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EVIDENCE FOR CHEMICAL DEFENSE IN TROPICAL GREEN ALGA *Caulerpa ashmeadii* (CAULERPACEAE: CHLOROPHYTA): Isolation of New Bioactive Sesquiterpenoids

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Abstract—Results of field feeding preference studies with 12 species of tropical green algae of the genus *Caulerpa* showed that *C. ashmeadii* was preferred least by herbivorous fishes. Chemical investigations of *C. ashmeadii* demonstrated the presence of high concentrations of sesquiterpenoid metabolites. The chemical isolation and structural elucidation of five major *C. ashmeadii* metabolites, as well as the results of field feeding preference, antimicrobial, and ichthyotoxicity assays demonstrating the biological activities of these metabolites are reported here.

Key Words—Herbivory, chemical defense, marine algae, allelochemicals, *Caulerpa*, sesquiterpenoids.

INTRODUCTION

Some secondary metabolites from tropical algae have been implicated in chemical defense against grazing fishes and invertebrates in herbivore-rich tropical waters (Sun and Fenical, 1979; McConnell et al., 1982; Paul and Fenical, 1982, 1983). Other investigators have shown distinct feeding preferences in laboratory

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and field feeding assays (Ogden, 1976; Hay, 1981, 1984; Littler et al., 1983; Lewis, 1985). However, few studies have tried to relate these known feeding preferences with the presence of chemical deterrents in algae (Norris and Fenical, 1982; Hay, 1984). In this study, *Caulerpa ashmeadii* was the most resistant alga to herbivorous fishes of 12 *Caulerpa* species and, in some cases, was almost completely avoided in feeding preference assays. Chemical investigation of this same alga led to the isolation of five new sesquiterpenoid metabolites which showed a high degree of biological activity in antimicrobial and ichthyotoxicity assays. The presence of these biologically active terpenoids, which have not been found in any other species of *Caulerpa*, appears to explain the resistance of *C. ashmeadii* to grazing fishes.

Other species of *Caulerpa* have been studied and found to contain terpenoid metabolites, many of which possess the novel bisenol acetate functionality unique to green algae of the related families Caulerpaceae and Udoteaceae (Blackman and Wells, 1978; Amico et al., 1978; Capon et al., 1981, 1983; Paul and Fenical, 1982, 1985). A major sesquiterpenoid metabolite, caulerpenyne (**6**) has been isolated from 10 different species of *Caulerpa* in varying concentrations (Amico et al., 1978; Paul, 1985). Other nonterpenoid nitrogenous compounds such as caulerpin (**7**) and caulerpicin have also been isolated from several different species of *Caulerpa* and have been proposed to be responsible for the biological activity of these algae (Maiti et al., 1978; Mahendran et al., 1979: Doty and Aguilar-Santos, 1966, 1970; Lobel and Ogden, 1981; Vest et al., 1983).

In this study, we posed the following questions: (1) Can the low susceptibility of *Caulerpa ashmeadii* to grazers be related to the presence of bioactive secondary metabolites? (2) How do the biological activities of caulerpenyne and caulerpin compare with the activities of the *C. ashmeadii* metabolites? (3) What are the molecular structures of the *C. ashmeadii* metabolites and can the structural features of these molecules explain the observed biological activities?

METHODS AND MATERIALS

Study Area. Looe Key (24°37′18″N, 81°24′24″W) is located 12.9 km southwest of Big Pine Key, Monroe County, Florida, and was established as a National Marine Sanctuary in 1981. Within the 18.2-km^2 sanctuary lies an inner "core" area of about 1.7 km^2 that includes rich seagrass, coral, and macroalgal assemblages. There are several distinctive habitats: (1) the seaward-most intermediate fore-reef at about 15 m depth; (2) the fore-reef including the spur and groove system (10 m deep to intertidal); (3) the reef flat (1–2 m deep); and (4) the extensive back-reef system (1–10 m deep). Spearfishing is not permitted at Looe Key and, consequently, herbivorous fish populations (especially the parrotfishes and surgeonfishes) are both exceptionally large and diverse (Bohnsack

et al., NOAA draft report). Littler, Littler, and LaPointe (NOAA draft report) were of the opinion that herbivory by reef fishes is the dominant direct controller of algal standing stocks throughout the reef flat and fore-reef slopes.

