

**A BIPHENYL, A DIHYDROPHENANTHRENE AND A XANTHONE
FROM *CLUSIA PARALYCOLA***

Franco Delle Monache,^{*,a} Giuliano Delle Monache,^a Bruno Botta,^b and
Eszter Gacs-Baitz^c

^aCentro Chimica Recettori, Università Cattolica, Roma, Italy

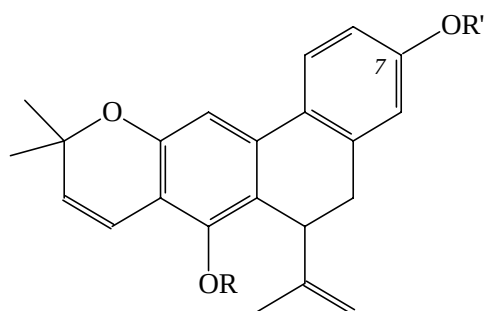
E-mail: f.dellemonache@uniserv.ccr.rm.cnr.it

^bDipartimento di Chimica e Tecnologia delle Sostanze Biologicamente
Attive, Università La Sapienza, Roma, Italy

^cResearch Center for Chemistry, H-1525 Budapest, Hungary

Abstract – A prenylated biphenyl was isolated in a re-examination of the roots of *Clusia paralycola* and attributed the structure (**5a**) and the name clusiaparalycoline D for the similarity with other biphenyls isolated from the same source. A second product, isomeric with the previously isolated paralycolin A, was assigned a 9,10-dihydrophenanthrene structure (**6a**) and named paralycolin B.

In a previous paper¹ we reported the isolation from the roots of *Clusia paralycola* (Guttiferae) of a compound, named paralycolin A, which was erroneously assigned the structure 2*H*-pyran[2,3:6,5]-4,5,6-trihydroxy-9,10-dihydrophenanthrene. The correct structure 2*H*-pyran[2,3:5,6]-1,7,8-trihydroxy-9,10-dihydrophenanthrene (**1a**) was revealed by the identity of the permethyl derivative (**1b**) of paralycolin A with the corresponding product obtained from cedrelin A (**1c**) and B (**1d**), two metabolites isolated from *Cedrelinga catenaeformis* Duke (Leguminosae).²



	R	R'	R''
1a	H	H	H
1b	Me	Me	Me
1c	H	H	Me
1d	Me	H	Me

On the other hand, three new prenylbiphenyl derivatives, namely clusiaparalycoline A (**2**), B (**3**) and C (**4**), have been recently isolated from the roots of *Clusia paralycola* by a biosay-directed fractionation.³

Table 1. ^1H and ^{13}C spectral data of compound (**5a**)

Carbon	δ_{C}	δ_{H}	$^n\text{J}_{\text{H,C}}$ connected carbons			
3	153.69	-				
5	150.91	-				
10	143.40	-				
1	141.39	-				
9	139.86	-				
3''	134.54	-				
7	133.03	-				
3'	132.46	-				
12	131.59	-				
14	128.99	5.60 d (10)	$^3\text{J}_3$	$^3\text{J}_5$	$^3\text{J}_{15}$	$^2\text{J}_4$
8	125.66	-				
2''	123.76	5.10 br t (7)				
2'	122.26	5.12 br t (7)				
13	116.36	6.65 dd (10, 0.7)	$^3\text{J}_4$	$^3\text{J}_{16}$	$^3\text{J}_{17}$	$^2\text{J}_{15}$
11	113.21	6.68 s	$^3\text{J}_7$	$^3\text{J}_9$	$^3\text{J}_{2''}$	$^2\text{J}_{10}$
2	111.31	6.19 dd (1.4, 0.7)	$^3\text{J}_4$	$^3\text{J}_6$	$^3\text{J}_7$	$^2\text{J}_3$
6	109.75	6.04 d (1.4)	$^3\text{J}_2$	$^3\text{J}_4$	$^3\text{J}_7$	$^2\text{J}_5$
4	108.02	-				
15	76.00	-				
1'	31.99	2.97 d (7)	$^3\text{J}_7$	$^3\text{J}_{11}$	$^3\text{J}_3$	$^2\text{J}_5$
16-Me	27.87	1.45 s				
1''	27.85	3.12 d (7)	$^3\text{J}_7$	$^3\text{J}_9$	$^3\text{J}_3$	$^2\text{J}_8$ $^2\text{J}_{2''}$
17-Me	27.64	1.43 s				
t-Me	25.74	1.69 d (1)				
t-Me	25.74	1.65 d (1)				
c-Me	17.74	1.64 br s				
c-Me	17.57	1.46 br s				

