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XANTHONES FROM *SCHULTESIA LISIANTHOIDES*

CHRISTIAN TERREAUX, MARC MAILLARD, MAHABIR P. GUPTA* and KURT HOSTETTMANN†

Institut de Pharmacognosie et Phytochimie, Université de Lausanne. BEP, CH-1015 Lausanne, Switzerland; *CIFLORPAN, Faculdad de Farmacia, Universidad de Panama, Panama, Republica de Panama

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Key Word Index—*Schultesia lisianthoides*; Gentianaceae; xanthone aglycones; 5-hydroxy-1,2,3-trimethoxyxanthone.

Abstract—Nine xanthone aglycones and one xanthone glycoside have been identified in *Schultesia lisianthoides*. Seven of the aglycones have been isolated and their structures elucidated by UV, mass and NMR spectroscopy; three xanthones were identified on-line by HPLC–UV and HPLC–mass spectrometry. 5-Hydroxy-1,2,3-trimethoxyxanthone is a new natural product. The other constituents have already been encountered in different Gentianaceae species.

INTRODUCTION

Phytochemical investigation of *Schultesia lisianthoides* (Griseb.) Benth & Hook was undertaken as part of our study of the Gentianaceae, known as the main producer of xanthone secondary metabolites. Xanthones form a class of natural products with an increasing interest, mainly due to their monoamine oxidase inhibitory and antioxidant properties [1]. *S. lisianthoides* is a herb growing below 1000 m in Panama, which is also distributed throughout Latin America. To our knowledge, only one report on the phytochemistry of this genus has appeared and this does not concern xanthones [2]. HPLC-UV analysis of the dichloromethane extract of the whole plant showed a rich content in xanthone aglycones. This report describes the isolation procedure and the structure elucidation of most of them. The methanol extract was also analysed by HPLC–UV and HPLC–mass spectrometry (MS) and this study revealed the presence of a secoiridoid as the major constituent, together with a xanthone glycoside and several flavonoids.

RESULTS AND DISCUSSION

Analysis of the dichloromethane extract of the whole plant of *S. lisianthoides* by HPLC combined with photodiode array UV detection revealed nine compounds with spectra showing the characteristic absorption bands of xanthones (Fig. 1). Further HPLC–MS analysis of the extract using a thermospray interface (TSP) showed the $[M + H]^+$ pseudomolecular ions for the peaks characteristic of xanthones and allowed the attribution of their molecular weights (M_r 258, 272, 274, 288 and 302) (Fig. 1). The UV spectra obtained on-line and the retention times of the products were compared, by means of a computerized databank, with those of reference compounds previously isolated in our laboratory. This analysis allowed the identification of four xanthones (i.e. compounds **3**, **4**, **7** and **8**). The aim of the isolation procedure was to obtain the five xanthone aglycones which had not been identified on-line (**1**, **2**, **5**, **6** and **9**).

Fractionation of the dichloromethane extract was undertaken in order to isolate these xanthones. Combination of centrifugal partition chromatography (CPC), Lobar chromatography on reversed-phase columns and gel filtration on Sephadex LH 20 afforded compounds **1**, **2**, **3**, **5**, **6**, **7** and **9**. The EI mass spectrum of **1** exhibited a $[M]^+$ ion at *m/z* 302, which represented the molecular weight of a tetrasubstituted xanthone with one hydroxyl and three methoxyl groups. Its ^{1}H NMR spectrum showed three aromatic methoxyls (δ4.03, 3.95, 3.91), one isolated aromatic proton singlet at δ6.78 and three aromatic protons forming an ABX system (δ7.81, 7.29, 7.20; J = 1.9, 7.8 Hz). The ^{13}C NMR spectrum displayed resonances of two *ortho*-disubstituted aromatic methoxyls (δ62.0, 61.5) and a third methoxyl at high field (δ56.3), which had one or two protons in *ortho* position [3]. The chemical shift recorded for the carbonyl group (δ175.3) indicated that it was not chelated by a OH group in *peri* position. The low field shift of the proton at C-8 (δ7.81), together with other NMR considerations, suggested a 1,2,3,5-substitution, with two methoxyl groups at C-1 and C-2. Measurements of UV spectra with different shift reagents [4] were in accord with the proposed substitution pattern and also fixed the position of the third methoxyl at C-3. Indeed, an unchanged spectrum upon addition of $AlCl_3$ confirmed that the carbonyl was not chelated. A bathochromic shift was observed in the presence of a strong base [sodium methoxide (NaOMe)], while a weak base [sodium acetate (NaOAc)] did not

