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Sulfonated xanthones from *Hypericum sampsonii*

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Abstract

Xanthones, 1,3-dihydroxy-5-methoxyxanthone-4-sulfonate and 1,3-dihydroxy-5-*O*-β-D-glycopyranosylxanthone-4-sulfonate, together with nine known compounds were obtained from *H. sampsonii*. This is the first report of sulfonated xanthonoids. Furthermore, compounds **1** and **2** exhibited significant cytotoxicity against the P388 cancer cell line.

Keywords: *Hypericum sampsonii*; Guttiferae; Xanthonoid sulfonates; Cytotoxicity

1. Introduction

Hypericum species are well known to contain a variety of oxygenated and prenylated xanthones (Ishiguro et al., 1995, 1996, 1997; Rath et al., 1996). During the course of our search for biologically active substances in the whole plant of *Hypericum sampsonii*, we have isolated a number of new polyisoprenyl benzophenones (Hu and Sim, 1998, 1999a,b), among which sampsonones A and I showed cytotoxic properties. Further investigations on the chemical constituents of the whole plant of the title plant resulted in the isolation of two new xanthone sulfonates (**1** and **2**) and nine known compounds (**3**–**11**). We report herein the isolation and the structural determination of the eleven compounds.

2. Results and discussion

The EtOH extract was partitioned between chloroform and water and afforded an insoluble residue, which showed cytotoxicity against the P388 cell line. The residue was dissolved in EtOH and further subjected to Sephadex LH-20 chromatography, being eluted with EtOH/H_2O (1:1) to yield compounds **1**, **2** and mangiferin (**3**). The water-soluble part was chromatographed on a silica gel column, affording six fractions. The fractions then yielded one benzophenone glycoside (**4**), two flavonoids (**5** and **6**), three xanthones (**7**–**9**) and two simple aromatic acids (**10** and **11**) after further chromatographic purification.

Compounds **3**–**11** were determined by detailed NMR & MS analysis as mangiferin (**3**) (Chen and Chen, 1985), 2-β-D-glucopyranosyl-4,6-dihydroxyphenyl phenyl ketone (**4**) (Huang et al., 2001), luteolin (**5**) (Youssef and Frahm, 1995), quercetin 3-*O*-β-D-galactopyranoside (6) (Glennie and Jain, 1980), 2-hydroxyxanthone (**7**) (Han et al., 1996), euxanthone (**8**) (Della Monache et al., 1983), neolancerin (**9**) (Schaufelberger and Hostettmann, 1988), 3,4-dihydroxycinnamic acid (**10**) and 3,4-dihydroxybnzoic acid (**11**) (Charles and Behnke, 1992).

Compound **1** was isolated as yellow needles, readily soluble in water, and had a high melting point (>360 °C). The UV and IR spectra suggested a xanthone derivative [IR ν_{max}^{KBr} cm^{-1}: 3534, 3473, 1659, 1570, 1497, 1424, 1269, 1146; UV λ_{max}^{EtOH} nm (log ε): 206 (4.16), 224 (4.15),

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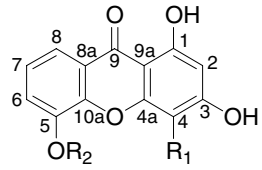
248 (4.28), 310 (4.04)]. Atomic absorption data of **1** indicated the presence of K^+. The molecular formula of $C_{14}H_9O_8KS$ was established from the characteristic ion peaks at *m/z* 414.9281 $[M + K]^+$ ($C_{14}H_9O_8K_2S$, calcd. 414.9292) 398.9529 $[M + Na]^+$ ($C_{14}H_9O_8KNaS$, calcd. 398.9553) and 376.9714 $[M + H]^+$ ($C_{14}H_{10}O_8KS$, calcd. 376.9734) in its HRFABMS (positive mode) spectrum, which was confirmed by the characteristic ion peaks at *m/z* 791 $[2M + K]^+$ and 415 $[M + K]^+$ in its ESI mass spectrum.