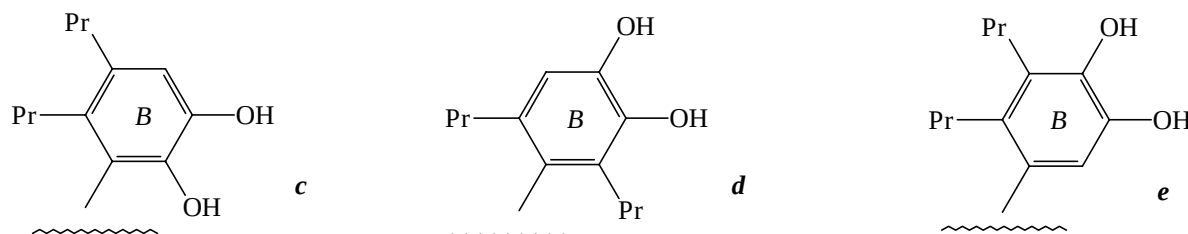
*400 and 100 MHz, respectively; in CDCl_3 , relative to TMS as int. reference.

The signals showed the appropriate integrated intensities. Coupling constants (in Hz) are given in parentheses. $^1\text{J}_{\text{H,C}}$ connectivities were established by an HETCOR measurement; long-range connectivities were determined by a series of INEPTL experiments.⁴

The addition of a $\text{D}_2\text{O}/\text{H}_2\text{O}$ (1:1) solution caused in the ^{13}C NMR spectrum of **5a**, besides the expected splittings of three lowfield signals (at δ 150, 143 and 139), corresponding to the hydroxylated carbons, the doubling of the resonances at δ 109 and 113.

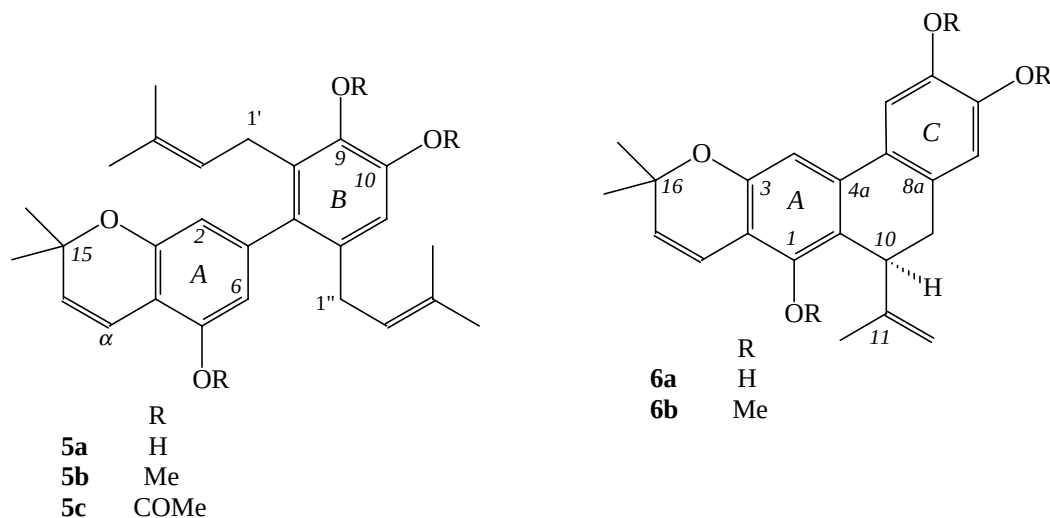
Since the signal at δ 111 (δ 6.19 in the ^1H NMR spectrum) gave no splitting, the partial structure (**b**) was excluded and the A ring was assigned structure (**a**). The diamagnetic shift ($\Delta\delta$ 0.25 ppm) of the α -chromene proton in the ^1H NMR spectrum of the acetyl derivative (**5c**) confirmed the *peri* relationship between the phenolic OH and the chromene ring.⁵ The splitting after deuteration of the carbon signal at δ 113 requires that one of the ortho-related (δ 143 and 139) hydroxyl groups is adjacent to the isolated

