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PHENALENE METABOLITES FROM *EICHHORNIA CRASSIPES*

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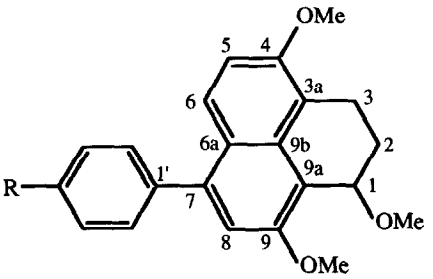
Abstract Four aromatic metabolites with the phenalene skeleton have been isolated from the aquatic plant *Eichhornia crassipes*. The structure have been attributed on the basis of the spectroscopic features of their permethylated derivatives. Their *in vitro* antialgal activity was tested.

Eichhornia crassipes Solms, commonly known as water hyacinth, is a widely distributed aquatic plant and its rapid growth disturbs navigation and fisheries. A part from the mechanical effects, floating mats of water hyacinth limit the growth of subemerged plants and phytoplankton. At the present it has not ascertained if this interference on other organisms involves only competition or if it can also be caused by allelopathic effects[1].

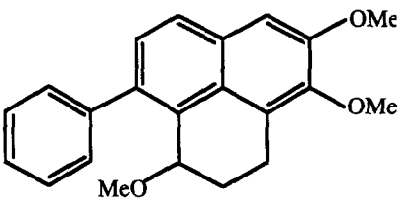
In pursuing our search for bioactive compounds from aquatic plants[2] we have already isolated from *Eichhornia crassipes* a new antimicotic benzoindenone[3] and some sterols which inhibited the growth of *Raphanus sativus* L.[4].

In this paper we report the isolation from the ethyl acetate extract of the plant of four aromatic metabolites with the phenalene skeleton which, tested *in vitro* against several unicellular freshwater algae, showed inhibitory activity on the alga *Porphyridium aerugineum*.

All the compounds were treated with methanolic CH_2N_2 and characterized as methyl ether derivatives. The first compound isolated was identified as the already known 2,6-dimethoxy-9-phenylphenalenone (lachnanthtocarpone red dimethyl ether) (**1**) by comparison of its UV, MS and ^{1}H-NMR data with those described by B. Laundon *et al.*[6]. The ^{13}C-NMR data of **1**, reported in table 2, have been assigned on the basis of DEPT and 2D heterocorrelated experiments.



2 R = H **3** R = OMe



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Compound **2**, $[\alpha]_D$ + 52° (C 0.9, $CHCl_3$), was attributed structure 4,9-dimethoxy-7-phenyl-2,3-dihydrophenalen-1-olo-O methyl ether. Its HR-EIMS spectrum showed a molecular ion at *m/z* 334.1562 according to

a molecular formula $C_{22}H_{22}O_3$. The spectrum exhibited also peaks at *m/z* 319.1342 $[M - Me]^+$ and 291.1376 $[M - Me - CO]^+$ due to the presence of methoxyl groups and an intense peak at *m/z* 257.1169 $[M - C_6H_5]^+$ due to the presence of a phenyl group.

The ^{1}H-NMR spectrum showed five aliphatic protons at δ 2.09, 2.19, 2.49, 3.10 and 4.88, three methoxyl groups at δ 3.48, 3.91 and 3.95, three aromatic protons at δ 7.10, 7.20 and 7.63 beside five further aromatic protons of the phenyl group at δ 6.96 (2H) and in the 7.10 - 7.16 (3H) ppm range (Table 1).

Table 1. NMR data of **2** in $CDCl_3$.

Position	DEPT	δ ^{13}C	H-C one bond	δ ^{1}H	H-C long - range	δ ^{13}C
1	CH	38.4		4.88 br s		126.9, 128.0, 150.7
2	CH_2	29.2	---	2.09 m 2.19 m	--	120.9, 128.0
3	CH_2	18.1	---	2.49 ddd 3.10 dt	--	120.9, 126.9, 151.8
3a	C	120.9		-		-
4	C	151.8		-		-
5	CH	112.2		7.20 d		120.9, 126.2
6	CH	125.1		7.63 d		126.9, 146.1, 151.8
6a	C	126.2		-		-
7	C	146.1		-		-
8	CH	105.7		7.10 s		126.2, 128.0
9	C	150.7		-		-
9a	C	128.0		-		-
9b	C	126.9		-		-
1-OMe	CH_3	60.7		3.48 s		-
4-OMe	CH_3	56.3		3.91 s		151.8
9-OMe	CH_3	55.4		3.95 s		150.7

