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A HERBICIDAL FATTY ACID PRODUCED BY *LYNGBYA AESTUARII*

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Key Word Index—*Lyngbya aestuarii*; Oscillatoriaceae; marine alga; herbicide; fatty acid; 2,5-dimethyldodecanoic acid.

Abstract—A herbicidal component isolated from ethanolic extracts of *Lyngbya aestuarii* was identified as 2,5-dimethyldodecanoic acid. It inhibited the growth of *Lemna minor* at concentrations higher than 200 ng/ml. Growth inhibition was strongly pH dependent.

INTRODUCTION

Certain algae produce phytotoxic agents that may be useful as herbicides [1]. The treatment of crop plants with seaweed extracts has been shown to produce beneficial effects such as increased growth rate and yield [2]. These studies indicate that algae may be a rich source of potential agrochemicals. To detect such growth regulating agents, we are using a test system based on the growth response of *Lemna minor* [3–5].

During the course of screening crude extracts of marine algae for regulators of plant growth, an extract of a psammophilous, shallow-water variety of the marine cyanophyte *Lyngbya aestuarii* was discovered to possess appreciable herbicidal activity against *Lemna*. We report here the isolation, chemical structure and biological activity of the phytotoxic principle.

RESULTS AND DISCUSSION

The purified herbicide was obtained by extraction of the algal material followed by solvent partitioning, gel filtration, chromatography on silica gel and finally HPLC. High resolution mass spectrometry indicated that the herbicide had the molecular formula $C_{14}H_{28}O_2$. The compound was a carboxylic acid as it formed a methyl ester with diazomethane. Doublets at δ 1.167 and 0.843 (J = 6.7 Hz) in the ^{1}H NMR spectrum suggested that there were two methyl branches. One of the methyl groups was on a methine adjacent to the carboxylic acid group and to a methylene group. The mass spectrum showed a McLafferty rearrangement ion (base peak) at m/z 74, typical for 2-methylalkanoic acids. The second methyl group had to be attached to C-5, since fragment ions were present at m/z 101 (fission of C-4–C-5 bond) and 129 (cleavage of C-5–C-6 bond). The acid was therefore a 2,5-dimethyldodecanoic acid **1**. The optical rotation of **1** was laevorotatory, $[\alpha]_D$ −9.4° in methanol, denoting *R* stereochemistry at C-2 [6]. The absolute configuration of C-5 was not determined.

The purified fatty acid strongly inhibited the growth of *Lemna minor* grown at pH 5.0 (ED_{50} = 0.5 mcg/ml). On further investigation, the herbicidal activity was found to be pH dependent (Table 1). A similar influence of pH of the culture medium on fungitoxicity of fatty acids has been noted previously [7]. At lower pH values the acids are less ionized and enter the cell membrane more readily, resulting in greater toxicity. In an attempt to increase the membrane permeability of **1** at pH 7, the methyl ester was prepared and tested for activity. Although less susceptible to the effects of pH, the ester was found to be markedly less inhibitory than the free acid (Table 1).

EXPERIMENTAL

^{1}H NMR spectra were obtained at 300 MHz and 600 MHz in $CDCl_3$ and CD_2Cl_2; ^{13}C NMR data at 75 MHz in $CDCl_3$. ^{1}H chemical shifts are reported in δ units (ppm) relative to TMS (δ0) and $CHDCl_2$ (δ5.320) as int. standards; ^{13}C chemical shifts are reported relative to $CDCl_3$ (δ77.0). EIMS were obtained at 70 eV.

Isolation. Lyngbya aestuarii Liebmann ex Gomont was collected at Kamalo Jetty, Molokai, Hawaii. Algal material (6 kg), separated from surrounding matrix by filtration on a coarse mesh screen, was extracted twice with 70% EtOH. The extract was partitioned between 70% EtOH and hexane. The EtOH layer was dil to 50% and extracted twice with CH_2Cl_2. The hexane and CH_2Cl_2 layers were combined to give 8.55 g of an oil which was applied to a 40 × 2 cm column of Sephadex LH-20 with *iso*-PrOH–CH_2Cl_2 (1:1). The active fraction was then chromatographed on silica gel (350 ml) to give 160 mg of crude fatty acid. Final purification was achieved by HPLC on Whatman ODS-2 (MeOH–H_2O, 9:1) and Dupont Zorbax CN 9 (*iso*-PrOH–hexane, 1:49) to yield 54 mg (9×10^{-4}% based on wet wt alga) of (2*R*)-2,5-dimethyldodecanoic acid (**1**); $[\alpha]_D^{22}$ −9.4° (MeOH, *c* 4.4); ^{1}H NMR ($CDCl_3$): δ2.413 (1:5:10:10:5:1 *hextet*, J = 6.7 Hz, H-2), 1.634 ($m \rightarrow ddd$ on irr. at 2.413, J = −13.5, 11.6, 4.9 Hz, H-3), 1.448 ($m \rightarrow ddd$ on irr. at 2.413, J = −13.5, 11.6, 4.9 Hz, H-3), 1.38–1.1 (complex multiplets for 15H), 1.167 (*d*, J = 6.7 Hz, Me on C-2), 0.869 (*t*, J = 6.7 Hz, 3H

