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ALLELOCHEMICAL ACTIVITY OF PHENYLPROPANES FROM *ACORUS GRAMINEUS**

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Key Word Index—*Acorus gramineus*; Araceae; algae; allelochemicals; phenylpropanes.

Abstract—Six phenylpropanes have been isolated from the aquatic plant *Acorus gramineus* and characterized on the basis of their physical features. The most abundant compounds, tested for their allelochemical properties, were found to inhibit some green and blue-green algae.

INTRODUCTION

An investigation on the allelopathic influences of aquatic plants on algae has been planned in our laboratories. Recently reports of allelopathic interactions in aquatic communities have increased [1–3]; however more attention has been focussed on the detrimental effect of algae on the aquatic macrophytes, while less evidence has been presented for the reverse.

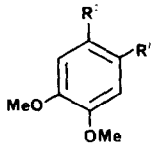
In a continuation of our studies of the chemical composition of aquatic plants [4] we have now considered the genus *Acorus*. Among the species belonging to this genus, the paludal rhizomatous herb *Acorus calamus* L. has been extensively studied from a chemical point of view [5, 6] and has revealed insecticide and antimicrobial activities [7, 8], while the less widespread species *Acorus gramineus* Soland has been only partly examined [9] and the presence of spasmolytic substances has been reported. This paper deals with the isolation of antialgal substances from this species.

RESULTS AND DISCUSSION

Acorus gramineus, collected in the Botanical Garden of the University of Naples, was air-dried and extracted at room temperature with hexane in a Soxhlet apparatus. The extract was separated by conventional procedures into acidic and neutral fractions and this latter was chromatographed on silica gel to give, after TLC and HPLC purification processes, the phenylpropanes 1,2-dimethoxy-4-(1′*Z*-propenyl)benzene (**1**), 1,2-dimethoxy-4-(*E*-3′-methyloxiranyl)benzene (**2**), 1,2,4-trimethoxy-5-(1′*Z*-propenyl)benzene (**3**), 1,2,4-trimethoxy-5-(2′-propenyl)benzene (**4**) and 1,2,4-trimethoxy-5-(*E*-3′-methyloxiranyl)benzene (**5**) besides traces of 1,2,4-trimethoxy-5-(1′*E*-propenyl)benzene (**6**). Compounds **3**, **4** and **6** were identified on the basis of their physical properties [5] and by comparison with authentic material whereas **1**, **2**, and **5** were characterized mainly on the basis of their MS, ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) data. The oxiranyl compounds **2** and **5** were also compared with racemic samples obtained by *m*-chloroperbenzoic acid epoxidation of *trans*-isoeugenol methylether and α-asarone.

The presence of phenylpropanes is characteristic of *A. gramineus* and *A. calamus*. In the latter species the geometric isomers α- (**6**) and β-asarone (**3**) are the main metabolites, whereas *trans*-isoeugenol methylether (**7**) and γ-asarone (**4**) are present in small amounts; in addition the α:β asarones ratio is *ca* 6:1.

In *A. gramineus* *cis*-isoeugenol methylether (**1**) is present and the ratio α:β asarones is 1:7; in addition the epoxides **2** and **5** arising from the *trans* isomers **7** and **6**, are also present. The isolation of **2** and **5** in an optically active form seems to exclude them as artefacts and suggests an enzymatic oxidation process specific only for the *trans* isomers in *A. gramineus*.



1	R¹ CH=CH CH₃ (Z)	R² H
2	R¹ CH—CH CH₃ (E) (O)	R² H
3	R¹ CH=CH CH₃ (Z)	R² OMe
4	R¹ CH₂ CH=CH₂	R² OMe
5	R¹ CH—CH CH₃ (E) (O)	R² OMe
6	R¹ CH=CH CH₃ (E)	R² OMe
7	R¹ CH=CH CH₃ (E)	R² H

*Part 12 in a series of 'Studies on Aquatic Plants'. For part 11 see ref. [4].

