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CYTOTOXICITY FLAVONES FROM *DESMODIUM OXYPHYLLUM*

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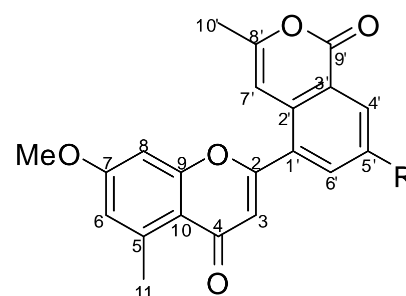
Abstract –Two new flavones, oxyphyllumflavone A and B (**1** and **2**) were isolated from *Desmodium oxyphyllum*. Their structures were elucidated by spectroscopic methods including extensive 1D- and 2D-NMR techniques. Compounds **1** and **2** are the first naturally occurring flavones possessing a six-membered 6-methyl-α-pyrone ring. Compounds **1** and **2** were also evaluated for their cytotoxicity against NB4, A549, SHSY5Y, PC3, and MCF7 cell lines, and they showed cytotoxicity with IC_{50} values in the range of 4.2 – 9.6 μM.

The phytochemical studies showed that the flavanoids are the principal bioactive constituents isolated from the genus *Desmodium* (Leguminosae), some of which exhibited considerable bioactivities such as cytotoxicity,[1,2] anti-MRSA activity,[3] inhibitory activity against the film-forming growth of *Zygosaccharomyces rouxii* F51,[4] and antifungal activity effects.[5]

Desmodium oxyphyllum (Leguminosae) is a sub-shrub, which grows to a height between 30 cm and 1.5 m in south China. It has been used in the folk medicine to treat febrile diseases, cough, asthma, hepatitis and bleeding wounds.[6,7] Some new flavonols,[8] Isoflavones,[9] and coumaronochromones were reported on the previously phytochemical studies of this plants. Motivated by a search for new bioactive metabolites from local plants, our group investigated the chemical constituents of the whole plant of *D. oxyphyllum* growing in Honghe Prefecture, which led to the isolation and characterization of two new flavones (**1** and **2**), with five known compounds luteolin,[10] apigenin,[10] desmodol,[11] quercetin,[10] and kaempferol.[10] This paper deals with the isolation, structural



Structures of compounds **1**,**2**

characterization of new compounds, and their cytotoxicity against five human tumor cell lines. The known compounds were determined by comparison of their NMR data with those reported in literatures.