aromatic proton in the B ring, as confirmed by the presence of only one lowfield (δ 60)⁶ methoxyl resonance in the ¹³CNMR spectrum of the trimethyl derivative (**5b**). Therefore, only the partial structures (**c**), (**d**) and (**e**) were taken into consideration.



INEPTL⁴ experiments (Table 1), however, evidenced the proximity of one methylene (proton signal at δ 3.12) with one hydroxyl group (carbon signal at δ 139) as well as the vicinity of the other methylene (proton signal at δ 2.97) with the aromatic CH (carbon signal at δ 113). The first requirement is not consistent with structure (**c**), whereas the second excluded structure (**e**).

The combination of partial structures (**a**) with (**d**) gives the final structure (**5a**), namely clusiaparalycoline D.



The second metabolite was isolated only as a methyl derivative (**6b**) from fractions containing a mixture of **6a** and paralycolin A (**1a**) after treatment with CH₂N₂.

¹H and ¹³C NMR spectra and molecular peak (m/z 392) in the mass spectrum indicated for **6b** a molecular formula C₂₅H₂₈O₄, suggesting the structure of a 9,10-dihydrophenanthrene isomeric with trimethyl paralycolin A (**1b**).¹

In details, the NMR spectral data (Table 2) confirmed the presence of the same substituents (a 2,2-dimethyl-2H-pyran ring, an isopropenyl chain and three methoxy groups) as in **1b**. The signals at δ 7.18 and 6.49 of two *para* related protons suggested a 6,7-dimethoxy substitution for the C ring. As a confirm

DIF NOE experiments disclosed the vicinity of the following couples: one methoxyl group and the isopropenyl substituent; H-4 and H-5 protons; the aromatic proton at δ 6.49 and the 9-methylene. INEPTL experiments, in summary, supported the carbon signals assignment and evidenced, in particular, the connectivities C(1)-C(10a)-C-10 and C(8)-C(8a)-C(9). Since in the NMR spectra of the original mixture (**1a/6a**) no signal for methoxyl groups was present, the compound (**6a**) was attributed the structure 2*H*-pyran[2,3:5,6]-1,6,7-trihydroxy-9,10-dihydrophenanthrene (paralycolin B).

Table 2. ^1H and ^{13}C spectral data of compound (**6b**)