The protons H-2' and H-6', centered at δ 6.96, are correlated to the carbons at δ 125.9; the protons H-3' and H-5', in the 7.10 -7.16 ppm range, are correlated to the carbons at δ 128.0; the H-4' proton in the 7.10 - 7.16 ppm range is correlated to the carbon at δ 125.7. C-1' is at δ 145.6.

The broad singlet at δ 4.88 was attributed to the H-1 proton geminal with the methoxyl group at δ 3.48 according to the nOe effect between these signals. In a H-H one-bond COSY the H-1 proton showed cross peaks with the signals at δ 2.09 and 2.19, attributed to the H-2 protons which, in turn, were correlated to the signals at δ 2.49 and 3.10, assigned to the H-3 protons. The H-H long-range COSY evidenced a cross peak between the H-1 proton and the H-3 proton at δ 3.10. This interaction across four bonds, investigated through decoupling experiments, was due to a coupling of 1.8 Hz between the protons. The value was indicative of a W conformation of the equatorial protons in a rigid semichair ring and fixed axial the methoxyl group at C-1.

The doublet at δ 7.20 was coupled with the doublet at δ 7.63 and showed nOe effect with the protons of the methoxyl group at δ 3.91. This group was linked to the carbon at δ 151.8 on the basis of a H-C long range experiment. The H-H long-range correlation of the proton at δ 7.63 with the aliphatic H-3 protons and the H-C long-range correlation of all these protons with the carbon at δ 151.8 univocally assigned the methoxyl group to the C-4 and the protons at δ 7.20 and 7.63 to the C-5 and C-6 positions respectively. The nOe effect between the methoxyl group at δ 3.95 and the aromatic singlet at δ 7.10, correlated to the carbon at δ 105.7, proved their *ortho* position and, accordingly, the methine carbon was upfield shifted. This shift of about 20 ppm excluded the C-7 position for the proton as a methoxyl group at C-8 should shift of only 10 ppm the C-7 carbon[7]. On the other hand the C-7 position was unaccetable also for the methoxyl group owing to the chemical shifts at δ 7.63 and δ 125.1 of the H-6 and C-6 signals: in fact a methoxyl group in *peri* position should shift to a value higher than 8.4 ppm the proton H-6[8] and should shift upfield the carbon of about 6 ppm, owing to a γ effect[9]. On the basis of these considerations the phenyl group was located at C-7 and the methoxyl group at C-9. The H-C long-range experiments confirmed the assigned structure evidencing three bond couplings of the H-5 and H-8 protons with the same carbon 6a at δ 126.2 as well as cross peaks of the H-8 and H-1 protons with the carbon 9a at δ 128.0.

Compound **3**, 4,9-dimethoxy-7-(4'-methoxy-phenyl)-2,3-dihidro-phenalen-1-olo-O methyl ether had $[\alpha]_D$ +49° (C 1.0, $CHCl_3$) and in HR-EIMS spectrum a molecular peak at *m/z* 364.1667 according to a molecular formula $C_{23}H_{24}O_4$.The ^{1}H-NMR spectrum was rather similar to that of **2**. In the aliphatic region, besides the H-1 proton at δ 4.85, the H-2 protons at δ 2.08 and 2.18, the H-3 protons at δ 2.50 and 3.10 and the C-1, C-4, C-9 methoxyl methyls at δ 3.48, 3.91 and 3.95, was present a methyl at δ 3.75, assigned to the C-4' position. Accordingly in the aromatic region, besides the H-5, H-6 and H-8 protons at δ 7.21, 7.61 and 7.09, was present an AA'BB' system with two doublets at δ 6.73 and 6.89. The ^{13}C-NMR data (Table 2), obtained by one resonance and DEPT experiments, were assigned by comparison with the data of **2** and confirmed the presence of a *p*-methoxy phenyl group with the C-1' at δ 137.8, C-2' and C-6' at δ 128.8, C-3' and C-5' at δ 113.9 and C-4' at δ 159.1.