†Author to whom correspondence should be addressed.

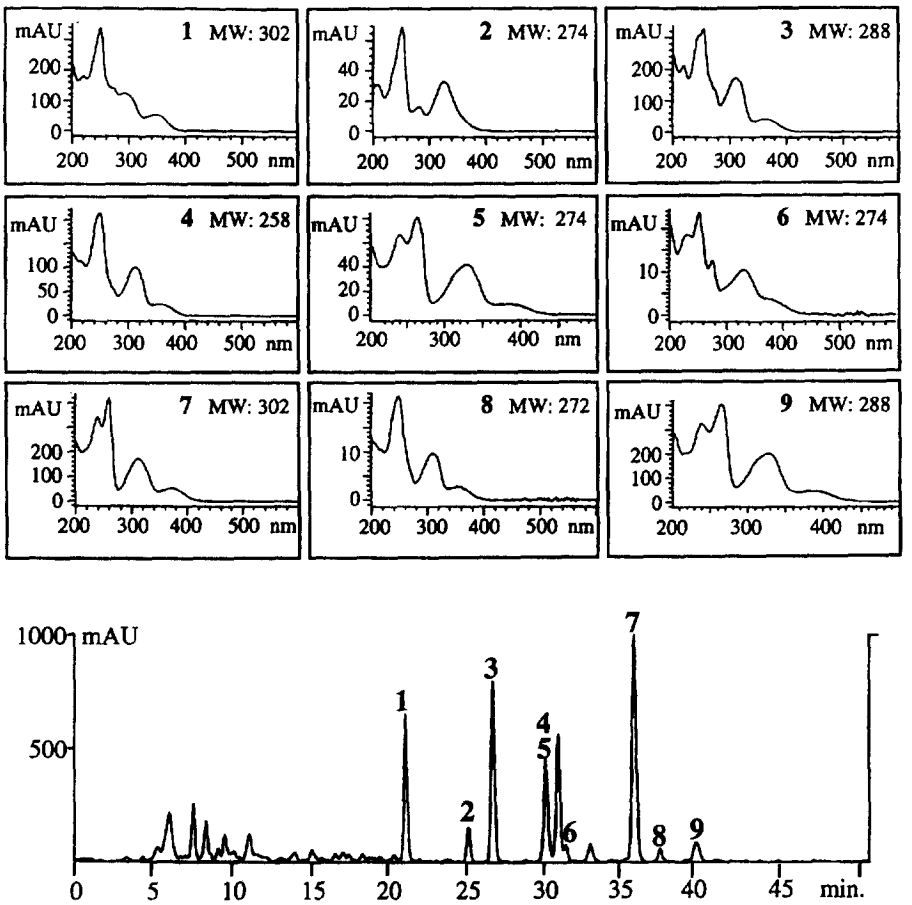
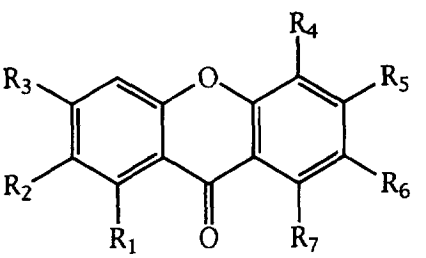


Fig. 1. Information obtained by on-line HPLC–UV and HPLC–MS analysis of the dichloromethane extract of *S. lisianthoides*.



