

A Xanthone Dixylopyranoside from *Swertia thomsonii*

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Swertia thomsonii, Gentianaceae, Xanthone Xylopyranoside

A xanthone dixylopyranoside has been isolated from *Swertia thomsonii* C. B. Clarke and its structure established as 1,7-dihydroxy-3,8-dimethoxyxanthone 1-O- $[\beta$ -D-xylopyranosyl-(1 \rightarrow 4)- β -D-xylopyranoside on the basis of spectral evidence, particularly 2D NMR studies. Three known compounds, 1,7-dihydroxy-3,8-dimethoxyxanthone, swertianin (1,7,8-trihydroxy-3-methoxy-xanthone) and ursolic acid were also isolated from this species.

Introduction

The plants of the genus *Swertia* (family *Gentianaceae*) are rich sources 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 investigations have shown that some xanthonoids possess a marked hypoglycemic activity when administered to rats [2, 3]. Moreover some species of *Swertia* 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]. In continuation of our work on this species we now report the isolation and characterization of a new xanthone dixylopyranoside in addition to three previously reported xanthonoids [8].

Results and Discussion

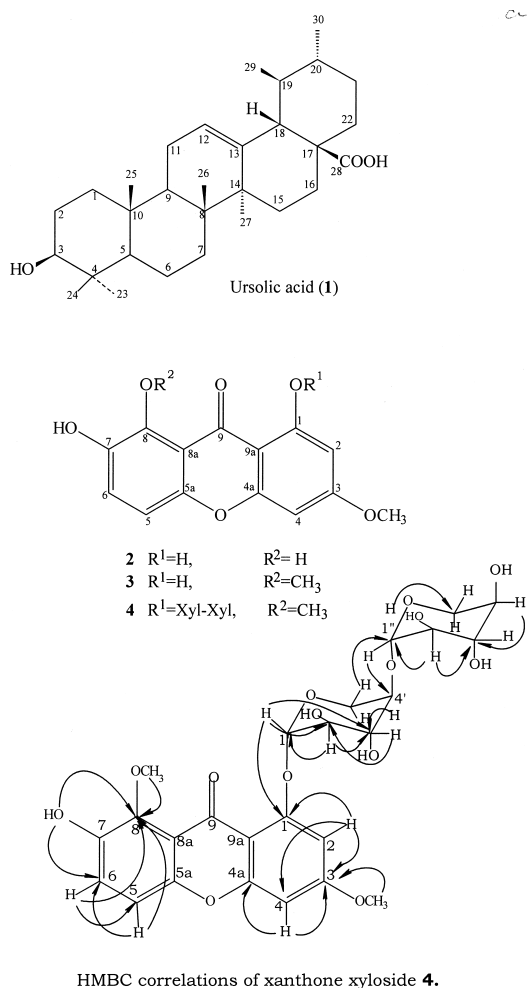
The ethanol extract of *S. thomsonii*, after drying and treatment with aqueous acetic acid, was filtered and washed with the acid. The residue, was crystallized from methanol/1,4-dioxane solvent mixture which afforded **4**, m.p. 215 °C. The FAB (+ve) mass spectrum of **4** showed the molecular ion peak at $m/z = 553$ ($M+1$)⁺. The EIMS of the compound showed the molecular ion peak of the aglycone ($M-264$)⁺ at $m/z = 288$. A base peak at