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Table 1. ^{1}H NMR data of phenylpropanes

H	1	2	5
3	6.72 *d* (8.2)	6.71 *d* (8.3)	6.85 *s*
4	6.81 *dd* (2.8, 8.2)	6.82 *dd* (2.9, 8.3)	
6	6.71 *d* (2.8)	6.70 *d* (2.9)	6.54 *s*
1′	6.45 *dq* (1.8, 10.6)	3.42 *d* (2.1)	3.59 *d* (1.8)
2′	5.66 *dq* (6.8, 10.6)	2.76 *dq* (2.1, 4.9)	2.91 *dq* (1.8, 5.3)
3′	1.88 *dd* (1.8, 6.8)	1.37 *d* (4.9)	1.41 *d* (5.3)
OMe	3.85 *s*	3.88 *s*	3.82 *s*
OMe	3.85 *s*	3.88 *s*	3.84 *s*
OMe			3.91 *s*

Coupling costant values (Hz) are reported in parentheses.

The most abundant phenylpropanes **2**, **3** and **5** were tested for their antialgal activities by using the filter-paper-petri dish bioassay [10, 11]. Fourteen strains of blue-green and green algae were employed for the inhibition tests (Table 3). Preliminary tests with different amounts of phenylpropanes were carried out to establish the range of inhibition on each strain. The concentrations of the compounds which gave an inhibition zone of 10–30 mm were chosen. Among the algae tested seven strains were sensitive to the three phenylpropanes. Amounts of 2 μmol of these substances were required to prevent the growth of the sensitive strains. However the most active phenylpropane was the oxiranyl **5**, while the other two compounds showed similar antialgal activity. It is noteworthy that each strain tested was either sensitive to the three compounds or not at all. From a taxonomic point of view no significant differences in sensitivity to the phenylpropanes tested were observed between green and blue-green algae. The allelochemical activity of the phenylpropanes was compared with that of the well known algicide copper sulphate. As can be seen in Table 3 the growth of the sensitive strains was inhibited by 0.5–10 μmol of copper sulphate, about the same amounts at which the phenylpropanes showed inhibitory growth effects.

Further investigations may ascertain the possible use of phenylpropanes as selective algal growth inhibitors in natural environments affected by algal blooms.

Table 2. ^{13}C NMR data of phenylpropanes

C	1	2	5
1	147.9 *s*	147.6 *s*	141.5 *s*
2	148.0 *s*	149.6 *s*	149.6 *s*
3	110.9 *d*	111.6 *d*	97.7 *d*
4	121.3 *d*	119.9 *d*	151.4 *s*
5	130.1 *s*	130.6 *s*	118.1 *s*
6	112.1 *d*	112.6 *d*	113.1 *d*
1′	129.5 *d*	59.0 *d*	58.5 *d*
2′	125.4 *d*	55.7 *d*	55.3 *d*
3′	14.6 *q*	17.7 *q*	17.8 *q*
OMe	55.9 *q*	55.8 *q*	55.8 *q*
OMe	55.9 *q*	55.8 *q*	56.2 *q*
OMe			56.4 *q*

EXPERIMENTAL

^{1}H (270 MHz) and ^{13}C NMR (67.88 MHz) spectra were obtained from $CDCl_3$ solns. MS were determined at 70 eV with the source at 150°.

Isolation and purification of phenylpropanes. *A. gramineus* Soland. was collected at the vegetative stage of growth at the Botanical Garden of the University of Naples. Air-dried specimen (3 kg) were extracted with hexane at room temp. to give, after evapn of the solvent *in vacuo*, a residue (1.2 g) which was dissolved in Et_2O and treated with 2 M NaOH to eliminate the acidic compounds. The organic layer was evapd and the resulting neutral material (850 mg) was subjected to CC over silica gel. Hexane eluted a mixture of phenylpropanes (100 mg) which was rechromatographed on prep. TLC (hexane) to give *cis*-isoeugenol methylether (**1**) (7 mg), γ-asarone (**4**) (8 mg), a mixture of α- (**6**) (5 mg) and β-asarone (**3**) (37 mg) resolved by prep. $AgNO_3$ TLC, and a mixture of oxiranylpropanes which was subjected to reverse phase C-18 HPLC (MeOH–H_2O 17:3) to give **2** (10 mg) and **5** (12 mg).