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
1	OMe	OMe	OMe	OH	H	H	H
1a	OMe	OMe	OMe	OAc	H	H	H
2	OH	H	OMe	OH	OH	H	H
3	OH	OMe	OMe	OH	H	H	H
4	OH	H	OMe	OH	H	H	H
5	OH	H	OH	H	H	OMe	OH
6	OH	H	OH	OMe	H	H	OH
7	OH	H	OMe	H	H	OMe	OMe
8	OH	H	OMe	OMe	H	H	H
9	OH	H	OMe	H	H	OMe	OH

modify the concerned maximum. This behaviour could only be explained by substitution at C-3 by the third methoxyl group. Thus, the hydroxyl group was at C-5. This was confirmed by acetylation of **1** in standard conditions to yield the monoacetate **1a**. Comparison of the ^{1}H NMR spectra of **1** and **1a** showed typical downfield shifts [5] of the signals attributable to H-6 (Δ 0.13 ppm), H-7 (Δ 0.13 ppm) and H-8 (Δ 0.37 ppm). It confirmed the acetylation of the hydroxyl at C-5 in **1a**. Thus, **1** is 5-hydroxy-1,2,3-trimethoxyxanthone, which is a new natural product.

The ^{1}H NMR measurements of **2** showed one chelated OH at C-1 (δ 11.82) and two coupled pairs of aromatic protons (J = 2.2 Hz, *meta* coupling, and J = 8.6 Hz, *ortho* coupling). The EI mass spectrum gave a molecular ion $[M]^+$ at m/z 274. This indicated that the four substituents were three hydroxyl groups and one methoxyl group. Comparison of the UV spectrum of **2** with spectra of different tetrasubstituted xanthones [6] showed that **2** had a 1,3,5,6-substitution pattern, which was in agreement with the ^{1}H NMR data mentioned above. The position of the methoxyl group was assigned by UV analysis. The absorption band at λ_{max} 323 nm was shifted upon addition of $AlCl_3$ (λ_{max} 389 nm) and $AlCl_3$ + HCl (λ_{max} 349.5 nm), proving the presence of both an *ortho*-dihydroxyl group (OH in positions 5 and 6) and a hydroxyl at C-1 chelated with the carbonyl group. Spectra observed with NaOMe and NaOAc (λ_{max} 350 nm), as well as the bathochromic shift in the presence of NaOAc + H_3BO_3 (λ_{max} 340 nm) also showed the presence of an *ortho*-dihydroxyl group. Therefore, **2** is 1,5,6-trihydroxy-3-methoxyxanthone. This xanthone was first described in *Canscora decussata* (Gentianaceae) [7], then isolated from *Hypericum androsaemum* (Guttiferae) [8]. Its presence in *Chironia krebsii* (Gentianaceae) was shown by on-line analysis of the extract of the plant [9].

The structures of **5** and **6** were established by UV and ^{1}H NMR data. The small amounts isolated (1 mg of each compound) did not allow ^{13}C NMR measurements. Both compounds had two OH in the *peri* positions of the carbonyl (1 and 8), as shown in the ^{1}H NMR spectra, where two signals for chelated hydroxyl groups were seen (δ 12.25, 11.70 in **5** and δ 11.80, 11.46 in **6**). Two pairs of protons, *meta* and *ortho* coupled, were present in **5** and **6**, meaning that both products could be 1,3,5,8- or 1,3,7,8-substituted xanthones. Comparison of their UV spectra with literature data [6] resulted in the attribution of a 1,3,7,8-substitution for **5** and a 1,3,5,8-substitution for **6**. For both **5** and **6**, the EI MS spectrum displayed a molecular ion at m/z 274 and it was assumed that these compounds had only one methoxyl group. Their relative positions were determined by means of UV shift reagents. The bathochromic shifts observed with a weak base (NaOAc, nearly 30 nm), as well as with a strong base (NaOMe) in the spectrum of both compounds, indicated that their C-3 was hydroxylated. Considering all these data, the structures of these two xanthones were established as 1,3,8-trihydroxy-7-methoxyxanthone (**5**) and 1,3,8-trihydroxy-5-methoxyxanthone (**6**). Product **5** has already been isolated from *C. decussata* [10]. Xanthone **6**, known as isobellidifolin, was described from *Gentiana bellidifolia* [11] and is quite widespread in the Gentianaceae.

