, neurological disorders, premature ejaculation and to make tonic. D. gangeticum is known to be rich in flavonoids, alkaloids, sterols and glycolipids with antioxidant, antibacterial, antidiabetic, antiulcer activities. To date, over 30 compounds were found from this plant in the world [1, 2]. However, in Vietnam, very few studies on chemical constituents of D. gangeticum have been reported. Only two publics of Nguyen Tiet Dat et al. isolated 4 phenolic glucosides from the leaves of D. gangeticum along with few other researches on qualitative identification of coumarin and flavonoid. [3,4,5,6] To
clearly chemical constituents of *D. gangeticum* Vietnam, in this paper we report the isolation and structural identification of four other glycosides such as (6S,9R)-roseoside (1), kaempferol-3-O-rutinoside (2), quercetin-3-O-rutinoside (3), β-sitosterol-3-O-β-D-glucopyranoside (4), and another compound protocatechuic acid (5) from methanol extract of the leaves of *D. gangeticum*.

### 2. EXPERIMENTAL

#### 2.1. Plant materials

The leaves of *D. gangeticum* were collected at Melinh, Hanoi, Vietnam in June 2017. The scientific name was identified by Dr Bui Van Thanh, Institute of Ecology and Biological Resources, VAST. The voucher specimen no. TL-DG20062017 is preserved at Institute of Natural Product Chemistry, Vietnam Academy of Science and Technology.

#### 2.2. General experimental procedures

The $^1$H-NMR (500MHz) and $^{13}$C-NMR (125MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and tetramethylsilane was used as an internal standard. Column chromatography (CC) was performed using a silica gel (0.040 – 0.063 mm) and YMC RP-18 resins (30 – 50 μm). Thin layer chromatography (TLC) used pre-coated silica gel 60 F$_{254}$ and RP-18 F$_{545}$S plates. Compounds were visualized by UV light at 254 and 365 nm, and by spraying with the solution of 10 % H$_2$SO$_4$ in ethanol and heating for 1-3 minutes.

#### 2.3. Extraction and isolation

The dried leaves of *D. gangeticum* (1.5 kg) were powdered and extracted in turn with n-hexane, ethyl acetate, methanol at 50°C (3 times x 2 hours per time) on heated ultrasonic machine. Filtered extracts were combined and concentrated under low pressure to give n-hexane (24 g), ethyl acetate (52 g) and methanol (85 g) extracts. The methanol extract (50 g) was separated on a silica gel column eluted with ethyl acetate: methanol (100:1 – 1:100, v:v) to obtain 6 fractions (M1→M6). The fraction M1 was chromatographed on a silica gel column eluted with chloroform: methanol (15:1, v:v) to give 1 (9.0 mg). The M2 was fractioned on a silica gel column using ethyl acetate: methanol (25:1, v:v) to obtain 4 subfractions (M2.1→M2.4). The M2.1 was purified on an YMC RP-18 column eluted with methanol : water (2:1, v:v) to give 5 (12.0 mg). The M2.3 was passed through on a silica gel column using chloroform: methanol (10:1, v:v) to give 2 (10.5 mg). The fraction M3 was chromatographed on a silica gel column eluted with dichlomethane: methanol (15:1, v:v) to give 4 (16.5 mg). The fraction M5 was separated on a silica gel column and eluted with chloroform : methanol : water (8:1:0.05, v:v:v) to obtain 5 fractions (M5.1→M5.5). The M5.3 was purified on an YMC RP-18 column eluted with methanol : water (1:2, v:v) to obtain 3 (15.0 mg).

