Sikokianin D, A New C-3/C-3''-Biflavanone from the Roots of Wikstroemia indica

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Abstract: A new 3,3''-biflavanone, sikokianin D (1), was isolated from the roots of Wikstroemia indica, together with two known compounds. Their structures were elucidated by chemical evidence and spectral analyses, including HR-ESI-MS, and 1D- and 2D-NMR techniques.

Keywords: Wikstroemia indica; sikokianin D; C-3/C-3''-biflavanone

1. Introduction

Wikstroemia indica (Linn.) C. A. Mey., a shrub of the Thymelaeaceae family, is wildly distributed in the southeast of China. Known as Liaogewang, it has long been used as a folk medicine in southern China for treating arthritis, tuberculosis, syphilis and pertussis [1]. Moreover, W. indica has antifungal, anti-inflammatory, anti-cancer, antiviral and antimalarial effects [2–7]. The chemical constituents of the roots have been investigated previously, leading to the identification of groups of flavonoid, coumarin and lignan compounds [2–9]. In previous paper [10], we have reported several C-3/C-3''-biflavanones from the roots of Stellera chamaejasme L. (Thymelaeaceae) collected in Yunnan. C-3/C-3''-Biflavanones have been shown to exhibit a wide range of pharmacological activities, such as antibacterial, anti-inflammatory, antimalarial, and antitumor activities [4,11–14]. In connection with these interesting biflavanones, we examined the chemical constituents of other Thymelaeaceae plants and one new C-3/C-3''-biflavanone, sikokianin D (1), together with two known compounds, namely sikokianin B (2)
and sikokianin A (3) (Figure 1) was isolated from the roots of *Wikstroemia indica*. This paper describes the isolation and structure elucidation of these compounds.

![Figure 1. Chemical Structures of 1–3.](image)

2. Results and Discussion

Compound 1 was obtained as a pale yellow amorphous powder with optical activity ([α]_D^{20} : +231). The HR-ESI-MS of 1 exhibited a quasi-molecular-ion peak ([M+H]^+) at m/z 557.1442 (calc. 557.1448), corresponding to the molecular formula C_{31}H_{24}O_{10}. Moreover, this compound showed positive reaction with HCl-Mg reagent, indicating that it is a flavonoid. The ^1H-NMR spectrum of 1 (Table 1) displayed signals of one methoxyl group (δ_H 3.79, s, 3H), two H-atoms corresponding to H-2 (δ_H 5.57, 1H, d, J = 5.0 Hz) and H-2" (δ_H 5.19, 1H, d, J = 9.5 Hz), and two H-atoms corresponding to H-3 (δ_H 3.19, 1H, br s) and H-3" (δ_H 3.26, 1H, dd, J = 9.5, 3.0 Hz) at the rings C and C' of the biflavone. In the ^1H- and ^13C-NMR established by ^1H-^1H COSY and HMQC experiments (Table 1), the spectra showed its structural fragments to include two sets of typical 5,7-dioxygenated A rings (δ_H 5.74, 5.77, each 1H, d, J = 2.0 Hz; δ_H 5.78, 5.98, each 1H, d, J = 2.0 Hz), and two sets of para-oxygenated B rings (δ_H 7.22, 6.90, each 2H, d, J = 8.5 Hz; δ_H 6.93, 6.63, each 2H, d, J = 8.5 Hz). From the ^13C-NMR data (Table 1), two carbonyl groups (δ_C 198.5, 196.1) were also observed. These structural fragments were connected to form the given carbon framework of 1 as a dimer of flavanonol derivatives. The partial (-CH-CH-CH-CH-) structure inferred from the ^1H-^1H COSY spectrum (bold line in Figure 2) suggested that the linkage of the two flavanones was possible only at the C-3 and C-3" positions, which was supported by the comparison of the ^1H- and ^13C-NMR data of 1 with those of known 3,3"-biflavones [4,6,8,10], and further confirmed by the HMBC correlations of H-2 (δ_H 5.57) with C-3" (δ_C 51.0). The B ring could be located at C-2, based on the observation of the clear cross-peaks of H-2' and H-6' (δ_H 7.22) with C-2 (δ_C 81.2). In the same way, linkage of the B' ring to C-2" of the C' ring was deduced by the correlations of H-2" and H-6"(δ_H 6.93) with C-2" (δ_C 83.3). The HMBC cross-peak between the methoxyl group and C-4' on the B ring indicated that the methoxyl group was connected to C-4'.