Xanthone **9** showed a molecular ion $[M]^+$ of m/z 288, indicating a substitution with two hydroxyl and two methoxyl groups. The ^{1}H NMR spectrum of **9** showed the presence of two hydroxyls chelated with the carbonyl (OH at C-1 and C-8), which was confirmed by the ^{13}C NMR low-field chemical shift of the carbonyl (δ 185.3). Two pairs of coupled protons (similar to xanthone **5**) were also observed in the ^{1}H NMR spectrum, and the feature of the UV spectrum determined a 1,3,7,8-substitution for **9** [12]. As the position of the two OH was already fixed, the structure of **9** was established as 1,8-dihydroxy-3,7-dimethoxyxanthone. This xanthone, known as methylswertianin, is frequently encountered in the Gentianaceae and was isolated first from *Swertia japonica* [13].

Xanthones **3** and **7**, previously identified on-line by comparison with reference substances, were isolated. Measurements of their UV, MS, ^{1}H and ^{13}C NMR spectral data confirmed the on-line identification. The EI MS spectrum revealed a molecular weight of 288 for **3**, and the ^{1}H NMR spectrum displayed similar signals to those of the spectrum of **1**. The only difference was the presence of a chelated proton at δ 12.78. Thus, this xanthone has a hydroxyl group at C-1 instead of the methoxyl group observed in **1**. UV measurements in the presence of $AlCl_3$ and NaOMe confirmed xanthone **3** to be 1,5-dihydroxy-2,3-dimethoxyxanthone as determined by the on-line analysis. It has already been described as a constituent of plants from different families: Gentianaceae (*Halenia elliptica* [14]), Guttiferae (*Calophyllum walkeri* [15]) and Polygalaceae (*Monnina sylvatica* [16]). Xanthone **7** exhibited a molecular ion $[M]^+$ at m/z 302, meaning that it was substituted by three methoxyl groups and one hydroxyl group. The ^{1}H NMR spectrum confirmed this supposition and a signal at δ 13.25 showed the presence of a chelated hydroxyl group. The presence of two pairs of coupled protons (*meta* and *ortho* coupling) and typical UV absorption bands of 1,3,7,8-substituted xanthones [12] proved **7** to be 1-hydroxy-3,7,8-trimethoxyxanthone, as determined by the on-line identification. This xanthone, known as decussatin, is a common xanthone encountered in many species of Gentianaceae. It was first described in *Swertia decussata* as 8-hydroxy-3,4,7-trimethoxyxanthone [17], but its structure was then revised as the actual one [18].

Xanthones **4** (1,5-dihydroxy-3-methoxyxanthone) and **8** (1-hydroxy-3,5-dimethoxyxanthone) were not isolated. Their presence in the extract was shown by the on-line analysis. Both are trisubstituted xanthones described for the first time in Brazilian Guttiferae (*Kielmeyera* sp.) [19] and then isolated from many species of the Gentianaceae including *Chironia krebsii* [20].