$m/z = 243$ arises due to the xanthone nucleus ($C_{13}H_7O_5$). The high resolution mass spectrum (HRMS) afforded the molecular formula of the aglycone as $C_{15}H_{12}O_6$ (288.0613). The HRMS also afforded the fragments $C_{14}H_{10}O_6$, $C_{15}H_{12}O_5$ (272.0704) and $C_{14}H_9O_5$ (257.0376), characteristic of the loss of methyl, hydroxy and methoxy groups, respectively from the parent molecule. The data suggested the presence of one hydroxy and two methoxy groups in **4**. UV absorption maxima at 300 and 246.5 nm, in methanol, indicate a xanthone chromophore. There was no bathochromic shift induced by the addition of sodium hydroxide. This established that in **4** there is neither a hydroxy group at C-3 nor at C-6 [9, 10]. The IR spectrum of the compound showed bands at 1610 cm^{-1} and 3450 cm^{-1} suggesting the presence of carbonyl and hydroxy groups in the molecule [11]. The ¹H NMR spectrum, in DMSO-*d*₆, showed two pairs of aromatic proton signals, of which two were *ortho* coupled and two protons *meta* coupled, revealing a tetraoxygenated pattern of the xanthone nucleus. Signals of a pair of *meta* coupled doublets ($J = 2.0$ Hz) at $\delta = 6.80$ and 6.82 were assigned to the protons 2-H and 4-H whereas the two signals of a pair of *ortho* coupled doublets ($J = 7.5$ Hz) at $\delta = 7.33$ and 7.41 were assigned to protons 5-H and 6-H, respectively. Two singlets appeared at $\delta = 3.94$ and 3.91 (3H each) and were assigned to methoxy groups attached at C-3 and C-8 carbon atoms of the xanthone nucleus, respectively. The presence of a phenolic group in **4** was

confirmed by the positive colour reaction with dilute FeCl_3 solution. Its location was assignable to C-7 carbon due to its upfield chemical shift (-2 ppm) as compared to those of bellidifolin, norswertianin and swertianin **2** [12]. This indicated “no chelation” with the carbonyl group of the xanthone nucleus assigning its connectivity at C-7, the only suitable location in the ring. Two doublets appearing at $\delta = 4.18$ ($J = 6.7$ Hz) and 4.88 ($J = 6.3$ Hz) arose due to two anomeric protons of the sugar residue. This suggested the presence of two sugar fragments in the compound. The connectivity of the sugar moiety was established with C-1 of xanthone nucleus which was the only possible

place for attachment. As there was no further vacancy in the ring for attachment of a second sugar fragment, it was therefore assumed that two sugar units could be bonded to one another through an oxygen atom. The ^{13}C NMR spectrum of **4** in DMSO-d_6 showed 25 carbon resonances (Table 1). The values were assigned to different carbon atoms on the basis of DEPT experiments and comparison with other tetraoxygenated xanthones [13–15]. The chemical shift due to C-1 of the xanthone moiety at $\delta = 159.19$ ppm was shifted upfield (-2.6) as compared with swertianin [12] indicating attachment of sugar at this position. Also the signal due to a carbonyl carbon (C-9) was shifted upfield (-9.6 ppm) to $\delta = 174.48$ suggesting no chelation with the hydroxy group at C-1 or C-8 positions [16, 17]. These data clearly established that the xylopyranosyl residue in **4** was attached to the C-1 of the xanthone nucleus through an oxygen atom. To establish the exact locations of substituents on the xanthone nucleus HMQC and HMBC experiments [18] were carried out (Table 2). The HMQC data of **4** showed that C-2, C-4, C-5 and C-6 carbon atoms of xanthone moiety possess hydrogen atoms while the other carbon atoms were quaternary in nature. HMBC experiments [18] of **4** established the 3-OMe and 4-OMe substitution (Table 2). The 7-OH substitution was established on the basis of equivalent long-range interaction of the OH proton ($\delta = 9.2$) with C-6 ($\delta = 123.80$) and C-8 ($\delta = 147.87$). The connectivity of the dixylopyranoside residue at C-1 can be assigned on the basis of interaction of the anomeric proton ($1'\text{-H}$) at $\delta = 4.88$ with the carbon at $\delta = 159.19$ (C-1), in addition to its interactions with C-2' ($\delta = 73.42$). The HMBC spectrum also showed an interaction of $1''\text{-H}$ ($\delta = 4.18$) with C-4' ($\delta = 69.87$), indicating $1''\rightarrow 4'$ linkage between the two xylose units.

