A Novel Pterocarpan Derivative From the Roots of *Sophora flavescens*

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Abstract

Flavescensin A (1), a novel rearrangement derivative of pterocarpan with an unusual spirotetrahydrofuran ring, along with 7 known pterocarpans were isolated from the roots of *Sophora flavescens* using several different chromatographic separations. The planar structure of 1 was elucidated by their nuclear magnetic resonance spectroscopic and high-resolution electrospray ionization mass spectrometry data, and the absolute configuration of 1 was determined on the basis of electronic circular dichroism data. Putative biosynthetic pathway toward 1 was proposed. In addition, all of the compounds were evaluated for their anti-influenza virus and anti-inflammatory activities.

Keywords

*Sophora flavescens*, pterocarpans, flavescensin A, anti-influenza virus, anti-inflammatory

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*Sophora flavescens* Alton, known as “Kushen” in China, grows mainly in mainland China, Japan, Korea, Russia, and India. The roots of *S. flavescens* are commonly used as the traditional Chinese medicine for the treatment of pruritus, dysentery, trichomonas vaginitis, eczema, and pyogenic infections of the skin.¹² Phytochemical studies have revealed that the main active constituents in *S. flavescens* are quinolizidine alkaloids, prenylated flavonoids, and pterocarpans.³⁴ Pharmacological and chemical research on the quinolizidine alkaloids and prenylated flavonoids have been quite thorough, whereas study of the pterocarpans components remains relatively limited. However, pterocarpans have attracted much scientific attention because of their broad range of biological activities and unique structural characteristics.² Thus, the isolation of much more pterocarpans from medicinal plants is a worthwhile objective.

In the course of screening for anti-influenza virus and anti-inflammatory constituents from the root of *S. flavescens*, a novel rearrangement derivative of pterocarpan featuring a 6/5/5/6/5 pentacyclic ring system, named flavescensin A (1), along with 7 known pterocarpans (2-8) were isolated (Figure 1). Herein, we reported the isolation, structure elucidation, and biological activities of them.

Results and Discussion

Flavescensin A (1), obtained as a white amorphous powder, had a molecular formula of C₁₆H₁₅O₆ as deduced from its negative-ion high-resolution electrospray ionization mass spectrometry (HRESIMS) at *m/z* 303.0872 [M-H]- (calculated for C₁₆H₁₅O₆ 303.0874), requiring 9 index of hydrogen deficiency. The infrared (IR) spectrum revealed the existence of hydroxy (3355 cm⁻¹), carbonyl (1693 cm⁻¹), and phenyl (1614, 1499 cm⁻¹) groups. The ¹H nuclear magnetic resonance (NMR) data (Table 1) of compound 1 indicated the presence of a methylenedioxy group (δ_H 5.90 [1H, d, J = 1.2], 5.88 [1H, d, J = 1.2]), 4 methylenes (δ_H 4.22 [1H, dd, J = 9.0, 7.6 Hz], 3.88 [1H, dd, J = 9.0, 4.5 Hz]; 2.80 [1H, d, J = 14.8 Hz], 2.22 [1H, d, J = 14.8 Hz]; 2.63 [1H, m], 2.34 [1H, m]; 2.28 [1H, m], 1.98 [1H, m]), 3 methines (δ_H 5.43 [1H, d, J = 9.4 Hz], 4.07 [1H, m], 3.99 [1H, m]), and a 1,2,4,5-tetra-substituted phenyl group (δ_H 6.56 [1H, s], 6.34 [1H, s]). The ¹³C NMR data (Table 1) and heteronuclear single-quantum coherence spectra of compound 1 showed 16 carbon signals, including 1 sp³ oxygenated tertiary (δ_C 70.0), 3 sp³ methine (δ_C 47.7, 66.9, 88.4), 4 sp³ methylene (δ_C 28.1, 36.7, 43.3, 72.9, 101.5), 1 sp² quaternary (δ_C 119.0), 3 sp² oxygenated tertiary (δ_C 142.5, 148.3, 154.3), 2 sp² methine (δ_C 93.2,114.2), 1 methylenedioxy group (δ_C 101.5), and 1 carbonyl (δ_C 207.8) carbons.

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The above-mentioned NMR data indicated that 1 was a pterocarpan derivative very similar to stachyodin A, which was previously isolated from Indigofera stachyodes. Their spectral difference was almost entirely due to the D-ring. A methylenedioxy signal at δH 5.90 and 5.88 (each 1 H, d, J = 1.2 Hz) and 2 aromatic singlets at δH 6.56 (s, H-7) and 6.34 (s, H-10) were detected, while the ABX coupling system and methoxy signals disappeared, which revealed that the methylenedioxy group should be connected between C-8 and C-9. This deduction was further confirmed by correlation peaks of the proton at δH 6.56 (s, H-7) with the carbons at δC 47.7 (d, C-6a), 142.5 (s, C-8), and 148.3 (s, C-9), and the proton signals of the methylenedioxy with the carbons at δC 142.5 (s, C-8) and 148.3 (s, C-9) in heteronuclear multiple bond correlation (HMBC). The planar structure of compound 1 was further determined by 1H-1H correlation and HMBC spectra (Figure 2).

