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Structures of new phenylphenalene-related compounds from *Eichhornia crassipes* (water hyacinth)

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ABSTRACT

Two new compounds identified as methyl derivatives of phenyl naphthalenedicarboxylic acid and phenyl naphthalenecarboxylic acid linked to a phenylphenalene unit were isolated from the extract of *Eichhornia crassipes* (water hyacinth). The structures have been determined on the basis of spectroscopic analyses, mainly using 2D NMR techniques.

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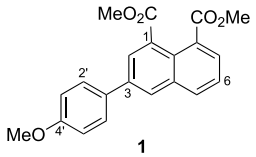
1. Introduction

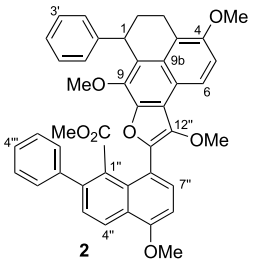
Eichhornia crassipes (water hyacinth) is invasive in much of the world where it often jams rivers and lakes with thousands of tons of floating plant matter.[1] In our search for compounds from aquatic plants, phenylphenalene metabolite derivatives were isolated from the ethyl acetate extract of *E. crassipes* and characterized as methyl ether derivatives obtained by treatment with methanolic CH_2N_2.[2] Recently, based on detailed spectroscopic analysis we revised the structures of some of them.[3] Further examination of the ethyl acetate extract has afforded two new compounds with a phenyl naphthalenedicarboxylic ester and a dimeric oxidized phenylphenalene skeletons.

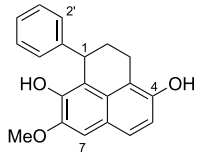
Phenylphenalenones represent a class of phenylpropanoid derived natural products that are of special interest because of their potential role as phytoalexins and phytoanticipins.[3–8]

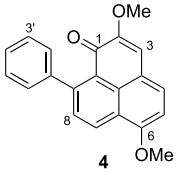
2. Results and discussion

The ethyl acetate extract of *E. crassipes* was concentrated in vacuo and fractionated into acidic and neutral fractions. The crude acidic residue was treated with methanolic CH_2N_2 and chromatographed on silica gel and Sephadex LH-20 columns and pure new compounds **1** and **2** were purified by HPLC.









Compound **1** showed the molecular ion peak at *m/z* 350 $[M]^+$ and significant fragments at *m/z* 319 $[M-CH_3O]^+$, 291 $[M-C_2H_3O_2]^+$ in the EIMS spectrum. Its molecular formula was deduced to be $C_{21}H_{18}O_5$ from a molecular ion peak at *m/z* 350.1150 in HREIMS spectrum and NMR spectral data. The ^1H and ^{13}C resonances of **1** were assigned by combination of COSY, DEPT, HSQC, and HMBC experiments (Table 1). The ^1H NMR spectrum showed two narrow doublets at δ 8.26 and 8.13 (J=1.5), two doublets at δ 8.04 and 7.98 (J=8.5), one triplet at δ 7.56 (J=8.5), two doublets at δ 7.68 and 7.04 (J=8.5), and two singlets at δ 3.94 and 3.88. These signals were attributed to a 1,3,8 trisubstituted naphthalene and a 1,4 disubstituted benzene by a COSY experiment.

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Table 1
NMR spectral data of compound **1** in $CDCl_3$, 500 MHz (^1H) and 125 MHz (^{13}C)