Acid hydrolysis of **4** was achieved by refluxing with sulfuric acid (10%) at 100°C for 2 h [4]. The aglycone obtained in chloroform was compared and found identical with **3** through TLC under different solvent systems and mixed TLC. The sugar residue, obtained in the aqueous hydrolyzate was identified as xylose through paper chromatography. On the basis of these spectral and chemical evidences the compound was assigned structure **4**. To the best of our knowledge, it has not been encountered before in nature.



Scheme 1.

Carbon atom	2		3		4	
	δ [ppm]	Type	δ [ppm]	Type	δ [ppm]	Type
1	161.83	C	162.65	C	159.19	C
2	97.05	CH	96.72	CH	100.80	CH
3	166.97	C	166.52	C	164.80	C
4	92.66	CH	91.81	CH	95.72	CH
4a	157.62	C	157.25	C	158.33	C
5	105.83	CH	113.07	CH	115.84	CH
5a	147.02	C	149.70	C	153.71	C
6	124.09	CH	124.38	CH	123.80	CH
7	140.42	C	144.80	C	144.70	C
8	147.91	C	147.25	C	147.87	C
8a	107.29	C	105.72	C	108.59	C
9	184.12	C=O	185.38	C=O	174.48	C=O
9a	101.68	C	103.25	C	106.92	C
Ar-OMe	56.08	OMe	56.01	OMe	56.18	OMe
Ar-OMe	–	–	61.01	OMe	56.24	OMe
1'	–	–	–	–	102.55	CH
2'	–	–	–	–	73.42	CH
3'	–	–	–	–	75.94	CH
4'	–	–	–	–	69.87	CH
5'	–	–	–	–	65.62	CH ₂
1''	–	–	–	–	104.09	CH
2''	–	–	–	–	73.37	CH
3''	–	–	–	–	76.54	CH
4''	–	–	–	–	69.54	CH
5''	–	–	–	–	68.67	CH ₂

Table 1. ¹³C NMR spectral data of **2**, **3** and **4** (125 MHz, DMSO-*d*₆).

¹ H NMR, δ [ppm]	Adjacent interacting carbon, δ [HMQC]	Neighbouring interacting carbons, δ [HMBC]
3.91 (H; 8-OMe)	56.18 (C; OMe)	147.87 (C-8)
3.94 (H; 3-OMe)	56.24 (C; OMe)	164.80 (C-3)
6.80 (2-H)	100.80 (C-2)	159.19 (C-1), 164.80 (C-3), 95.07 (C-4), 106.92 (C-9a)
6.82 (4-H)	95.72 (C-4)	100.80 (C-2), 164.80 (C-3), 158.33 (C-4a)
7.41 (5-H)	115.84 (C-5)	123.80 (C-6), 144.70 (C-7), 147.87 (C-8)
7.33 (6-H)	123.80 (C-6)	115.84 (C-5), 147.87 (C-8)
9.20 (H; 7-OH)	–	123.80 (C-6), 147.87 (C-8)
4.88 (1'-H)	102.55 (C-1')	159.19 (C-1), 73.42 (C-2')
3.45 (2'-H)	73.42 (C-2')	102.55 (C-1'), 75.94 (C-3')
3.33 (3'-H)	75.94 (C-3')	73.37 (C-2')
3.25 (4'-H)	69.87 (C-4')	75.94 (C-3')
3.0, 3.7 (5'-H)	65.62 (C-5')	104.09 (C-1''), 76.54 (C-3'')
4.18 (1''-H)	104.09 (C-1'')	68.67 (C-5''), 69.87 (C-4'')
2.97 (2''-H)	73.37 (C-2'')	104.09 (C-1''), 76.54 (C-3'')
3.08 (3''-H)	76.54 (C-3'')	73.37 (C-2'')
3.62 (4''-H)	69.54 (C-4'')	104.09 (C-1''), 76.54 (C-3'')
3.97, 3.65 (5''-H)	68.67 (C-5'')	–

Table 2. 2D-NMR Spectral data of **4** (¹H–¹³C interactions).