Herbivory Studies. The suspended-line bioassay method of Littler et al. (1983) was used in the grazing experiments. Clumps of 12 *Caulerpa* species (~ 10 cm^2 in area), representative of the spectrum of available forms, were placed between twists of a three-stranded, 2-mm-thick, white nylon line at 0.5-m intervals in a randomized pattern. The lines were placed in three of the major reef habitat types (intermediate fore-reef, fore-reef, and reef-flat) which have been shown to have large herbivorous fish populations (Bohnsack et al., NOAA draft report), on June 19 and 21, 1984.

The technique yields insights into the differential resistances of the various species and forms of *Caulerpa* to herbivory. For all habitats on both days, three separate lines were used, each containing three clumps per species. A fourth control line was placed in the nearby back reef, where herbivores were few, as a control for losses other than by grazing. The lines were photographed, then suspended ca. 0.3 m above the bottom with each end tied to a coral head for a 3-hr daylight period. Surgeonfishes and parrotfishes were not wary of the lines and began feeding as soon as the divers moved away. Fish typically moved from clump to clump taking small bites, becoming more persistent as they located a particularly palatable clump. After 3 hr, the lines and algae were returned to the boat, rephotographed, and subsequently quantified in the laboratory by the point intercept method (Littler et al., 1983). Losses of all control algae were negligible over the identical 3-hr period. In this technique, the percent thallus area (two-dimensional) lost to grazing for each specimen was calculated from the color slides (Kodachrome 64) by projecting the transparencies onto a grid of dots (at stratified randomized intervals) that were directly related to surface area. Results were compared using Duncan's multiple-range test (Steel and Torrie, 1960).

Chemical Analyses. Samples of *Caulerpa ashmeadii* were collected near Big Pine Key, Florida, in November 1984. The algae were placed directly into ethanol and subsequently extracted with dichloromethane. Metabolites were purified by open-column Florisil chromatography and by silica gel high-performance liquid chromatography (HPLC) with ethyl acetate–isooctane mixtures.

IR spectra were recorded with a Perkin-Elmer model 137 spectrophotometer and UV spectra were recorded in methanol with a Beckman Mk IV instrument. Proton nuclear magnetic resonance (NMR) spectra were recorded with a 360-MHz Nicolet-Oxford Magnetics FT spectrometer and [^{13}C]NMR spectra were recorded at 50 MHz with a Nicolet-200 instrument. High-resolution mass spectra were obtained through the Mass Spectrometry Service Laboratory at the University of Minnesota.

Laboratory Assays. Each metabolite was examined for antimicrobial activity against three marine bacteria and one marine fungus by standard agar plate-

assay disk methods. Zones of inhibition (clear zones) were measured for each filter disk in triplicate against each microorganism tested.

Tropical damselfishes, *Pomacentrus coeruleus* and *Dascyllus aruanus* (ca. 10–15 g average weight), were used to assay for toxicity of the algal metabolites toward marine fishes (Paul et al., 1980). For each assay, compounds were dissolved in a small amount of ethanol and stirred into seawater (~25°C) at known concentrations (100 μl EtOH in 200 ml seawater). A damselfish was placed into the seawater and observed for 1.5 hr. A solvent control was always run simultaneously. Toxicity was measured as the death of the fish within this time. Each compound was tested against at least three fish at the minimum effective concentrations.

RESULTS

Herbivory Studies. All loss of algal material from the lines resulted from grazing by herbivorous fishes. This was confirmed by the characteristic grazing scars and by extensive observations of the suspended thalli on the lines by divers. This method measured the relative vulnerability of each species to grazing by natural populations of herbivorous fishes. We could not identify the fish species responsible for grazing nor could we determine the preference of any one fish species.

The most palatable species (Table 1, Figure 1) were *Caulerpa prolifera* v. *obovata* and *C. lanuginosa*, forms that occur cryptically among beds of the seagrass *Thalassia testidinum* which grow on sand-covered rock. The most consistently herbivore-resistant alga across all habitats was *C. ashmeadii*, with less than half of its area being grazed by fishes (Figure 1). This contrasts with a mean 89.5% loss shown by the other 11 species. *Caulerpa ashmeadii* was significantly more resistant to fish herbivory ($P < 0.05$, Duncan's multiple-range test, Steel and Torrie, 1960) than all other species (Figure 1) with the exception of *C. sertulariodes* which lost 71.3% of its thallus area during the experiments.