Position	^1H δ[a] (m, *J*/Hz)	NOESY	δ_C	HMBC[b]
1			131.0 (q)[c]	
2	8.26 (d, 1.5)	2′/6′	129.8 (t)	1′, 4, 9, 1-*C*OOMe
3			138.0 (q)	
4	8.13 (d, 1.5)	2′/6′	128.8 (t)	1′, 9
5	8.04 (d, 8.5)		132.6 (t)	4, 7, 9, 10
6	7.56 (t, 8.5)		125.6 (t)	7, 8, 10
7	7.98 (d, 8.5)		129.8 (t)	5, 9, 8-*C*OOMe
8			132.0 (q)	
9			126.6 (q)	
10			135.7 (q)	
1′			132.5 (q)	
2′/6′	7.68 (d, 8.5)	2, 4	128.3 (t)	3, 4′, 3′/5′
3′/5′	7.04 (d, 8.5)	4′-OMe	114.5 (t)	1′, 4′
4′			159.8 (q)	
4′-OMe	3.88 s	3′/5′	57.3 (p)	4′
1-COO*Me*	3.94 s		52.1 (p)	1-*C*OOMe
1-*C*OOMe			169.6 (q)	
8-COO*Me*	3.94 s		52.1 (p)	8-*C*OOMe
8-*C*OOMe			169.6 (q)	

[a] ^1H chemical shift values (δ ppm from $SiMe_4$); given in parentheses multiplicity and coupling constant (*J* in Hz).
[b] HMBC correlations from H to C.
[c] Letters p, s, t, and q, in parentheses indicate, respectively, the primary, secondary, tertiary, and quaternary carbons, assigned by DEPT.

In the ^{13}C NMR spectrum (Table 1) 16 carbon signals were evident. The DEPT spectrum indicated the presence of two methyl and six methine signals. The HSQC experiment allowed the assignment of the protons to the corresponding carbons.

The HMBC spectrum (Table 1) showed cross peaks of the H-2 and H-7 protons (δ 8.26 and 7.98, respectively), with the C-9 and the carboxyl carbons (δ 126.6 and 169.6, respectively), the C-9 carbon was also correlated to the H-4 and H-5 (δ 8.13 and 8.04). In the same spectrum the H-2 and H-4 gave correlations with C-1′ (δ 132.5) and H-6 (δ 7.56) with C-10 (δ 135.7). Finally, the H-2′/H-6′ protons (δ 7.68) gave cross peaks with the C-3 and C-4′ carbons and the H-3′/H-5′ protons gave cross peaks with the C-1′ carbon. These data led to the structure of dimethyl 3-(4-methoxyphenyl)naphthalene-1,8-dicarboxylate for compound **1**. The assignment of the methoxyl at C-4′ was confirmed by NOE between the methoxyl at δ 3.88 and the H-3′/H-5′ protons. Compound **1** could be formed by oxidation of 2-hydroxy-8-(4-hydroxyphenyl)phenalen-1-one, bearing the aryl substituent in the unusual 8-position, previously identified from *E. crassipes*.[9]

Compound **2** had in the HREIMS spectrum a molecular ion peak at *m*/*z* 664.2459 consistent with a molecular formula $C_{43}H_{36}O_7$. The structure of compound **2** was obtained using two-dimensional NMR techniques (COSY, NOESY, HSQC, and HMBC, Table 2). The ^1H NMR and COSY spectra revealed three sets of *ortho*-coupled aromatic protons (δ 8.14 and 7.62; 7.90 and 7.36; 7.66 and 6.78) and 10 aromatic protons of a monosubstituted phenyl ring (δ 7.90, 7.45, and 7.38; 7.21, 7.16, and 7.02). The COSY spectrum indicated that the signals located at δ 2.02/2.19 and 2.50/3.15 corresponded to two vicinal methylenes and the first was coupled directly to a benzylic methine, whose signal was located at δ 4.80. Furthermore, the ^1H NMR spectrum showed the presence of five methoxyl groups (δ 3.97, 3.94, 3.75, 3.65, and 3.25).

The ^{13}C NMR spectrum (Table 2) showed only 19 signals, that were identified by a DEPT experiment, as five methyls, two methylenes, and 11 methines.

