PERGAMON

Phytochemistry 61 (2002) 415–420

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Xanthones from *Swertia punctata*

Nebojša Menković[a], Katarina Šavikin-Fodulović[a], Vanja Bulatović[a], Ivana Aljančić[d], Nenad Juranić[c], Slobodan Macura[c], Vlatka Vajs[b], Slobodan Milosavljević[d,*]

[a]*Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Yugoslavia*
[b]*Institute for Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Yugoslavia*
[c]*Department of Biochemistry and Molecular Biology, Mayo Foundation, 200 First Street, SWGugg. CL009B, Rochester, MN 55905, USA*
[d]*Faculty of Chemistry, University of Belgrade, Studentski trg 16, PO Box 158, 11001 Belgrade, Yugoslavia*

Received 21 March 2002; received in revised form 11 June 2002

Abstract

Isolation of 1-*O*-primeverosyl-3,8-dihydroxy-5-methoxyxanthone and 1-*O*-gentiobiosyl-3,7-dimethoxy-8-hydroxyxanthone, along with five known xanthones, isobellidifolin, methylbellidifolin, isoswertianin, methylswertianin and norswertianin-1-*O*-β-D-glucoside, from the roots of *Swertia punctata* is reported. In the aerial parts four xanthones, bellidifolin, methylbellidifolin, swertianolin and mangiferin, and flavone-*C*-glucoside, isoorientin were identified. The chemotaxonomic and pharmacological significance of these results is discussed.

Keywords: *Swertia punctata*; Gentianaceae; Tetraoxygenated xanthones; 1-*O*-Primeverosyl-3,8-dihydroxy-5-methoxyxanthone; 1-*O*-Gentiobiosyl-3,7-dimethoxy-8-hydroxyxanthone

1. Introduction

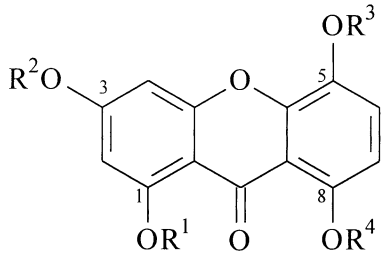
Due to numerous pharmacological properties of its constituents, genus *Swertia*, comprising ca. 170 species, has received considerable attention so far (Neerja et al., 2000; Peres et al., 2000). Some members of the genus such as *S. japonica*, *S. chirata*, *S. hookeri*, *S. macrosperma*, *S. petiolata* and *S. calycina* have been used in traditional medicine of the Far East for many years. Among their active principles xanthones (mostly 1,3,7,8- and 1,3,5,8-tetraoxygenated) occupy an important role, exhibiting various activities, e.g. antidepressant, antileukaemic, antitumor, antitubercular, choleretic, diuretic, antimicrobial, antifungal, antiinflammatory, antiviral, cardiotonic, hypoglycaemic, etc. (Neerja et al., 2000; Basnet et al., 1994). The study of xanthones, the secondary metabolites occurring in a limited number of families, is also interesting from a chemosystematic point of view. They were found to be more useful chemotaxonomic markers in comparison to co-occurring secoiridoids and flavone-*C*-glucosides. The oxidation pattern of xanthones is generally uniform within a particular section and is of prime importance for chemotaxonomy.

Among the Europaean *Swertia* species, only *S. perennis* is officially recognized, whereas to *S. punctata* Baumg. (together with *S. alpestris* and *S. obtusa*) a provisional status has been assigned (Tutin, 1972). On the other hand, Vladimirov and Tan (1998) claimed recently that *S. punctata* growing on the moisty terrains of the Western Stara Planina mountain, situated between Bulgaria and Yugoslavia, is the well and precisely defined plant species differing from *S. perennis* occuring in Rila, Vitosha and Pirin mountains (Bulgaria). *S. punctata* is also described by Jovanović-Dunjić (1973) as the only species of the genus occurring in Serbia.