**6S,9R)-roseoside (1):** Colorless resin. $^1$H-NMR (500 MHz, MeOD), δ (ppm): 1.05 (3H, s, CH$_3$-12); 1.06 (3H, s, CH$_3$-11); 1.31 (3H, d, J = 6.0 Hz, CH$_3$-10); 1.94 (3H, brs, CH$_3$-13); 2.18 (1H, d, J = 17.0 Hz, H$_5$-2); 2.55 (1H, d, J = 17.0 Hz, H$_5$-2); 3.20 (1H, m, H-2'); 3.24 (1H, m, H-5'); 3.29 (1H, m, H-4'); 3.35 (1H, m, H-3'); 3.65 (1H, m, H$_5$-6'); 3.87 (1H, d, J = 2.0, 15.0 Hz, H$_5$-6'); 4.36 (1H, d, J = 7.5 Hz, H-1'); 4.44 (1H, m, H-9); 5.87 (1H, m, H-8); 5.88 (1H, brs, H-4); 5.89 (1H, m, H-7). $^{13}$C-NMR (125 MHz, MeOD), δ (ppm): 201.36 (C-3); 167.34 (C-5); 135.26 (C-7); 131.55 (C-8); 127.18 (C-4); 102.72 (C-1'); 80.01 (C-6); 78.08 (C-3'); 78.00 (C-5'); 77.33 (C-9); 75.24 (C-2'); 71.63 (C-4'); 62.79 (C-6'); 50.69 (C-2); 42.43 (C-1); 24.67 (C-12); 23.42 (C-11); 21.19 (C-10); 19.57 (C-13).

**kaempferol-3-O- rutinoside (2):** Yellow powder. $^1$H-NMR (500 MHz, MeOD), δ (ppm): 8.09
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(2H, d, J = 8.0 Hz, H-2’,6’); 6.92 (2H, d, J = 8.0 Hz, H-3’,5’); 6.43 (1H, d, J = 2.5 Hz, H-8); 6.24 (1H, d, J = 2.0 Hz, H-6); 5.15 (1H, d, J = 7.5 Hz, H-1’); 4.54 (1H, brs, H-1’); 3.40 – 3.90 (10H, rhamnose and glucose); 1.14 (3H, d, J = 6.5 Hz, H-6”). 13C-NMR (125 MHz, MeOD), δ (ppm): 179.40 (C-4); 166.71 (C-7); 162.98 (C-5); 161.60 (C-4’); 159.56 (C-9); 158.68 (C-2); 135.51 (C-3); 132.41 (C-2’,6’); 122.08 (C-1’); 116.20 (C-3’,5’); 104.72 (C-10); 102.48 (C-1”,1’’); 100.30 (C-6); 95.12 (C-8); 77.80 (C-3’’); 77.20 (C-5’’); 75.71 (C-2’’); 73.91 (C-4’’); 72.30 (C-4’’); 72.10 (C-2’’); 71.53 (C-3’’); 69.78 (C-5’’); 68.70 (C-6’’); 17.90 (C-6’’).

quercetin-3-O-rutinoside (3): Yellow powder. 1H-NMR (500 MHz, MeOD), δ (ppm): 7.69 (1H, d, J = 2.5 Hz, H-2’); 7.65 (1H, dd, J = 8.5, 2.0 Hz, H-6’); 6.90 (1H, d, J = 8.5 Hz, H-5’); 6.42 (1H, d, J = 2.0 Hz, H-8); 6.23 (1H, d, J = 2.0 Hz, H-6); 5.13 (1H, d, J = 7.5 Hz, H-1’); 4.54 (1H, s, H-1’’); 3.40 – 3.90 (10H, rhamnose and glucose); 1.14 (3H, d, J = 6.0 Hz, H-6”). 13C-NMR (125 MHz, MeOD), δ (ppm): 179.42 (C-4); 166.01 (C-7); 162.97 (C-5); 159.34 (C-9); 158.51 (C-2’); 149.80 (C-4’); 145.83 (C-3’); 135.63 (C-3’); 123.56 (C-6’); 123.14 (C-1’); 117.71 (C-2’); 116.07 (C-5’); 105.64 (C-10); 104.71 (C-1’’); 102.42 (C-2’’); 99.96 (C-6’); 94.88 (C-8); 78.19 (C-3’’); 77.22 (C-5’’); 75.73 (C-2’’); 73.95 (C-4’’); 72.26 (C-3’’); 72.10 (C-3’’); 71.41 (C-4’’); 69.71 (C-5’’); 68.56 (C-6’’); 17.87 (C-6’’).