All of the protons were correlated to the respective carbons using an HSQC experiment. The connectivity of these fragments was made through the analysis of HMBC data, that was essential to obtain the structure of the compound **2**. The benzyl proton (δ 4.80) and the methylene (δ 2.02 and 2.19) attributed to the H-2 gave cross peaks with the carbons at δ 145.7 and 117.4 (C-1′ and C-9a), both the H-2 and H-3 methylenes were correlated to the carbon at δ 122.7

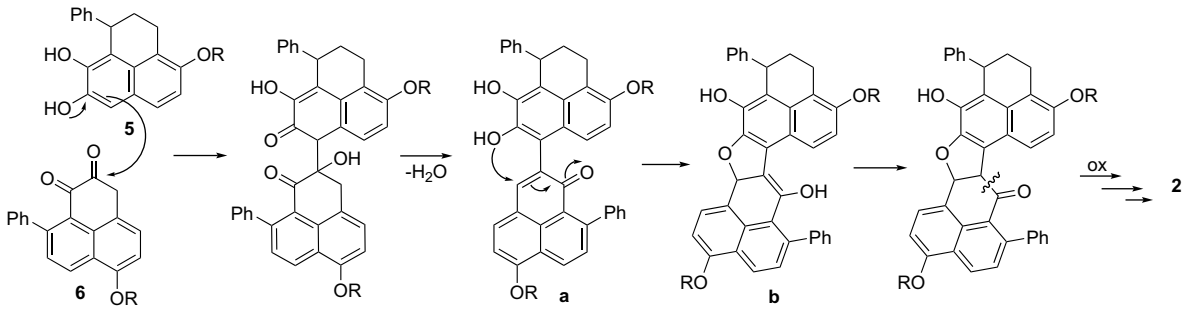
Table 2
NMR spectral data for compound **2** in $CDCl_3$, 500 MHz (^1H) and 125 MHz (^{13}C)

Position	^1H δ[a] (m, *J*/Hz)	NOESY	δ_C	HMBC[b]
1	4.80 (dd, 4.2, 2.8)	2′/6′	39.8 (t)[c]	2, 3, 9, 9a, 9b, 1′, 2′/6′
2*ax*	2.02 dddd (13.6, 13.6, 4.2, 3.9)		29.0 (s)	1, 3, 3a, 9a, 1′
2*eq*	2.19 dddd (13.6, 3.9, 2.8, 2.8)			
3*ax*	2.50 ddd (17.6, 13.6, 3.9)		18.1 (s)	1, 2, 3a, 4, 9b
3*eq*	3.15 ddd (17.6, 3.9, 2.8)			
3a			122.7 (q)	
4			154.6 (q)	
5	6.78 (d, 8.0)	4-OMe	105.4 (t)	3a, 4, 6a
6	7.66 (d, 8.0)		129.0 (t)	4, 7, 9b
6a			133.6 (q)	
7			115.0 (q)	
8			144.5 (q)	
9			141.8 (q)	
9a			117.4 (q)	
9b			138.8 (q)	
1′			145.7 (q)	
2′/6′	7.02 (d, 7.5)	1, 2, 9-OMe	128.3 (t)	1, 3′/5′, 4′
3′/5′	7.21 (t, 7.5)		129.0 (t)	1′, 2′/6′
4′	7.16 (t, 7.5)		125.4 (t)	2′/6′, 3′/5′
4-OMe	3.97 s	5	55.0 (p)	4
9-OMe	3.65 s	2′/6′	59.5 (p)	9
1″			134.8 (q)	
2″			138.0 (q)	
3″	7.62 (d, 8.5)		129.1 (t)	1″, 10″, 1‴
4″	8.14 (d, 8.5)		121.8 (t)	2″, 5″, 9″
5″			152.4 (t)	
6″	7.36 (d, 8.5)	5″-OMe	112.5 (t)	5″, 7″, 8″, 10″
7″	7.90 (d, 8.5)		121.5 (t)	5″, 8″, 9″, 11″
8″			122.0 (q)	
9″			131.2 (q)	
10″			122.8 (q)	
11″			138.0 (q)	
12″			125.8 (q)	
1‴			140.3 (q)	
2‴/6‴	7.90 (d, 8.0)	1″-COO*Me*	129.8 (t)	2″, 3‴/5‴, 4‴
3‴/5‴	7.45 (t, 8.0)		127.3 (t)	1‴, 2‴/6‴
4‴	7.38 (t, 8.0)		127.3 (q)	
5″-OMe	3.94 s	6″	55.0 (p)	5″
12″-OMe	3.25 s	7″	52.6 (p)	12″
1″-COO*Me*	3.75 s	2‴/6‴	52.0 (p)	1″-*C*OOMe
1″-*C*OOMe			171.9 (q)	

