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Secondary Metabolites of Smilax Nantoensis

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ABSTRACT

*A new flavonoid, 5,8-dihydroxy-7,3',4'-trimethoxyflavone (**1**), along with four flavonoids, 5-hydroxy-7,2',3',5'-tetramethoxyflavone, 5-hydroxy-7,3', 4'-trimethoxyflavone, 5,3'-dihydroxy-7,4'-dimethoxyflavone and 5,4-dihydroxy-7,3'-dimethoxyflavone, four benzenoids, vanillic acid, p-hydroxybenzoic acid, methylparaben and caffeic acid, and three steroids, β-sitosterol, β-sitostenone and stigmastenone were isolated from the aerial part of Smilax nantoensis (Liliaceae). The structure of the new flavonoid was elucidated from chemical and physical evidence.*

Keywords

Smilax nantoensis, Liliaceae, Aerial part, Flavonoid.

Introduction

The genus *Smilax* (Liliaceae) includes about 300 species and is widely distributed in tropical and temperate regions throughout the World, especially in East Asia and North America [1]. Many of them have been long used as medicinal herbs, especially in China as Traditional Chinese Medicines (TCM). As one of the most popular and important TCM in the genus, *Smilax glabra*, is an evergreen vine widely distributed in southern China [1]. The rhizomes of *S. glabra*, known as Tufuling in China, are used as a TCM for detoxication, clearing heat, relieving dampness and easing joint movement [1]. Modern pharmacological research showed that the *S. glabra* extracts possessed anti-inflammatory, immunomodulatory, protective against hepatocyte damage and anti-tumor effects [2,3]. The 95% ethanol and ethyl acetate extracts of this herb were reported to show antibacterial activity *in vitro* using the K-B paper dispersion and the broth dilution methods [4]. Previous phytochemical investigations have shown that the main constituents in the rhizomes of *S. glabra* include flavonoids, phenylpropanoids and phenolic acids [3]. Astilbin was thought to be the main bioactive constituent and reported to have antibacterial, antitumor, anti-inflammatory, selective immunosuppressive and antioxidant properties [5]. The chemical constituents and the biological activity of *S. nantoensis* have not yet been reported. The MeOH extract of its aerial part was subjected to solvent partitioning and chromatographic separation to afford twelve pure substances. The chemical constituents in the aerial part of *S. nantoensis* were separated with column chromatography. These extracts contain one new flavonoid, 5,8-dihydroxy-7,3′,4′-trimethoxyflavone (**1**), and the following 11 known compounds: four flavonoids, (5-hydroxy-7,2′,3′,5′-tetramethoxyflavone [6], 5-hydroxy-7,3′, 4′-trimethoxyflavone [7], 5,3′-dihydroxy-7,4′-dimethoxyflavone [8] and 5,4′-dihydroxy-7,3′-dimethoxyflavone [9]), four benzenoids (vanillic acid [10], *p*-hydroxybenzoic acid

[11], methylparaben [12], and caffeic acid [13]), and three steroids (β-sitosterol [14], β-sitostenone, and stigmastenone [15]). All of these compounds were isolated for the first time from this plant.

Compound **1**, $C_{18}H_{17}O_7$ $[M + Na]^+$ at *m/z* 367, yellow needle crystals from methanol, mp. 211-213 ℃, was isolated first. In the IR spectrum, bands for hydroxyls (3600 cm^{-1}) and hydrogen-bonded carbonyl (1665 cm^{-1}) were observed. The UV spectrum disclosed an absorption maximum at 268 nm attributable to the benzoyl moiety in A-ring of a flavonoid among other bands [6]. Its ^1H NMR spectrum showed flavone-nucleus protons with two singlets (1H each) at δ 6.59 and 6.55 for C-6 and C-3 respectively plus an ABX pattern at δ 7.19 (C-5′, J = 8.0 Hz), 7.49 (C-2, J = 2.0 Hz) and 7.61 (C-6′, J = 8.0, 2.0 Hz). Resonances between δ 3.82 and 3.93 due to three methoxyl groups were clearly observed when ^1H NMR spectrum was determined in $CDCl_3$. The presence of a hydrogen-bonded hydroxyl proton signal at δ 12.63 indicated that the hydroxyl group must be at C-5. A signal at δ 182.4 assignable to carbonyl carbon was discernible in the ^{13}C NMR spectrum. The sequential correlation of the NOESY spectrum were successfully established as shown in Figure 1. Thus the structure of this compound was determined to be 5,8-dihydroxy-7,3′,4′-trimethoxyflavone (**1**), which was further confirmed by HMBC experiments.

Experimental

General

UV spectra were obtained in MeCN, IR spectra were measured on a Hitachi 260-30 spectrophotometer. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), HETCOR, HMBC, COSY and NOESY spectra were obtained on a Varian (Unity Plus) NMR spectrometer. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Silica gel 60 (Merck, 70~230 mesh, 230~400 mesh) was used for column chromatography. Precoated Silica gel plates (Merck, Kieselgel 60 F-254), 0.20 mm and 0.50 mm, were used for analytical TLC and preparative TLC, respectively, and visualized with 50% H_2SO_4.

Plant material

The specimen of *S. nantoensis* was collected from Nantou County, Shuili Township, Taiwan in May 2020. A voucher specimen was identified by Professor Fu-Yuan Lu (Department of Forestry and Natural Resources College of Agriculture, National Chiayi University) and was deposited in the School of Medical and Health Sciences, Fooyin University, Kaohsiung, Taiwan.

