Asian Journal of Chemistry; Vol. 27, No. 6 (2015), 2019-2021

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17657

A New Isoflavanone from *Desmodium oxyphyllum* and Its Cytotoxicity

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Received: 24 March 2014; *Accepted*: 26 August 2014; *Published online*: 17 March 2015; AJC-16950

A new isoflavanone, (3*R*) 4',7-methoxy-5-methoxycarbonyl-isoflavanone (**1**) was isolated from *Desmodium oxyphyllum*. This new compound was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) and it showed potential cytotoxicity against NB4 and PC3 cell with IC_{50} values of 5.6 and 7.2 μM.

Keywords: Isoflavanone, *Desmodium oxyphyllum*, Cytotoxicity.

INTRODUCTION

The genus *Desmodium* is a large member of the Leguminosae family. It contains about 350 species which is mainly distributed in tropical and subtropical regions of the world and about 28 species in China[1]. Many species in this genus have a long history of medicinal use in traditional Chinese medicine[2]. The water decoction of *Desmodium* plants has been widely used in China to treat various diseases like asthma, typhoid fever, inflammations, malaria, infantile malnutrition, dysentery, pyrexia, *etc*[3]. Due to their versatile medicinal traditional uses, an increasing number of phytochemical studies have been carried out on *Desmodium* plants. Flavonoids and alkaloids are regarded as the major constituents and perhaps responsible for most of the activities shown by plants of this genus[4-7]. *Desmodium oxyphyllum* (Leguminosae) is a sub-shrub and grows to a height between 30 cm and 1.5 m. This genus extends from India, Nepal, Myanmar, North Korea, Japan to South China[1,2]. It has been used in the folk medicine to treat febrile diseases, cough, asthma, hepatitis and bleeding wounds[1,8]. Previous phytochemical studies of *D. oxyphyllum* revealed the presence of flavonols[2], isoflavanones[9] and coumaronochromones[9].

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 Dehong Prefecture, which led to the isolation and characterization of a new isoflavone (**1**). This paper deals with the isolation, structural characterization of this new compound and its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7).

EXPERIMENTAL

Optical rotations were measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer and CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS spectra were measured on a VG Auto Spec-3000 MS spectrometer. ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker DRX-500 instrument with TMS as internal standard. Column chromatography was performed on silica gel (200-300 mesh), or on silica gel H (10-40 μm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

The whole plant of *D. oxyphyllum* was collected in Dehong Prefecture, Yunnan Province, P.R. China, in September 2010. The identification of the plant material was verified by Dr. N. Yuan of Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (YNNU 2010-10-22) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered *D. oxyphyllum* (2.8 kg) were extracted four times with 70 % aqueous acetone (4 × 3 L) at room temperature and filtered. The filtrate was evaporated under reduced pressure and the crude extract (260 g) was decolourized by MCI. The 90 % methanol part (96 g) was chromatographed on a silica gel column eluting with a chloroform-methanol gradient system (9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. The further separation of fraction A (9:1, 22.2 g) by silica gel column chromatography, eluted with petroleum ether-acetic ester (9:1-

1:2), yielded subfractions A1-A6. Subfraction A4 (1:1, 7.35 g) was subjected to silica gel column chromatography using petroleum ether-acetone and preparative HPLC (58 % MeOH-H_2O, flow rate 12 mL/min) to give compound **1** (15.6 mg).

(3R) 4',7-Methoxy-5-methoxycarbonyl-isoflavanone (1): $C_{19}H_{18}O_6$, pale yellow gum; $[\alpha]_D^{25.2}$ -33.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 308 (3.68), 245 (3.41), 210 (4.08) nm; CD (c = 0.2, MeOH) λ_{max} (nm, Δε): 250 (+1.85), 348 (+1.32); IR (KBr, ν_{max}, cm^{-1}) 3387, 2956, 2880, 1695, 1652, 1608, 1516, 1457, 1432, 1365, 1268, 1051, 946, 860; ^1H and ^{13}C NMR data (500 and 125 MHz), (Table-1); ESIMS *m/z* 351; HRESIMS *m/z* 365.1008 $[M + Na]^+$ (calcd $C_{19}H_{18}O_6Na$ for 365.1001).

RESULTS AND DISCUSSION

A 70 % aqueous acetone extract prepared from dry powder of the whole *D. oxyphyllum* plant was subjected repeatedly to column chromatography on silica gel, Sephadex LH-20, RP-18 and preparative HPLC to afford compound **1**, The structure of the compound **1** is shown in Fig. 1 and the ^1H and ^{13}C NMR data of compounds **1** and **2** are listed in Table-1.