Chemical Analyses. The major *Caulerpa ashmeadii* metabolite, **1** (Figure 2), was isolated as 20% of the organic extract after purification by silica gel HPLC (15% EtOAc–isooctane) ($[\alpha]_D^{25} = -48°$, $c = 1.4$, $CHCl_3$). This metabolite was analyzed for $C_{21}H_{30}O_6$ by high-resolution mass spectrometry (318.1846 for M^+-HOAc) in combination with consideration of its [^{13}C]NMR spectral features. This molecular formula required seven degrees of unsaturation. The presence of the *E,E*-1,4-diacetoxybutadiene functional group was evident based upon UV absorption at λ_{max} 248 nm ($\epsilon = 17{,}000$); IR absorptions at 2940, 1720, 1440, and 1210 cm^{-1}; and characteristic [^{1}H]- and [^{13}C]NMR spectral features (see Tables 2 and 3) (Blackman and Wells, 1978; Sun and Fenical, 1979). In addition to the bisenol acetate functionality, compound **1** was recognized to possess an additional secondary acetate group and a methyl-

TABLE 1. COMPARISON OF 12 TAXA OF *Caulerpa* TO GRAZER SUSCEPTIBILITY (MEANS ARE GIVEN IN PERCENT CONSUMED)[a]

Species	Mean	SD	CI	SE	*N*
Caulerpa prolifera (Forssk.) Lamour. v. *obovata* J. Ag.	97.4	5.6	3.2	1.5	14
Caulerpa lanuginosa J. Ag.	95.8	15.6	8.1	3.7	15
Caulerpa cupressoides (West) J. Ag. v. *cupressiodes*	95.1	7.3	4.1	1.9	15
Caulerpa racemosa (Forssk.) J. Ag.	93.7	5.9	3.8	1.7	12
Caulerpa verticillata J. Ag.	92.0	14.5	8.0	3.7	15
Caulerpa cupressoides (West) C. Ag. v. *mamillosa* (Montagne) Wever-van Bosse	90.4	13.8	7.6	3.6	15
Caulerpa mexicana (Sonder) J. Ag.	87.5	12.6	7.0	3.3	15
Caulerpa paspaloides (Bory) Greville v. *wurdemanni* Wevver-van Bosse	87.2	14.9	11.4	5.0	9
Caulerpa paspaloides (Bory) Greville v. *compressa* Weber-van-Bosse	87.0	14.2	7.8	3.6	15
Caulerpa mexicana (Sonder) J. Ag. F. *pectinata* (Kützing) Taylor	86.9	12.1	7.0	3.2	15
Caulerpa sertularioides (Gmel.) Howe	71.3	37.5	21.7	10.0	14
Caulerpa ashmeadii Harv.	46.9	37.6	20.8	9.7	15

[a] SD = standard deviation; CI = 95% confidence interval; SE = standard error; *N* = number of lines.

trisubstituted olefin as deduced by further [^{1}H]- and [^{13}C]NMR resonances (Tables 2 and 3). These functionalities accounted for six of the seven degrees of unsaturation, thus indicating one carbocyclic ring to be present in the structure. Comparisons of **1** with the *C. flexilis* var. *muelleri* metabolite **8** (Tables 2 and 3) showed both to possess similar spectral features and carbon skeletons. Proton NMR decoupling studies allowed the complete assignment of **1**, including a secure placement of the secondary acetate at C-4.

Compound **2** was isolated as 10% of the organic extract after final purification by silica gel HPLC (15% EtOAc–isooctane) ($[\alpha]_D^{25} = -98°$, $c = 1.4$, $CHCl_3$). The molecular formula of the metabolite was indicated as $C_{17}H_{24}O_3$ by high-resolution mass spectrometry ($M^+ = 276.1740$), a formula which requires six degrees of unsaturation. IR absorptions at 2940, 1760, 1680, 1650, 1440, 1210, 1110 cm^{-1} and UV absorption at λ_{max} 240, $\epsilon = 22{,}000$, when considered together with ^{1}H and ^{13}C spectral features, indicated the presence of an enol acetate, an unsaturated carbonyl, and a methyl-trisubstituted olefin.