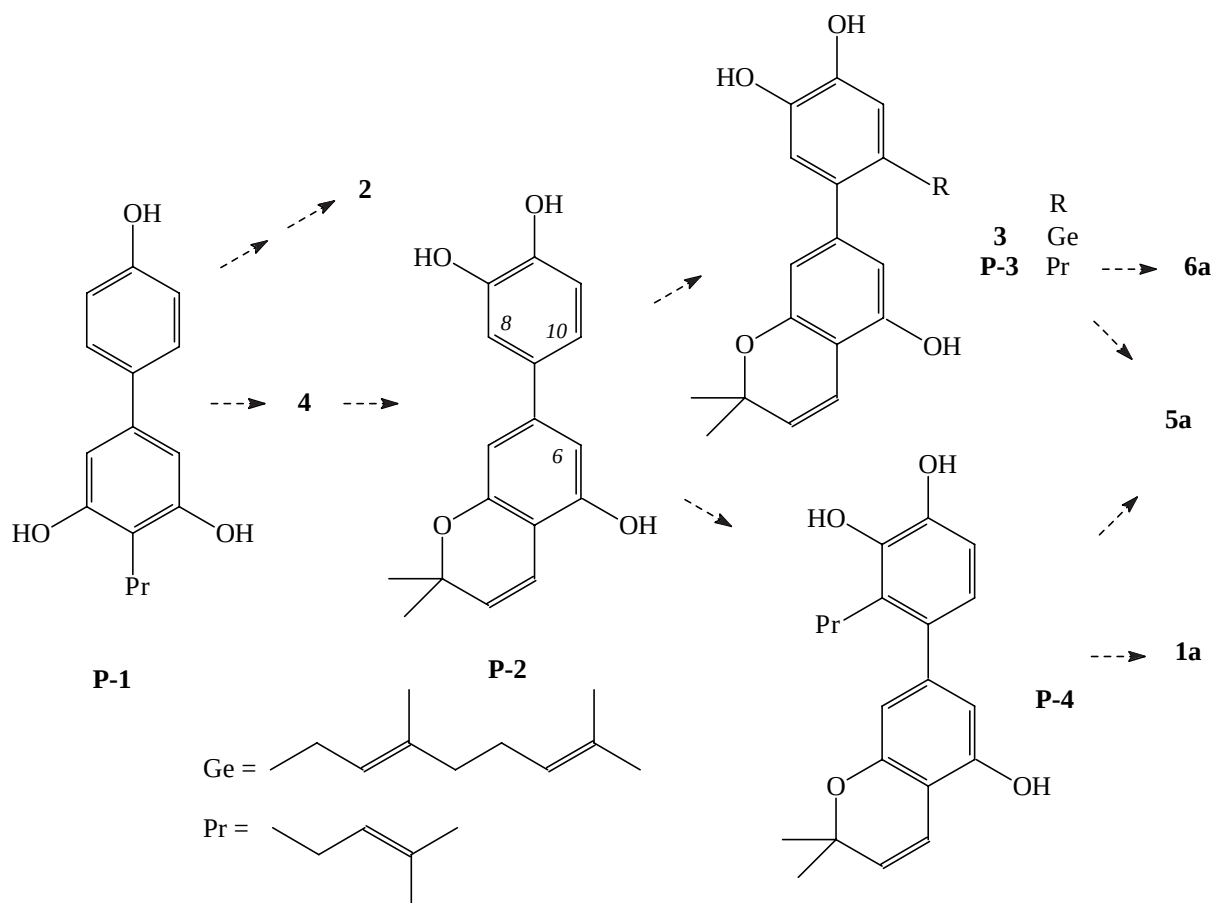
Carbon	δ_{C}	δ_{H}	$^n\text{J}_{\text{H,C}}$	connected carbons
1	154.51	-		
3	153.75	-		
7	150.07	-		
6	149.17	-		
11	146.58	-		
4a	136.88	-		
15	129.80	5.40 d (10)		
8a	128.13	-		
4b	127.15	-		
10a	124.02	-		
14	118.02	6.65 br d (10)		
2	113.85	-		
		4.72 br s		
12	112.78	4.62 br s		
8	112.76	6.49 s	$^3\text{J}_{4\text{b}}$ $^3\text{J}_6$ $^3\text{J}_9$	$^2\text{J}_7$
5	108.23	7.18 s	$^3\text{J}_{4\text{a}}$ $^3\text{J}_7$ $^3\text{J}_{8\text{a}}$	$^2\text{J}_6$
4	107.93	7.36 br s	$^3\text{J}_2$ $^3\text{J}_{4\text{b}}$ $^3\text{J}_{10\text{a}}$	$^2\text{J}_3$
16	75.82	-		
1-OMe	61.97	3.61 s	$^3\text{J}_1$	
6-OMe		3.39 s		
	55.51			
7-OMe		3.37 s		
10	38.60	3.99 dd (6.5, 2) 2.98 dd (15, 6.5)	$^3\text{J}_1$ $^3\text{J}_{4\text{a}}$ $^3\text{J}_{8\text{a}}$ $^3\text{J}_{12}$ $^3\text{J}_{13}$ $^3\text{J}_{4\text{b}}$ $^3\text{J}_8$ $^3\text{J}_{10\text{a}}$ $^3\text{J}_{11}$	$^2\text{J}_9$ $^2\text{J}_{11}$ $^2\text{J}_{8\text{a}}$ $^2\text{J}_{10}$
9	33.36	2.77 dd (15, 2)	$^3\text{J}_{4\text{b}}$ $^3\text{J}_8$ $^3\text{J}_{11}$	$^2\text{J}_{8\text{a}}$ $^2\text{J}_{10}$
17-Me	28.05	1.38 s		
18-Me	27.97	1.41 s		
13-Me	21.92	1.72 br s		

