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Chemical constituents of *Spatholobus suberectus*

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[ABSTRACT] AIM: To investigate chemical constituents of *Spatholobus suberectus* Dunn. **METHODS:** Isolation and purification were carried out by column chromatographic methods. Compounds were characterized based on their physical characteristics and spectra data. **RESULTS:** Seventeen compounds were isolated from ethanol extract of *S. suberectus*. The structures were elucidated as prestegane B (**1**), (2*R*, 3*R*)-buteaspermanol (**2**), (+)-medioresinol (**3**), (2*R*, 3*R*)-3,7-dihydroxyflavanone (**4**), benzeneethanol (**5**), 4, 7, 2′-trihydroxy-4′-methoxyisoflavanol (**6**), naringenin (**7**), blumenol A (**8**), protocatechuic acid ethyl ester (**9**), liquiritigenin (**10**), 7, 4'-dihydroxy-8-methoxy-isoflavone (**11**), 3, 5, 7, 3', 5'-pentahydroxyflavanone (**12**), protocatechuic acid (**13**), glycyroside (**14**), 8-methylretusin-7-*O*-*β*-D-glucopyranoside (**15**), 3, 3', 4', 5, 6, 7, 8-heptahydroxyflavan (**16**), and dulcisflavan (**17**). **CONCLUSION:** All compounds are firstly isolated from the title plant and compounds **1**, **3** were isolated from the *Spatholobus* genus for the first time.
[KEY WORDS] *Spatholobus suberectus*; Flavonoids; Chemical constituents

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1 Introduction

The family Leguminosae comprises more than 750 genera (in 40 tribes) and 18 000 species distributed around the world[1]. The phytochemical studies of the family Leguminosae are considerably extensive. Several types of compounds including alkaloids, non-protein amino acids, amines, flavonoids, isoflavonoids, coumarins, phenylpropanoids, anthraquinones, di-, sesqui- and triterpenes, cyanogenic glycosides and lectins have been described in this family[1]. *Spatholobus suberectus* Dunn (Leguminosae), as a traditional Chinese herbal medicine, is mainly distributed in Fujian Province and Guangxi Zhuang Autonomous Region of China[2]. It has been extensively used to promote blood circulation and treat rheumatism, anemia, menoxenia, arthralgia and other disorders clinically[3]. This paper reports the isolation and structure elucidation of 17 compounds, all of which were isolated for the first time from the title plant.

2 Apparatus and Reagents

^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury-plus 400-NMR spectrometer at 400 MHz and 100 MHz. ESI-MS spectra were recorded on the Bruker esquire/HCT Series Ion Trap. Semi-preparative HPLC was also employed. Column chromatography was performed on silica gel (48−75 μm, Qingdao Marine Chemical, Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech., Hong Kong, China). Silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd.) were used for TLC.

3 Plant Material

The vine stems of *S. suberectus* were collected from Guangxi Province, China, in March 2009, and identified by Prof. GUO De-An. A voucher specimen (No. 20090302) is deposited in Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.

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These authors have no any conflict of interest to declare.

4 Extraction and Isolation

The dried stems of *S. suberectus* (4.5 kg) were successively refluxed with 95% and 70% ethanol three times at boiling temperature for 2 h. All the above extracts were combined and concentrated in vacuum to obtain the total extracts (600 g). The extracts (600 g) were subsequently suspended in water and partitioned successively with petroleum ether, chloroform, ethyl acetate, and *n*-butanol respectively. The chloroform fraction (26 g) was fractionated by CC (silica gel, petroleum ether/Me_2CO, 20 : 1 to 0 : 1) to give fractions 1-3. Fr. 1 (1.8 g) was subjected to CC (silica gel, petroleum ether/Me_2CO, 10 : 1 to 0 : 1) and purified by CC (Sephadex LH-20, petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1) to provide compounds **1** (13.0 mg) and **2** (10.6 mg). Fr. 2 (3.4 g) was separated by CC (silica gel, petroleum ether/Me_2CO, 20 : 1 to 0 : 1), and CC (Sephadex LH-20, petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1) giving compounds **3** (5.5 mg), **4** (4.4 mg) and **5** (4 mg). Fr. 3 (5 g) was subjected to CC (silica gel, petroleum ether/Me_2CO, 10 : 1 to 0 : 1), then purified by CC (Sephadex LH-20, petroleum ether/$CHCl_3$ /MeOH, 2 : 1 : 1) and semi-preparative HPLC (Agilent Eclipse XDB-C_{18}, 5 mm, 9.4 mm × 250 mm, 1.5 mL·min^{-1}, UV detection at 250 nm), to afford compounds **6** (35.5 mg), **7** (5.5 mg) and **8** (34.6 mg).