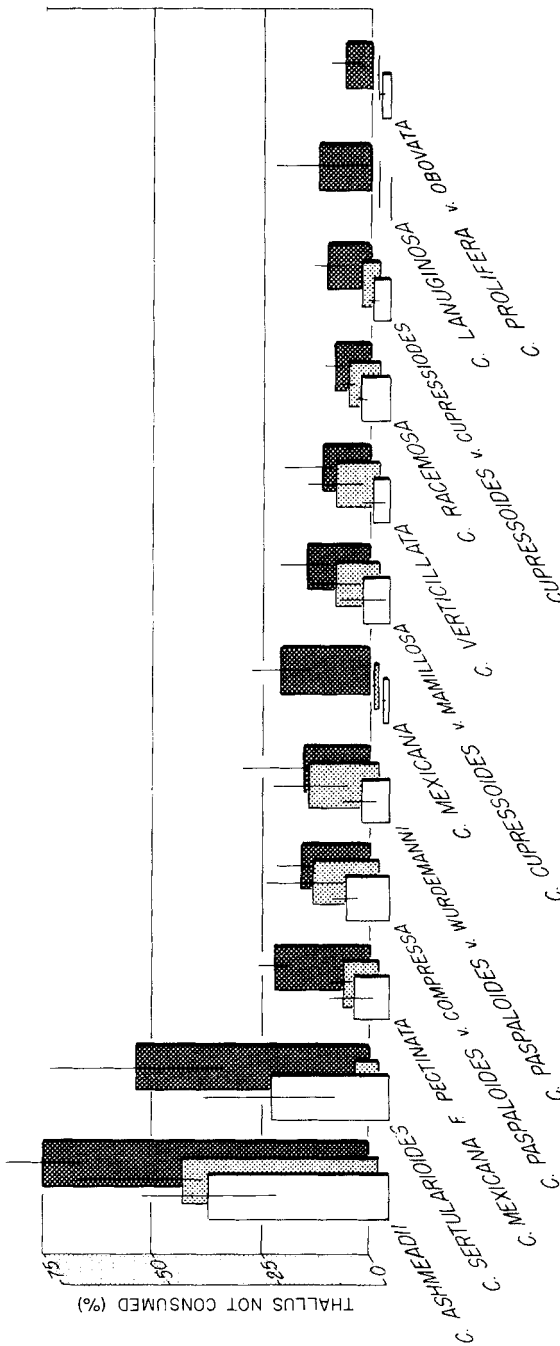

FIG. 1. Comparison of the three major habitats from deep to shallow (front, light = intermediate fore-reef; middle, gray = fore-reef; back, dark = reef flat) in terms of overall herbivory, and grazer resistance of 12 *Caulerpa* forms, in order from highest to lowest means for all habitats. Standard error is given by vertical lines.