daucosterol (4): White crystals. 1H-NMR (500MHz, DMSO-d6), δ (ppm): 0.78 (3H, s, CH3-18); 0.81 (3H, d, J = 7.0 Hz, CH3-27); 0.83 (3H, d, J = 7.0 Hz, CH3-26); 0.84 (3H, t, J = 7.0 Hz, CH3-29); 0.91 (3H, d, J = 6.5 Hz, CH3-21); 0.97 (3H, s, CH3-19); 3.00 (1H, m, H-2’); 3.04 (1H, m, H-4’); 3.08 (1H, m, H-5’); 3.14 (1H, m, H-3’); 3.43 (1H, m, H-6’a); 3.47 (1H, m, H-3’); 3.67 (1H, m, H-6’b); 4.22 (1H, d, J = 7.0 Hz, H-1’); 5.33 (1H, br d, J = 5.0 Hz, H-6). 13C-NMR (125MHz, DMSO-d6), δ (ppm) 37.2 (C-1’); 29.5 (C-2’); 70.1 (C-3’); 38.7 (C-4’); 140.2 (C-5’); 122.1 (C-6’); 31.8 (C-7’); 31.8 (C-8’); 50.1 (C-9’); 36.6 (C-10); 21.0 (C-11); 39.7 (C-12’); 42.2 (C-13’); 56.7 (C-14’); 24.2 (C-15’); 28.1 (C-16’); 55.9 (C-17’); 11.8 (C-18’); 19.6 (C-19’); 36.0 (C-20’); 19.1 (C-21’); 33.9 (C-22’); 26.0 (C-23’); 45.8 (C-24’); 29.1 (C-25’); 18.9 (C-26’); 18.6 (C-27’); 23.0 (C-28’); 11.7 (C-29’); 101.0 (C-1’’); 75.6 (C-2’’); 76.3 (C-3’’); 73.4 (C-4’’); 79.1 (C-5’’); 61.8 (C-6’’).

protocatechuic acid (5): White crystals. 1H-NMR (500 MHz, MeOD), δ (ppm): 6.79 (1H, d, J = 7.5 Hz, H-5’); 7.43 (1H, d, J = 8.0, H-6’); 7.47 (1H, s, H-2’). 13C-NMR (125 MHz, MeOD), δ (ppm): 168.01 (C-7’); 150.45 (C-4’); 145.73 (C-3’); 123.62 (C-6’); 123.60 (C-1’); 117.87 (C-5’); 115.54 (C-2’).

3. RESULTS AND DISCUSSION

Compound 1 was obtained as colorless resin. The 13C-NMR spectra of 1 showed 19 carbon signals. Six of them were assigned as glucose moiety with the anomeric carbon at δc 102.72. Four carbon signals at δc 167.34 (C-5’); 135.26 (C-7’); 131.55 (C-8’); 127.18 (C-4’) and three proton signals at 5.87-5.9 ppm suggested the present of two double bonds in its structure. In proportion to 13C-NMR, the 1H-NMR spectra of 1 displayed signals of four methyl groups and 6 protons of glucose moiety at δH 3.20-3.87 with anomeric proton at δH 4.36 (J = 7.5 Hz). The coupling constant at 7.5 Hz established glucose attached to aglycone by a β-D-glycoside linkage. From the above evidences and comparison with spectral data of (6S,9R)-roseoside in literature [6], compound 1 was identified as (6S,9R)-roseoside.