*400 and 100 MHz, respectively; in CDCl_3 , relative to TMS as int. reference.

The signals showed the appropriate integrated intensities. Coupling constants (in Hz) are given in parentheses. $^1\text{J}_{\text{H,C}}$ connectivities were established by an HETCOR measurement; long-range connectivities were determined by a series of INEPTL experiments.⁴

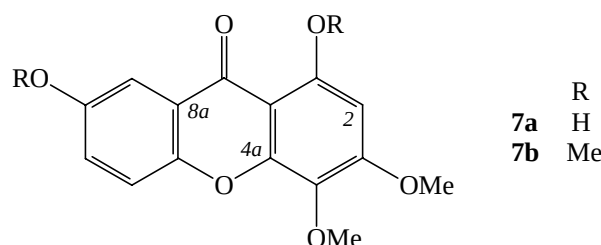
The co-occurrence of **5a** with **6a** supports our prediction that dihydrophenanthrenes may derive from prenylated biphenyl precursors.¹ In Scheme 1 a possible biogenetic pathway, involving paralycolin A (**1a**) and B (**6a**), the biphenyls (**2-4**) and (**5a**) and the precursors (**P-1**) to (**P-4**), is summarized. In this pathway the biphenyl (**4**) is the intermediate between the precursors (**P-1**) and (**P-2**). The attachment of an isopropenyl group to **P-2** either at 10- or 8-position gives the precursors (**P-3**) and (**P-4**), respectively. Conversely the attachment of a geranyl group at C-10 affords compound (**3**). On the other hand, **P-3** and **P-4** by cyclization onto C-6 may give **6a** and **1a**, respectively, whereas the introduction of a second prenyl group either in **P-3** or **P-4** affords the biphenyl (**5a**). Finally, compound (**2**) may be obtained from **P-1** by the same sequence of reactions as going from **4** to **3**.

Scheme 1



A new xanthone (**7a**) was also separated from the co-occurring paralycolin A (**1**) by precipitation from CHCl_3 . UV, NMR and MS spectra suggested the structure of a xanthone with 1,3,4,7-tetraoxygenation due to two methoxyl and two hydroxyl groups. The proton signal (at δ 12.77) of one hydroxyl and the carbon signal (at δ ca. 60)⁶ of one methoxyl revealed a 1-hydroxy-4-methoxy substitution. The second methoxyl group was placed on C-3, because the UV spectrum of **7a** did not show any bathochromic shift by addition of AcONa and excluded a 3-hydroxy substitution. Physical and spectral data of the permethylated (with

CH₂N₂) derivative (**7b**) were coincident with those of 1,3,4,7-tetramethoxyxanthone isolated from *Garcinia eugeniifolia* (Guttiferae).⁷



EXPERIMENTAL

General

Melting points (uncorrected): Kofler apparatus. ¹H and ¹³CNMR (400 MHz and 100 MHz, TMS as internal standard): Varian XL 400. EIMS (direct inlet) and HRMS: VG7070 EQ spectrometer.

Extraction and isolation of the compounds

Roots of *Clusia paralycola* G. Mariz (subfam. Clusioidae, fam. Guttiferae) were collected in Gravatà (Pe), Brazil, and identified by Dr. Alda Chiappeta: a voucher specimen is maintained in the Herbarium of Departamento de Antibioticos under the cypher 5421.

