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

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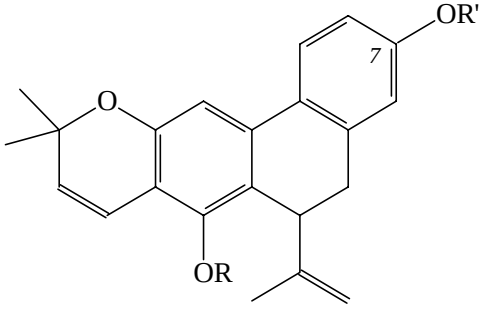
Abstract – A prenylated biphenyl was isolated in a re-examination of the roots of *Clusia paralycola* and attributed the structure (**5a**) and the name clusiaparalycoline D for the similarity with other biphenyls isolated from the same source. A second product, isomeric with the previously isolated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

In a previous paper[1] 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

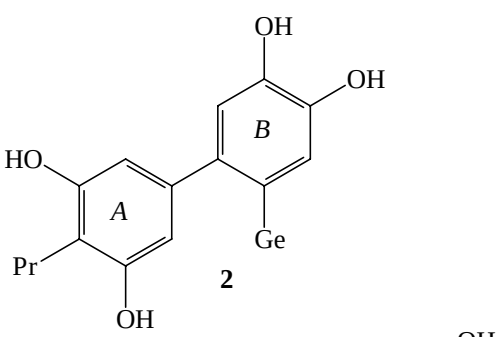
On the other hand, three new prenylbiphenyl derivatives, namely clusiaparalycoline A (**2**), B (**3**) and C (**4**), have been recently isolated from the roots of *Clusia paralycola* by a biossay-directed fractionation.[3]

We report herein the structure elucidation of three further compounds, isolated in a re-examination of the same plant, *i. e.* a biphenyl (**5a**), a dihydrophenanthrene (**6a**) and a xanthone (**7a**).

The formation of a trimethyl derivative (**5b**) with CH_2N_2 and a triacetyl derivative (**5c**) with pyridine/Ac_2O suggested the presence of three hydroxyl groups in compound (**5a**).

The ^{1}H- and ^{13}C-NMR spectral data -resumed in Table 1- and molecular peak at *m/z* 420 in the MS spectrum were consistent with a molecular formula $C_{27}H_{32}O_4$. In details, the NMR spectra showed the signals for a 2*H*-dihydropyran ring (C_5H_8O) and two γ,γ-dimethylallyl chains (2 x C_5H_9) as substituents.

If we ideally subtract from the molecular formula the elements of these substituents, giving back one H for each position made free, we obtain the skeleton of a trihydroxybiphenyl ($C_{12}H_{10}O_3$).



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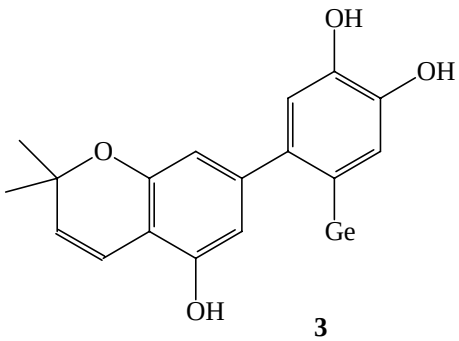

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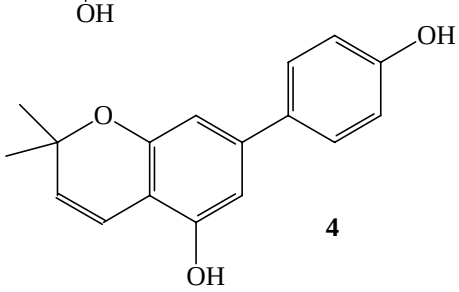