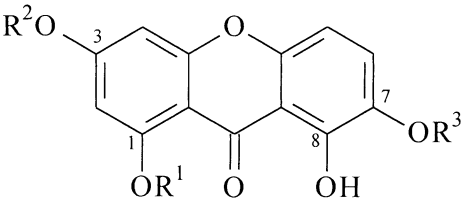
Our preliminary LC/DAD investigations (Šavikin-Fodulović et al., 2002) of the xanthone content of *S. punctata* originating from Stara Planina, revealed a number of 1,3,7,8- and 1,3,5,8,-tetraoxygenated xanthones in roots and also in the aerial parts (Fig. 1). Due to a possible chemosystematic and pharmacological importance of these findings, we have repeated more thoroughly the investigation of both aerial parts and roots of *S. punctata* collected at the same locality as before.

* Corresponding author. Tel.: +381-11-630-474; fax: +381-11-636-061.
E-mail address: smilo@chem.bg.ac.yu (S. Milosavljević).

0031-9422/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00231-5



	R^1	R^2	R^3	R^4
1	H	Me	H	H
2	H	H	Me	H
3	H	Me	Me	H
4	Glc^6-Xyl	H	Me	H
5	H	Me	H	Glc



	R^1	R^2	R^3
6	H	H	Me
7	H	Me	Me
8	Glc^6-Glc	Me	Me
9	Glc	H	H

Fig. 1. Xanthones isolated from the aerial parts and roots of *Swertia punctata*.

2. Results and discussion

A combination of different preparative chromatographic techniques applied on MeOH extracts of the roots afforded five tetraoxygenated xanthones, isobellidifolin (**2**) (Terreaux et al., 1995), methylbellidifolin (**3**) (Ishimaru et al., 1990; Basnet et al., 1994; Miana and El-Hazimi, 1984), isoswertianin (**6**) (Terreaux et al. 1995; Gottlieb et al., 1970), methylswertianin (**7**) (Terreaux et al., 1995; Basnet et al., 1994; Miana and El-Hazimi, 1984) and norswertianin-1-*O*-β-D-glucoside (**9**) (Hostettmann et al., 1974) together with two new xanthones.

Table 1
UV maxima, nm (log ε), of **4** and **8**

Compound	MeOH	+ NaOAc	+ $AlCl_3$	+ $AlCl_3$/HCl
4	235 (4.18) 248 sh	250 (4.04)	256 (4.05)	241 (4.07)
	278 (3.92)	265 (3.99)	289 (4.02)	288 (3.97)
	314 (3.88)	356 (4.03)	347 (3.95)	342 (3.89)
	367 (3.48)		433 (3.32)	430 (3.23)
8	240 (4.90)	267 (3.90)	244 (3.79)	243 (3.85)
	268 (3.83)	311 (3.76)	280 (3.91)	280 (3.90)
	312 (3.70)	379 (2.84)	341 (3.79)	338 (3.78)
	377 (2.96)		430 (2.91)	430 (2.93)

The similar procedure applied on the MeOH extract of the aerial parts revealed four xanthones, bellidifolin (**1**) (Ishimaru et al., 1990; Basnet et al., 1994), swertianolin (**5**) (Kaldas et al., 1974; Ishimaru et al., 1990), mangiferin, (**10**), and **3**, together with flavone, isoorientin (**11**) (Hostettmann and Jacott-Guillarmod, 1976; Bellmann and Jacot-Guillarmod, 1973). Compounds **1**–**3**, **5**–**7**, **9** and **11** were identified by comparison of their spectral data to those published. Mangiferin (**10**) was identified by LC/DAD of the crude extract using coinjection ($t_R = 24.44$ min) of the standard sample isolated previously from *Gentiana lutea* (Menković et al., 2000) and identified by spectral data. Among the isolated compounds, **7** and **9** were found previously in the roots, while xanthones **1**, **5** and **10**, together with flavone **11** were identified previously in the aerial parts of *S. perennis* (Rivaille et al., 1969; Hostettmann and Jacot-Guillarmod, 1976).