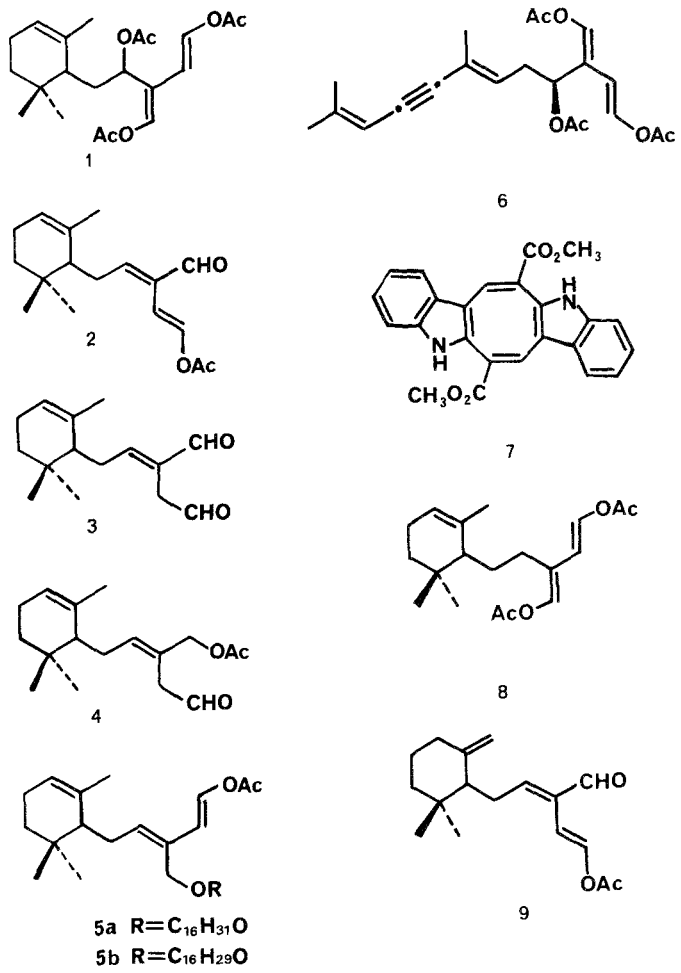

FIG. 2. Metabolites from *Caulerpa ashmeadii* and related species of *Caulerpa*.

Spectral comparisons could be readily made with the related metabolites **1** and **8** and the known metabolite onchidal (**9**) (Ireland and Faulkner, 1978). Thus, compound **2** was assigned as a double-bond isomer of onchidal.

Sesquiterpenoid **3** was isolated as 5% of the organic extract after silica gel HPLC (15% EtOAc–isooctane) ($[\alpha]_D^{25} = -104°$, $c = 1.0$, $CHCl_3$). Compound **3** was determined to be $C_{15}H_{22}O_2$ by high-resolution mass spectrometry (234.1622 for M^+). The presence of two aldehydes (one α, β unsaturated) was readily apparent from IR absorptions at 2960, 1720, 1730, 1580, 1450, 1220 cm^{-1}; from UV absorption at λ_{max} 232, ϵ = 16,500; and by [^{1}H]- and [^{13}C]NMR spectral features (Tables 2 and 3). Full proton NMR decoupling and

TABLE 2. [^{1}H]NMR DATA FOR *Caulerpa ashmeadii* AND RELATED METABOLITES[a]

Carbon	Metabolite						
	1	2	3	4	5a	8[b]	9
1	7.60, d, J = 12.5 Hz	8.19, d, J = 12.5	9.53, bs	9.50, t, J = 2 Hz	7.34, d, J = 12.6 Hz	7.37, d, J = 13 Hz	8.26, d, J = 14 Hz
2	5.73, d, J = 12.5 Hz	5.97, d, d, J = 12.5, 1.7 Hz	3.40, 3.30, J_{AB} = 14.5 Hz	3.11, t, J = 2 Hz	6.13, d, J = 12.6 Hz	5.92, d, J = 13 Hz	6.10, dd, J = 14, 1 Hz
3							
4	5.90, t, J = 7 Hz	6.42, t, J = 7 Hz	6.78, t, J = 7 Hz	5.81, t, J = 7 Hz	5.58, t, J = 7 Hz	n.r.[c]	6.41, t, J = 7 Hz
5	1.90, mult	2.51, t, J = 7 Hz	2.43, *mult*	2.18, *mult*	2.20, mult	n.r.	2.05 m
6	1.90, mult	2.00, mult	1.71, mult	1.68, mult	2.18, mult	n.r.	2.57 m
7							
8	5.18, mult	5.41, mult	5.43, mult	5.33, mult	5.28, mult	5.33, mult	
9	1.50, *mult*	1.38, *mult*	1.40, *mult*	1.48, mult	1.99, mult	n.r.	1.3–2.05 m
10	1.29, mult	1.23, mult	1.21, mult	1.13, mult	1.42, 1.15, mult	n.r.	
11							
12	7.20, s	9.37, d, J = 1.7 Hz	9.39, s	4.45, s	4.54, mult	7.17, s	9.40, d, J = 1
13	1.61, s	1.68, s	1.69, s	1.65, s	1.61, s	1.71, s	4.81, s, 4.50, s
14	0.88, s	0.88, s	0.90, s	0.88, s	0.83, s	0.89, s	0.89, s
15	1.08, s	0.91, s	0.93, s	0.92, s	0.83, s	1.00, s	0.99, s
OAc	2.17, s	2.13, s		1.98, s	2.07, s	2.16, s	2.16, s
	2.03, s					2.14, s	
	2.01, s						
Ester					1.28, bs (28 H)		
					0.82, s (3 H)		

[a] ^{1}H spectra recorded at 360 MHz in CCl_4 solution.
[b] Compound 8 recorded at 90 MHz in $CDCl_3$.
[c] n.r., Values not reported in literature.