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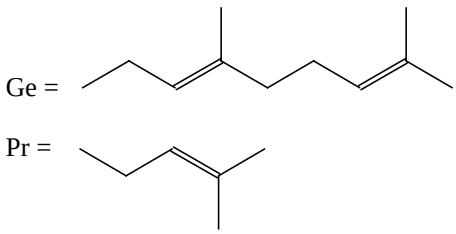
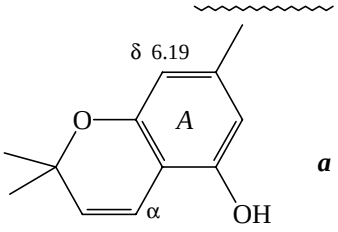
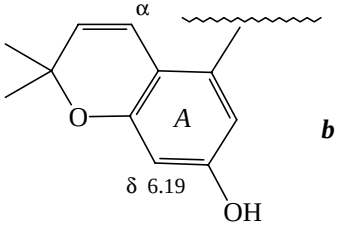


The multiplicity of the proton signal at δ 6.19 revealed a *meta* coupling (*J* = 1.4 Hz) with the aromatic proton at δ 6.04 and a long-range coupling (*J* = 0.7 Hz) wih the α-proton of the chromene ring. These findings, supported by decoupling experiments, require the two aromatic protons to be only in the relative positions of the two partial structures (***a***) and (***b***).



a



b

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

Carbon	δ_C	δ_H	$^{n}J_{H,C}$ 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)	$^{3}J_3$ $^{3}J_5$ $^{3}J_{15}$ $^{2}J_4$
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)	$^{3}J_4$ $^{3}J_{16}$ $^{3}J_{17}$ $^{2}J_{15}$
11	113.21	6.68 s	$^{3}J_7$ $^{3}J_9$ $^{3}J_{2"}$ $^{2}J_{10}$
2	111.31	6.19 dd (1.4, 0.7)	$^{3}J_4$ $^{3}J_6$ $^{3}J_7$ $^{2}J_3$
6	109.75	6.04 d (1.4)	$^{3}J_2$ $^{3}J_4$ $^{3}J_7$ $^{2}J_5$
4	108.02	-	
15	76.00	-	
1'	31.99	2.97 d (7)	$^{3}J_7$ $^{3}J_{11}$ $^{3}J_3$ $^{2}J_5$
16-Me	27.87	1.45 s	
1"	27.85	3.12 d (7)	$^{3}J_7$ $^{3}J_9$ $^{3}J_3$ $^{2}J_8$ $^{2}J_{2"}$
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	

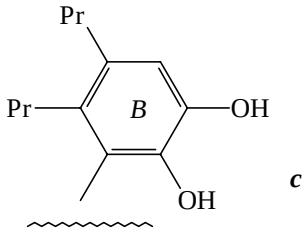
*400 and 100 MHz, respectively; in $CDCl_3$, relative to TMS as int. reference.

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

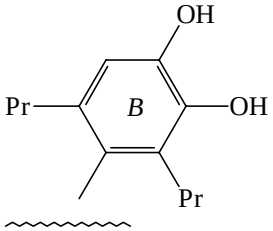
The addition of a D_2O/H_2O (1:1) solution caused in the ^{13}CNMR spectrum of **5a**, besides the expected splittings of three lowfield signals (at δ 150, 143 and 139), corresponding to the hydroxylated carbons, the doubling of the resonances at δ 109 and 113.

Since the signal at δ 111 (δ 6.19 in the ^{1}HNMR spectrum) gave no splitting, the partial structure (**b**) was excluded and the A ring was assigned structure (***a***). The diamagnetic shift (Δδ 0.25 ppm) of the α-chromene proton in the ^{1}H 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

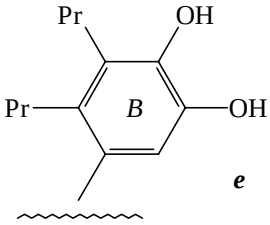
aromatic proton in the B ring, as confirmed by the presence of only one lowfield (δ 60)[6] methoxyl resonance in the ^{13}CNMR spectrum of the trimethyl derivative (**5b**). Therefore, only the partial structures (**c**), (**d**) and (**e**) were taken into consideration.