Compound **4**, a yellow crystalline material, showed in ESIMS $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ ions at *m*/*z* 569, 591 and 607, respectively, corresponding to molecular formula $C_{25}H_{28}O_{15}$. The UV absorption of **4** (Table 1) was typical for 1,3,5,8-tetraoxygenation. This type of substitution was also confirmed by the ^{1}H NMR data (Table 2) containing resonances of two *meta* (δ 6.69, H-2 and δ 6.52, H-4; $J_{2,4} = 2.4$ Hz) and two *ortho*-coupled protons (δ 7.37, H-6 and δ 6.66, H-7; $J_{6,7} = 9.0$ Hz). The observed bathochromic shift of band at 314 nm to 356 nm with the increase in intensity revealed free OH group at C-3 (Corrêa et al., 1970). The presence of an additional (chelated) phenolic OH (at C-1 or C-8) was evident from a low field singlet (δ 12.55) and the bathochromic shifts observed in the UV spectrum in the presence of $AlCl_3$/HCl (Table 1). The ^{1}H NMR further revealed signals of a methoxy group (δ 3.86) and a disaccharide moiety. Acid catalyzed hydrolysis of **4**, yielding 1,3,8-trihydroxy-5-methoxyxanthone (isobellidifolin, **2**), also isolated from this extract, indicated C-5 as the position bearing the methoxy group. This was also supported by the occurrence of NOE (in NOESY) and ROE (in ROESY) between the methoxy protons and H-6. Additional evidence of 5-methoxy substitution was gained from HMBC correlation between the methoxy

Table 2
^{1}H (500 MHz) and ^{13}C (50 MHz) NMR data[a] of **4** and **8** in DMSO-d_6

H/C	**4**		**8**	
	δ_H	δ_C	δ_H	δ_C
1	–	159.4	–	159.5[b]
2	6.69, *d* (2.4)	100.0	6.80, *d* (2.4)	99.1
3	–	164.9[c]	–	166.0
4	6.52, *d* (2.4)	96.5	6.76, *d* (2.4)	94.9
4a	–	159.0	–	159.3[b]
5	–	139.3	6.93, *d* (9.0)	104.9[c]
6	7.37, *d* (9.0)	119.5[d]	7.46, *d* (9.0)	120.6
7	6.66, *d* (9.0)	108.9[b]	–	142.7
8	–	154.0	–	150.5
8a	–	108.5[b]	–	108.8
9	–	179.9	–	181.5
9a	–	104.3[c]	–	104.9[c]
10a	–	144.1	–	148.4
OMe	3.86, *s*	56.9	3.93, *s*, 3.83, *s*	56.7, 56.5
OH	12.55, *s* (8-OH)		13.31, *s* (8-OH)	
Glc-1				
1′	4.95, *d* (7.8)	102.0	5.13, *d* (7.5)	100.8
2′	3.40, *dd* (4.2, 7.8, 8.4)	73.0	3.44, *ddd* (4.0, 7.5, 8.5)	73.5
3′	3.32[b]	76.4	3.32[b]	76.6
4′	3.30[b]	69.8	3.25, *ddd* (5, 9.0, ~9.0)	69.8
5′	3.55, *m*	76.3	3.74 *brdd* (~8, 9.0)	75.8
6′	(A) 3.63, *dd* (6.0, 11.4); (B) 3.95, *brd* (11.4)	68.7	(A) 3.65;[b] (B) 4.00, *brd* (10.0)	69.0
Glc-2				
1″			4.23, *d* (7.5)	103.9
2″			2.97, *ddd* (4.5, 7.5, ~8)	73.7
3″			3.13, *ddd* (5.0, ~8, ~8)	76.9
4″			3.06[b]	70.3
5″			3.06[b]	77.1
6″			(A) 3.43, *ddd* (5.5, 6.0,11.0); (B) 3.65[b]	61.2
Xyl				
1″	4.18, *d* (7.8)	104.2[c]		
2″	3.00, *dd* (7.2)	73.6		
3″	3.08, *brdd* (7.8, 7.8)	76.5		
4″	~3.30[b]	69.8		
5″	(A) 2.98, *dd* (11.4, 11.4); (B) 3.68, *dd* (5.4, 11.4)	65.8		
OH[d]	5.06, *d* (4.2), 5.15, *d* (5.4), 5.16, *d* (5.4), 4.91, *d* (4.8), 4.93 *brs*		5.18, *d* (4.5), 5.17, *d* (5.0), 5.13, *d* (4.0), 4.92, *d* (5.0), 4.89, *d* (4.5), 4.83, *d* (4.5), 4.45, *t* (6.0)	