Compound 2 was obtained as yellow powder. The 1H-NMR spectra of 2 displayed 6 aromatic protons including 2 protons at δH 8.09 (d, J = 8.0 Hz, H-2’,6’), 2 protons at δH 6.92 (d, J = 8.0 Hz, H-3’,5’), one proton at δH 6.43 (d, J = 2.5 Hz, H-8), and the other at δH 6.24 (d, J = 2.0 Hz, H-6) along with sugar signals from δH 3.40 to 3.90 suggested 2 is a flavonoid glycoside. In addition, the 13C-NMR spectra of 2 showed 15 carbon signals belonging the flavonoid part
and 12 carbon signals belonging sugar moiety. The $^1$H- and $^13$C-NMR spectral data of 2 were the same as those of kaempferol-3-O-rutinoside [7]. Thus, compound 2 was identified as kaempferol-3-O-rutinoside (nicotiflorin).

Compound 3 was obtained as yellow powder. The $^1$H- and $^13$C-NMR spectral data of 3 were similar to those of 2 except the signals in B rings. Compound 3 has three dissymmetrical protons at $\delta_H$ 7.69 (1H, d, $J = 2.5$ Hz, H-2'); 7.65 (1H, dd, $J = 8.5$, 2.0 Hz, H-6'); 6.90 (1H, d, $J = 8.5$ Hz, H-5'). These signals of 3 were compatible with those of quercetin. In addition, the signals of sugar moiety of 3 were similar to those of 2 and identified as rutinose. From these spectral data comparison with a literature [8], compound 3 was determined as quercetin-3-O-rutinoside (rutin).

Compound 4 was obtained as white crystals. The $^1$H-NMR, $^13$C-NMR and DEPT data of 4 revealed 6 methyl groups in aglycone moiety CH$_3$-18, CH$_3$-19, CH$_3$-21, CH$_3$-26, CH$_3$-27, CH$_3$-29 at $\delta_H$ 0.78, 0.97, 0.91, 0.83, 0.81, 0.84 and $\delta_C$ 11.8, 19.6, 19.1, 18.9, 18.6, 11.7, a double bond (C-5, C-6) at $\delta_H$ 5.33 and $\delta_C$ 140.2, 122.1. These signals were appropriate to spectral data of a known alcyone $\beta$-sitosterol. The remaining signals were identified as glucose moiety with the anomeric carbon at $\delta_C$ 101.0 and $\delta_H$ 4.22. From the above evidences and comparison with those reported in literature [9], compound 4 was deduced to be $\beta$-sitosterol-3-O-$\beta$-D-glucopyranoside.

Compound 5 was obtained as white crystals. The $^1$H-NMR of 5 showed three aromatic proton signals. Two doublet signals at $\delta_H$ 7.43 and 6.79 with coupling constant 8.0 and 7.5 suggested they are ortho- protons. The other was a singlet signal at $\delta_H$ 7.47. According to $^13$C-NMR of 5, seven carbon signals from $\delta_C$ 115.54 to 168.01 were seen. By comparing these data with those reported [10], compound 5 was identified to be protocatechuic acid.

![Figure 1. The structure of five isolated compounds from the leaves of D. gangeticum.](image_url)

**4. CONCLUSION**

From the methanol extract of of the leaves of D. gangeticum (Fabaceae), four glycosides were isolated and indentifieded structures such as (6S,9R)-roseoside (1), kaempferol-3-O-rutinoside (nicotiflorin) (2), quercetin-3-O-rutinoside (rutin) (3), $\beta$-sitosterol-3-O- $\beta$-D-glucopyranoside (4) along with protocatechuic acid (5). This is the first time kaempferol-3-O-rutinoside (2) was isolated from Desmodium gangeticum and (6S,9R)-roseoside (1) was reported from the genus Desmodium. Both of them are typical flavonoids which have been reported to express many valuable bioactivities especially antioxidant, antidiabetic, anti-inflammatory, neuroprotective, hepatoprotective, vasoprotective properties, anticancer, etc. [11, 12]. Follow-up investigations of chemical constituents and biological properties of D. gangeticum are still continuing to carry out by us.
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