c



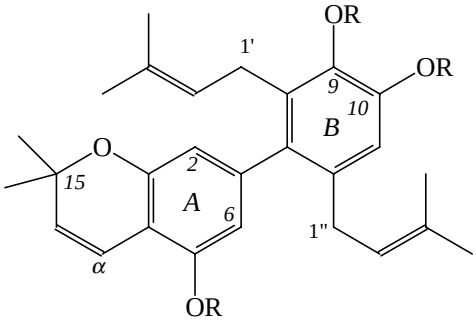
d



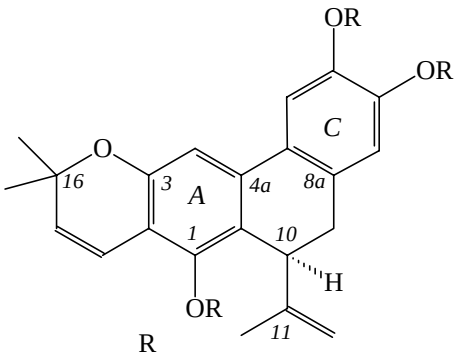
e

INEPTL[4] 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.



	R
5a	H
5b	Me
5c	COMe



	R
6a	H
6b	Me

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 CH_2N_2.

^{1}H and ^{13}C NMR spectra and molecular peak (*m/z* 392) in the mass spectrum indicated for **6b** a molecular formula $C_{25}H_{28}O_4$, 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-2*H*-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. ^{1}H and ^{13}C spectral data of compound **(6b**)