The relative configuration of compound 1 was further confirmed by the rotating-frame Overhauser effect spectroscopy (ROESY) correlations. In the ROESY spectra, the correlations of H-6α/H-4β, H-4β/H-2β, H-2α/H-11b, H-4α/H-11b, and H-11a/H-1β were clearly detected, suggesting the β-orientations of H-11a and 11b-OH, and δ8 configuration for C-4a (Figure 2). In addition, the coupling constant of 9.4 Hz of the H-6a and H-11a resonances, indicating a cis configuration (cf. a trans configuration, J = 14.0 Hz).9

The absolute configuration of compound 1 was confirmed by comparison of calculated and experimental electronic circular dichroism (ECD) spectra (Figure 3). The experimental spectrum of 1 exhibited negative Cotton effect (CE) at 211, 274 nm, and positive CE at 247, 308 nm, which agreed with the calculated spectrum of the (4aS, 11bS, 6aR, 11aR) enantiomer. Therefore, the structure of 1 was finally elucidated.

It is worth mentioning that flavescensin A (1) is an unusual pterocarpan derivative featuring a 6/5/5/6/5 pentacyclic ring system. We think that the cooccurring maackiain (7) might be a precursor of 1. So, putative biosynthetic pathway toward 1 was proposed (Scheme 1).

The 7 known compounds were identified as 11b-hydroxy-11β,1-dihydro-maackiain (2),10 11β-hydroxy-11β,1-dihydro medicarpin (3),10 pterocarpadiol B (4),11 ariabilin (5),12 3-hydroxymedicarpin (6),13 maackiain (7),12 anhydroglycinol

Table 1. NMR data of 1 in Chloroform-d.

| Position | δH (mult., J, Hz) | δC (mult.) | Position | δH (mult., J, Hz) | δC (mult.) |
|----------|------------------|------------|----------|------------------|------------|
| 1        | 2.28 (m); 1.98 (m) | 28.1 (t) | 7        | 6.56 (s)         | 114.2 (d) |
| 2        | 2.63 (m); 2.34 (m) | 36.7 (t)  | 8        | 142.5 (s)        | 148.3 (s) |
| 3        | 207.8 (s)         | 9         | 10       | 6.34 (s)         | 93.2 (d)  |
| 4        | 2.80 (d, 14.8); 2.22 (d, 14.8) | 43.3 (t) | 10       | 90.0 (s)         | 154.3 (s) |
| 6        | 4.22 (dd, 9.0, 7.6); 3.88 (dd, 9.0, 4.5) | 72.9 (t) | 11a      | 5.37 (d, 9.4)    | 88.4 (d)  |
| 6a       | 4.07 (m)          | 47.7 (d)  | 11b      | 3.99 (m)         | 66.9 (d)  |
| 6b       | 119.0 (s)         | OCH2O     |          | 5.90 (d, 1.2); 5.88 (d, 1.2) | 101.5 (t) |

Figure 1. Chemical formulas of 1-8.
respectively, by comparing their NMR data with literature values.

Compounds 1-8 were evaluated for their anti-influenza virus activities in MDCK cells and anti-inflammatory activities in RAW 264.7 cells. It is a great pity that none of them were active toward these assays.

Experimental

General Experimental Procedures

Optical rotations, Jasco DIP-370 digital polarimeter; ultraviolet (UV), Shimadzu UV-2401A spectrophotometer; IR, Tenor 27 spectrophotometer; CD, Applied Photophysics spectropolarimeter; 1D- and 2D-NMR, Bruker AVANCE III-600 spectrometer; HRESIMS, Agilent 6530Q of spectrometer; medium-pressure liquid chromatography (MPLC), Büchi Labortechnik AG CH-9230 Flawil; preparative high-performance liquid chromatography (HPLC), Agilent 1100; enzyme labeling instrument, Thermo Fisher Scientific; Silica gel, Qingdao Marine Chemical Factory; sephadex LH-20; mersham Biosciences AB; column, Soochow High Tech chromatography Co., Ltd.; ODS-C_18, YMC Co., Ltd.; precoated silica gel plates, Qingdao Marine Chemical Factory.

Plant Material

The dried roots of *Sophora flavescens* were collected in June 2019 from Honghe County, Yunnan Province, China, and identified by one of the authors (Xuan-Qin Chen). A voucher specimen (number: KUMST20190608) has been deposited at the Key Laboratory of Phytochemistry (Kunming University of Science and Technology).