Extraction and isolation

The aerial part (0.6 kg) of *S. nantoensis* were chipped and air-dried and extracted repeatedly with MeOH (2 L × 3) at room temperature. The combined MeOH extracts (19.8 g) were then evaporated and further separated into 5 fractions by column chromatography on silica gel (2.8 kg, 70-230 mesh) with gradients of *n*-hexane/CH_2Cl_2/acetone/MeOH. Part of fraction 1 (2.5 g) was subjected to silica gel chromatography by eluting with *n*-hexane-acetone (80:1), enriched with acetone to furnish five further fractions (1-1−1-5). Fraction 1-2 (0.9 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to obtain 5-hydroxy-7,3′,4′-trimethoxyflavone (5.6 mg). Part of fraction 1-4 (0.4 g) was subjected to silica gel chromatography, by eluting with *n*-hexane-acetone (70:1), enriched gradually with acetone, to furnish four fractions (1-4-1−1-4-4). Fraction 1-4-3 (0.1 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to yielded 5,3′-dihydroxy-7,4′-dimethoxy- flavone (5.8 mg) and 5,4′-dihydroxy-7,3′-dimethoxyflavone (7.2 mg). Part of fraction 2 (3.8 g) was subjected to silica gel chromatography by eluting with *n*-hexane-acetone (50:1), enriched with acetone to furnish three further fractions (2-1−2-3). Fraction 2-1 (0.5 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to yielded mixture of β-sitostenone and stigmastenone (11.4 mg). Fraction 2-2 (1.1 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to obtain β-sitosterol (15.6 mg). Fraction 2-3 (0.8 g) was further purified on a silica gel column using *n*-hexane/acetone mixtures to obtain vanillic acid (3.2 mg) and *p*-hydroxybenzoic acid (2.7 mg). Part of fraction 3 (4.4 g) was subjected to silica gel chromatography by eluting with CH_2Cl_2–MeOH (70:1), enriched with MeOH, to furnish three fractions (3-1–3-3). Fraction 3-2 (1.7 g) was further purified on a silica gel column using CH_2Cl_2/MeOH mixtures to obtain methylparaben (7.2 mg). Fraction 3-3 (1.6 g) was further purified on a silica gel column using CH_2Cl_2/MeOH mixtures to obtain caffeic acid (13.8 mg). Fraction 4 (6.2 g) was subjected to silica gel chromatography, eluting with CH_2Cl_2-MeOH (60:1), and enriched gradually with MeOH, to obtain three fractions (4-1–4-3). Fraction 4-2 (1.7 g) was further purified on a silica gel column using CH_2Cl_2/MeOH mixtures to obtain 5-hydroxy-7,2′,3′,5′-tetra- methoxyflavone (4.7 mg). Fraction 5 (1.6 g) was subjected to silica gel chromatography, eluting with CH_2Cl_2-MeOH (50:1), and enriched gradually with MeOH, to obtain three fractions (5-1–5-3). Fraction 5-2 (0.8 g) was further purified on a silica gel column using CH_2Cl_2/MeOH mixtures to obtain 5,8-dihydroxy-7,3′,4′-trimethoxyflavone (**1**) (4.2 mg).

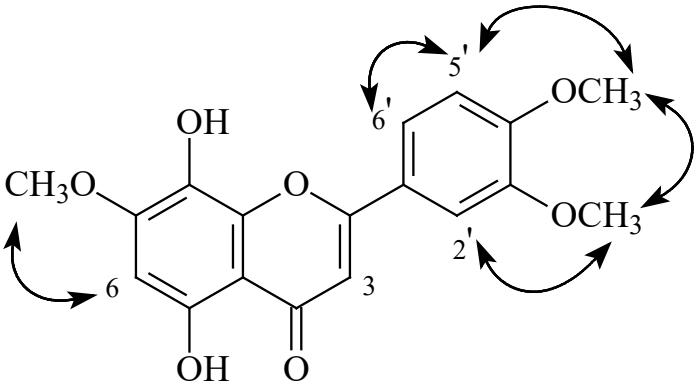

Figure 1: NOESY experiments of 5,8-dihydroxy-7,3′,4′-trimethoxy-flavone (**1**).

5,8-Dihydroxy-7,3′,4′-trimethoxyflavone (1)

Yellow needles (MeOH), mp 211-213 ℃, UV λ_{max} 268, 300, 355 nm, IR ν_{max} 3600, 1660, 1590 cm^{-1}, ESI-MS *m/z* 367 $[M + Na]^+$; HR-ESI-MS *m/z* 367.0792 $[M + Na]^+$ (calcd for $C_{18}H_{16}O_7Na$,

367.0794). ^{1}H NMR (400 MHz, $CDCl_3$) : δ 3.82 (3H, *s*, $C_{4'}$-OCH_3), 3.85 (3H, *s*, $C_{3'}$-OCH_3), 3.93 (3H, *s*, C_7-OCH_3), 6.55 (1H, *s*, H-3), 6.59 (1H, *s*, H-6), 7.19 (1H, *d*, *J* = 8.0 Hz, C-5′), 7.49 (1H, *d*, *J* = 2.0 Hz, C-2′), 7.61 (1H, *dd*, *J* = 8.0, 2.0 Hz, C-6′), 12.63 (1H, *br s,* C_5-OH). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 55.6 ($C_{4'}$-O*C*H_3), 55.7 ($C_{3'}$-O*C*H_3), 56.5 (C_7-O*C*H_3), 97.9 (C-6), 105.6 (C-4a), 109.2 (C-5′), 111.7 (C-2′), 113.5 (C-3), 119.3 (C-6′), 121.8 (C-1′), 148.8 (C-3′), 152.3 (C-4′), 157.9 (C-8), 158.1 (C-8a), 162.2 (C-5), 163.2 (C-2), 165.5 (C-7), 182.4 (C-4).

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