In the ^1H NMR spectrum (in DMSO-d_6), the presence of two chelated hydroxyl groups [δ 13.18 and 12.98 (each 1 H, *s*)] and a methoxyl group [δ 4.00 (3 H, *s*)] were observed. In addition, an aromatic proton [δ 6.20 (1 H, *s*)] and three other *ortho*-coupled protons [δ 7.52 (1 H, *dd*, $J = 8.0$, 1.4 Hz), 7.41 (1 H, *t*, $J = 8.0$ Hz) and 7.71 (1 H, *dd*, $J = 8.0$, 1.4 Hz)] were observed. All protonated carbons were assigned by HMQC spectral analysis. In the HMBC spectrum, the methoxyl protons (δ 4.00) showed a ^3J coupling to an oxygenated aromatic carbon (δ 148.3), which was further correlated to one of the three *ortho*-coupling protons at δ 7.41. Furthermore, another proton of the three *ortho*-coupling protons at δ 7.71 was correlated to the carbonyl carbon at δ 180.1. The above showed that the methoxyl group was attached to C_5 of the xanthone. The orientation of the other aromatic ring was determined as follows. In the HMBC spectrum, the hydrogen of the chelated hydroxyl at δ 13.18 was correlated to three aromatic carbons at δ 98.3, 102.1 and 162.4. The carbon at δ 98.3 was further correlated to the hydrogen of the other chelated hydroxyl at δ 12.98. The carbon at δ 98.3 was also observed to have correlation with an aromatic proton at δ 6.20 in the HMQC spectrum. Furthermore, the enhancement of the two chelated hydroxyl signals in NOE spectrum was observed when the aromatic proton at δ 6.20 was irradiated (Fig. 1). These results indicated that **1** was a 1,3-dihydroxy-5-methoxyxanthone derivative. Therefore the last substituent SO_3K was determined to be located at C_4. To the best of our knowledge, this is the first sulfonated xanthone isolated from plants.

Acid hydrolysis of **1** yielded **1a** and sulfonate, the latter being detected in the water layer as a white precipitate when $BaCl_2$ was added (Sanchez-Contreras et al., 2000). **1a** had a $[M]^+$ of *m/z* 258.0518 in the HREIMS, which corresponds to $C_{14}H_{10}O_5$ (calcd. 258.0528). Its structure was determined as 1,3-dihydroxy-5-methoxyxanthone from analysis of its ^1H NMR spectrum. Thus, **1** was elucidated as 1,3-dihydroxy-5-methoxyxanthone-4-sulfonate.

Compound **2** was isolated as a yellow powder, readily soluble in water and had a high melting point (>360 °C); an atomic absorption analysis indicated the presence of K^+. Its molecular formula was established as $C_{19}H_{17}O_{13}SK$ by HRFAB mass spectral analysis (positive mode) (*m/z* 562.9658 $[M + K]^+$, $C_{19}H_{17}O_{13}SK_2$,



1. R_1=SO_3K, R_2=CH_3
1a. R_1=H, R_2=CH_3
2. R_1=SO_3K, R_2=β-D-glucopyranosyl

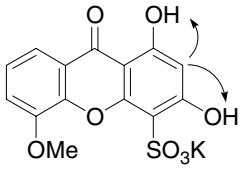


Fig. 1. NOE-Difference correlations of **1**.

calcd. 562.9664); and 525.0121 $[M + H]^+$ (*m/z* $C_{19}H_{18}O_{13}SK$, calcd. 525.0105) and from its ESI mass spectrum (*m/z* 1087 $[2M + K]^+$, 1071 $[2M + Na]^+$, 563 $[M + K]^+$ and 547 $[M + Na]^+$. The UV and IR spectra were similar to those of **1**, which suggested that **2** was also a 1,3,5-trioxygenated xanthone-4-sulfonate derivative. The ^1H NMR spectrum resembled close that of **1** except that the methoxyl signal at C_5 was replaced by a sugar moiety. Acid hydrolysis of **2** yielded D-glucose. The β configuration of the D-glucoside was established from the large coupling constant value ($J = 7.4$ Hz) of the anomeric proton at δ 4.91. Analysis of the ^{13}C NMR data (Table 1) allowed assignment of the pyranose form of the D-glucose. A ^3J correlation between this anomeric proton at δ 4.91 and the aromatic carbon at δ 146.6 (C_5) determined that the β-D-glucopyranosyl moiety was attached to the xanthone at C_5. Hence, compound **2** was determined to be 5-*O*-β-D-glucopyranosyl-1,3-dihydroxyxanthone-4-sulfonate.

Compounds **1** and **2** were evaluated for cytotoxicity proportion against the P388 cell line, and were found to be moderately active (ED_{50} of 3.46 and 15.69 μmol/L, respectively). By contrast, VP-16 (positive control) had an ED_{50} of 0.064 μmol/L).

Accordingly, as results of this investigation, the structures of two new sulfonated xanthones from *H. sampsonii* were identified, with each having moderate cytotoxicity against P388 cancer cell line. To our knowledge, this is the first report of sulfonated xanthonoids.

