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Dimeric Phenalene Metabolites from *Eichhornia crassipes*

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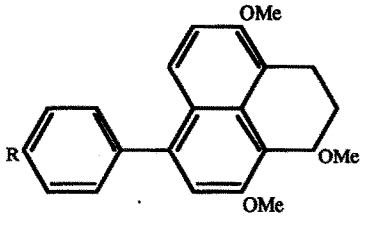
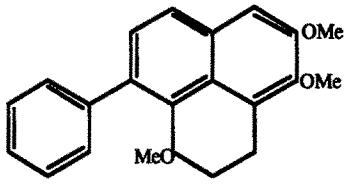
Key Words: *Eichhornia crassipes*; phenalenes; dimeric phenalenols.

Abstract. Ten aromatic metabolites with the phenalene skeleton have been isolated as permethylated derivatives from the ethyl acetate extract of the aquatic plant *Eichhornia crassipes*. Beside the already described monomeric lachnanthocarpone red dimethyl ether, 4,9-dimethoxy-7-phenyl-2,3-dihydro-phenalen-1-ol-O-methyl, 4,9-dimethoxy-7-(4'-methoxy-phenyl)-2,3-dihydro-phenalen-1ol-O-methyl and 4,5-dimethoxy-9-phenyl-2,3-dihydro -phenalen-1-ol-O-methyl, six new dimeric compounds have been characterized. The structures have been deduced on the basis of the data of these compounds.

Eichhornia crassipes is an aquatic plant, commonly known as water hyacinth, which we are investigating in connection with a study of the allelopathic interactions between freshwater macrophytes and microalgae[1]. From the ethyl acetate extract of the plant ten aromatic compounds with the phenalene skeleton have been isolated as permethylated derivatives. Besides the known lachnanthocarpone red dimethyl ether[2], present in the neutral fraction of the extract, the following have been isolated from the acidic fraction: the already characterized[3] 4,9-dimethoxy-7-phenyl-2,3-dihydro-phenalen-1-ol-O-methyl (**1**), 4,9-dimethoxy-7-(4'-methoxy-phenyl)-2,3-dihydro-phenalen-1-ol-O-methyl (**2**), 4,5-dimethoxy-9-phenyl-2,3-dihydro-phenalen-1-ol-O- methyl (**3**), and six new dimeric compounds **4 - 9**.



1 R = H **2** R = OMe



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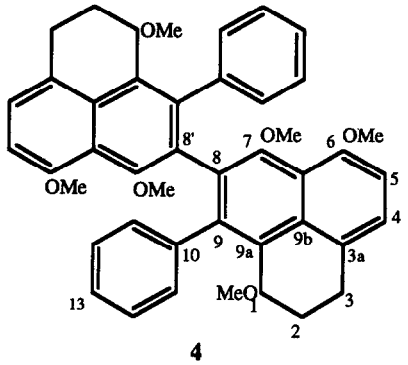
Compound **4**, λ_{max} 239 (ε 25,000) was attributed the molecular formula $C_{44}H_{42}O_6$ according to a molecular ion peak at *m/z* 666.2893 in its HR-MS spectrum. The presence in the ^{13}C-NMR spectrum of only 19 signals

(Table 1), attributable on the basis of an inverse gated decoupling experiment to 22 different carbons, suggested a dimeric structure. The ^{1}H-NMR spectrum showed five aliphatic signals centered at δ 2.08, 2.20, 2.47, 3.12 and 4.86 attributable to ten protons, three signals at δ 3.58, 3.61 and 3.86 for six methoxyl groups, two doublets at δ 7.00 and 7.12 of four aromatic protons besides ten protons, in the 7.06 - 7.24 ppm range, attributable to two phenyl groups corroborated by the presence of a peak at *m/z* 589 $[M - C_6H_5]^+$ in the MS spectrum. The similarity of the aliphatic signals in the ^{1}H-NMR spectrum with those of the monomeric 2,3-dihydro-phenalen-1-ol methyl ethers[3] **1 - 3** allowed the attribution of the signals at δ 4.86 to the H-1 protons, the signals at δ 2.08 and 2.20 to the H-2 protons and those at δ 2.47 and 3.12 to the H-3 protons of two linked identical phenalene moities.

