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A New Cytotoxic Xanthone from *Hypericum chinense*

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A new xanthone, 1,8-dihydroxy-4-(2-hydroxyethyl)-3-methoxy-9*H*-xanthen-9-one (**1**) was isolated from the leaves and stems of *Hypericum chinense*. Its structure was elucidated by spectroscopic methods, including extensive 1D- and 2D NMR techniques. Compound **1** was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) and it showed high cytotoxicity against PC3 and SHSY5Y cell with IC_{50} values of 3.5 and 2.4 μM, respectively.

Keywords: Cytotoxic, Xanthone, *Hypericum chinese*.

INTRODUCTION

The family Clusiaceae is a rich source of xanthones, which show various bioactivities[1,2]. These xanthones show various bioactivities, including, anti-hepatitis B virus[3], anti-tobacco mosaic virus[4], antibacterial[5,6], antioxidant[7,8], antiinflammatory[9], tumor-promoting inhibition[10] and cytotoxicity[11,12]. The genus hypericum belonging to clusiaceae is distributed widely in temperate regions and has been used for traditional medicines in various parts of the world. In China, *Hypericum chinese* is used as a folk medicine for treatment of female disorders[13]. Previous phytochemical investigations on *H. chinese* resulted in the isolation of xanthones[12], acylphloroglucinols[14], lactones[15] and norlignans[16] from this species.

With the aim of multipurpose utilization of herb plants and identify bioactive natural products from this genus, the phytochemical investigation on *H. chinese* was carried out. As a result, a new xanthone (**1**) was isolated from this plant. It structure was elucidated on the basis of spectroscopic methods, including extensive 1D- and 2D NMR techniques. In addition, the cytotoxicity of compound **1** was evaluated. The details of the isolation, structure elucidation and cytotoxicity of this new compound are reported in this article.

EXPERIMENTAL

UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS were measured on a VG Auto Spec-3000 MS spectrometer. ^1H, ^{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 mm, Qingdao Marine Chemical Inc., China). Second separate was used an Agilent 1100 HPLC equipped with ZORBAX-C18 (21.2 mm × 250 mm, 7.0 mm) column and DAD detector.

The leaves and stems of *Hypericum chinense* L. were collected in Honghe Prefecture, Yunnan Province, People's Republic of China, in September 2010. The identification of the plant material was verified by Prof. Ren P.Y. (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2010-9-23) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves and stems of H. chinense (2 kg) were extracted 4 times with 70 % acetone (4 × 3 L) at room temperature and filtered. The crude extract (136 g) was applied to silica gel (200-300 mesh) column chromatography, eluting with a $CHCl_3$-acetone gradient system (9:1, 8:2, 7:3, 6:4, 5:5), to give five fractions A-E. The further separation of fraction A (9:1, 16.8 g) by silica gel column chromatography, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures A1-A5. The subfraction A3 (7:3, 4.21 g) was subjected to preparative HPLC (55 % MeOH, flow rate 12 mL/min) to give compound **1** (11.4 mg).

1,8-Dihydroxy-4-(2-hydroxyethyl)-3-methoxy-9*H*-xanthen-9-one (1): Obtained as a yellow gum; UV (MeOH)

λ_{max} (log ε) 210 (4.13), 242 (3.42), 305 (3.87) nm; IR (KBr, ν_{max}, cm^{-1}) 3438, 3075, 2942, 2880, 1652, 1600, 1542, 1465, 1370, 1183, 1062, 882, 768; ESIMS *m/z* (positive ion mode) 325 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 325.0682 $[M + Na]^+$ (calcd. $C_{16}H_{14}NaO_6$ for 325.0688).

RESULTS AND DISCUSSION

A 70 % aq. acetone extract prepared from the leaves and stems of *H. chinense* was subjected repeatedly to column chromatography on Silic gel, Sephadex LH-20, RP-18 and Preparative HPLC to afford compound **1**. The structure of **1** was shown in Fig. 1 and its ^1H and ^{13}C NMR data were listed in Table-1.