The roots (500 g) were extracted with acetone and the residue was partitioned between H₂O/hexane and H₂O/EtOAc. The EtOAc residue (45 g) was suspended in CHCl₃ (450 mL) at 4 °C overnight and filtered, the precipitate being essentially betulinic acid. The CHCl₃ soluble fraction (8 g) by column chromatography on silica gel with benzene/EtOAc mixtures gave four fractions, CP-I to CP-IV. The main constituent of CP-I (300 mg) was identified with friedelin, but the fraction was not further processed. CP-II (250 mg) by column chromatography on silica gel with CHCl₃ afforded compound (**5a**) (64 mg). The fraction CP-III (800 mg) washed with CHCl₃ gave the xanthone (**7a**) as a precipitate, whereas the filtrate by cc on silica gel with CHCl₃/MeOH, 95:5 yielded paralicolin A (**1a**, 294 mg). Finally, CP-IV (2.1 g) was suspended in CHCl₃ to give crude betulinic acid as a precipitate. The soluble fraction was treated with a saturated solution of CH₂N₂ in Et₂O and the reaction mixture by column chromatography on silica gel with benzene/EtOAc, 1:1, afforded **1b** (170 mg) and **6b** (60 mg).

Clusiachromene D (**5a**)

2*H*-Pyran-[3,4:6,5]-5,9,10-trihydroxy-8,12-bis[γ,γ-dimethylallyl]biphenyl: vitreous solid; ¹H and ¹³C NMR in Table 1. EIMS *m/z* (rel. int.): 420 [M]⁺ (49), 405 [M-Me]⁺ (100), 403 [M-OH]⁺ (22), 377 [M-C₃H₇]⁺ (13), 365 [M-C₄H₇]⁺ (14), 364 [M-C₄H₈]⁺ (16), 361 [405-C₃H₈]⁺ (12), 349 [405-C₄H₈]⁺ (43), 335 [405-C₅H₁₀]⁺ (28), 321 [364-C₃H₇]⁺ (23), 309 [364-C₄H₇]⁺ (17), 293 [349-C₄H₈]⁺ (23); HRMS

Calcd for C₂₇H₃₂O₄: 420.2301. Found: 420.2305. *Methyl derivative (5b)*: oil; ¹H NMR: δ 6.79 (1H, d, J = 10 Hz, H-α), 6.78 (1H, s, H-6), 6.24 (1H, s, H-2), 6.15 (1H, br s, H-11), 5.57 (1H, d, J = 10 Hz, H-β), 5.15, 5.03 (1H each, br t, J = 7 Hz, 2 x =CH), 3.88, 3.82, 3.74 (3H each, s, 3 x OMe), 3.16, 3.09 (1H each, dd, J = 13 and 7 Hz, CH₂), 3.04 (2H, d = 7 Hz, CH₂), 1.66, 1.57, 1.45, 1.37 (3H each, br s, 4 x Me), 1.44, 1.43 (3H each, s, 2 x Me); ¹³C NMR: δ 153.35 (s, C-3), 151.68 (s, C-5), 146.10 (s, C-10), 145.07 (s, C-9), 141.08 (s, C-1), 135.41, 134.58 (s each, 2 x CH₂), 130.32 (s, C-14), 128.57 (d, C-α), 123.99, 123.84 (d each, 2 x =CH), 123.58 (s, C-8), 116.87 (d, C-β), 111.45 (d, C-11), 110.49 (d, C-2), 108.91 (s, C-4), 105.60 (d, C-6), 75.71 (s, C-15), 60.48 (q, 9-OMe), 55.63, 55.54 (q each, 2 x OMe), 32.55 (t, CH₂), 27.82 (q, Me), 27.65, 27.29 (t each, 2 x CH₂), 25.71 (q, 2 x *t*-Me), 17.64 (q, 2 x *c*-Me). *Acetyl derivative (5c)*: ¹H NMR : δ 6.92 (1H, s, H-11), 6.46 (1H, br s, H-2), 6.41 (1H, s, H-6), 6.35 (1H, d, J = 10 Hz, H-α), 5.66 (1H, d, J = 10 Hz, H-β), 5.10, 4.89 (1H each, br t, J = 7 Hz, 2 x =CH), 3.04 (4H, d = 7 Hz, 2 x CH₂), 2.30, 2.28, 2.27 (3H each, s, 3 x OAc), 1.66, 1.57, 1.46, 1.35 (3H each, br s, 4 x Me), 1.44 (6H, s, 2 x Me); ¹³C NMR: δ 168.78, 168.51, 168.42 (s each, 3 x OCO), 153.54 (s, C-3), 151.48 (s, C-5), 146.17 (s, C-10), 145.07 (s, C-9), 141.08 (s, C-1), 133.62, 132.72 (s each, 2 x C=), 131.54 (s, C-14), 131.14 (d, C-α), 123.37, 121.82 (d each, 2 x =CH), 123.58 (s, C-8), 120.88 (d, C-11), 116.10 (d, C-β), 115.94 (d, C-2), 115.52 (d, C-6), 112.99 (s, C-4), 76.27 (s, C-15), 32.27 (t, CH₂), 27.86 (q, Me), 27.83, 27.68 (t each, 2 x CH₂), 25.64, 25.48 (q each, 2 x *t*-Me), 20.79 (q, 2 x COMe), 20.36 (q, COMe), 17.57, 17.44 (q each, 2 x *c*-Me).