The methoxyl groups at δ 3.86, linked to the carbon at δ 152.4 on the basis of H-C long range data, showed an nOe effect with the doublets at δ 7.00 which were coupled with the doublet at δ 7.12. This latter signal showed in the H-C long range spectrum cross peaks with the signals at δ 152.4 and 125.1 with this latter also correlated with the H-1 and H-3 protons. These data univocally assigned the signal at δ 125.1 to the C-9b carbons and consequently, the protons at δ 7.12 and 7.00 to the C-4 and C-5 positions and the methoxyl group at C-6.



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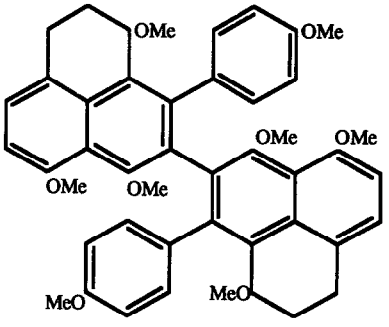

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On biogenetic grounds, the phenyl groups could be located at C-7 or at C-9[4]. However the C-7 *peri* position was excluded not only because the *ortho* and *meta* carbons of the phenyl groups had the same chemical shifts[5] but also because a phenyl in the *peri* position should have shifted the methoxyl group at C-6 to a value lower than 3.2 ppm[6]. Accordingly, the signal at δ 148.8, assigned to the C-9 carbon, had heterocorrelations with both the protons H-1 and the protons at δ 7.06 of the phenyl groups. The chemical shift of the C-9 carbons then excluded the presence of a methoxyl group at the *ortho* position[7] so that these groups were located at C-7 and the two monomeric moieties were linked through the carbons C-8 and C-8' at δ 130.9.

Compound **5**, λ_{max} 239 (ε 25,000), was different from **4** in the presence of *p*-methoxyphenyl groups instead of the phenyl groups. In fact the MS spectrum had a molecular ion at *m/z* 726.3201 corresponding to a molecular formula $C_{46}H_{46}O_8$ and it showed a peak at *m/z* 619 due to the loss of a *p*-methoxyphenyl residue. Accordingly, the ^{1}H-NMR spectrum showed a methoxyl signal at δ 3.80 and an AA'BB' system at δ 6.84 and

7.01 instead of the phenyl protons and the ^{13}C-NMR spectrum showed a signal for C-10 at δ 137.7, the C-11 and C-15 signals at δ 128.9, the C-12 and C-14 signals at δ 113.5 and the C-13 at δ 157.7.

Compound **6**, λ_{max} 234 (ε 21,000) had a molecular formula $C_{46}H_{46}O_8$ corresponding to the molecular ion at *m/z* 726.3190 in its MS spectrum. Also in this case, the presence of only 23 carbons in an inverse gated decoupling experiment was diagnostic of the presence of two identical moieties in the molecule. The ^{1}H-NMR spectrum besides the H-1 protons at δ 4.92, the H-2 protons at δ 2.21 and 2.26 and the H-3 protons at δ 2.56 and 3.19 showed eight methoxyl signals at δ 3.65 (12H), 3.80 (6H) and 3.86 (6H), two aromatic AA'BB' systems at δ 6.83 and 7.02 and four protons as a singlet at δ 7.05. The peak at m/z 619 in the MS spectrum and the nOe effect of the methoxyl at δ 3.80 on the protons at δ 6.83 were indicative of the presence of *p*-methoxyphenyl groups which were located at the C-9 positions owing to the nOe effect of the C-1 methoxyl group at δ 3.63 on the H-11 and H-15 protons at δ 7.02. The aromatic signal at δ 7.05 had nOe