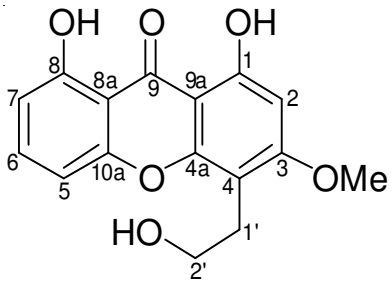

Fig. 1. Structure of compound **1**

Compound **1** was isolated as a yellow gum. The HRESIMS of **1** gave the pseudomolecular $[M + Na]^+$ ion at *m/z* 325.0682, corresponding to a molecular formula of $C_{16}H_{14}O_6$. Its UV spectrum showed the maximum absorption at 305, 242 and 210 nm. Strong absorption bands accounting for ν(OH) (3438 cm^{-1}), ν(C=O) (1652 cm^{-1}) and aromatic groups (1600, 1542, 1465 cm^{-1}) could also be observed in its IR spectrum. The ^1H- and ^{13}C NMR spectrum (Table-1) displayed signals for all 16 carbons and 14 protons, including a xanthones skeleton[17] (C-1 - C-9, C-4a, C-8a - C-10a; H-2, H-5 - H-7), one methoxy group (δ_C 55.9 q, δ_H 3.80 s), a hydroxyethyl unit[11] [δ_C 34.3 t, 63.2 t; δ_H 2.50 t (7.2), 3.62 t (7.2)] and two phenolic hydroxy groups (δ_H 13.46 s and 13.14 s). The HMBC correlation (Fig. 2) of the methoxy proton signal (δ_H 3.80) with C-3 (δ_C 161.4) showed that the methoxy group was located at C-3. The long-range correlations of H_2-1' (δ_H 2.50) to C-3 (δ_C 161.4), C-4 (δ_C 108.3) and C-4a (δ_C 155) were observed in **1**. This led us to conclude that the hydroxyethyl unit was located at C-4. Finally, HMBC correlations between the hydroxy proton (δ_H 13.46) and C-1 (δ_C 161.5), C-2 (δ_C 98.6) and C-9a (δ_C 104.6), as well as those between the other hydroxy proton (δ_H 13.14) and C-7 (δ_C 108.6), C-8 (δ_C 162) and C-8a (δ_C 109.2), led to the assignment of the phenolic groups at C-1 and C-8. The typical proton signals of ring A [δ_H 6.82 d (8.3), 7.49 t (8.3), 6.68 d (8.3)] and ring B (δ_H 6.62 s) also supported that **1** should be a 1,3,4,8-tetrasubstituted xanthone[17]. Thus, compound **1** was assigned as 1,8-dihydroxy-4-(2-hydroxyethyl)-3-methoxy-9*H*-xanthen-9-one.

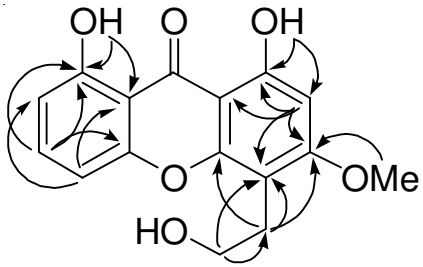

Fig. 2. Key HMBC (↷) correlations of **1**

Since xanthones are known to exhibit potential cytotoxicity[2,11,12]. Compound **1** was tested for its cytotoxicity against five human tumor cell lines (NB4, A549, SHSY5Y, PC3 and MCF7) using the MTT method as reported previously[18]. Taxol was used as the positive control. The results showed that compound **1** exhibited high cytotoxicity against PC3 and SHSY5Y cell with IC_{50} values of 3.5 and 2.4 μM, respectively.

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TABLE-1
^1H AND ^{13}C NMR DATA OF COMPOUND **1** (δ IN ppm, 500 AND 125 MHz, IN C_5D_5N)

No.	δ_C (m)	δ_H (m, J, Hz)	No.	δ_C (m)	δ_H (m, J, Hz)
1	161.5 s		4a	155.0 s	
2	98.6 d	6.62 s	8a	109.2 s	
3	161.4 s		9a	104.6 s	
4	108.3 s		10a	158.4 s	
5	110.5 d	6.82 d (8.3)	1′	34.3 t	2.50 t (7.2)
6	135.2 d	7.49 t (8.3)	2′	63.2 t	3.62 t (7.2)
7	108.6 d	6.68 d (8.3)	3-OMe	55.9 q	3.80 s
8	162.0 s		1-Ar-OH		13.46 s
9	182.5 s		8-Ar-OH		13.14 s

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