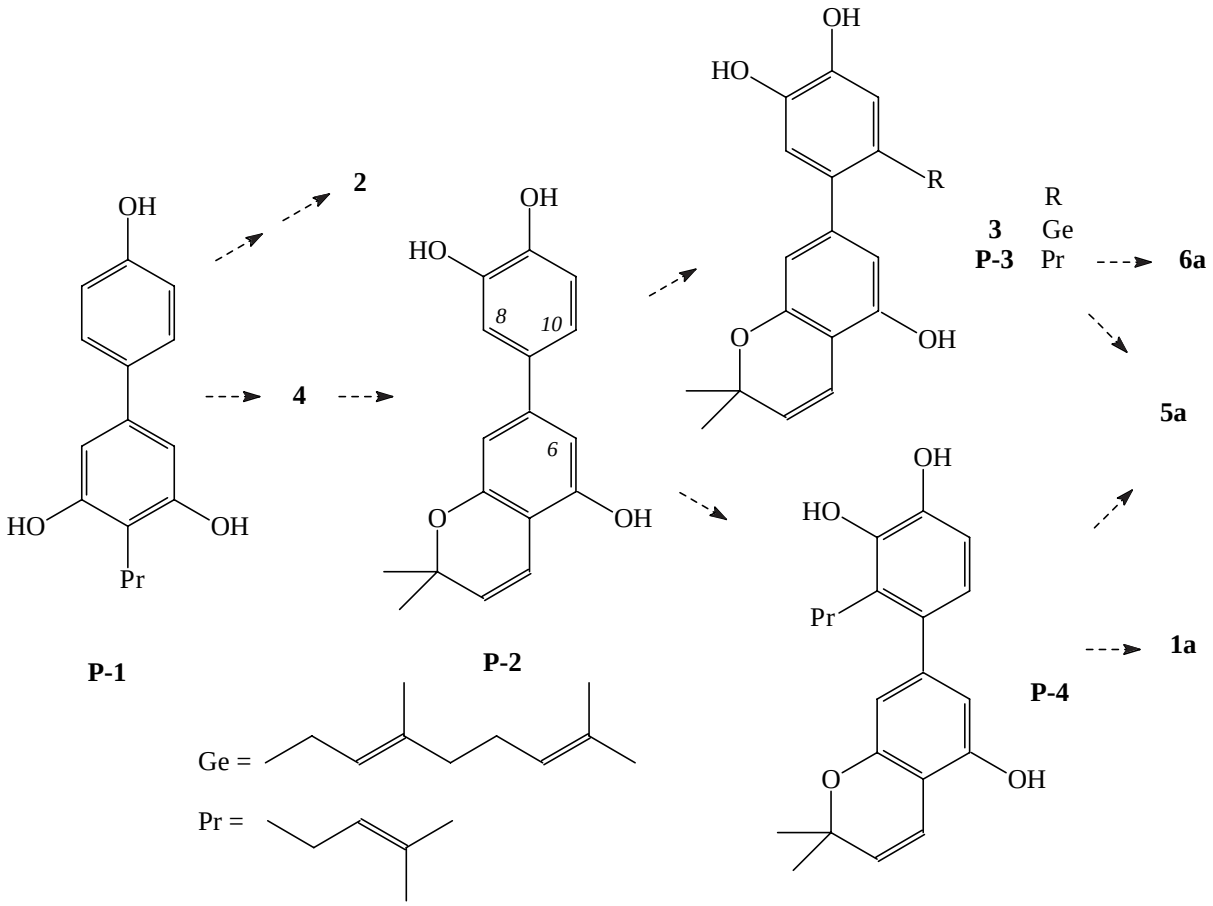
Carbon	δ_C	δ_H	$^{n}J_{H,C}$ connected carbons	
1	154.51	-		
3	153.75	-		
7	150.07	-		
6	149.17	-		
11	146.58	-		
4a	136.88	-		
15	129.80	5.40 d (10)		
8a	128.13	-		
4b	127.15	-		
10a	124.02	-		
14	118.02	6.65 br d (10)		
2	113.85	-		
		4.72 br s		
12	112.78			
		4.62 br s		
8	112.76	6.49 s	$^{3}J_{4b}$ $^{3}J_{6}$ $^{3}J_{9}$	$^{2}J_{7}$
5	108.23	7.18 s	$^{3}J_{4a}$ $^{3}J_{7}$ $^{3}J_{8a}$	$^{2}J_{6}$
4	107.93	7.36 br s	$^{3}J_{2}$ $^{3}J_{4b}$ $^{3}J_{10a}$	$^{2}J_{3}$
16	75.82	-		
1-OMe	61.97	3.61 s	$^{3}J_{1}$	
6-OMe		3.39 s		
	55.51			
7-OMe		3.37 s		
10	38.60	3.99 dd (6.5, 2)	$^{3}J_{1}$ $^{3}J_{4a}$ $^{3}J_{8a}$ $^{3}J_{12}$ $^{3}J_{13}$	$^{2}J_{9}$ $^{2}J_{11}$
		2.98 dd (15, 6.5)	$^{3}J_{4b}$ $^{3}J_{8}$ $^{3}J_{10a}$ $^{3}J_{11}$	$^{2}J_{8a}$ $^{2}J_{10}$
9	33.36			
		2.77 dd (15, 2)	$^{3}J_{4b}$ $^{3}J_{8}$ $^{3}J_{11}$	$^{2}J_{8a}$ $^{2}J_{10}$
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. $^{1}J_{H,C}$ connectivities were established by an HETCOR measurement; long-range connectivities were determined by a series of INEPTL experiments.[4]

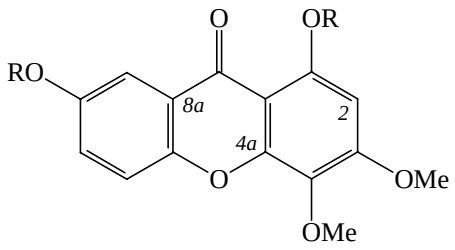
The co-occurrence of **5a** with **6a** supports our prediction that dihydrophenanthrenes may derive from prenylated biphenyl precursors.[1] In Scheme 1 a possible biogenetic pathway, involving paralycolin A(**1a**) and B (**6a**), the biphenyls (**2-4)** and (**5a**) and the precursors (**P-1**) to (**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