[a] ^1H chemical shift values (δ ppm from $SiMe_4$); given in parentheses multiplicity and the coupling constant (*J* in Hz).
[b] HMBC correlations from H to C.
[c] Letters p, s, t, and q, in parentheses indicate, respectively, the primary, secondary, tertiary, and quaternary carbons, assigned by DEPT.

(C-3a). The H-3 protons were also correlated with the carbons at δ 154.6 and 138.8 (C-4 and C-9b). Furthermore, the H-5 and H-6 protons were correlated to the C-4 and C-6a (δ 133.6) carbons and the H-6 proton was also correlated to C-7 (δ 115.0). Beside the already reported correlations, the H-3″ proton was correlated to the C-1″ (δ 134.8), C-10″ (δ 122.8), and C-1‴ (δ 140.3) carbons. The H-4″ proton was correlated to the carbons at δ 138.0, 152.4, and 131.2 assigned to C-2″, C-5″, and C-9″, respectively. Both H-6″ and H-7″ protons were correlated to the carbons C-5″ and C-8″ (δ 122.0), the first proton was also correlated to the C-10″ carbon and the second to the C-9″ and C-11″ (δ 138.0) carbons.

The analysis of the NOESY spectrum (Table 2) showed NOEs between the H-2′/H-6′ protons with H-1, H-2, and 9-OMe, the H-5 proton with 4-OMe, the H-6 with 12″-OMe, the H-6″ with 5″-OMe, the H-2‴/H-6‴ with 1″-COOMe. These data confirmed the structure of compound **2** as methyl 5-methoxy-2-phenyl-8-(3,7,10-trimethoxy-6-phenyl-5,6-dihydro-4*H*-phenaleno[2,1-*b*]furan-9-yl)-1-naphthoate. This compound could be derived from coupling of



Scheme 1. Hypothetical pathway for metabolite **2** formation.

phenylphenalene (**5**) and phenylphenalenones (**6**, lachnanthocarpone), whose related compounds were found in *E. crassipess*.[2b,3] A hypothesis for the formation of this compound is described in Scheme 1. Phenylphenalene adds to lachnanthocarpone (1,2-dione form) and subsequent re-aromatization and loss of a water molecule gives the intermediate (**a**). 1,4-Addition to the enone of hydroxyl gives cyclic intermediate (**b**). Oxidation of the **b** tautomer at α carbonyl linkage gives compound **2**.

The crude neutral fraction was fractionated by silica-gel column chromatography and the fractions were purified by preparative thin-layer chromatography yielding pure compounds **3** and **4**. The spectroscopic data of compound **3** corresponded to 2,3-dihydro-4,9-dihydroxy-8-methoxy-1-phenylphenalene isolated from *Musa texlis*.[10] Compound **4** was identified as 2,6-dimethoxy-9-phenyl-1*H*-phenalen-1-one (also known as lachnanthocarpone dimethyl ether).[2a]

3. Experimental section

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were run on a Varian INOVA 500 NMR spectrometer at 500 and 125 MHz, respectively. Electronic ionization mass spectra (EIMS) were obtained with an HP 6890/5973-N GC/MS. HPLC was performed on an Agilent 1100 by using an UV detector. Silica gel 60 (230–400 mesh, E. Merck) and Sephadex LH-20 (Pharmacia) were used for CC. Preparative HPLC was performed using RP-8 (LiChrospher 10 μm, 250×10 mm i.d., Merck) column.

3.2. Isolation of compounds

Plant of *E. crassipes* were collected in the Botanical Gardens of the University of Federico II and identified by Prof. Antonino Pollio of the Dipartimento di Biologia Vegetale of the University of Naples.