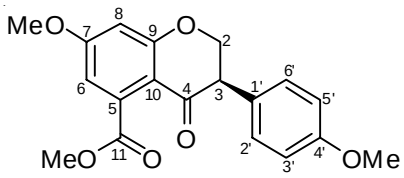

Fig. 1. Structure of compound **1**

Compound **1** was obtained as pale yellow gum. The HRESIMS showed the quasi-molecular ion peak at *m/z* 365.1008 $[M + Na]^+$ (calcd $C_{19}H_{18}O_6Na$ for 365.1001), in accordance with the molecular formula $C_{19}H_{18}O_6Na$, which indicated 11 degrees of unsaturation. Its UV spectrum showed the maximum absorption at 308, 245 and 210 nm. Strong absorption bands accounting for hydroxyl (3387 cm^{-1}), carbonyl (1695, 1652 cm^{-1}) and aromatic groups (1608, 1516 and 1457 cm^{-1}) could be observed in its IR spectrum. The ^1H and ^{13}C NMR spectra of compound **1** (Table-1) displayed signals for all 19 carbons and 18 protons, including two aromatic ring (δ_C 135.2 s, 108.7 d, 167.8 s, 104.2 d, 156.6 s, 109.2 s, 128.4 s, 131.8 d (2C), 116.5 d (2C), 162.2 s) with six aromatic protons [δ_H 6.96, d (2.2 Hz); 6.72, d (2.2 Hz); 7.48, d (8.7 Hz), 2H; 7.03 d (8.7 Hz), 2H], one O-bearing methylene [δ_C 73.5; δ_H 4.62 dd (11.2, 6.4 Hz), 4.83 t (11.2 Hz)]; one methine [δ_C 48.6; δ_H 4.28 dd (11.2, 6.4)], one carbonyl group (δ_C 197.8), one methoxycarbonyl group (δ_C 169.8, 53.7; δ_H 4.08 s) and two methoxy group (δ_C 55.9, 56.1; δ_H 3.84, 3.79 s). The proton signals at δ_H 4.83, t (11.2 Hz); 4.62, dd, (11.2, 6.4 Hz) and 4.28, dd (11.2, 6.4 Hz), combined with the carbon signals at δ_C 197.8 (C-4), 73.5 (C-2), 48.6 (C-3) in the ^{13}C NMR (Table-1), implied compound **1** possessed an isoflavanone skeleton[10]. The HMBC correlation (Fig. 2) between one methoxy proton (δ_H 3.84) and C-4' (δ_C 162.2) suggested one methoxy group at C-4'. The other methoxy group located at C-7 was supported by the HMBC correlation of the methoxy proton (δ_H 3.79) with C-7 (δ_C 167.8). The methoxycarbonyl group at C-5 was supported by HMBC correlations of H-6 (δ_H 6.96) with the ester carbonyl carbon (δ_C 169.8 s) and the fact that no correlation was observed between H-8 (δ_H 6.72) and the carbonyl. The typical protons signals [δ_H 6.96, d (2.2 Hz); 6.72, d (2.2 Hz); 7.48, d (8.7 Hz), 2H; 7.03 d (8.7 Hz), 2H] also supported the 5,7-disubstituted for ring B and 4'-monosubstituted for ring C. The *R* configuration at C-3 was assigned by the comparison of NMR, optical rotation and CD data with those of the known compounds[11,12]. Thus, compound **1** was determined as (3*R*) 4',7-methoxy-5-methoxy-carbonyl-isoflavanone.

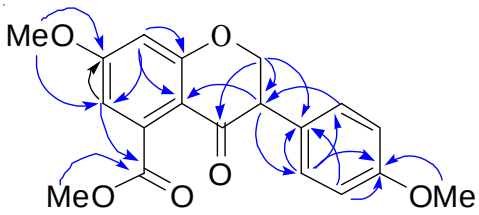

Fig. 2. Selected HMBC (⌒) correlations of **1**

Isoflavanones are known to exhibit cytotoxic effects. The cytotoxicity of **1** was tested using a previously reported procedure[13]. All treatments were performed in triplicate. In the MTT assay, the IC_{50} was defined as the concentration of the test compound resulting in a 50 % reduction of absorbance compared with untreated cells. The results showed that compound **1** showed potential cytotoxicity against NB4 and PC3 cell with IC_{50} values of 5.6 and 7.2 μM.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 21302164), the Excellent Scientific and Technological Team of Yunnan High School (2010CI08),

TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1** (δ in ppm, in C_5D_5N, 500 MHz)

No.	δ_C (m)	δ_H (m, *J*, Hz)	No.	δ_C (m)	δ_H (m, *J*, Hz)
2	73.5 t	4.62 dd (11.2, 6.4)	10	109.2 s	-
-	-	4.83 t (11.2)	11	169.8 s	-
3	48.6 d	4.28 dd (11.2, 6.4)	1′	128.4 s	-
4	197.8 s	-	2′, 6′	131.8 d	7.48 d (8.7)
5	135.2 s	-	3′, 5′	116.5 d	7.03 d (8.7)
6	108.7 d	6.96 d (2.2)	4′	162.2 s	-
7	167.8 s		11-OMe	53.7 q	4.08 s
8	104.2 d	6.72 d (2.2)	4′-OMe	55.9 q	3.84 s
9	156.6 s	-	7-OMe	56.1 q	3.79 s

the Yunnan University of Nationalities Green Chemistry and Functional Materials Research for Provincial Innovation Team (2011HC008).

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