2 $[\alpha]_D$ +24° ($CHCl_3$; *c* 0.3); EIMS *m/z* 194 (22%), 165 (36), 151 (100). **5** $[\alpha]_D$ +32° (*c* 0.7 in $CHCl_3$; EIMS *m/z* 224 (17%), 195 (31), 181 (100).

Epoxidation of **6** *and* **7**. Pure samples of **6** (25 mg) and **7** (20 mg) were dissolved in C_6H_6 (10 ml) and kept at room temp with *m*-chloroperbenzoic acid (50 mg) for 3 hr. Work-up of the reaction mixtures gave the racemic epoxides **2** (16 mg) and **5** (14 mg).

Antialgal bioassay. Growth inhibitory activity of phenylpropanes isolated from *A. gramineus* was tested against the algae listed in Table 3 obtained from Cambridge Collection of Algae and Protozoa, Texas Algal Collection, and Naples Algal Collection of the Dipartimento di Biologia Vegetale of the University.

All the strains were separately cultivated on Bold basal medium (BBM). The growth of each strain was followed with a colorimeter (Bausch & Lomb Spectronic 20). During late exponential phase (*ca* 0.7 u.a.) 1 ml of each algal culture was inoculated on a petri dish containing 30 ml of BBM solidified with 15% agar. The phenylpropanes **2**, **3** and **5** were dissolved in Me_2CO (80 μl/mg). Then filter paper disks (Difco Bacto Concentration Disks, 6 mm) were impregnated with a known quantity of each soln to be tested for inhibitory activity. After evapn of the solvent, the filter papers were placed on each dish previously inoculated. A blank paper dish containing only Me_2CO was also prepared. The sensitivity of the strains used in the agar plating bioassay was tested by using the algal inhibitor $CuSO_4$. Sterile solns of $CuSO_4$ ranging in concns from 10^{-1} to 10^{-3} M were prepared by serial dilutions. Each soln was applied aseptically onto a sterile filter disk to give final amounts of 0.5 to 10 μmol of $CuSO_4$.

Table 3. Antialgal activity of phenylpropanes **2**, **3** and **5** compared with copper sulphate

No strain	Species	Phenylpropanes 2	3	5	$CuSO_4$ 10 μmol	0.5 μmol
C 202–7a	*Ankistrodesmus braunii*	+ +	+ + +	+ + +	+ + +	—
C 211–8h	*Chlorella emersonii*	+ +	+ +	+	+ + +	—
C 249–1	*Muriella aurantiaca*	+ +	+ +	+ + +	+ +	—
C 379–1c	*Stichococcus bacillaris*	—	—	—	+ + +	—
C 1224–5a	*Euglena gracilis*	—	—	—	—	—
N 188	*Pseudococcomixa simplex*	—	—	—	+ + +	—
T 76	*Scenedesmus quadricauda*	—	—	—	+ +	—
T 119	*Chlorococcum hypnosporum*	+ +	+ + +	+ +	+ + +	—
T 268	*Coccomixa elongata*	—	—	—	+ + +	—
T 293	*Chlamydomonas sphagnophila*	+	+ +	+ + +	+ + +	—
T 397	*Chlorella vulgaris*	—	—	—	+ + +	—
T 584	*Nostoc commune*	—	—	—	+ + +	+
T 625	*Synecococcus leopoliensis*	+	+ +	+ +	+ + +	+ + +
T 1444	*Anabaena flos-aquae*	+	+	+ +	+ + +	+ + +

+ + + Diameters of inhibition zone from 23 to 30 mm.
+ + Diameters from 15 to 22 mm.
+ Diameters from 6 to 14 mm.
— Growth was observed.

The plates were incubated upside down at 20° with a total irradiance of 390 $\mu E/m^2$. sec provided by daylight fluorescent lamps from below the plates. The photoperiod was 16 hr light:8 hr dark.

Observations for inhibition of growth of the plated algae around the filter disk were made daily as soon as growth became visible. The inhibition is given as diameter of the zone of no growth.

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