3. Experimental

3.1. General

EIMS were observed using a Micromass VG 7035 mass spectrometer at 70 ev, Whether ESIMS were recorded using a LCQ™ mass spectrometer and FABMS

Table 1
NMR Spectroscopic data for compounds **1**, **1a** and **2**

Position	1			2			1a	
	H[a]	^{13}C[b]	HMBC[c]	^{1}H[a]	^{13}C[b]	HMBC[c]	^{1}H[d]	^{1}H[a]
1		162.4			162.6			
2	6.20 *s*	98.3	1, 3, 4, 9a	6.23 *s*	98.2	1, 4, 9a	6.29 *d* (2.0)	5.95 *d* (1.6)
3		162.4			162.2			
4		110.6			110.0		6.50 *d* (2.0)	6.13 *d* (1.6)
4a		154.3			154.0			
5		148.3			146.6			
6	7.52 *dd* (8.0, 1.6)	120.4	5, 8, 10a	7.75 *dd* (7.9, 1.0)	122.8	8, 10a	7.46 *dd* (8.0, 1.0)	7.43 *dd* (8.0, 1.0)
7	7.41 *t* (8.0)	124.5	5, 6, 8a	7.43 *t* (8.0)	124.5	5, 8a	7.37 *t* (8.0)	7.32 *t* (8.0)
8	7.71 *dd* (8.0, 1.6)	116.1	8a, 9, 10a	7.80 *dd* (8.0, 1.2)	118.0	6, 9, 10a	7.74 *dd* (8.0, 1.0)	7.62 *dd* (8.0, 1.0)
8a		119.0			120.3			
9		180.1			179.9			
9a		102.1			102.1			
10a		146.0			146.1			
1-OH	13.18 *s*		1, 2, 9a	12.95 *s*		1, 2, 9a	12.92 *s*	12.86 *s*
3-OH	12.98 *s*		2, 3, 4	12.57 *s*		2, 3, 4		
5-OCH_3	4.00 *s*	57.4	5				4.21 *s*	3.96 *s*
5-Glu								
G1				4.91 *d* (7.4)	104.0	5		
G2				3.39 *m*	73.9			
G3				3.29 *m*	75.3			
G4				3.19 *m*	69.8			
G5				3.39 *m*	77.4			
G6				3.78 *dd* (11.0, 5.6)	60.9			
				3.52 *dd* (11.0, 6.0)				

[a] Recorded in DMSO-d_6 at 300 MHz.
[b] Recorded in DMSO-d_6 at 75 MHz.
[c] Carbons that correlated with the proton resonance.
[d] Recorded in acetone-d_6 at 500 MHz.

on a MAT 95XL-T mass spectrometer. NMR spectra were acquired on a Bruker ACF 300 [300 MHz (^{1}H) and 75 MHz (^{13}C)] and AMX 500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] instruments using DMSO-d_6 and acetone-d_6 solutions with TMS as an internal standard. IR spectra were recorded on a Bio-Rad FTIR spectrophotometer, whereas UV spectra were obtained on a Hewlett–Packard 8452A diode array spectrophotometer. Atomic absorption analyses were carried out using a Hitachi Z-5000 spectrometer. Chromatographic separations utilised Sephadex LH-20 (25–100 μm, Merck, Darmstadt, Germany).

3.2. Plant material

The whole plant of *H. sampsonii* was collected from Jinhua, Zhejiang Province, PR China in August 1997 and was identified by Associate Professor Jin-Gui Shen of the Shanghai Institute of Materia Medica. A voucher specimen was deposited at the herbarium of National Center for Drug Screening (Accession No. PC-1997-2H), Chinese Academy of Sciences, Shanghai, PR China.

3.3. Extraction and isolation

The whole air-dried ground plant material (5.0 kg) was extracted at room temperature with EtOH–H_2O (95:5), for seven days and the extract was concentrated in vacuo. The concentrate was partitioned between H_2O and $CHCl_3$ and afforded insoluble residue (10 g), which displayed cytotoxicity against the P388 cell line. An aliquot (1 g) of the residue was dissolved in EtOH and subjected to Sephadex LH-20 chromatography, with EtOH/H_2O (1:1) as eluent to yield compounds **1** (37 mg), **2** (6 mg) and mangiferin (**3**, 425 mg). The water-soluble portion (40.0 g) was then separated into six fractions by a silica gel cc, eluted with different proportions of $CuCl_3$–MeOH (1:0, 20:1, 10:1, 5:1, 1:1, 0:1). Fraction 1 was subjected to Sephadex LH-20 cc, eluted with EtOH–H_2O (95:5) to give **7** (7 mg) and **8** (113 mg). Whereas, fraction **3** was fractionated in an identified, manner to give **5** (70 mg). Fraction 4 cc gave mixtures I and II following the same chromatographic procedure. Further preparation of mixture I by ODS column chromatography, eluted with acetone–H_2O (65:35) affected **10** (7 mg), **11** (5 mg) and **4** (83 mg), respectively. Mixture II was separated on an ODS column, eluted with acetone–H_2O (55:45) to give **6** (4 mg) and **9** (8 mg).

