A New Xanthone from *Swertia Thomsonii*

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Summary: A new xanthone has been isolated from *Swertia thomsonii* C.B Clarke and its structure established as 1,7-dihydroxy-3,8-dimethoxyxanthone on the basis of spectral evidence, particularly 2D NMR. Two known compounds, swertianin (1,7,8- trihydroxy-3-methoxyxanthone) and ursolic acid have also been isolated from the same specie for the first time.

Introduction

The plants of the genus *Swertia* (family *Gentianaceae)* is a rich source of xanthonoids, flavonoids, irridoids and terpenoids. The herbs of this genus are extensively used as bitter tonic and febrifuge in the Ayurvedic system of medicine [1]. The extracts of a number of species have long been used in folk medicine for the treatment of hepatitis, cholecystitis, pneumonia, dysentery and spasm whereas recent investigation have shown that some xanthones possess a marked hypoglycemic activity when administered to rats [2,3]. Moreover some *swertiae* are reported to possess CNS-depressant [4,5] and antihepatitis principles [6]. *Swertia thomsonii* is a perennial herb, widely distributed in the northern areas of Pakistan [7]. Inspite of its extensive usage in folk medicine [1,2,8] no attempt was made to characterize its chemical constituents. In this paper we report the isolation and characterization of a new xanthone (1) in addition to two know compounds viz: 1,7,8-trihydroxy-3-methoxyxanthone (2) [9] and ursolic acid (3)[10].

Results and discussion

The ethanol extract of whole plant of *S. thomsonii,* after drying, was treated with aqueous acetic acid [4], filtered and washed. The filtrate was extracted with ether which on fractional crystallization yielded compound **1**.

The EIMS spectrum of compound **1** gave $[M^+]$ at m/z 288 which was further confirmed by FD mass spectrometry. The high resolution EI mass spectrum of **1** afforded the molecular formula $C_{15}H_{12}O_6$ (288.0705) suggesting ten double bond equivalents in the molecule. The peak at m/z 270.0453 resulted by the loss of water molecule from the molecular ion peak, has the composition $C_{15}H_{10}O_5$. The UV spectrum showed absorption maxima at 239, 261,310 and 378 nm in ethanol, indicated the presence of xanthone nucleus [11,12], and it was very useful in locating the oxygen substituents in rings A and C, particularly the presence of free hydroxyl groups at C-3 and C-6. It is known that if the hydroxyl group is present at C-3 or C-6

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positions then the addition of few drops of NaOH or NaOAc in ethanol gives rise to a bathochromic shift with increase in intensity (300 - 330 nm) in the UV spectrum [11-13]. However if the C-3 and C-6 positions are blocked (by methoxy or glycosyl groups) then no change occurs in the UV curve by the addition of the base. Thus the UV spectrum of **1** was recorded after addition of a few drops of NaOH solution in ethanol which showed no bathochromic shift. This established that in **1** there is neither hydroxyl group at C-3 not at C-6. The IR spectrum showed bands at 1660 and 3450 cm^{-1} suggesting carbonyl and hydroxyl groups in the molecule. The tetraoxygenated pattern in the xanthone nucleus was revealed by its ^1H NMR spectrum in which two *meta* coupled proton signals appeared at δ 6.29 and 6.49 (J=2.0 Hz) and two *ortho* coupled proton signals at δ 7.19 and 7.34 (J=9.1 Hz), in addition to two each three proton singlets at δ3.80 and 3.85 due to methoxyl groups and two singlets at δ9.55 and 13.26 due to phenolic hydroxyl groups. The downfield value of 13.26 indicated the presence of a chelated phenolic hydroxyl group which could be located at C-1 or C-8. The ^{13}C NMR spectrum of compound **1** (125 MHz, DMSO-d_6) showed 15 carbon resonances (Table-1). The multiplicities were determined by DEPT experiment [14,15] which revealed the presence of two methyls, four methines and nine quaternary carbon resonances. The values (ppm) were assigned to different carbon atoms on the basis of comparison with other tetraoxygenated xanthones [16,17]. Four methine resonances at δ96.72, 91.81, 113.07 and 124.38 were ascribed to C-2, C-4, C-5 and C-6 respectively on the basis of DEPT and HMQC experiments (Table-2).