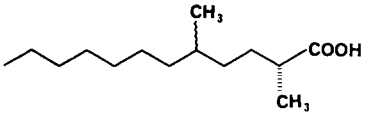

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Table 1. Effect of 2,5-dimethyldodecanoic acid and its methyl ester on growth of *Lemna minor*

Treatment	Concentration (μg/ml)	Frond production pH 5	pH 6	pH 7
Control		122±7.6	129±11.9	114.9±24
Free acid	0.2	65.4±4.5 (53)	95.6±14.3 (74)	116.2±15.7 (101)
	0.5	42±2.4 (34)	89.2±11 (69)	100.2±8.1 (87)
	1.0	29±3.2 (23)	64.2±13.2 (49)	119±10.6 (103)
	5.0	9.6±8.9 (7)	17.8±10.3 (13)	60.8±6.6 (52)
Methyl ester	0.5	132±11.9 (108)	124±14.9 (96)	99±19.5 (86)
	1.0	145±14.1 (118)	114±11.4 (88)	106±11.8 (92)
	5.0	53±10.6 (43)	95.2±10.3 (73)	113±14.2 (98)

Frond production is expressed as the mean number of fronds per culture ± standard error. The same value, expressed as a percentage of the control, is shown in parentheses. Experimental conditions are described in the text.

on C-12), 0.843 (*d*, $J = 6.7$ Hz, Me on C-5); ^{13}C NMR ($CDCl_3$): δ183.32 (C-1), 39.69 (C-2), 36.88 (C-6), 34.34 (C-4), 32.75 (C-5), 31.92 (C-10), 30.01 (C-8), 29.95 (C-9), 29.38 (C-3), 27.03 (C-7), 22.69 (C-11), 19.54 (Me on C-5), 16.77 (Me on C-2), 14.12 (C-12); MS *m/z* (rel. int.) 228 $[M]^+$ (3), 171 (12), 155 (7), 129 (6), 101 (7), 74 (100); high resolution MS *m/z* 228.2082 (calc. for $C_{14}H_{28}O_2$, 228.20894); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2930, 1720.

Preparation of Me ester. A 0.1 M Et_2O soln of CH_2N_2 was added to a soln of 15 mg 1 in 2 ml Et_2O until a slight yellow colour persisted. Evapn left a colourless oil which was dried *in vacuo* over P_4O_{10} to give 15.8 mg (99% yield) of Me (2*R*)-2,5-dimethyldodecanoate; $[\alpha]_D^{22} = -15°$ (CCl_4; *c* 0.77); ^{1}H NMR (CD_2Cl_2): δ3.629 (3H, *s*), 2.378 (1H, *dq*, $J = 7.0$ Hz), 1.59 (1H, *m*), 1.43 (1H, *m*), 1.35–1.23 (15H, *br*), 1.113 (3H, *d*, $J = 7.0$ Hz), 0.880 (3H, *t*, $J = 6.5$ Hz), 0.845 (3H, *d*, $J = 6.5$ Hz); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2940, 1740; high resolution EIMS *m/z* 242.2255 (calc. for $C_{15}H_{30}O_2$, 242.2246).

Bioassay. Stock and experimental cultures of *Lemna* were maintained in Hoagland's medium [8], either unbuffered (pH 5) or supplemented with 3 mM 3-(*N*-morpholino)propanesulphonic acid (pH 7). All experimental cultures were started with a single 4-frond colony in 125 ml Erlenmeyer flasks, each containing 50 ml of sterile nutrient soln. The temp was maintained at 25 ± 1°. Light was provided from cool-white fluorescent tubes at an incident intensity of 200 μE/m²/sec. Illumination was provided for 16 hr, followed by 8 hr darkness. Treatments were terminated by removing the cultures after 10 days of incubation and counting the number of fronds present. Five replicate cultures for each herbicide concn were tested.

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