[a] Assigned by means of DQF COSY, PS NOESY, TOCSY, HSQC and HMBC and the analogy with ^{1}H NMR data of the related compounds.
[b] Overlapped signals.
[c] Not visible in ^{13}C{^{1}H} NMR spectrum; detected via HSQC and/or HMBC.
[d] OH signals of both sugar units.

protons and C-5 (δ 139.3). The sugar residue of **4** showing 11 signals in the ^{13}C NMR spectrum was identified as primeverosyl by the similarity of ^{13}C chemical shifts (Table 2) to those reported for xanthone heterozides bearing this group (Otsuka, 1999). The NOE of the anomeric proton (H-1′) of the glucose subunit (δ 4.95 *d*, $J=7.8$ Hz) to H-2, as well as HMBC correlation of between H-1′ and C-1 (δ 159.4) were in accordance with the attachment of the primeverosyl moiety to the oxygen at C-1. Consequently the structure **4** was assigned as 1-*O*-primeverosyl-3,8-dihydroxy-5-methoxyxanthone.

Xanthone **8**, obtained as yellow crystals, exhibited in ESIMS $[M+H]^+$ and $[M+Na]^+$ ions at m/z 613 and 635, respectively, corresponding to molecular formula $C_{27}H_{32}O_{16}$. The UV absorption of **8** (Table 1) was typical for 1,3,7,8-tetraoxygenated xanthone, the substitution pattern, usual in *Swertia* (Neerja et al., 2000). The ^{1}H NMR spectrum of **8** (see Table 2), exhibiting signals of four protons, two *meta* (δ 6.76, H-2 and δ 6.80, H-4; $J_{2,4}=2.4$ Hz) and two *ortho*-coupled (δ 6.93, H-5 and δ 7.46, H-6; $J_{5,6}=9.0$ Hz), also confirmed this type of substitution. The ^{1}H NMR spectrum also revealed two methoxy groups (δ 3.93 and 3.83), a chelated phenol hydroxyl (δ 13.31) and a disaccharide moiety containing six doublets of secondary hydroxyls (δ 4.8–5.5) and a triplet (δ 4.45, $J=6.0$ Hz) of a primary hydroxyl (6″-OH). Two anomeric protons were detected as doublets (J ca. 7.5 Hz) at δ 5.13 (H-1′) and 4.23 (H-1″). The ^{1}H NMR, together with the ^{13}C NMR spectrum (Table 2), containing 12 signals of the sugar residue at the chemical shifts similar to those reported for xanthone gentiobiosides (Otsuka, 1999), indicated the gentiobiosyl structure of this group. This was also confirmed by acid-catalysed hydrolysis yielding glucose. The aglycone obtained by the hydrolysis was identified as **7**, also isolated from the same extract. The 3,7-dimethoxy pattern was also in accordance with NOEs of the methoxy groups to *ortho*-positioned protons, one of them (at higher field) dipolarly coupled to H-6 and the other to H-4. The NOE observed between H-2 and the anomeric proton of the inner glucose unit (H-1′) indicated a 1-*O*-gentiobioside structure. The UV data measured in the presence of shift reagents (NaOAc and $AlCl_3$/HCl, Table 1) are also in agreement with the hydroxyl at *peri*-position (C-1). According to the above evidence, the structure of **8** was assigned as 1-*O*-gentiobiosyl-3,7-dimethoxy-8-hydroxyxanthone.