The ethyl acetate fraction (55 g) and *n*-BuOH-soluble fraction (250 g) were isolated by similar procedures as described above obtaining compounds **9-17** (**9**: 12.8 mg, **10**: 3.0 mg, **11**: 8.0 mg, **12**: 7.0 mg, **13**: 73.8 mg, **14**: 6.1 mg, **15**: 3.2 mg, **16**: 10.0 mg, and **17**: 12.8 mg) respectively.

5 Identification

Compound 1 White crystal ($CHCl_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.7 (C-9'), 148.9 (C-3'), 147.7 (C-3), 146.6 (C-4'), 144.4 (C-4), 130.3 (C-1), 129.4 (C-1'), 122.0 (C-6'), 120.5 (C-6), 114.0 (C-2'), 111.6 (C-2), 111.4 (C-5'), 111.1 (C-5), 71.2 (C-9), 55.8 (OCH_3-4'), 55.7 (OCH_3-4), 46.5 (C-8'), 40.8 (C-8), 38.1 (C-7), 34.6 (C-7'). Compound **1** was characterized as prestegane B by comparison of ^{13}C NMR data with the literature[4].

Compound 2 Greenish crystal ($CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.55 (2H, m, H-2', 6'), 7.48-7.41 (3H, m, H-3', 4', 5'), 7.19 (1H, s, H-5), 6.43 (1H, s, H-8), 5.68 (1H, br d, *J* = 3.7 Hz, OH-3), 5.14 (1H, d, *J* = 11.6 Hz, H-2), 4.53 (1H, dd, *J* = 11.6, 3.7 Hz, H-3), 3.80 (3H, s, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 192.5 (C-4), 157.5 (C-9), 155.3 (C-7), 144.3 (C-1'), 137.9 (C-6), 128.8 (C-2'), 128.5 (C-3', C-5', C-6'), 128.3 (C-4'), 111.0 (C-10), 107.6 (C-5), 103.6 (C-8), 84.0 (C-3), 72.9 (C-2), 56.3 (OCH_3). Compound **2** was characterized as (2*R*, 3*R*)-buteaspermanol by comparison of ^{1}H NMR and ^{13}C NMR data with the literature[5].

Compound 3 White powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.58 (4H, s, H-2', H-2", H-6', H-6"), 5.54 (2H, s, 4'-OH, 4"-OH), 4.73 (2H, d, *J* = 3.8 Hz, H-2, H-6), 4.28 (2H, dd, *J* = 8.8, 6.6 Hz, H-4a, H-8a), 3.92 (2H, d, *J* = 3.1 Hz, H-4b, H-8b), 3.89 (9H, s, -OCH_3 × 3), 3.10 (2H, m, H-1, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.1 (C-3', C-5'), 146.7 (C-3"), 145.3 (C-4"), 134.8 (C-4'), 132.2 (C-1"), 131.4 (C-1'), 118.5 (C-6"), 115.1 (C-5"), 110.8 (C-2"), 103.6 (C-2', C-6'), 85.2 (C-2), 85.1 (C-6), 71.0 (C-4), 70.8 (C-8), 56.3 (-OCH_3×2), 56.0 (-OCH_3), 54.4 (C-1), 54.1 (C-5). Compound **3** was characterized as (+)-medioresinol by comparison of ^{1}H NMR and ^{13}C NMR data with the literature[6].