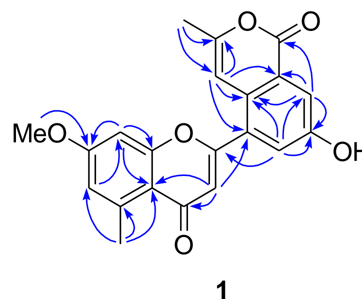


Figure 1. Selected HMBC ( ) correlations of **1**

Compound **1** was obtained as a yellow gum, and its HRESIMS revealed a peak at *m/z* 387.0840 $[M+Na]^+$ indicative of molecular formula $C_{21}H_{16}O_6$, corresponding to 14 degrees of unsaturation. The IR spectrum indicated the presence of hydroxy (3428 cm^{-1}), carbonyl (1738, 1657 cm^{-1}) and aromatic (1603, 1562, and 1463 cm^{-1}) groups. ^1H, ^{13}C, and DEPT NMR spectra showed signals for 21 carbons and 16 hydrogen atoms, a typical pattern of flavone system [δ_C 160.7 s, 107.7 d, 180.1 s, 143.0 s, 116.0 d, 162.0 s, 101.8 d, 158.0 s, 114.2 s, 121.2 s, 139.8 s, 131.3 s, 105.3 d, 157.4 s, 101.2 d] with four aromatic protons [δ_H 6.63 s, 1H, 6.39 s, 1H, 7.47 s, and 7.00 s], one methy group (δ_C 22.8 q; δ_H 2.32 s), a propenyl group [δ_C 106.0 d, 155.3 s, 19.7 q, δ_H 6.02 s], and a carbonyl group (162.8 s). Long-range HMBC correlations (Figure 1) of H-7′ (δ_H 6.02 s) to C-1′ (δ_C 121.2 s), C-2′ (δ_C 139.8 s), C-3′ (δ_C 131.3 s), C-8′ (δ_C 155.3 s) and C-10′ (δ_C 19.7 q), of H-4′ (δ_H 7.47 s) to C-9′ (δ_C 162.8 s), C-2′ (δ_C 139.8 s), and C-3′ (δ_C 131.3 s), were observed in **1**. This indicated that the propenyl and carbonyl group were attached to the aromatic ring at positions C-2′ and C-3′ respectively, and formed a six-member 6-methyl-*α*-pyrone ring. The HMBC correlations of methyl protons (δ_H 2.32 s) with C-5 (δ_C 143.0 s), C-6 (δ_C 116.0 d), and C-10 (δ_C 114.2 s) revealed this $-CH_3$ was located at C-5. The -OH located at C-5′ was supported by HMBC correlations of the -OH proton (δ_H 9.20 brs) with C-4′ (δ_C 105.3 d), C-5′ (δ_C 157.4 s), and C-6′ (δ_C 101.2 d). The HMBC correlations of -OMe proton signal (δ_H 3.90) with C-7 (δ_C 162.0 s) placed the -OMe at C-7. Five singlets (δ_H 6.39 s, 1H, 6.52 s, 1H, 6.63 s, 1H, 7.00 s, 1H, and 7.47 s, 1H) in the ^1H NMR spectrum also supported the substituent positions in compound **1**. Thus, the structure of **1** was established as shown, and it was given the name of oxyphyllumflavone A.

Table 1. ^1H and ^{13}C NMR Data of Compounds **1**–**2** (δ in ppm, in $CDCl_3$)

No.	1		2	
	δ_C (m)	δ_H (*J* in Hz)	δ_C (m)	δ_H (*J* in Hz)
2	160.7 s		161.4 s	
3	107.7 d	6.52 s	107.0 d	6.48 s
4	180.1 s		179.4 s	
5	143.0 s		142.7 s	
6	116.0 d	6.63 s	116.3 d	6.60 s
7	162.0 s		162.1 s	
8	101.8 d	6.39 s	101.0 d	6.32 s
9	158.0 s		158.4 s	
10	114.2 s		114.6 s	
11	22.8 q	2.32 s	23.8 q	2.28 s
1′	121.2 s		120.6 s	
2′	139.8 s		139.6 s	
3′	131.3 s		130.9 s	
4′	105.3 d	7.47 s	104.4 d	7.52 s
5′	157.4 s		160.8 s	
6′	101.2 d	7.00 s	99.1 d	7.03 s
7′	106.0 d	6.02 s	105.7 d	6.08 s
8′	155.3 s		155.1 s	
9′	162.8 s		162.9 s	
10′	19.7 q	2.00 s	20.4 q	2.02 s
7-OMe	55.7 q	3.90 s	55.8 q	3.90 s
5′-OMe			56.0 q	3.94 s
5′-OH		9.20 brs		

Compound **2** was assigned the molecular formula $C_{22}H_{18}O_6$ by HRESIMS. The comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed that the only difference between them was due to the appearance of a methoxy group (δ_C 56.0 q, δ_H 3.94 s) and disappearance of a hydroxy group (δ_H 9.20 brs) in **2**. The HMBC correlation of methoxy proton signal (δ_H 3.94) with C-5′ (δ_C 160.8) placed the methoxy group at C-5′. Thus, the structure of compound **2** was determined as shown, and this compound was given the name of oxyphyllumflavone B.