Table 2. ^{13}C-NMR Chemical Shifts in $CDCl_3$ of compounds **1**, **3** and **4**.

Position	1	3	4	Position	1	3	4
1	179.8	38.3	38.7	9	143.3	150.8	148.8
2	152.1	29.1	29.3	9a	126.8	128.1	128.9
3	112.6	18.1	18.3	9b	125.0	126.7	125.0
3a	121.9	121.2	120.7	1'	148.7	137.8	145.7
4	131.3	151.6	149.3	2'	128.2	128.8	128.1
5	105.2	111.9	152.5	3'	128.2	113.9	128.1
6	156.9	125.3	111.9	4'	125.9	159.1	125.8
6a	124.9	126.2	126.2	5'	128.2	113.9	128.1
7	128.8	146.1	127.9	6'	128.2	128.8	128.1
8	130.2	105.5	124.8	1-OMe	_	60.5	60.8

1 2-OMe δ 55.1, 6-OMe δ 56.0. **3** 4-OMe δ 56.3, 9-OMe δ 55.4, 4'-OMe δ 55.2. **4** 4–OMe δ 56.2, 5–OMe δ 60.6.

Compound **4** , $[\alpha]_D$ + 53° (C 0.8, $CHCl_3$), was assigned structure 4,5-dimethoxy-9-phenyl-2,3-dihydro-phenalen-1-ol-O methyl ether. The occurence in the HR-EIMS spectrum of an intense peak $[M - 1]^+$ at *m/z* 333.1483 was indicative of a molecular formula $C_{22}H_{22}O_3$. The ^{1}H-NMR spectrum showed the H-1

proton at δ 4.87, the H-2 protons at δ 2.14 and 2.19, the H-3 protons at δ 2.46 and 3.09 and three methoxyl methyls at δ 3.63, 3.65 and 3.86 respectively. In the aromatic region were present a singlet at δ 7.06, two coupled doublets at δ 7.12 and 7.29 and five protons at δ 7.09 (2H) and in the 7.17 - 7.31 (3H) ppm range. The ^{13}C-NMR spectrum with four methine signals at δ 128.1, a methine at δ 125.8 and a carbon at δ 145.7 suggested the presence of a phenyl group. The nOe effect of the methoxyl group at δ 3.65 with the H-1 proton and the protons of the phenyl group at δ 7.09 suggested that the methoxyl group was at the C-1 position and unambiguosly located the phenyl group at C-9.

Furthermore, in the ^{13}C-NMR spectrum was present only an aromatic upfield shifted methine at δ 111.9: this shift was justified by the *ortho* position of a methoxyl group and, accordingly, a nOe experiment evidenced effects between the proton at δ 7.06, related to the carbon at δ 111.9, and the methoxyl group at δ 3.86. The lack of nOe interactions for the methoxyl group at δ 3.63 suggested its location *ortho* to the methoxyl group at δ 3.86. As the C-6 position was not allowed to a methoxyl group owing to the lack of *peri* effect on the C-7 proton, the only possibility was to locate the proton at C-6 and, consequently, the methoxyl groups at the C-4 and C-5 carbons.

The compounds were tested for their antialgal activity with the filter paper-Petri dish bioassay[9] against twenty algal strains using as reference standard the algicide $CuSO_4$. All the algal strains were cultivated on Bold basal medium (BBM)[10] and the growth of each alga was followed at 550 nm with a colorimeter. During late exponential phase (0.7 absorbance units), each algal culture (1ml) was inoculated on a Petri dish containing BBM (30 ml) solidified with agar (1.5%). Each compound was dissolved in acetone (80 μl/mg) and a known quantity of each solution was absorbed on a sterile paper disk (Difco Bacto Concentration Disks, 6mm). After evaporation of the solvent, the filter papers were placed on each inoculated Petri dish. Only the alga *Porphyridium aerugineum* was sensitive and an amount of 2 μmol of the compounds **2** - **4** caused a diameter of inhibition zone of 25 mm. The same amount of the algicide copper sulphate gave a comparable inhibition (diameter of inhibition zone of 28 mm).

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