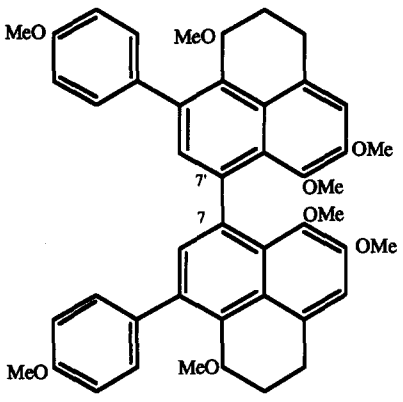
Table 1. ^{13}C NMR chemical shifts of compounds **4** - **9**

C	**4**	**5**	**6**	**7**	**8**	**9**
1 1'	38.7	38.6	38.5	38.7	38.6	38.7 37.8
2 2'	29.4	29.3	29.3	29.4	29.2	29.4
3 3'	18.3	18.3	18.1	18.2	18.0	18.2
3a 3a'	120.5	120.6	120.7	120.5	120.4	120.5
4 4'	124.9	124.9	111.9	124.8	124.9 111.9	124.9
5 5'	111.9	111.9	152.4	111.8	111.9 152.4	111.8
6 6'	152.4	152.4	149.2	152.4	152.4 149.2	152.4
6a 6a'	126.2	126.2	126.1	126.0	125.8	126.2
7 7'	148.8	148.3	149.0	128.8	149.3 128.7	128.8 148.7
8 8'	130.9	130.9	124.6	149.7	124.7	149.2 130.9
9 9'	149.3	149.3	148.7	130.9	148.4	130.9 149.3
9a 9a'	128.8	128.8	128.4	128.3	128.2	128.3 128.9
9b 9b'	125.1	125.1	124.8	125.0	124.7 126.1	125.1
10 10'	145.7	137.7	137.8	137.7	137.8 145.7	137.7 145.7
11 11'	128.1	128.9	128.9	128.9	128.9 128.1	128.9 128.0
12 12'	128.1	113.5	113.4	113.5	113.5 128.1	113.4 128.0
13 13'	125.8	157.7	157.7	157.7	157.7 125.7	157.7 125.8
14 14'	128.1	113.5	113.4	113.5	113.5 128.1	113.4 128.0
15 15'	128.1	128.9	128.9	128.9	128.9 128.	128.9 128.0

4 1-OMe = δ 60.8, 6-OMe = δ 56.1, 7-OMe = δ 60.4.
5 1-OMe = δ 60.8, 6-OMe = δ 56.0, 7-OMe = δ 60.3, 13-OMe = δ 55.4.
6 1-OMe = δ 60.8, 5-OMe = δ 53.6, 6-OMe = δ 54.1, 13-OMe = δ 55.2.
7 1-OMe = δ 60.8, 6-OMe = δ 56.0, 8-OMe = δ 60.4, 13-OMe = δ 55.2.
8 1-OMe = δ 60.8, 6-OMe = δ 53.5, 7- OMe = δ 54.1, 13-OMe = δ 55.3, 1'-OMe = δ 61.1, 5'-OMe = δ 56.1, 6'-OMe = δ 60.3.
9 1-OMe = δ 60.8, 6-OMe = δ 56.0, 7-OMe = δ 60.3, 13-OMe = δ 55.2, 1'-OMe = δ 60.7, 6'-OMe = δ 56.0, 8'-OMe = δ 60.4.

interaction with the methoxyl signal at δ 3.86 and gave cross peaks in the H-C long range COSY with the signal at δ 120.7 and the signal at δ 152.7, bearing the methoxyl group at δ 3.64. As the signal at δ 120.7 was also correlated to the H-1 and H-3 protons, it was assigned to the C-9b carbon and, consequently, two of the protons at δ 7.05 were located at C-4 while the methoxyl groups were located at the C-5 and C-6 positions. Finally the residue protons at δ 7.05 were assigned at C-8 on the basis of their long range correlation with the 6a carbons at δ 126.1 and the 9a carbons at δ 128.4 thus setting at C-7 and C-7' the linkage of the two moieties of the molecule.