3.3.1. 1,3-Dihydroxy-5-methoxyxanthone-4-sulfonate (*1*)

Yellow needles, m.p. > 360 °C. HRFABMS: *m/z* 414.9281 $[M + K]^+$, $C_{14}H_9O_8K_2S$ requires 414.9292; 398.9529 $[M + Na]^+$, $C_{14}H_9O_8KNaS$ requires 398.9553

and 376.9714 $[M + H]^+$, $C_{14}H_{10}O_8KS$ requires 376.9734. FAB-MS (positive mode, matrix: 3-nitrobenzyl alcohol) *m/z*: 415, 399, 377, 345, 286, 231, 192. ESIMS *m/z*: 791, 415, 171. UV λ_{max}^{EtOH} nm (log ε): 206 (4.16), 224 (4.15), 248 (4.28), 310 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3534, 3473, 1659, 1570, 1497, 1424, 1269, 1146. For ^1H and ^{13}C NMR spectral data, see Table 1.

3.3.2. Acidic hydrolysis of **1**

To a solution of **1** (5 mg) MeOH (2 mL), was added 3% HCl (5 mL), with the whole heated until reflux began this being maintained for 30 min. After evaporation of the MeOH, the **1a** so formed was extracted with EtOAc and purified by preparative TLC of silica gel eluted with $CHCl_3$–MeOH (10:1). The sulfonate was detected in the water layer as a white precipitate when adding $BaCl_2$.

3.3.3. 1,3-Dihydroxy-5-methoxyxanthone (**1a**)

Yellow needles, m.p. 228–229 °C (dec.). HREIMS: *m/z* 258.0518 $[M]^+$, $C_{14}H_{10}O_5$ requires 258.0528. EIMS *m/z*: 258, 243, 229, 215, 187, 129, 57. UV λ_{max}^{EtOH} nm (log ε): 207 (4.06), 241 (3.77), 313 (3.33). IR ν_{max}^{KBr} cm^{-1}: 3433, 3219, 1645, 1613, 1573, 1508, 1292, 1274. For ^1H NMR spectral data, see Table 1.

3.3.4. 1,3-Dihydroxy-5-O-β-D-glucopyranosylxanthone-4-sulfonate (**2**)

Yellow powder, m.p. > 360 °C. $[\alpha]_D^{31.2}$ + 38.10 (*c*, 0.033, MeOH). HRFABMS: *m/z* 562.9658 $[M + K]^+$, $C_{19}H_{17}O_{13}K_2S$ requires 562.9664 and 525.0121 $[M + H]^+$, $C_{14}H_{10}O_8KS$ requires 525.0105. FABMS (positive mode, matrix: thioglycerol) *m/z*: 563, 547, 525, 475, 433, 401, 325, 295, 253, 187. ESIMS *m/z*: 1087, 1071, 563, 365, 171. UV λ_{max}^{EtOH} nm (log ε): 306 (4.15), 246 (4.43), 222 (4.32), 206 (4.28). IR ν_{max}^{KBr} cm^{-1}: 3455, 1644, 1578, 1065, 1026. For ^1H and ^{13}C NMR spectral data, see Table 1.

3.3.5. Acid hydrolysis of **2** (Hu et al., 1996)

A MeOH solution of **2**, together with the standard glucose, was applied at points about 1 cm from the bottom of a HPTLC Si gel plate and hydrolyzed with HCl vapour for 2 h at 50 °C. The plate was then heated at 60 °C for 2 h to remove residual HCl, and developed using $CHCl_3/CH_3OH/H_2O$ (8:2:0.1) as the eluent. The plate was sprayed with 10% H_2SO_4 (in EtOH), and then heated at 110 °C.

3.4. Bioassay

The P388 (mouse lymphocytic leukemia) cell line was used, with cell survival evaluated using the MTT-tetrazolium assay as described previously (Mosmann, 1983).

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