The stereochemistry at the C-2/C-3 and C-2"/C-3" positions in 1 was determined as cis-trans by comparison of the J values (J_{H-H} = 5.0 Hz and J_{H-H'} = 9.5 Hz) with those of the known 3,3"-biflavanones. The key NOESY correlations between H-2" (δ_H 5.19) with H-2'(H-6') (δ_H 7.22)
further confirmed the conclusion above. The relative stereochemistry of compound 1 was confirmed as shown in Figure 1 and the compound named sikokianin D.

Compound 2 was first reported as sikokianin B of which the location of MeO group was unsettled [8], and the exact configuration was elucidated by Nunome [4]. Sikokianin B and sikokianin C were determined by comparing their $^1$H- and $^{13}$C-NMR and MS data with published values.

Table 1. NMR data of sikokianin D (1) in CD$_3$OD (500 MHz for $^1$H, 125 MHz for $^{13}$C).

| No. | $\delta_H$ Mult (J = Hz) | $\delta_C$ |
|-----|--------------------------|------------|
| 2   | 5.57 d (5.0)             | 81.2 d     |
| 3   | 3.19 br s                | 49.3 d     |
| 4   | 5.74 d (2.0)             | 96.0 d     |
| 5   |                         | 165.0 s    |
| 6   |                         | 168.1 s    |
| 7   |                         | 165.0 s    |
| 8   | 5.77 d (2.0)             | 97.0 d     |
| 9   |                         | 165.0 s    |
| 10  |                         | 103.6 s    |
| 1'  |                         | 130.0 s    |
| 2'  | 7.22 d (8.5)             | 128.4 d    |
| 3'  | 6.90 d (8.5)             | 114.9 d    |
| 4'  |                         | 160.0 s    |
| 5'  | 6.90 d (8.5)             | 114.9 d    |
| 6'  | 7.22 d (8.5)             | 128.4 d    |
| 2'' | 5.19 d (9.5)             | 83.3 d     |
| 3'' | 3.26 dd (9.5, 3.0)       | 51.0 d     |
| 4'' |                         | 196.1 s    |
| 5'' |                         | 165.3 s    |
| 6'' | 5.78 d (2.0)             | 97.0 d     |
| 7'' |                         | 167.9 s    |
| 8'' | 5.98 d (2.0)             | 96.4 d     |
| 9'' |                         | 163.9 s    |
| 10''|                         | 105.1 s    |
| 1'''|                         | 128.9 s    |
| 2'''| 6.93 d (8.5)             | 130.3 d    |
| 3'''| 6.63 d (8.5)             | 116.1 d    |
| 4'''|                         | 158.9 s    |
| 5'''| 6.63 d (8.5)             | 116.1 d    |
| 6'''| 6.93 d (8.5)             | 130.3 d    |
| 4'-OCH$_3$ |            | 3.79 s    | 55.7 q |
3. Experimental

3.1. General

Melting points were measured on a Thermal Values analytical microscope and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet FI-IR 200SXY spectrophotometer. The spectra of high resolution-electrospray ionization-mass spectrometry (HR-ESI-MS) were acquired with a Micromass Q-TOF mass spectrometer (Waters Corporation USA). $^1$H- and $^{13}$C-NMR spectra were measured in CD$_3$OD with TMS as the internal standard on a Bruker DMX-500 NMR instrument. Silica gel G$_{254}$ and H (Qingdao Sea Chemical Factory, China) were used for TLC and column chromatography, respectively.