Table 1:^{13}C NMR sepctral data of compound **1** and **2** (500 MHz;DMSO-d_6):

	1		2	
C.No.	^{13}C-value (ppm)(BB)	C-State (DEPT)	^{13}C-Value (ppm)(BB)	C-State (DEPT)
1.	162.65	C	161.83	C
2.	96.72	CH	97.05	CH
3.	166.52	C	166.97	C
4.	91.81	CH	92.66	CH
4a.	157.25	C	157.62	C
5.	113.07	CH	105.83	CH
5a.	149.70	C	147.02	C
6.	124.38	CH	124.09	CH
7.	144.80	C	140.42	C
8.	147.25	C	147.91	C
8a.	105.72	C	107.29	C
9.	185.38	C=0	184.12	C=0
9a.	103.25	C	101.68	C
Ar-OMe	56.01	OMe	56.08	OMe
Ar-OMe	61.01	OMe		

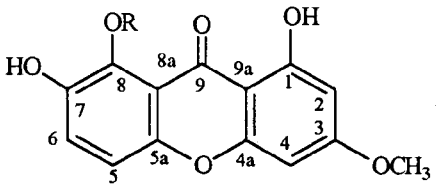
Table-2:2D-NMR spectral data of **1** (^1H-^{13}C coupling) (125 MHz; DMSO-d_6)

^{1}HNMR (δ)	Adjacent interacting carbon (δ) (HMQC)	Neighbouring interacting carbons (δ) (HMBC)
3.80 (H; OMe)	61.01 (C;OMe)	144.80(C-7) 105.72 (C-8a),
3.84 (H; OMe)	56.01 (C; OMe)	166.52 (C-3)
6.29 (H-2)	96.72 (C-2)	162.65 (C-1), 166.52 (C-3), 91.81 (C-4), 103.25 (C-9a)
6.49 (H-4)	91.81 (C-4)	96.72 (C-2), 166.52 (C-3), 157.25 (C-4a), 103.25 (C-9a)
7.19 (H-5)	113.07 (C-5)	149.70 (C-5a), 124.38 (C-6), 147.25 (C-8)
7.34 (H-6)	124.38 (C-6)	113.07 (C-5), 149.70 (C-5a) 144.80 (C-7)
9.55 (H;7-OH)	-	124.38 (C-6), 144.80 (C-7), 147.25 (C-8)
13.25 (H;1-OH)	-	162.65 (C-1), 96.72 (C-2), 103.25 (C-9a)

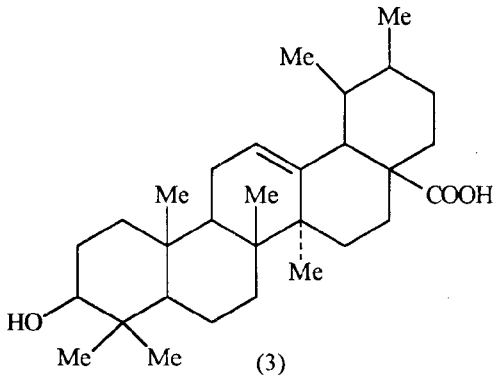
In order to establish the positions of methoxyl and hydroxyl groups, HMBC ($^1H/^{13}C$ long range shift correlation) experiment [18] was carried out (Table 2) which showed that the proton at δ 13.26 (1-OH) was coupled to carbons at δ 162.6 (C-1), 103.25 (C-9a) and 96.72 (C-2) whereas the proton at δ 9.55 (7-OH) was coupled with the carbons at δ 124.38 (C-6), 144.80 (C-7) and 147.25 (C-8) assigning the placement of these hydroxyl groups at C-1 and C-7 respectively. The methoxyl protons at δ 3.80 (8-OCH_3) showed coupling with the carbons at δ 144.80 (C-7) and 105.72 (C-8a) assigning its location at C-8, while the methoxyl protons at δ 3.84 (3-OCH_3) showed coupling with the carbon at δ 166.52 assigning its attachment at C-3. Similarly the proton at δ 6.29 (H-2) showed interactions with carbons at δ 162.65 (C-1), 166.52 (C-3), 91.81 (C-4) and 103.25 (C-9a) whereas the proton at δ 6.49 (H-4) showed coupling with the carbons at δ 96.72 (C-2), 166.52 (C-3), 157.25 (C-4a) and 103.25 (C-9a), which confirmed their assignments. On the other hand the proton at δ 7.19 (H-5) have cross peaks exhibiting interactions with carbons at δ 149.70 (C-5a) and 124.38 (C-6) while the proton at δ 7.34 (H-6) have interactions with carbons at δ 113.07 (C-5) and 144.80 (C-7) strengthening their attributions. On the basis of these spectral data the structure of compound **1** has been elucidated as 1,7-dihydroxy-3,8-dimethoxyxanthone, which has not been reported earlier. Its exact ^1H and ^{13}C NMR spectral assignment has been done with the aid of 2D-NMR experiments particularly HMQC and HMBC (Table-2).