The methanol extract of *S. lisianthoides* was also submitted to HPLC–UV and HPLC–TSP–MS analysis. The analysis revealed the presence of one major metabolite in this extract. The pseudomolecular ion $[M + H]^+$ at m/z 374, the UV spectrum and the retention time on

reversed-phase HPLC suggested this compound to be the iridoid-glycoside swertiamarin. This secoiridoid is very widespread in the Gentianaceae and was first described in *Swertia japonica* [21]. Among the minor compounds of the methanolic extract, one xanthone glycoside was identified on-line by comparison with reference compounds: lancerin (1,3,7-trihydroxy-4-*O*-glucosylxanthone), which was previously isolated first from *Tripterospermum lanceolatum* (Gentianaceae) [22]. HPLC–UV and HPLC–TSP–MS analysis of this extract showed the presence of flavone glycosides, according to the UV spectra and the mass fragmentations observed. Moreover, some of the xanthone aglycones isolated from the dichloromethane extract were also identified in the methanol extract (**2**, **3** and **7**). In order to search for the presence of *C*-glycosides, a part of the methanol extract was submitted to acidic hydrolysis. The residue was extracted by chloroform and *n*-butanol, successively. Analysis of the butanolic fraction showed the absence of *C*-glycosides in this extract.

As the interest for xanthones is continuously increasing due to their monoamine oxidase inhibitory and antioxidant properties, it is important to isolate new derivatives of this type. This report shows the efficiency of the preliminary HPLC–UV and –MS analysis in saving time and targeting the fractionation of the unidentified xanthones. Four xanthones were rapidly identified and the isolation of two of them confirmed the correct on-line identification. Testing of the IMAO activities of the isolated compounds is currently in progress and the results will be published elsewhere.

EXPERIMENTAL

General. Mps: uncorr. Analyt. HPLC was carried out on an instrument equipped with a photodiode array detector and with TSP–MS detection. MS detection was performed on a quadrupole triple-stage MS instrument, TSQ 700 Finnigan MAT. The extracts, frs and pure compounds were analysed on a Nova-Pak C_{18} column (4 μm, 150 × 3.9 mm i.d., Waters) at a flow rate of 1 ml min^{-1}, using a gradient of 5–65% MeCN in 50 min. UV spectra were recorded in MeOH. CPC was performed on a Pharma-Tech CCC-1000 apparatus at 1000 rpm in the head-to-tail mode, with a Knauer UV detector. The solvent system was cyclohexane–EtOAc–MeOH–H_2O (4:5:3:3). Lobar sepns were realized on a Lobar RP-18 column (40–63 μm, 310 × 25 mm i.d., Merck). Sepns on Sephadex LH 20 were performed with ($CHCl_3$–MeOH, 1:1) as mobile phase. ^{1}H and ^{13}C NMR spectra were measured at 200.06 and 50.31 MHz, respectively, in DMSO-d_6 or $CDCl_3$. TMS was used as int. standard.

Plant material. The whole plant of *S. lisianthoides* (Griseb.) Benth & Hook was collected in May 1992 at El Tecal, Calzada Larga, Province of Panama, Panama. The plant material was identified by C. Galdames and Prof. M. Correia at the Herbarium of the University of Panama, where a voucher sample of the plant is deposited.

Extraction and isolation. The powder of the whole plant (650 g) was extracted at room temp. successively with CH_2Cl_2 and MeOH. The CH_2Cl_2 extract (15 g) was filtered on a Sephadex LH 20 column, yielding two frs. CPC sepn of the xanthone-containing part (6 g) yielded 5 frs. Fr. 1 (3 g) was sepd on Sephadex LH 20 and gave 7 frs (1.1 to 1.7). Fr. 1.4 was purified by precipitation to give **7** (50 mg). Lobar RP-18 chromatography (MeOH 70%) of fr. 1.6 (150 mg) afforded **3** (20 mg), **6** (1 mg) and **9** (9 mg). Purification of the third fr. of this Lobar column on Sephadex LH 20 yielded **5** (1 mg). Fr. 2 (400 mg) of the CPC sepn was also submitted to Lobar RP-18 chromatography (MeOH 60%) and four frs were obtained. Purification of the second one with a Sephadex LH 20 column led to the isolation of xanthones **1** (8 mg) and **2** (12 mg). Xanthone aglycones **2**, **3**, **5**–**7** and **9** have already been isolated from different plants. Spectral data not already described in the lit. are included here.