2.1. Chemotaxonomic and pharmacological significance

According to Neerja et al. (2000), 79 simple oxygenated xanthones have been isolated from the genus *Swertia* till the end of 1998. They contained three types of substituents, namely OH, OMe and O-sugar. The only exception is mangiferin (**10**), bearing a *C*-glucose unit, and occurring in a large number of families. They are divided in three groups: trioxygenated (9%), tetraoxygenated (64%) and pentaoxygenated (27%). The eight positions in their skeleton are substituted with following abundances: C-1 (100%), C-2 (13%), C-3 (95%), C-4 (28.2%), C-5 (41%), C-6 (6.5%), C-7 (58%) and C-8 (77%). The oxygenation pattern, i.e. 1,3,5,8- and 1,3,7,8-tetrasubstitution, in the xanthones isolated from *S. punctata* fits very well to these. As far as the type of substitution is concerned the xanthone complex of *S. punctata* is quite similar to that of the previously studied *S. perennis*, originating from French (Rivaille et

al., 1969; Rivaille and Raulais, 1969) and Swiss Alps (Hostettmann and Jacot-Guillarmod, 1976; Hostettmann and Miura, 1977).

As reviewed by Neerja et al. (2000) xanthones **1**, **3**, **7** and **10** exhibited various biological activities such as: hypoglycemic (**1**,**3**,**7**), hepatoprotective (**1**,**3**), antituberculous (**7**), antioxidant (**7**), antimalarial (**7**) and antiinflammatory (**10**). This makes *S. punctata* attractive as a source of medicinal raw material, but since its population is scarce and endangered, as reported recently in the Red Book of Serbian Flora (Jovanović, 1999), our efforts are now concentrated in finding out an alternative way for biomass production.

3. Experimental

3.1. General

The spectra were recorded with the following instruments: IR, Perkin-Elmer FT-IR Spectrometer 1725 X; ^{1}H and ^{13}C NMR 1D and 2D NMR, Varian Gemini 2000 (200 MHz for ^{1}H) and Bruker AMX 500 (500 MHz for ^{1}H); UV, G113AA HP 8543 advanced UV-Vis spectrometer; DCIMS (150 eV, isobutane), Finnigan MAT Mass Spectrometer 8230, double focusing (BE geometry); ESIMS (a sample, dissolved in MeOH–H_2O, 1:1) Finnigan MAT 900, double focusing (EB geometry) equipped with a Finnigan MAT electrospray interface. Optical rotations, Perkin-Elmer 141 MC polarimeter. Melting points (not corrected), Boetius PHMK apparatus. Analytical HPLC, Hewlett Packard HPLC model 1090, DAD detector (HP 1040), column, Lichrospher RP-18 (5 ì), 250×4 mm I.D. (Merck), flow rate, 0.8 ml/min, mobile phase, A (MeCN) + B (H_2O containing 1% 0.1 N H_3PO_4), elution, combination of gradient and isocratic modes: 98–90% B, 0–5 min, 90% B, 5–18 min, 90–85% B, 18–20 min, 85% B, 20–25 min, 85–70% B, 25–30 min, 70–30% B, 30–40 min, 30–0% B, 40–50 min. Preparative medium pressure chromatography (MPLC), Lobar column (silica gel Si 60, size A or B). Dry-column flash chromatography (DCFC), Silica gel Si 60; MN Polyamide DC 6. TLC, 0.2 mm, silica gel 60 F 254 Merck, detection, under UV or by heating after spraying with 50% H_2SO_4. Column chromatography (CC), polyamide-6-powder (polycaprolactam); Sephadex LH-20. Elemental C,H-analysis, combustion (Pregl) method.

3.2. Plant material

Plant material was collected at the locality Ivankovica, Stara Planina (1850 m) in October 1999. A voucher specimen (No. 11673) has been deposited in the herbarium at the Faculty of Biology, Botanical Garden "Jevremovac", Belgrade.