Compound **7**, λ_{max} 238 (ε 22,500), had a molecular formula $C_{46}H_{46}O_8$, according to the peak at *m/z* 726.3212 in the HR-MS spectrum. The ^{1}H-NMR spectrum was similar to that of **4** with the H-1 protons at δ 4.97, the H-2 protons at δ 2.24 and 2.29, the H-3 protons at δ 2.55 and 3.20, eight methyls at δ 3.58 (6H), 3.63 (6H), 3.77 (6H) and 3.85 (6H), an AA'BB' system at δ 6.78 and 6.96 and an AB system at δ 7.00 and 7.12. By contrast, remarkable differences were found in the ^{13}C-NMR spectrum which exhibited the C-7, C-9 and C-9b carbons shifted upfield to δ 138.8, 130.9 and 120.5 respectively and the C-6a and C-9a carbons shifted downfield to δ 125.0 and 135.2 respectively. These differences suggested a structure with a methoxyl group at C-8 and the linkage between the two moieties at C-7 and C-7'.



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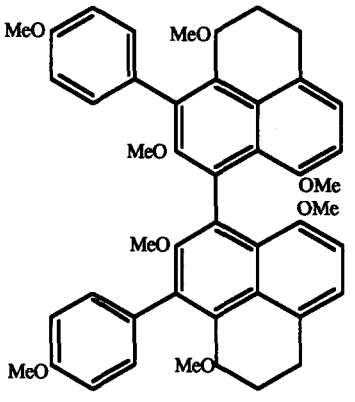

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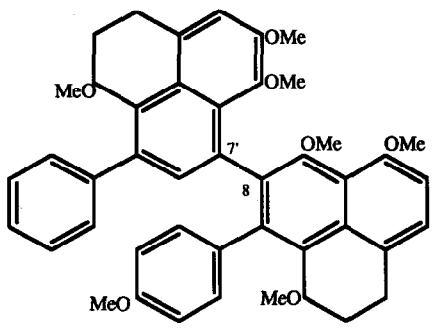
Compound **8**, λ_{max} 238 (ε 24,000), had a molecular formula $C_{45}H_{44}O_7$ according to the molecular ion peak at *m/z* 696.3104 in the HR-MS spectrum. The ^{13}C-NMR spectrum showed 28 signals attributable through an inverse gated decoupling experiment to 45 carbons. These data suggested a structure with two different phenalene moieties.

The ^{1}H-NMR spectrum showed the H-1 and H-1' protons at δ 4.92 and 4.98, the four H-2 and H-2' protons centered at δ 2.27, the H-3_{ax} and H-$3'_{ax}$ at δ 2.57, the H-3_{eq} and H-$3'_{eq}$ at δ 3.19, seven methyls at δ 3.62, 3.65 (9H), 3.80 and 3.86 (6H). In the aromatic region of the spectrum an AA'BB' system at δ 6.83 and 7.01, an AB system at δ 7.11 and 7.28, a singlet at δ 7.06 were detectable besides five protons of a phenyl group in the 7.06 - 7.24 ppm range. NOe experiments showed interaction of the methyl at δ 3.62 with the H-1 proton and the H-

11 and H-15 protons at δ 7.01, interaction of the methyl at δ 3.86 with the H-4' proton at δ 7.06, interaction of the methyl at δ 3.80 with the H-12 and H-14 protons at δ 6.83 and, finally, interaction of the methyl at δ 3.65 with the H-1' proton. These data and the ^{13}C-NMR chemical shifts suggested a dimeric structure with a 6,7-dimethoxy-9-phenyl-2,3-dihydro-phenalen-1-ol-O-methyl linked at the C-8 carbon with the C-7' carbon of a



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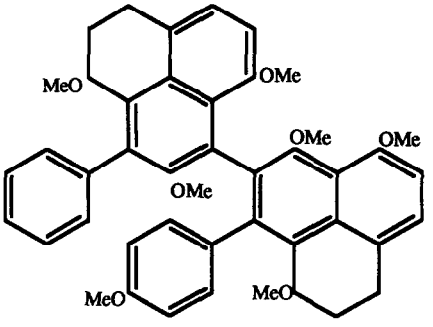

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5,6-dimethoxy-9-phenyl-2,3-dihydro-phenalen-1-ol-O- methyl with a *p*-methoxyl group at one of the phenyl groups.