On the basis of EIMS, ^1H NMR, ^{13}C NMR (Table-1) and 2D NMR data (Table- 3) another

xanthone (2), isolated from the $CHCl_3$ extract of the whole plant has been identified as 1,7,8-trihydroxy-3-methoxyxanthone [9], while ursolic acid (3) obtained from the n-hexane extract of whole of the plant was identified on the basis of EIMS and ^1H NMR spectral correlations [10,19,20]. These two compounds were isolated for the first time from *Swertia thomsonii*.



(1) R = CH_3
(2) R = H



(3)

Experimental

Plant material

Swertia thomsonii (whole plant), collected in the month of August from Skurdu, NWFP, Pakistan, was identified by Mr. Shahid Farooq, Plant Texonomist, PCSIR, Peshawar, and a voucher specimen (No: 9704) of the plant was deposited in the Herbarium of PCSIR Peshawar.

Melting points were determined on electrothermal melting point apparatus and are uncorrected. UV spectra were recorded in ethanol on a Shimadzu UV-160 spectrophotometer. I.R. spectra were recorded in KBr on a Unican SP 1000 instrument. HREIMS spectra were determined on a JEOL JMS-110 mass spectrometer. EIMS and FDMS spectra were recorded on Varian MAT 112 and 312 double focusing mass spectrometer. NMR spectra including DEPT and 2D experiments were recorded in $CDCl_3$ and d_6-DMSO using TMS as internal standard, on Bruker AM-400 and 500 instruments, operating at 400 and 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. Silica gel used for column chromatrography was kieselgel 60 (70-230 mesh; Merck) and for preparative TLC was Kieselgel 60 $PF_{254+366}$ (Merck). TLC was conducted on precoated silica gel F_{254} aluminum sheets (0.25 mm). The TLC chromatograms were visualized by UV light, exposure to I_2 vapour and spraying with 1% ceric sulphate in 1M H_2SO_4.

Table-3:2D-NMR spectral data of **2** (^1H-^{13}C coupling) (125 MHz; DMSO-d_6)

^1H NMR (δ)	Adjacent interacting carbon (δ) (HMQC)	Neighbouring interacting carbons (δ) (HMBC)
3.87 (H;OMe)	56.00 (C; OMe)	166.97 (C-3)
6.35 (H-2)	97.05 (C-2)	161.8 (C-1), 166.9 (C-3), 92.6 (C-4), 101.68 (C-9a)
6.56 (H-4)	92.66 (C-4)	97.05 (C-2), 166.9 (C-3) 157.62 (C-4a), 101.68 (C-9a)
6.87 (H -5)	105.83 (C-5)	147.02 (C-5a), 140.42 (C-7) 107.29 (C-8a)
7.28 (H-6)	124.09 (C-6)	147.02 (C-5a), 140.42 (C-7) 147.91 (C-8)
9.36 (H;7-OH)	-	140.42 (C-7)
11.63 (H;1-OH)	-	161.83 (C-1), 97.05 (C-2) 101.68 (C-9a)
11.85 (H;8-OH)	-	140.42 (C-7), 107.29 (C-8a)

Isolation

Air dried ground material of whole plant of *S. thomsonii* (0.6 kg) was extracted with n-hexane, followed by extraction with $CHCl_3$ and EtOH and each extract was concentrated *in vacuo*. The residue obtained from the hexane extract was divided into $CHCl_3$ soluble and insoluble fractions. The $CHCl_3$ soluble portion, on subjecting to preparative TLC (Kieselgel 60 $PF_{254+366}$), using $CHCl_3$/pet. ether (9:1) solvent system afforded **3** (300 mg).

The residue obtained from the chloroform extract was chromatographed on a silica gel column using Kieselgel 60, 70-230 mesh. The column was first eluted with $CHCl_3$ and then $CHCl_3$/MeOH (9:1) solvent mixture. No elution of compounds occured at this stage when the fractions were checked by TLC. The polarity was then enhanced by applying $CHCl_3$/MeOH solvent mixture in the ratio of 85:15 and allowed two litre solvent mixture to pass through the column. After collection of the two litre solvent mixture, a half litre fraction was collected and

evaporated, which on crystallization from MeOH afforded **2** (200 mg).

The ethanol extract was dried (70 g), treated with aq. AcOH (5%, pH-6) and allowed to stand for 48 hours. The solid mass was filtered and the filtrate was extracted with diethyl ether. The ether part showing three spots, was concentrated (50%) and allowed to cool. Some solid residue was obtained which was removed by filteration. The mother liquor was concentrated again and filtered similarly. Further concentration of the mother liquor afforded **1** which was further purified by recrystallization from methanol (100 mg).