CH_2N_2) derivative (**7b**) were coincident with those of 1,3,4,7-tetramethoxyxanthone isolated from *Garcinia eugeniifolia* (Guttiferae).[7]



	R
7a	H
7b	Me

EXPERIMENTAL

General

Melting points (uncorrected): Kofler apparatus. ^{1}H and ^{13}CNMR (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. Clusioideae, 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 H_2O/hexane and H_2O/EtOAc. The EtOAc residue (45 g) was suspended in $CHCl_3$ (450 mL) at 4 °C overnight and filtered, the precipitate being essentially betulinic acid. The $CHCl_3$ soluble fraction (8 g) by column chromathography 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 chromathography on silica gel with $CHCl_3$ afforded compound (**5a**) (64 mg). The fraction CP-III (800 mg) washed with $CHCl_3$ gave the xanthone (**7a**) as a precipitate, whereas the filtrate by cc on silica gel with $CHCl_3$/MeOH, 95:5 yielded paralicolin A (**1a**, 294 mg). Finally, CP-IV (2.1 g) was suspended in $CHCl_3$ to give crude betulinic acid as a precipitate. The soluble fraction was treated with a saturated solution of CH_2N_2 in Et_2O and the reaction mixture by column chromathography on silica gel with benzene/EtOAc, 1:1, afforded **1b** (170 mg) and **6b** (60 mg).

Clusiachromene D (5a)

2H-Pyran-[3,4:6,5]-5,9,10-trihydroxy-8,12-bis[γ,γ-dimethylallyl]biphenyl: vitreous solid; ^{1}H and ^{13}C NMR in Table 1. EIMS *m/z* (rel. int.): 420 $[M]^+$ (49), 405 $[M\text{-}Me]^+$ (100), 403 $[M\text{-}OH]^+$ (22), 377 $[M\text{-}C_3H_7]^+$ (13), 365 $[M - C_4H_7]^+$ (14), 364 $[M - C_4H_8]^+$ (16), 361 $[405 - C_3H_8]^+$ (12), 349 $[405 - C_4H_8]^+$ (43), 335 $[405 - C_5H_{10}]^+$ (28), 321 $[364 - C_3H_7]^+$ (23), 309 $[364 - C_4H_7]^+$ (17), 293 $[349 - C_4H_8]^+$ (23); HRMS

Calcd for $C_{27}H_{32}O_4$: 420.2301. Found: 420.2305. *Methyl derivative* (**5b**): oil; ^{1}H 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, CH_2), 3.04 (2H, d = 7 Hz, CH_2), 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); ^{13}C 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, CH_2), 27.82 (q, Me), 27.65, 27.29 (t each, 2 x CH_2), 25.71 (q, 2 x *t*-Me), 17.64 (q, 2 x *c*-Me). *Acetyl derivative* (**5c**): ^{1}H 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 CH_2), 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); ^{13}C 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, CH_2), 27.86 (q, Me), 27.83, 27.68 (t each, 2 x CH_2), 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 (CH_2Cl_2/heptane): ^{1}H and ^{13}C NMR spectra in Table 2. EIMS *m/z* (rel. int.): 392 $[M]^+$ (43), 377 $[M - Me]^+$ (100), 361 $[M - OMe]^+$ (14), 351 $[M – C_3H_5]^+$ (14), 321 $[351 – OCH_2]^+$ (21), 196 $[M/2]^{+2}$ (17), 188.5 $[M –Me/2]^{+2}$ (30); HRMS Calcd for $C_{25}H_{28}O_4$: 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; (+ $AlCl_3$): 282, 328, 450; (+ $AlCl_3$/HCl): 281, 324, 445; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) : δ 12.77 (1H, s, exchanged with D_2O, 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); ^{13}C 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 $C_{15}H_{12}O_6$: 288.0634. Found: 288.0639.

1,3,4,7-Trimethoxyxanthone (**7b**): obtained by methylation of **7a** with CH_2N_2: mp 189-190 °C, lit.,[7] mp 189-191; UV and ^{1}H NMR spectra in agreement with published data.[7]

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