Compound **9**, λ_{max} 238 (ε 23,000) had molecular formula $C_{45}H_{44}O_7$. The ^{1}H-NMR spctrum showed four protons of an AA'BB' system at δ 6.78 and 6.96, four protons as two coincident AB systems at δ 6.99 and 7.11 and five protons of a phenyl group in the 7.03 - 7.24 ppm range. The ^{13}C-NMR spectrum had all the signals present in the compounds **4** and **7** suggesting a structure with a 6,7-dimethoxy-9-phenyl-phenalen-1-ol-O-methyl moiety linked to a 6,8-dimethoxy-9-phenyl-phenalen-1-ol-O- methyl through the C-8 and C-7' carbons respectively with a *p*-methoxyl group at one of the phenyl groups.

EXPERIMENTAL

General procedures. UV spectra were obtained on a Perkin-Elmer LAMBDA 7 spectrophotometer in EtOH solutions. EIMS spectra were performed on a Kratos MS 50 apparatus at 70 eV. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM 400 spectrometer in $CDCl_3$ solutions. One-bond and long-range H-C COSY experiments were performed with the XHCORR microprogramme using delays corresponding to $J_{C,H}$ 160 Hz and 8 Hz respectively. The CD curves were measured in ethanol with a Jasco J-500 dichrograph. The apparatus for HPLC consisted of a pump module (Varian Vista 5500), an UV detector (Varian UV 100) and a recorder (Varian Linear 1200). Hibar LiChrosorb RP-18 (5 μm, 250 x 4 mm i.d.) and Hibar LiCHrosorb RP-18 (7 μm, 250 x 10 mm i.d.) columns from Merck were used for analytical and preparative purposes respectively.

Isolation of compounds. The plants of *E. crassipes* (dry weight 11 Kg) were treated with ethyl acetate to give a crude extract (70 g) which was separated into a neutral (62 g) and an acidic fraction (6 g) by conventional procedures. An aliquot of the acid material (3 g) was treated with methanolic CH_2N_2 to give a

mixture of permethylated derivatives which were fractionated on Si gel column with light petroleum-ethyl acetate. Elution with a mixture 9:1 gave the monomeric **1 - 3,** which were separated through C-18 reverse phase HPLC (MeOH - H_2O 17:3) while a mixture 4:1 gave the dimeric **4 - 9,** separated through C-18 reverse phase HPLC (MeOH-H_2O 9:1). **4** (21 mg) had $[\alpha]_D$ - 4° (c 0.9 in $CHCl_3$); CD: $[\theta]_{236}$ + 45,000 $[\theta]_{251}$ - 70,000; EIMS: *m/z* 666, 651, 623, 589. **5** (16 mg) had $[\alpha]_D$ - 3° (c 1.1 in $CHCl_3$); CD: $[\theta]_{233}$ + 41,000 $[\theta]_{246}$ - 63,000; EIMS: *m/z* 726, 711, 683, 619. **6** (5 mg) had $[\alpha]_D$ + 4° (c 0.8 in $CHCl_3$); CD: $[\theta]_{235}$ + 20,000 $[\theta]_{247}$ -17,000; EIMS: *m/z* 726, 711, 683, 619. **7** (11 mg) had $[\alpha]_D$ - 5° (c 1.0 in $CHCl_3$); CD: $[\theta]_{232}$ - 37,000 $[\theta]_{245}$ + 26,000; EIMS: *m/z* 726, 711, 683, 619. **8** (14 mg) had $[\alpha]_D$ + 8° (c 1.2 in $CHCl_3$); CD: $[\theta]_{233}$ + 71,000 $[\theta]_{246}$ - 99,000; EIMS: *m/z* 696, 681, 653, 619, 589. **9** (15 mg) had $[\alpha]_D$ - 5° (c 1.1 in $CHCl_3$); CD: $[\theta]_{232}$ - 36,000 $[\theta]_{246}$ + 68,000; EIMS: *m/z* 696, 681, 653, 619, 589.

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