3.3. Extraction and isolation of the compounds

3.3.1. Roots

Air-dried roots (75 g) were extracted twice at room temperature with MeOH (2×300 ml) for 24 h. The combined extracts were concentrated in vacuo to yield oily brown residue (*R*, 19.1 g) which was suspended in water (150 ml). Successive extraction with Et_2O (3×120 ml), EtOAc (3×120 ml), and *n*-BuOH (3×120 ml) afforded, after evaporation in vacuo fractions *RE* (1.2 g), *RA* (0.4 g) and *RB* (3.2 g), respectively. After evaporation of the water layer the residue *RW* (14.2 g) was obtained. Only *RE* and *RB*, containing appreciable content of xanthones and flavones (according to ^{1}H NMR) were further analyzed. All isolated known compounds were identified by comparison of their spectral data to those published. *RE* (1.1 g) was divided into six fractions ($RE_{1–6}$) by DCFC on silica gel, starting elution with toluene/EtOAc (9.5:0.5) and gradually increasing the polarity by addition of EtOAc (up to 7:3). Fractions RE_3 and RE_5 (toluene/EtOAc, 8:2) were identified as **3** (21 mg) and **7** (42 mg). Fraction RE_4 (80 mg), contained 1:1 mixture of **3** and **7**. DCFC on silica gel of RE_6 (84 mg, eluted with toluene/EtOAc 7:3), using toluene/EtOAc (9.5:0.5–9:1) afforded four subfractions ($RE_6^{1–4}$). RE_6^3 (35 mg, toluene/EtOAc, 9:1), after MPLC on silica gel (column size A, same eluent, 5 ml/min) yielded **2** (7.6 mg), t_R between 35 and 40 min, as yellow gum. RE_6^4 (15 mg, toluene/EtOAc, 9:1) was purified by PTLC on silica gel to yield **6** (2 mg), in form of yellow gum. *RB* (3.1 g) was divided into four fractions ($RB_{1–4}$) by DCFC on polyamide (100 g) changing polarity of the eluent (H_2O/MeOH) in a stepwise mode, from 9:1 to 100% MeOH. RB_2 (0.4 g, H_2O/MeOH, 6:4) rechromatographed on polyamide, H_2O/MeOH, 9:1–100% MeOH, to yield gentiobioside **8** (19 mg, H_2O/MeOH, 8:2), and primeveroside **4** (27 mg, H_2O/MeOH, 3:7). RB_3 (90 mg, H_2O/MeOH, 2:8) yielded, after the repeated CC on polyamide and filtration through Sephadex LH-20 (MeOH), additional quantity of **4** (5 mg). RB_4 (110 mg) was supended in MeOH, the insoluble fraction (mostly **7**) was filtered off, solute evaporated to dryness and crystallized from CH_2Cl_2–MeOH to yield **9** (33 mg).

3.3.2. Aerial parts

The same extraction procedure as above (2×150 ml MeOH) was applied on air dried aerial parts (42 g) to obtain oily dark green residue (*H*, 11 g). Successive extraction (3×60 ml) with Et_2O, EtOAc and *n*-BuOH of *H* suspended in H_2O (80 ml) gave fractions *HE* (1.2 g), *HA* (0.08 g) and *HB* (3.5 g), respectively. After evaporation of the water layer the residue *HW* (6.5 g) was obtained. *HE* (1.2 g) was divided into six fractions ($HE_{1–6}$) by DCFC on silica gel using toluene/EtOAc (9.5:0.5–7:3). HE_3 (toluene/EtOAc, 8:2) was identified as

3 (35 mg). HE_6 (77 mg), eluted with toluene/EtOAc, 7:3 yielded **1** (28 mg), upon crystallization from MeOH. DCFC on polyamide (H_2O/MeOH, 7:3–100% MeOH–MeOH/CH_2Cl_2, 3:2) of *HB* (2.3 g) afforded HB_1 (1.9 g, H_2O/MeOH, 7:3–100% MeOH) and HB_2 (0.24 g, MeOH/CH_2Cl_2, 3:2). Crystallization from MeOH of HB_2, followed by purification of the crystalline material on Sephadex LH 20 (MeOH), yielded **5** (20 mg). Evaporation of supernatant of HB_2 and purification of the residue (150 mg) on Sephadex LH 20 (MeOH) afforded a fraction (45 mg) containing **11** as the major constituent. Purification of an aliquot (15 mg) by PTLC (silica gel), EtOAc/MeOH/H_2O, 21:4:3 (three developments), followed by filtration through Sephadex LH-20, yielded **11** (3.2 mg).