Extraction and Isolation

The dried roots of *Sophora flavescens* (5.0 kg) were extracted with 95% EtOH (5 × 40 L, 24 hours, each). The EtOH extracts were evaporated to dryness (450 g) under reduced pressure, suspended in distilled H_2O, and partitioned with EtOAc and n-BuOH consecutively. The EtOAc fraction (124 g) was subjected to D-101 macroporous resin eluting with EtOH-H_2O (2:8 and 8:2) and obtained 2 fractions. The second part, the total flavonoid constituents (75 g) were subjected to silica-gel column chromatography (CC, 200-300 mesh) eluting with petroleum ether-EtOAc (4:1-1:1) and got 9 fractions (F_1-F_9).

F_1 (1.5 g) was separated by Sephadex LH-20 using MeOH as an eluent to afford 3 fractions (F_{1-1}, F_{1-2}, F_{1-3}) (400 mg) was chromatographed over silica-gel column (200, 300 mesh) and eluted with petroleum ether–CH_2Cl_2 (1:1) to obtain 8 fractions (F_{1-1}, F_{1-2}, F_{1-3}, F_{1-4}, F_{1-5}, F_{1-6}, F_{1-7}, F_{1-8}, F_{1-9}).

F_2 (2.2 g) was separated by silica-gel CC using petroleum ether–EtOAc (20:1) as eluent to give 5 (3.3 mg). F_{2-1} (362 mg) was chromatographed over silica-gel column (200, 300 mesh) and eluted with petroleum ether–CH_2Cl_2 (1:2) to obtain 2 fractions (F_{2-1-1}, F_{2-1-2}). Compound 7 (81.4 mg) was purified from F_{2-1-2} (153 mg) by repeated silica-gel column (petroleum ether–EtOAc 9:1).

Figure 2. Key 2-dimensional-nuclear magnetic resonance correlations of compound 1. COSY, correlation spectroscopy; HMBC, heteronuclear multiple bond correlation; ROESY, rotating-frame Overhauser effect spectroscopy.

![Figure 2](image-url)

Figure 3. Experimental and calculated electronic circular dichroism spectra of 1 in MeOH.

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![Figure 3](image-url)
divided into 4 subfractions (F7-3-1-F7-3-4). Compounds 4 (6.9 mg) and 8 (7.8 mg) were obtained from F7-3-2 (68 mg) by silica-gel column (200, 300 mesh) with CH2Cl2–MeOH (15:1).

Flavescensin A (1). White amorphous powder; [α]24 D = −52.93 (ε 0.08, MeOH); UV (MeOH) λ max (log ε): 196 (3.98), 264 (4.33), 310 (3.27) nm; IR (KBr) ν max: 3355, 2986, 2918, 1693, 1614, 1499, 1475, 1457, 1191, 1035 cm⁻¹; ECD (ε 2.21 × 10⁻⁴ M, MeOH), λ max (Δε) 211 (−5.86), 247 (+0.34), 274 (−0.06), 308 (+1.41) nm; 1H NMR (chloroform-d, 600 MHz) and 13C NMR (chloroform-d, 150 MHz); see Table 1; HRESIMS: m/z 303.0872 [M− H]⁻ (calculated for C16H15O6, 303.0874).

Anti-Influenza Virus Assay
In this study, influenza strain A/WSN/33/2009 (H1N1) was used. Oseltamivir as a positive control was used and purchased from LKT and Tszchem laboratories. The Madin–Darby canine kidney (MDCK) cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco, Grand Island, New York, USA) supplemented with 1% streptomycin (10 000 µg/mL)- penicillin (10 000 U/mL), 10% (v/v) fetal bovine serum, and together with 10 mmol/L N-(2-hydroxyethyl)-piperazine-N’-2-ethanesulfonic acid (HEPES) in a humidified atmosphere with 5% CO₂ at 37 °C. The anti-influenza virus activities of tested compounds were evaluated by previously reported methods. ¹⁵

Nitric Oxide Production Assay
The anti-inflammatory activities of all isolates were evaluated through measuring the level of nitrite accumulated in the culture medium. That was an important indicator of NO production according to the Griess reaction. RAW 264.7 cells (8 × 10⁴ cells/well) were seeded onto 96-well plate and pretreated with 3.125, 6.25, 12.5, 25, and 50 μM of 1-8, respectively. After 1 hour, it was treated with 1 μg/mL lipopolysaccharides (LPS). ¹⁶ Detailed experimental procedures and information could be found in the operating method of Beyotime’s Griess solution kits.

Conclusions
From Sophora flavescens, 1 new compound (1) and 7 known ones (2-8) were isolated. Compound 1 is a novel rearrangement derivative of pterocarpan featuring a 6/5/5/6/5 pentacyclic ring system with an unusual spirotetrahydrofuran ring, and putative biosynthetic pathway toward 1 was proposed.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material
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