TABLE 3. [^{13}C]NMR DATA FOR *Caulerpa ashmeadii* AND RELATED METABOLITES[a]

	Metabolite					
Carbon	1	2	3	4	5a	8[b]
1	134.8	142.0	195.9	196.9	137.7	134.2
2	109.6	105.1	39.2	44.2	110.3	113.3
3	119.7	134.6	134.7	125.3	130.2	122.4
4	70.2	155.8	157.6	135.8	135.9	29.4
5	34.7	30.3	30.1	29.2	30.1	26.1
6	45.3	49.4	49.2	49.4	49.6	49.3
7	136.5	135.0	135.1	135.0	135.4	136.3
8	120.6	122.2	122.3	121.6	121.6	120.4
9	31.6	31.8	31.7	31.6	31.8	31.8
10	23.4	23.2	23.2	23.3	23.3	23.1
11	32.6	32.6	32.6	32.5	32.3	32.6
12	137.6	192.6	192.6	69.1	66.5	135.9
13	27.2	27.5	27.4	27.6	27.7	27.3
14	23.4	23.4	23.4	23.7	23.7	23.2
15	27.4	27.5	27.4	27.7	27.7	27.6
OAc	169.2	167.0		165.5	168.5	167.9
	167.2	19.9		20.3	19.9	167.5
	166.3					20.6 (2C)
	20.5					
	19.9 (2C)					
Ester					172.2	
					30.1 (11C)	
					34.4	
					29.0	
					25.4	
					14.3	

[a] Spectra recorded at 50 MHz in benzene-d6 solution. Multiplicities determined by SFORD and GASPE experiments.
[c] Compound 8 recorded at 20 MHz in $CDCl_3$.

spectral comparisons with the known compound rhipocephanal (Sun and Fenical, 1979) led to the final structure assignment of **3.** The geometry of the C-3–C-4 olefin was assigned as *E* based on the characteristic shielding of the C-12 aldehyde in the [^{1}H]NMR (Table 2) and on comparison with model compounds (Faulkner, 1971; Wehrli and Nishida, 1979). The saturated aldehyde showed coupling to an adjacent methylene group. The compound is a double-bond isomer of the African termite metabolite ancistrodial (Baker et al., 1978) which acts in chemical defense against ants.

Another sesquiterpenoid produced by *Caulerpa ashmeadii* was compound **4,** which was isolated as 8% of the organic extract after silica gel HPLC (10% EtOAc–isooctane) ($[\alpha]_D^{25} = -111°$, $c = 0.8$, $CHCl_3$). Again, spectral com-

parisons with other related metabolites facilitated the structural elucidation of this compound (Tables 2 and 3). Five degrees of unsaturation were inherent in the molecular formula ($C_{17}H_{26}O_3$; 218.1653 for M^+-HOAc). These unsaturations could be accounted for by one aldehyde group, a primary acetate, two olefins, and a carbocyclic ring. These latter functionalities were identified by [^{1}H]- and [^{13}C]NMR spectral features as well as by IR absorptions at 2960, 1745, 1730, 1580, 1450, 1220 cm^{-1} and UV absorption at λ_{max} 233, ϵ = 2100. Proton NMR decoupling studies showed that the aldehyde on C-1 was coupled only to a methylene at δ3.11. The methylene protons adjacent to the acetate group showed no coupling; therefore the olefin was placed at C-3–C-4. Protons on carbons C-4–C-6 were also interrelated through decoupling studies to further establish the structure of **4.**