Acid hydrolysis of **4** and **8** (Ishimaru et al., 1990) yielded aglycones **2** and **7**, respectively, identified by identitity of their ^{1}H NMR spectra to those of the co-occurring compounds isolated from the same extract. Compound **9** was peracetylated with Ac_2O in pyridine at room temp. overnight. The ^{1}H NMR spectral data of this acetate were identical to those of peracetylated norswertianin-1-*O*-β-D-glucoside (Hostettmann et al., 1974).

3.4. 1-O-Primeverosyl-3,8-dihydroxy-5-methoxyxanthone (*4*)

Yellow crystals, mp 207–209 °C, $[\alpha]_D^{20} = -68.75$ (*c* 0.08, DMSO); UV: see Table 1; IR (KBr): $\nu_{max} = 3439$, 1636, 1401, 1262, 1106, 1083 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 2; ESIMS *m*/*z* : 569 $[M+H]^+$, 591 $[M+Na]^+$ and 607 $[M+K]^+$. Elemental analysis (Found: C, 52.91; H, 5.05. $C_{25}H_{28}O_{15}$ requires: C, 52.80; H, 4.97%).

3.5. 1-O-Gentiobiosyl-3,7-dimethoxy-8-hydroxyxanthone (*8*)

Yellow crystals, mp 184–186 °C; $[\alpha]_D^{20} = -33.63$ (*c* 0.33, DMSO); UV: see Table 1; IR (KBr): $\nu_{max} = 3428$, 1650 sh, 1619, 1583, 1498, 1459, 1401, 1285, 1249, 1083 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 2; ESIMS *m*/*z*: 613 $[M+H]^+$ and 635 $[M+Na]^+$. Elemental analysis (Found: C, 53.02; H, 5.33. $C_{27}H_{32}O_{16}$ requires: C, 52.93; H, 5.27%).

Acknowledgements

The authors are grateful to Professor V. Stevanović, Faculty of Biology, University of Belgrade for identification of the plant material. The authors from Yugoslavia also acknowledge their gratitude to Ministry for Science, Technologies and Development for financial support.

References

Basnet, P., Kadota, S., Shimizu, M., Namba, T., 1994. Bellidifolin a potent hypogycemic agent in streptozotocin (STZ)-induced diabetic rats from *Swertia japonica*. Planta Med. 60, 507–511.

Bellmann, G., Jacot-Guillarmod, A., 1973. Contribution à la phytochimie du genre *Gentiana*. I) Etude de composés flavoniques et xanthoniques dans les feuilles de *Gentiana. lutea* L. (1ère communication). Helvetica Chimica Acta 56, 284–294.

Corrêa, D.B., Fonseca e Silva, L.G., Gottlieb, O.R., Gonçalves, S.J., 1970. Quinone and xanthone constituents of *Kielmeyera rupestris*. Phytochemistry 9, 447–451.

Gottlieb, O.R., Mesquita, A.A.L., de Oliveira, G.G., Teixeira de Melo, M., 1970. Xanthones from *Kielmeyra speciosa*. Phytochemistry 9, 2537–2544.

Hostettmann, K., Jacot-Guillarmod, A., 1976. Identification de xanthones et de nouveaux arabinosides de *C*-glucosides flavoniques dans *Swertia perennis* L. Helvetica Chimica Acta 59, 1584–1591.

Hostettmann, K., Miura, I., 1977. A new xanthone diglucoside from *Swertia perennis* L. Helvetica Chimica Acta 60, 262–264.