Compound 1 (1,7 dihydroxy-3, 8-dimethoxy-xanthone)

Pale yellow needles, mp: 183°; TLC solvents: $CHCl_3$-C_6H_6 (6:4), Rf: 0.10 and C_6H_6-MeoH (4:1), Rf: 0.66; UV λ_{max}^{EtOH} nm: 239, 261, 310,378; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1665 (C=0), 1610, 1585 (C=C), 1510, 1470, 1320, 1280, 1170, 1060, 960; EIMS, m/z (rel. int.,%): 288(88) $[M]^+$, 270 (100), $[M-H_2O]^+$, 245 (43), 202 (16), 123 (8); **FDMS**; m/z: 288 $[M]^+$; **HREIMS**; m/z: 288:0705 (mol. formula: $C_{15}H_{12}O_6$,calc. for 288.0912), 270.0453 ($C_{15}H_{10}O_5$), 245.0413 ($C_{13}H_9O_5$); **^{1}H NMR** (400 MHZ, DMSO-d_6) δ:3.80(3H,s,8-OMe), 3.84 (3H, s, 3-OMe), 6.29 (1H,d,J=2.0 Hz, H-2), 6.49 (1H,d,J=2.0 Hz, H-4), 7.19 (1H,d,J=9.1Hz, H-5), 7.34 (1H,d, J= 9.1 Hz, H-6), 9.55 (1H, s, 7-OH), 13.25 (1H, s,1-OH); ^{13}C NMR: Table-1; HMQC and HMBC: Table-2.

Compound 2 (Swertianin)

Yellow neeldes, mp 220° (lit. 221° [9]); TLC solvents: $CHCl_3$:-pet.ether (8:2), Rf: 0.10; $CHCl_3$:-benzene (1:1), Rf: 0.16; benzene:-MeOH (4:1), Rf: 0.72; UV λ_{max}^{MeOH} nm: 203, 239, 268, 327, 390; IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 3320, 3210, 1665 (C=0), 1645, 1610, 1585 (C=C), 1510, 1470, 1325, 1285, 1210, 1170, 1155, 1090, 1060, 965; **HREIMS**, m/z (%): 274.0477 (mol. formula: $C_{14}H_{10}O_6$, calc. for 274.0754); **FDMS**, m/z: 274 $[M]^+$; **EIMS**, m/z: 274 $[M^+]$ (100), 245 (19), 231 (10), 136 (5), 123 (10); **^{1}H NMR** (DMSO-d_6); 500 MHz): δ 3.87 (3H,s, Ar-OMe), 6.35 (1H, d, J = 2.2 Hz, H-2), 6.56 (1H, d, J= 2.2 Hz, H-4), 6.87 (1H, d, J= 8.9 Hz, H-5), 7.28 (1H, d, J= 8.9 Hz, H-6), 9.36 (1H,s,7-OH at C-7), 11.63 (1H, s, 1-OH), 11.85 (1H, s, 8-OH) ^{13}C NMR; 125 MHz: Table 1; HMQC and HMBC: Table 3.

Compound 3 (ursolic acid)

Fine hair-like needles, mp 286-288° (lit. 285-288°) [10]; TLC solvents: $CHCl_3$: pet.ether (9:1), Rf: 0.40. UV λ_{max}^{MeOH} nm : 204; IR ν_{max}^{KBr} cm^{-1} : 3400 (OH), 1700 (C=O), 1475, 1035; **HREIMS**, m/z (%): 456.3596 (mol. formula: $C_{30}H_{48}O_3$, cal. for 456.3774); EIMS, m/z (rel. int. %): 456 $[M^+]$ (5), 248 (100), 207 (22), 203 (28), 189 (7), 133 (20), 69 (8); **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 0.71 (1H, dd, J= 1.7, 11.9 Hz, α H-5), 0.76 (3H, s, Me-26), 0.77 (3H, s, Me-24), 0.84 (3H, d, J= 6.4 Hz, Me-29), 0.91 (3H, s, Me-23), 0.94 (3H, d, J= 6.3 Hz, Me-30), 0.97 (3H, s, Me-25), 1.07 (3H, s, Me-27), 2.18 (1H, d, J= 11.1Hz, H-18), 3.20 (1H, dd, J = 4.9, 11.2 Hz, αH-3), 5.24 (1H, t, J= 3.7 Hz, H-12).

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