Compounds **5a** and **5b** were isolated as a mixture as 5% of the organic extract by final silica gel HPLC (5% EtOAc–isooctane) ($[\alpha]_D^{25}$ = −62°, c = 1.6, $CHCl_3$). The spectral features of these metabolites showed the presence of the familiar enol acetate functionality at C-1–C-2 and the same sesquiterpenoid monocyclic ring system as in compounds **1–4** (Tables 2 and 3). The presence of fatty acid ester residues positioned at C-12 were indicated by [^{1}H] and [^{13}C]NMR spectral features (Tables 2 and 3); IR absorptions at 2960, 1760, 1730, 1715, 1370, 1215, 1110 cm^{-1}; and UV absorption at λ_{max} 246, ϵ = 8200. High-resolution mass measurements of the acylonium ions (RCO^+) confirmed the presence of two fatty acid residues: 239.2392 for $C_{16}H_{31}O$ (17% intensity) and 237.2264 for $C_{16}H_{29}O$ (3.7% intensity). Compounds **5a** and **5b** were therefore estimated to compose 82% and 18% of the mixture, respectively. Other sesquiterpenoid esters possessing linear carbon skeletons have been reported from *C. prolifera* (De Napoli et al., 1983).

Laboratory Assays. All metabolites, except compounds **5a** and **5b,** showed antimicrobial activity toward at least one marine bacterium (Table 4). Only sesquiterpenoid **2** was active toward the marine fungus and compound **2** was the most active overall. Compounds **3** and **4** also showed activity toward all three bacteria.

All metabolites, except the fatty esters **5a** and **5b** and caulerpin **7,** were toxic to damselfish (Table 5) within 1.5 hr. The aldehydes **2, 3,** and **4** again showed the highest degree of biological activity in this assay.

DISCUSSION

This study demonstrates the strong avoidance of *Caulerpa ashmeadii* by reef herbivores and the presence of four biologically active compounds in the alga which are specific to this species of *Caulerpa* (Paul, 1985; Paul and Fenical, 1987). The unpalatability of *C. ashmeadii* relative to other *Caulerpa* species correlates with the presence of highly biologically active sesquiterpenoid

TABLE 4. RESULTS OF ANTIMICROBIAL ASSAYS FOR *Caulerpa* METABOLITES[a]

Compound	*Lagenidium callinectes*[b]	*Vibrio leignathi*[c]	*V. phosphoreum*[c]	SK-13[c]
1	−	−	−	+
2	+	+	+	+
3	−	+	+	+
4	−	+	+	+
5a, b	−	−	−	−
6	n.t.	+	−	n.t.

[a] + = inhibition of microbial growth, zone > 2 mm; − = no inhibition; n.t. not tested. All compounds tested at 200 μg/disk.
[b] Pathogenic marine fungus.
[c] Bacterial isolates, SK-13 is an unidentified strain of gram-positive spore-forming bacteria requiring Mn for growth.

metabolites which are unique to this species. Although most species of *Caulerpa* also produce bioactive sesquiterpenoids such as caulerpenyne (**6**) and nonterpenoid metabolites such as caulerpin (**7**) (Paul, 1985; Paul and Fenical, 1987), these compounds do not show the potent activities of the *C. ashmeadii* aldehydes. Since *C. ashmeadii* is biomechanically no tougher or stronger than the more palatable species (S. Armstrong, personal communication), it seems likely that its avoidance is the result of these secondary metabolites. In addition, comparable studies on the known toxic alga *Dictyota divaricata* Lamouroux (Gerwick, 1981; Norris and Fenical, 1982), in the same habitats of Looe Key (Littler, Littler, and Lapointe, NOAA draft report), showed similar resistance to grazing. Mean loss of *D. divaricata* was 42.6% per 3 hr, which is close to the recorded 46.9% loss per 3 hr for *C. ashmeadii*.

TABLE 5. RESULTS OF ICHTHYOTOXICITY ASSAYS FOR *Caulerpa* METABOLITES[a]

Compound	Toxicity[b]	Dosage (μg/ml)	Time (hr)
1	+	10.0	1.0
2	+	2.5	1.5
3	+	2.5	1.5
4	+	5.0	1.0
5a, b	−	20.0	1.5
6	+	20.0	1.0
7	−	20.0	1.5

[a] This bioassay used the tropical damselfish *Pomacentrus coeruleus* ($N = 4$ for each compound tested). Values expressed are the lowest dosages active within 1.5 hr for all four fish.
[b] + = death within time indicated; − = no death.