Hostettmann, K., Tabacchi, R., Jacot-Guillarmod, A., 1974. Contribution à la phytochimie du genre *Gentiana*. VI) Etude des xanthones dans les feuilles de *Gentiana bavarica* L. Helvetica Chimica Acta 57, 294–301.

Ishimaru, K., Sudo, H., Satake, M., Matsunaga, Y., Hasegawa, Y., Takemoto, S., Shimomura, K., 1990. Amarogentin, amaroswerin and four xanthones from hairy root cultures of *Swertia japonica*. Phytochemistry 29, 1563–1565.

Jovanović-Dunjić, R., 1973. *Swertia* L. In: Josifović, M. (Ed.), Flore de la Republique Socialiste de Serbie V. Academie Serbe des Sciences et des Arts, Beograd, pp. 407–408.

Jovanović, S., 1999. *Swertia perennis* L. In: Stevanović, V. (Ed.), The Red Book of Flora of Serbia 1. Extinct and Critically Endangered Taxa. Ministry of Environment of the Republic of Serbia, Faculty of Biology, University of Belgrade, Institution for Protection of Nature of the Republic of Serbia, pp. 261–263.

Kaldas, M., Hostettmann, K., Jacot-Guillarmod, A., 1974. Contribution à la phytochimie du genre *Gentiana*. IX) Etude de composés flavoniques et xanthoniques dans les feuilles de *Gentiana campestris* L. (1ère communication). Helvetica Chimica Acta 57, 279–280.

Menković, N., Šavikin-Fodulović, K., Savin, K., 2000. Chemical composition and seasonal variations in the amount of secondary compounds in *Gentiana lutea* leaves and flowers. Planta Medica 66, 178–180.

Miana, G.A., El-Hazimi, H.M.G., 1984. Xanthones of *Centaurium pulchellum*. Phytochemistry 23, 1637–1638.

Neerja, P., Jain, D.C., Bhakuni, R.S., 2000. Phytochemicals from genus *Swertia* and their biological activities. Indian Journal of Chemistry 39B, 565–586.

Otsuka, H., 1999. Triptexanthosides A-E: xanthone glycosides from aerial parts of *Tripterospermum japonicum*. Chem. Pharm. Bull. 47, 962–965.

Peres, M., Tanus, J.N., Fernando, de O., F., 2000. Tetraoxygenated naturally occurring xanthones. Phytochemistry 55, 683–710.

Rivaille, P., Massicot, J., Guyot, M., Plouvier, V., 1969. Les xanthones de *Gentiana Kochiana*, *Swertia decussata* et *S. perennis* (Gentianacees). Phytochemistry 8, 1533–1541.

Rivaille, P., Raulais, D., 1969. Recherches de xanthones et autres constituants chez *Gentiana* et *Swertia*: présence d'un triterpéne nouveau chez *Gentiana verna*. L. C. R. Acad. Sc. Paris Serie D. 269, 1121–1124.

Šavikin-Fodulović, K., Janković, T., Krstić, D., Menković, N., 2002. Xanthone compounds in some *Gentianaceae* species growing in Serbia and Montenegro. In: Májumdar, D.K., Govil, Y.N., Singh,

V.K. (Eds.), Recent Progress in Medicinal Plants, Vol. 8, Phytochemistry and Pharmacology Part II. Sci. Tech. Pub., USA, pp. 379–409.

Terreaux, C., Maillard, M., Gupta, M.P., Hostettmann, K., 1995. Xanthonoides from *Shultesia lisianthoides*. Phytochemistry 40, 1791–1795.

Tutin, T.G., 1972. *Gentiana* L. In: Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, Vol. 3. University Cambridge Press, pp. 59–63 [see also http://www.rbge.org.uk/forms/fe.html].

Vladimirov, V., Tan, K., 1998. *Swertia punctata* Baumg. in Bulgaria. IX OPTIMA Meeting (Abstracts) VI, Paris, p. 19.