Three more compounds isolated from *n*-hexane, chloroform and ethanol extracts of the whole plant were identified as ursolic acid (**1**), swertianin (**2**) and 1,7-dihydroxy-3,8-dimethoxy xanthone (**3**) through spectroscopic studies, respectively [8].

Experimental Section

General

Melting points were determined on an electrothermal melting point apparatus and are uncor-

rected. UV spectra were recorded in ethanol on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded in KBr on a Unicam 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 CDCl₃ and DMSO-d₆ 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 ¹³C NMR. Silica gel used for column chromatography was Kieselgel 60 (70–230 mesh; Merck), and for preparative TLC Kieselgel 60 PF₂₅₄₊₃₆₆ (Merck) was used. TLC was conducted on precoated silica gel F₂₅₄ aluminum sheets (0.25 mm). The TLC chromatograms were visualized by UV light, exposure to I₂ vapour and spraying with 1% ceric sulphate in 1 M H₂SO₄.

Plant material

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

Isolation

Air-dried whole plants (1.4 kg) were extracted with *n*-hexane, followed by extraction with CHCl₃ and EtOH and each extract was concentrated *in vacuo* (flow sheet). The residue obtained from the hexane extract was divided into CHCl₃ soluble and insoluble fractions. The CHCl₃ soluble portion, on subjecting to preparative TLC (Kieselgel 60 PF₂₅₄₊₃₆₆), using CHCl₃/petroleum ether (9:1) solvent system afforded **1** (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 CHCl₃ and then with CHCl₃/MeOH (9:1) solvent mixture. No elution of compounds occurred at this stage when the fractions were checked by TLC. The polarity was then enhanced by applying CHCl₃/MeOH solvent mixture in the ratio of 85:15 and the solvent mixture (2 l) was passed through the column. A half-liter 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 6.0) and allowed to stand

for 48 h. 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 filtration. The mother liquor was concentrated again and filtered similarly. Further concentration of the mother liquor afforded **3** which was further purified by recrystallization from methanol (100 mg).

The solid residue, obtained from aq. AcOH, was dissolved in 100 ml of MeOH/1,4-dioxane (1:1) solution and filtered. The filtrate was concentrated under vacuum which afforded **4**. The compound was further purified by recrystallization from methanol (200 mg).

1,7-Dihydroxy-3,8-dimethoxyxanthone 1-O-[[β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside (4)

Pale yellow needles, m.p. 215–216 °C; TLC solvent system: CHCl₃/MeOH (10:3), *R_f*: 0.34; UV (MeOH): λ_{max} = 246.5, 260, 300 nm. IR (KBr): ν_{max} = 3450 (OH), 1640 (C=O), 1610, 1580 (C=C), 1460, 1280, 1190, 1140, 1080, 1030 cm⁻¹. ¹H NMR (DMSO-d₆, 300 MHz): δ = 2.97–3.97 (br, 10H, sugar protons), 3.91 (3H, s, Ar-OMe), 3.94 (3H, s, Ar-OMe), 4.18 (1H, d, *J* = 6.7 Hz, 1''-H), 4.88 (1H, d, *J* = 6.3 Hz, 1'-H), 6.80 (1H, d, *J* = 2.0 Hz, 2-H), 6.82 (1H, d, *J* = 2.0 Hz, 4-H), 7.33 (1H, d, *J* = 7.8 Hz, 6-H), 7.41 (1H, d, *J* = 7.5 Hz, 5-H), 9.2 (1H, s, Ar-OH). HRMS *m/z*: 288.0613 (C₁₅H₁₂O₆); FAB (positive ion) *m/z*: 553 (M+1). EIMS *m/z* (rel. int. %) = (M⁺ absent), 288 (M-264)⁺ (7), 273 (29), 272 (82), 257 (10), 243 (100), 229 (19), 201 (11), 136 (22), 107 (16), 79 (16), 73 (42), 69 (15), 63 (13), 57 (20). ¹³C NMR (75 MHz): Table 1. HMQC and HMBC: Table 2.