Compound 4 White solid. ESI-MS *m/z* 255 [M − H]$^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.85 (1H, d, *J* = 8.4 Hz, H-5), 7.49 (2H, m, *J* = 7.6 Hz, H-2', 6'), 7.58 (2H, m, *J* = 8.4 Hz, H-3', 4', 5'), 6.60 (1H, d, *J* = 8.4 Hz, H-8), 6.44 (1H, s, H-6), 5.11 (1H, d, *J* = 12 Hz, H-2), 4.58 (1H, d, *J* = 12 Hz. H-3). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 192.5 (C-4), 163.7 (C-9), 163.7 (C-7), 136.5 (C-1'), 129.6 (C-5), 129.3 (C-4'), 128.7 (C-3', C-5'), 127.5 (C-2', C-6'), 112.3 (C-10), 111.2 (C-6), 103.5 (C-8), 83.9 (C-2), 73.2 (C-3). ^{1}H and ^{13}C NMR spectra suggested that **4** was a dihydroflavanol type of compound as H-2 and H-3 was observed at δ 5.11 (1H, d, *J* = 12 Hz; δ_C 83.9) and δ 4.58 (1H, dd, *J* = 12 Hz; δ_C 73.2), respectively. The substituent group of 7-OH was determined by comparison of ^{1}H NMR and ^{13}C NMR data with the literature [7]. The absolute configurations of C-2*R* and C-3*R* were elucidated by comparing the coupling constants (*J* = 12 Hz) between the protons of H-2 and H-3, and the CD spectrum with the literature data[8] (Fig. 1). Thus, **4** was was characterized as (2*R*,3*R*)-3,7-dihydroxyflavanone.

Compound 5 Colorless needle ($CHCl_3$), mp 180−181 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.89 (1H, d , *J* = 8.0 Hz, H-6), 6.7 (1H, br s, H-3), 6.6 (1H, br d, *J* = 8.0 Hz, H-5), 3.73 (3H, s, OCH_3), 3.56 (2H, m, H-2'), 2.79 (2H, m, H-1'). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 146.5 (C-2), 144. 2 (C-1), 130.2 (C-4), 121.5 (C-5), 114.4 (C-6), 111.5 (C-3), 63.7 (C-2'), 55.8 (-OCH_3), 38.7 (C-1'). Compound **5** was characterized as benzeneethanol by elucidation of its 1D (^{1}H and ^{13}C) and 2D (HMBC) NMR spectra (Fig. 2).

Compound 6 Colorless needle (MeOH), mp 195−197 °C, ^{1}H NMR (400 MHz, acetone-d_6) δ: 7.32 (1H, d, *J* = 8.4 Hz, H-5), 7.22 (1H, d, *J* = 8.4 Hz, H-6′), 6.57 (1H, dd, *J* = 2.4, 8.4 Hz, H-6), 6.45 (1H, dd, *J* = 2.4, 8.4 Hz, H-5′), 6.38 (1H, d, *J* = 2.4 Hz, H-3′), 6.37 (1H, d, *J* = 2.4 Hz, H-8), 5.50 (1H, d, *J* = 6.0 Hz, H-4), 4.26 (1H, dd, *J* = 9.6, 16.0 Hz, H-2b), 3.74 (3H, s, OCH_3-4′), 3.59 (1H, m, H-3), 3.58 (1H, m, H-2a). ^{13}C NMR (100 MHz, acetone-d_6) δ: 161.6 (C-4′), 161.4 (C-2′), 159.3 (C-7), 157.3 (C-9), 132.7 (C-5), 125.5 (C-6′), 120.0 (C-1′), 112.3 (C-10), 110.1 (C-6), 106.5 (C-5′), 103.5 (C-8), 96.8 (C-3′), 79.0 (C-4), 66.7 (C-2), 55.3 (OCH_3-4′), 39.9 (C-3). Compound **6** was characterized as 4, 7, 2′-trihydroxy-4′-methoxyisoflavanol by comparison of ^{1}H NMR and ^{13}C NMR data with the literature[9].

Compound 7 Pale yellow powder (MeOH), ESI-MS *m/z* 271 [M − H]$^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.29 (2H, d, *J* = 8.4 Hz, H-2', 6'), 6.78 (2H, d, *J* = 8.4 Hz, H-3', 5'),