3.2. Plant Material

The roots of *Wikstroemia indica* were purchased from a Chinese medicine pharmacy in Guangzhou, China, in September, 2011. The authentication process was carried out by Le Cai (Yunnan University). A voucher specimen was deposited in the Zhejiang University City College.

3.3. Extraction and Isolation

Air-dried powder roots (2.6 kg) of *W. indica* were extracted exhaustively with 95% aq. EtOH (9 L × 3) at r. t. After concentration in vacuo, a crude extract (270 g) was obtained, which was suspended in 1 L H$_2$O, and the suspension was extracted successively with petroleum ether (PE, 1 L × 3), EtOAc (1 L × 3), and BuOH (1 L × 3) to yield 34, 110, 89 g fractions, resp. The EtOAc extract was subjected to CC with PE/EtOAc gradient system of increasing polarity (9/1→5/5, 3600 mL) to give five fractions (Fraction 1–5). Fraction 3 was chromatographed repeatedly over SiO$_2$ column with MeOH/H$_2$O (7/3→9/1, 1,200 mL) to afford 3 (15 mg). Fraction 4 was subjected to MPLC on octadecyl silica gel (3.5 × 30 cm) eluting by gradient elution with MeOH-H$_2$O (5 mL/min, linear gradient, 50:50→90:10) to yield compounds 1 (28 mg) and 2 (36 mg).
**Sikokianin D (1).** Yellow amorphous powder, mp 213–215 °C; ([α]$_D^{20}$: +231 (c = 0.48, MeOH); IR (KBr, cm$^{-1}$): 3362, 1643; $^1$H-NMR and $^{13}$C-NMR data, see Table 1; HR-ESI-MS: $m/z$ 557.1442 [M+H]$^+$, calcd for C$_{31}$H$_{25}$O$_{10}$, 557.1448.

**Sikokianin B (2).** Yellow amorphous powder. $^1$H-NMR: $\delta_H$ 3.23 (1H, t, $J = 3.5$ Hz, H-3), 3.33 (1H, dd, $J = 9.5$, 3.0 Hz, H-3”), 3.76 (3H, s, OCH$_3$), 5.17 (1H, d, $J = 9$ Hz, H-2”), 5.53 (1H, d, $J = 4.5$ Hz, H-2), 5.75 (1H, d, $J = 2.0$ Hz, H-6), 5.84 (1H, d, $J = 2.0$ Hz, H-8), 5.86 (1H, d, $J = 2.0$ Hz, H-6”), 5.97 (1H, d, $J = 2.0$ Hz, H-8”), 6.74–7.16 (8H, m, H-Ar). HR-ESI-MS: $m/z$ 557.1446 [M+H]$^+$. Spectral data were in accordance with those reported in the literature [4,8], which confirmed that the isolated compound 2 was sikokianin B.

**Sikokianin A (3).** Yellow amorphous powder. $^1$H-NMR: $\delta_H$ 2.91 (1H, d, $J = 2.0$ Hz, H-3), 2.98 (1H, d, $J = 2.0$ Hz, H-3”), 3.82 (3H, s, OCH$_3$), 5.32 (1H, d, $J = 2.0$ Hz, H-2), 5.37 (1H, d, $J = 2.0$ Hz, H-2”), 5.75 (2H, d, $J = 0.5$ Hz, H-6, H-6”), 5.88 (2H, d, $J = 0.5$ Hz, H-8, H-8”), 6.63–7.04 (8H, m, H-Ar). HR-ESI-MS: $m/z$ 557.1448 [M+H]$^+$. Spectral data were in accordance with those reported in the literature [8], which confirmed that the isolated compound 3 was sikokianin A.

4. Conclusions

In conclusion, one new biflavanone, 5,5',7,7'-tetrahydroxy-2-(4-hydroxyphenyl)-2'-(4-methoxy-phenyl)-[3,3'-bichroman]-4,4'-dione (1), together with two known compounds, sikokianin B (2) and sikokianin A (3) was isolated from the EtOH extract of the roots of *Wikstroemia indica*.

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