Dry plant (11 kg) were extracted with AcOEt to give a crude extract (70 g), which was separated into neutral (62 g) and acidic fraction (6.0 g) by conventional procedures.

The acidic fraction was treated with methanolic CH_2N_2 and then chromatographed on SiO_2 column eluting with petroleum ether–AcOEt gradient. Fraction eluted with petroleum ether–AcOEt [(7:3), 200 mg] was rechromatographed on Sephadex LH-20 column eluting with EtOH to afford fractions A–F. Fraction D (56 mg) was purified by reversed-phase HPLC column [MeCN–H_2O (17:3)] to give pure compound **2** (2 mg). Fraction E (40 mg) was purified by reversed-phase HPLC column [CH_3CN–H_2O (17:3)], to give **1** (4 mg).

27.4 g of neutral fraction was chromatographed on SiO_2 column eluting with petroleum ether–AcOEt gradient. Fraction eluted with petroleum ether–AcOEt (17:3), 386 mg, was rechromatographed on SiO_2 column eluting with $CHCl_3$–acetone gradient. Fraction eluted with 100% $CHCl_3$, 56 mg, was purified on PLC eluent hexane–acetone (4:1) to give **3** (10 mg). Fraction eluted with petroleum ether–AcOEt (7:3), 120 mg, was rechromatographed on SiO_2 column eluting with $CHCl_3$–acetone gradient. Fraction eluted with 100% $CHCl_3$, 30 mg, was purified on PLC eluent hexane–acetone (4:1) to give **4** (15 mg).

*3.2.1. Dimethyl 3-(4-methoxyphenyl)naphthalene-1,8-dicarboxylate (**1**).* White solid; HREIMS *m*/*z* 350.1150 $[M]^+$ (calcd for $C_{21}H_{18}O_5$ 350.1154); ν_{max} (CH_2Cl_2) 3080, 1719, 1606, 1472, 1378, 1098, 910 cm^{-1}; UV λ_{max} ($CHCl_3$) nm (log ε): 231 (4.0), 280 (4.5); ^{1}H and ^{13}C NMR spectroscopic data are listed in Table 1.

*3.2.2. Compound **2**.* White solid; HREIMS *m*/*z* 664.2459 $[M]^+$ (calcd for $C_{43}H_{36}O_7$ 664.2461); $[\alpha]_D^{25}$ 0.0 (c 0.10, $CHCl_3$); ν_{max} (CH_2Cl_2) cm^{-1} 3046, 1814, 1635, 1601, 1469, 1095, 908; UV λ_{max} ($CHCl_3$) nm (log ε): 232 (1.4), 251 (4.3), 306 (0.6); ^{1}H and ^{13}C NMR spectroscopic data are listed in Table 2.

*3.2.3. 2,6-Dimethoxy-9-phenyl-1H-phenalen-1-one (**4**, lachnanthocarpone dimethylether).* Orange solid; EIMS *m*/*z* 316 $[M]^+$; ^{1}H NMR: δ 8.62 (1H, d, *J*=8.5 Hz, H-7), 7.59 (1H, d, *J*=8.0 Hz, H-4), 7.56 (1H, d, *J*=8.5 Hz, H-8), 7.42 (2H, br t, *J*=8.2 Hz, H-3′ and H-5′), 7.36 (3H, overlapped, H-2′, H-4′, and H-6′), 6.89 (1H, d, *J*=8.0 Hz, H-5), 6.81 (1H, s, H-3), 4.08 (3H, s, 2-OCH_3), and 3.85 (3H, s, 6-OCH_3); ^{13}C NMR: δ 179.8 (C-1), 157.5 (C-6), 152.1 (C-2), 148.5 (C-9), 143.0 (C-1′), 130.7 (C-4 and C-8), 128.4 (C-7), 127.8 (C3′/C-5′ and C-4′), 126.8 (C-9b), 126.3 (C-9a), 124.3 (C-6a), 121.4 (C-3a), 112.0 (C-3), 104.7 (C-5), 56.0 (6-OCH_3), and 55.4 (2-OCH_3), 28.1 (C-2′/C-6′).

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