5-*Hydroxy*-1,2,3-*methoxyxanthone* (**1**). Light yellow amorphous powder. Mp 192–195°. HPLC: R_t 21.4 min. UV λ_{max}^{MeOH} nm (log ε): 248.5 (4.41), 260sh, 285sh, 347 (3.66); (NaOMe) 213, 237, 278, 377; (NaOAc) 221, 278, 350; ($AlCl_3$) unchanged. EIMS *m/z* (rel. int. %): 302 $[M]^+$ (70), 287 (100), 259 (17), 244 (10). ^{1}H NMR (200.06 MHz, $CDCl_3$): δ7.81 (1H, *dd*, J = 1.9, 7.8 Hz, H-8), 7.29 (1H, *dd*, J = 1.9, 7.8 Hz, H-6), 7.20 (1H, *dd*, J = 7.8, 7.8 Hz, H-7), 6.78 (1H, *s*, H-4), 4.03 (3H, *s*, MeO-1), 3.95 (3H, *s*, MeO-3), 3.91 (3H, *s*, MeO-2). ^{13}C NMR (50.3 MHz, $CDCl_3$): δ153.8 (C-1), 139.8 (C-2), 158.7 (C-3), 95.9 (C-4), 153.6 (C-4a), 143.8 (C-4b), 144.3 (C-5), 119.2 (C-6), 123.8 (C-7), 117.6 (C-8), 123.0 (C-8a), 110.7 (C-8b), 175.3 (C=O), 62.0* (MeO-1), 61.5* (MeO-2), 56.3 (MeO-3).

Acetate (**1a**). ^{1}H NMR (200.06 MHz, $CDCl_3$): δ8.18 (1H, *dd*, J = 1.9, 7.9 Hz, H-8), 7.42 (1H, *dd*, J = 1.9, 7.9 Hz, H-6), 7.33 (1H, *dd*, J = 7.9, 7.9 Hz, H-7), 6.69 (1H, *s*, H-4), 4.02 (3H, *s*, MeO-1), 4.00 (3H, *s*, MeO-3), 3.92 (3H, *s*, MeO-2), 2.48 (3H, *s*, Ac-5).

1,5,6-*Trihydroxy*-3-*methoxyxanthone* (**2**). Yellow–brownish powder. Mp 273–279°. HPLC: R_t 25.0 min. UV, EIMS and ^{1}H NMR data in accord with lit. [7]. ^{13}C NMR (50.3 MHz, DMSO-d_6): δ162.6 (C-1), 96.6 (C-2), 165.8 (C-3), 92.5 (C-4), 157.1 (C-4a), 146.0 (C-4b), 132.6 (C-5), 152.7 (C-6), 113.2 (C-7), 115.9 (C-8), 112.6 (C-8a), 102.2 (C-8b), 179.7 (C=O), 55.9 (MeO-3).

1,5-*Dihydroxy*-2,3-*dimethoxyxanthone* (**3**). Yellow amorphous powder. Mp 243–246° (lit. 244–247° [16], 254–255° [15]). HPLC: R_t 27.2 min. UV, EIMS and ^{1}H NMR data in accord with lit. [16]. ^{13}C NMR (50.3 MHz, DMSO-d_6): δ153.1 (C-1), 131.1 (C-2), 159.9 (C-3), 91.3 (C-4), 152.6 (C-4a), 145.0 (C-4b), 146.4 (C-5), 120.4 (C-6), 124.0 (C-7), 114.1 (C-8), 120.4 (C-8a), 103.2 (C-8b), 180.8 (C=O), 59.9 (MeO-2), 56.4 (MeO-3).