Paralycolin B (6a)

2H-Pyran-[2,3:5,6]-1,6,7-trihydroxy-9,10-dihydrophenanthrene: isolated as methyl derivative (**6b**); mp 163-164 °C (CH₂Cl₂/heptane); ¹H and ¹³C NMR spectra in Table 2. EIMS *m/z* (rel. int.): 392 [M]⁺ (43), 377 [M - Me]⁺ (100), 361 [M - OMe]⁺ (14), 351 [M - C₃H₅]⁺ (14), 321 [351 - OCH₂]⁺ (21), 196 [M/2]⁺ (17), 188.5 [M - Me/2]⁺ (30); HRMS Calcd for C₂₅H₂₈O₄: 392.1988. Found: 392.1995.

1,7-Dihydroxy-3,4-dimethoxyxanthone (7a)

mp 291-293 °C (acetone); UV (MeOH) nm (log ε): 262 (4.64), 311 (4.14), 385 (3.90); (+ AcONa): 262, 311, 385; (+ AlCl₃): 282, 328, 450; (+ AlCl₃/HCl): 281, 324, 445; ¹H NMR (CDCl₃/DMSO-d₆, 2:1) : δ 12.77 (1H, s, exchanged with D₂O, 1-OH), 7.47 (1H, J = 2 Hz, H-8), 7.42 (1H, d, J = 8 Hz, H-5), 7.26 (1H, dd, J = 8 and 2 Hz, H-6), 6.42 (1H, s, H-2), 3.94 (6H, s, 2 x OMe); ¹³C NMR: δ 179.0 (s, C-9), 158.0, 157.1, 152.5 (s each, C-1, C-3, C-7), 147.9, 147.6 (s each, C-4a, C-4b), 126.7 (s, C-4), 123.0 (d, C-6), 118.9 (s, C-8a), 117.2 (d, C-5), 106.9 (d, C-8), 101.0 (C-8b), 92.8 (d, C-2), 59.5 (q, 4-OMe), 55.7 (q, OMe); EIMS *M/z* (rel. int.): 288 [M]⁺ (52), 273 [M - Me]⁺ (100), 245 (7), 243 (13); HRMS Calcd for C₁₅H₁₂O₆: 288.0634. Found: 288.0639.

1,3,4,7-Trimethoxyxanthone (7b): obtained by methylation of **7a** with CH₂N₂: mp 189-190 °C, lit.,⁷ mp 189-191; UV and ¹H NMR spectra in agreement with published data.⁷

ACKNOWLEDGEMENTS

This work was supported by grants from the Agreement between the Consiglio Nazionale delle Ricerche of Italy and the Hungarian Academy of Sciences.

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