Since some previously reported flavanoids from *Desmodium* plants exhibited cytotoxicity,[1,2] we tested the cytotoxicity for c five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) using the MTT method as reported previously.[12] Paclitaxel was used as the positive control. The results are depicted in Table 2. They showed cytotoxicity with IC_{50} values in the range of 4.2 – 9.6 μM.

Table 2. Cytotoxicity Data (IC_{50}, μM) for Compounds **1** and **2** from *D. oxyphyllum*

Compounds	NB4	A549	SHSY5Y	PC3	MCF7
1	5.6	4.2	6.2	5.8	8.2
2	7.5	6.8	4.5	6.4	9.6
Paclitaxel	0.03	0.02	0.2	0.2	0.1

EXPERIMENTAL

General. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on a DRX-500 NMR spectrometer with TMS as internal standard. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS was performed on a VG Autospec-3000 spectrometer. Semipreparative HPLC was performed on a Shimadzu LC-8A preparative liquid chromatograph with Zorbax PrepHT GF (21.2 mm × 25 cm) or Venusil MP C_{18} (20 mm × 25 cm) columns. Column chromatography (CC) was performed using silica gel (200–300 mesh, Qing-dao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and MCI gel CHP 20Y resin(75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 5% H_2SO_4 in EtOH.

Plant material. The whole plant of *D. oxyphyllum* was collected in Honghe Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Dr. Yuan. N. of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 2010-11-26) has been deposited in our laboratory.

Extraction and Isolation. The air-dried and powdered *D. oxyphyllum* (1.3 kg) plant material was extracted four times with 80% aqueous EtOH (4 × 5 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure, and the crude extract (120 g) was decolorized by MCI gel. The

portion of the extract soluble in 90% MeOH (30 g) was chromatographed on a silica gel column eluting with a $CHCl_3$-MeOH gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions (A–F). Separation of fraction B (9:1, 2.5 g) by silica gel CC, eluted with petroleum ether-acetone (9:1–1:2), yielded fractions B1–B7. Fraction B2 (8:2, 0.86 g) was subjected to silica gel CC using petroleum ether-acetone and semi-preparative HPLC (48% MeOH-H_2O) to give **1** (5.3 mg) and **2** (6.8 mg).

Oxyphyllumflavone A (1): yellow gum; UV (MeOH) λ_{max} (log ε) 370 (3.86), 282 (3.95), 210 (4.38) nm; IR (KBr) ν_{max} 3428, 1738, 1657, 1603, 1562, 1463, 1384, 1172, 1038, 873, 768 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 387 $[M+Na]^+$; positive HRESIMS *m/z* 387.0840 $[M+Na]^+$ (calcd for $C_{21}H_{16}O_6Na$, 387.0845).

Oxyphyllumflavone B (2): yellow gum; UV (MeOH) λ_{max} (log ε) 372 (3.80), 285 (3.92), 210 (4.45) nm; IR (KBr) ν_{max} 3438, 1740, 1655, 1605, 1568, 1457, 1376, 1166, 1043, 878, 765 cm^{-1}; ^{1}H and ^{13}C NMR data ($CDCl_3$, 500 and 125 MHz), see Table 1; positive ESIMS *m/z* 401 $[M+Na]^+$; positive HRESIMS *m/z* 401.1007 $[M+Na]^+$ (calcd for $C_{22}H_{18}O_6Na$, 401.1001).

Cytotoxicity Assay. Colorimetric assays were performed to evaluate cytotoxicity. NB4 (human acute promyelocytic leukemia cells), A549 (human lung adenocarcinoma), SHSY5Y (human neuroblastoma), PC3 (human prostate), and MCF7 (human breast adenocarcinoma) tumor cell lines were purchased from the American Type Culture Collection (ATCC). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO). Briefly, 100 μL of suspended adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition. In addition, suspended cells were seeded just before drug addition, with an initial density of 1×10^5 cells/mL in 100 μL of medium. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h; paclitaxel (Sigma, purity >95%) was used as a positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL of the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[13]

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