1,3,8-*Trihydroxy*-7-*methoxyxanthone* (**5**). Yellow amorphous powder. HPLC: R_t 30.5 min. UV λ_{max}^{MeOH} nm (log ε): 210 (3.66), 237 (3.74), 261.5 (3.74), 334 (3.48); (NaOMe) 210, 236.5, 262sh, 354.5; (NaOAc) 222.5, 268, 358.5; (NaOAc–H_3BO_3) 224, 261, 334.4; ($AlCl_3$) 209.5, 237sh,

*Assignments interchangeable.

275, 330.5, 364; ($AlCl_3$–HCl) 209.5, 237sh, 276, 330.5 364.5. EIMS *m/z* (rel. int. %): 275 $[M + H]^+$ (70), 259 (53), 200 (18), 159 (100). ^1H NMR (200.06 MHz, DMSO-d_6): δ12.25* (1H, *br s*, HO-1), 11.70* (1H, *br s*, HO-8), 7.38 (1H, *d*, $J = 7.8$ Hz, H-6), 6.86 (1H, *d*, $J = 7.8$ Hz, H-5), 6.07 (1H, *d* unres., H-4), 5.92 (1H, *d* unres., H-2), 3.92 (3H, *s*, MeO-7).

1,3,8-*Trihydroxy*-5-*methoxyxanthone* (**6**). Yellow amorphous powder. HPLC: R_t 32.2 min. UV λ_{max}^{MeOH} nm (log ε): 207.5 (4.01), 251.5 (4.14), 275sh, 329.5 (3.81); (NaOMe) 209.5, 231sh, 269, 359.5; (NaOAc) 219.5, 268, 358.5; ($AlCl_3$) ($AlCl_3$ – HCl) 207.5, 263, 285.5, 330, 368. EIMS *m/z* (rel. int. %): 274 $[M]^+$ (55), 259 (100), 245 (20), 229 (10). ^1H NMR (200.06 MHz, DMSO-d_6): δ11.80* (1H, *br s*, HO-1), 11.46* (1H, *s*, HO-8), 7.40 (1H, *d*, $J = 8.8$ Hz, H-6), 6.67 (1H, *d*, $J = 8.8$ Hz, H-7), 6.24 (1H, *d* unres., H-4), 6.07 (1H, *d* unres., H-2), 3.87 (3H, *s*, MeO-5).

1-*Hydroxy*-3,7,8-*trimethoxyxanthone* (**7**). Yellow amorphous powder. Mp 156–157° (lit. 157–158° [20]). HPLC: R_t 37.5 min. UV, EIMS, ^1H NMR and ^{13}C NMR data in accord with lit. [18, 20].

1,8-*Dihydroxy*-3,7-*dimethoxyxanthone* (**9**). Yellow amorphous powder. Mp 178–181° (lit. 190° [23]). HPLC: R_t 41.5 min. UV λ_{max}^{MeOH} nm (log ε): 208.5 (4.05), 239 (4.22), 262 (4.27), 314.5sh, 328.5 (3.99), 380 (3.40); (NaOMe) 210.5, 242, 273, 339, 400; (NaOAc) 220.5, 263sh, 328.5, 390; ($AlCl_3$) ($AlCl_3$–HCl) 208.5, 235.5, 275.5, 328.5, 366, 415sh. EIMS and ^1H NMR as ref. [23]. ^{13}C NMR (50.3 MHz, $CDCl_3$): δ162.9 (C-1), 97.2 (C-2), 167.4 (C-3), 92.9 (C-4), 158.1 (C-4a), 150.2 (C-4b), 105.6 (C-5), 120.4 (C-6), 142.9 (C-7), 149.5 (C-8), 107.6 (C-8a), 106.9 (C-8b), 185.1 (C=O), 57.1* (MeO-3), 55.9* (MeO-7).

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*Assignments interchangeable.