Several of the *Caulerpa* species examined here have been shown to be consumed at moderate to high rates in related studies (Littler et al., 1983; Hay, 1984; Lewis, 1985). The high percentage of individuals eaten (~80% for *C. ashmeadii* in Honduras (Hay, 1984) and its comparability to *C. prolifera* in palatability contrast markedly with the results of this study (Figure 1). Hay (1984) and Lewis (1985) contrastingly, noted a low to intermediate herbivore susceptibility for *C. cupressoides* which we found to be eaten readily (Table 1). These findings raise some questions regarding variation in the secondary metabolite composition of these species collected in different habitats. Alternatively, the results may indicate the presence of different herbivores with different grazing preferences or differences in grazing intensity among reefs. Paul (1985) showed that related tropical green algae (genera *Rhipocephalus*, *Udotea*, and *Penicillus*) showed between-population variation in the production of terpenoid metabolites. Concentrations of caulerpenyne (**6**) and related terpenoids may also vary in different populations of *Caulerpa* and be related to algal susceptibility to herbivory.

The nonterpenoid, nitrogenous metabolites caulerpin (**7**) and caulerpicin have often been proposed to function as chemical defenses in *Caulerpa* species (Doty and Aguilar-Santos 1966, 1970; Lobel and Ogden, 1981; Lewis, 1985). However, the sesquiterpenoid caulerpenyne (**6**), which often exists in concentrations of 40–50% of the organic extract in some species of *Caulerpa*, possesses much greater biological activity than caulerpin in ichthyotoxicity assays (Table 5). McConnell and coworkers (1982) also showed that caulerpenyne was responsible for almost all of the feeding deterrent activity of extracts of *C. prolifera* against the sea urchin *Lytechinus variegatus*. Caulerpin caused little, if any, deterrence in these assays. Similarly, Hodgson (1984) showed that caulerpenyne was primarily responsible for the antimicrobial and antineoplastic activities found in extracts of *C. prolifera*.

The *C. ashmeadii* metabolites and other *Caulerpa* terpenoid metabolites (Paul and Fenical, 1982, 1985) also show a much higher level of activity than caulerpin. Caulerpicin is a mixture of several related sphingosine derivatives which have been isolated in very small amounts from several species of *Caulerpa* (Doty and Aguilar-Santos, 1966, 1970; Maiti et al., 1978; Vest et al., 1983). Caulerpicin was not tested in these assays because it is a very minor *Caulerpa* metabolite. The major chemical defenses in *Caulerpa* species appear to be the terpenoid metabolites which contain aldehyde or enol acetate functional groups. These compounds are produced in large concentrations but are often not readily isolated because of their unstable and reactive chemical natures.

The sesquiterpenoid esters, **5a** and **5b**, showed little biological activity in our assays (Tables 4 and 5). Structurally similar terpenoid esters have been isolated from *Caulerpa prolifera* (De Napoli et al., 1983) and also from several

nudibranchs (Cimino et al., 1981, 1982, 1983; Okuda et al., 1983). The esters in the nudibranchs were also reported to be less toxic than the sesquiterpenoid aldehyde, polygodial, which was a major metabolite. The esters were found only in the digestive tract of the nudibranchs, whereas the mantle (which is more exposed to predators) contained the toxin polygodial which is reported to function in chemical defense (Cimino et al., 1981, 1983).

The molecular structures of the sesquiterpenoid aldehydes and enol acetates from *Caulerpa ashmeadii* also support the hypothesis that these metabolites are used in chemical defense. The aldehyde and enol acetate (a masked aldehyde) functional groups can be envisioned to react with proteins in a number of ways to affect or inactivate protein or enzyme function. The bioactive *C. ashmeadii* metabolites are related to numerous insect antifeedants such as ancistrodial (Baker et al., 1978), warburgenal (Kubo et al., 1976), and the iridoid aldehydes (Cavill and Hinterberger, 1960), which could function as defensive agents by identical chemical means.

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