Acid hydrolysis [4]

Compound **4** (10 mg) was hydrolyzed with H₂SO₄ (10%, 2 ml) by refluxing on a steam bath for 2 h. The resulting mixture was diluted with H₂O (5 ml) and extracted with CHCl₃ (10 ml). The solvent was evaporated and the resulting residue was crystallized from MeOH. Co-TLC, mixed TLC, m.p. mixed m.p. indicated that the aglycone was identical with **3** [8]. The aqueous hydrolyzate was subjected to paper chromatography using *n*-BuOH/ AcOH/H₂O (4:1:5) and the organic phase of *n*-BuOH/EtOH/H₂O/NH₃ (40:10:49:1) as solvent systems and found to be identical with D-xylose by comparison with an authentic sample. The spots were developed by spraying with silver nitrate/ammonia reagent and heating at 110 °C.

- [1] S. R. Dalal, S. Setha, R. C. Shah, *J. Ind. Chem. Soc.* **30**, 457 (1953).
- [2] B. Mukherjee, S. K. Bukherjee, *Int. J. Crude Drug Res.* **25**, 97 (1987).
- [3] P. Basnet, S. Kadota, M. Shimizu, T. Namba, *Planta Med.* **60**, 507 (1994).
- [4] S. Ghosal, P. V. Sharma, R. K. Chaudhuri, *J. Pharm. Sci.* **63**, 1286. (1974).
- [5] M. C. Ing, K. G. Hong, C. N. Lin, I. J. Chen, Kao-hsiung I Hsueh Ko' Hsuch Tsa Chih **2**, 131 (1986); *Chem. Abstr.* **105**, 91124u (1986).
- [6] D. Jing-Ye, S. Hungfa, *Chung Tsao Yao* **11**, 391 (1980); *Chem. Abstr.* **94**, 90124m (1981).
- [7] R. R. Stewart, in S. I. Ali and E. Nasir (eds): *Flora of West Pakistan. An Annotated Catalogue of the Vascular Plants of W. Pakistan and Kashmir*, p. 561, Fakhri Printing Press, Karachi (1972).
- [8] Inayat-ur-Rahman, M. Arfan, M. I. Choudhary, *J. Chem. Soc. Pak.* **20**, 155 (1998).
- [9] K. Hostettman, A. Jacot-Guillarmod, V. M. Chan, *Helv. Chim. Acta* **59**, 2592 (1976).
- [10] A. A. L. Mesquita, D. D. Correa, O. R. Gottlieb, M. T. Magalhaes, *Ann. Chem. Acta* **42**, 311 (1968).
- [11] H. M. Zhou, Y. L. Liu, G. Blasko, G. A. Cordell, *Phytochemistry* **28**, 3569 (1998).
- [12] H. Kanamori, I. Sakamoto, M. Mizuta, K. Hashimoto, O. Tanaka, *Chem. Pharm. Bull.* **32**, 2290 (1984).
- [13] I. Sakamoto, T. Tanaka, O. Tanaka, T. Tomimori, *Chem. Pharm. Bull.* **30**, 4088 (1982).
- [14] R. K. Asthana, N. K. Sharma, D. K. Kulshrestha, S. K. Chatterjee, *Phytochemistry* **30**, 1037 (1991).
- [15] A. Agrawal, J. Singh, *Phytochemistry* **27**, 3692 (1988).
- [16] T. Hayashi, T. Yamagishi, *Phytochemistry* **27**, 3696 (1988).
- [17] I. Miura, K. Hostettmann, K. Nakanishi, *Nouv. J. Chim.* **2**, 653 (1981).
- [18] M. R. Bendall, D. T. Pegg, D. M. Doddrell, *J. Magn. Reson.* **52**, 81 (1983).