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Isolation of New Xanthone from *Hypericum chinense* and Its Cytotoxicity

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A new xanthone, hypexanthone A (**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 cytotoxicity against NB4 and SHSY5Y cell with IC_{50} values of 5.2 and 6.3 μM, respectively.

Keywords: Xanthone, Hypexanthone A, *Hypericum chinense*.

INTRODUCTION

The family Clusiaceae is a rich source of xanthones[1,2]. These xanthones show various bioactivities, including, anti-hepatitis B virus[3], anti-tobacco mosaic virus[4], anti-bacterial[5,6], anti-oxidant[7,8], anti-inflammatory[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 *Hypericum 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 *Hypericum chinese* was carried out. As a result, a new xanthone, hypexanthone A (**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.

EXPERIMENTAL

Optical rotations were measured with a Horiba SEPA-300 polarimeter; 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-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector.

Leaves and stems of *Hypericum chinense* L. were collected in Dehong Prefecture, Yunnan Province, People's Republic of China, in September 2011. The identification of the plant material was verified by Prof. Ren P. Y (Xishuangbanna Botanical Garden). A voucher specimen (YNNI-2011-9-32) has been deposited in our laboratory.

Extraction and isolation: The air-dried and powdered leaves and stems of *Hypericum chinense* (2.2 kg) were extracted four times with 70 % acetone (4 × 3 L) at room temperature and filtered. The crude extract (108 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, 15.2 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 A2 (8:2, 3.65 g) was subjected to preparative HPLC (62 % MeOH, flow rate 12 mL/min) to give compound **1** (12.2 mg).

Hypexanthone A (1): Obtained as a yellow gum; $[\alpha]_D^{24.5}$ -53.8 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.47), 258 (3.82), 286 (3.97), 348 (3.75) nm; IR (KBr, ν_{max}, cm^{-1}) 3412, 3062, 2942, 2865, 1658, 1606, 1527, 1438, 1365,

TABLE-1
^{1}H 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	155.2 s	6.53 s	9a	105.2 s	
2	98.5 d		10a	158.4 s	
3	157.4 s		1′	32.6 t	2.96, 3.24 dd each (18.0, 5.0)
4	132.2 s		2′	69.8 d	4.08 t (5.0)
5	145.9 s		3′	80.5 s	
6	126.7 s		4′	22.1 q	1.58 s
7	124.3 d	7.12 d (8.0)	5′	25.6 q	1.52 s
8	118.5 d	7.76 d (8.0)	3-OMe	56.3 q	3.82 s
9	180.1 s		1-ArOH		12.8 s
4a	152.6 s		4-ArOH		13.2 s
8a	120.3 s				

1274, 1178, 1058, 868, 772; ESIMS *m/z* (positive ion mode) 381 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 381.0987 $[M + Na]^+$ (calcd $C_{19}H_{18}O_7Na$ for 381.0950).

RESULTS AND DISCUSSION

A 70 % aq. acetone extract prepared from the leaves and stems of *Hypericum chinense 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 ^{1}H and ^{13}C NMR data were listed in Table-1.

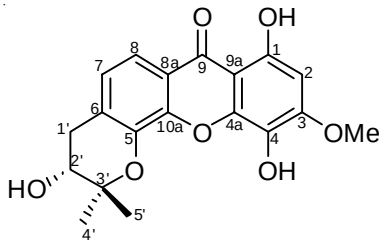

Fig. 1. Structure of compound **1**

Compound **1** was isolated as a yellow gum. The HRESIMS of **1** showed a pseudomolecular ion at *m/z* 381.0987 $[M + Na]^+$ corresponding to $C_{19}H_{18}NaO_7$. The UV spectrum exhibited four absorption bands characteristic of a xanthone (λ_{max} 235, 258, 286, 348 nm)[17]. Strong absorption bands accounting for hydroxy (3412 cm^{-1}), carbonyl (1658 cm^{-1}) and aromatic groups (1606, 1527, 1438 cm^{-1}) could also be observed in its IR spectrum. The ^{1}H- and ^{13}C NMR spectrum (Table-1) displayed signals for all 19 carbons and 18 protons, including a xanthones skeleton[17] (C-1- C-9, C-4a, C-8a-C-10a; H-2, H-7, H-8), one methoxy group (δ_C 56.3 q, δ_H 3.82 s), a 2′-hydroxy-3′,3′-dimethyl-1′,2′-dihydropyrane ring (C-1′-C-5′; H-1′, H2′, H-4′ and H-5′)[18] and two phenolic hydroxy groups (δ_H 12.8 s and 13.2 s). The HMBC correlation (Fig. 2) of the methoxy proton signal (δ_H 3.82) with C-3 (δ_C 157.4) showed that the methoxy group was located at C-3. The long-range correlations of H2-1' (δ_H 2.96, 3.24) to C-5 (δ_C 145.9), C-6 (δ_C 126.7) and C-7 (δ_C 124.3), of H-2' (δ_H 4.08) with C-6 (δ_C 126.7) and of H-7 (δ_H 7.12) with C-1′ (δ_C 32.6) were observed in compound **1**. This led us to conclude that the dihydropyrane ring was located at C-5 and C-6. Finally, HMBC correlations between the hydroxy proton (δ_H 12.8) and C-1 (δ_C 155.2), C-2 (δ_C 98.5) and C-9a (δ_C 105.2), as well as those between the other hydroxy proton (δ_H 13.2) and C-3 (δ_C 157.4), C-4 (δ_C 132.2) and C-4a (δ_C 152.6), led to the assignment of the phenolic hydroxy groups at C-1 and C-4. The typical proton signals of ring A [δ_H 7.12 d (8.0) and 7.76 d (8.0)] and ring B (δ_H 6.53 s) also supported that compound **1** should be a 1,3,4,5,6-pentasubstituted xanthone. The relative stereochemistry of compound **1** (Fig. 2) was deduced from its NOESY spectrum showing that Me-5' (δ_H 1.52), H-2′ (δ_H 4.08) and H-1' a (δ_H 2.96), on one hand and OH-2′ (δ_H 1.94), Me-4' (δ_H 1.58) and H-1' β (δ_H 3.24), on the other hand, were oriented on the same sides of the molecule. The absolute configuration of (2'*R*) for the OH-2' group was confirmed by the comparison of its optical rotation and coupling constants valves with these of known compounds[18]. Thus, the structure of compound **1** was established and gives the trivial name of hypexanthone A.

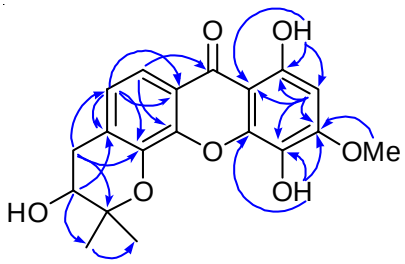

Fig. 2. Key HMBC (↷) correlations of compound **1**

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[19]. Taxol was used as the positive control. The results showed that compound **1** exhibited high cytotoxicity against NB4 and SHSY5Y cell with IC_{50} values of 5.2 and 6.3 μM, respectively.

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