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DISTRIBUTION OF THE PIGMENT CAULERPIN IN SPECIES OF THE GREEN ALGA *CAULERPA**

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Abstract—The distribution of the pigment caulerpin in the algal genus *Caulerpa* has been investigated. Specimens from Bermuda, Florida and Tasmania were examined directly and the results compared with literature reports of the occurrence or absence of caulerpin in various species from diverse locations. These surveys revealed a general correlation of the presence of caulerpin with the absence of peroxidase activity and the presence of bilateral rather than radial morphology.

INTRODUCTION

Caulerpin (**1**) is a unique pigment which was originally isolated from *Caulerpa racemosa* and reported to be a phenazine derivative [1]; it was subsequently found in other species of *Caulerpa* and correctly identified as **1** by Maiti *et al.* [2].

During a chemical -pharmacological screening of algae from saline cave pools in Bermuda, we found caulerpin in some species of *Caulerpa*, but not in others, and began to contemplate whether the distribution of this pigment might correlate with some feature of habitat or with accepted taxonomic and morphological distinctions in the genus. In order to consider a more valid and varied sampling of *Caulerpa* species, we expanded our survey to include Bermudian, Floridian and Tasmanian species and compared our data with those already published on caulerpin.

The ensuing report describes the results of our investigations into the distribution of **1** in the genus *Caulerpa*.

RESULTS AND DISCUSSION

Classical descriptions of the genus *Caulerpa* by Børgensen [3] and Svedelius [4] agree that the species may be divided into two major groups: (1) the bilateral leaf- or frond-like species, showing flattened ramuli in two rows, typically growing in quiet waters below the littoral; and (2) the radially branched species, with cylindrical, closely packed ramuli, characteristically found in shallower and brighter habitats of the littoral zone. There are exceptions to this scheme, however, the most notable being *Caulerpa sertularioides*, a shallow water, high-light plant which is bilateral, but possesses cylindrical ramuli. Interestingly, our evaluation revealed that this species has one of the highest caulerpin contents of all the species examined. The results of our investigation are summarized in Tables 1–3; a review of literature reports on caulerpin is presented in Table 4.

Our survey showed that caulerpin is generally associated with the radially branched, shallow water species which possess tightly whorled branchlets, while it has proved to be absent in most flattened, frond-like or sparingly branched species typical of deeper and quieter waters. One aberration from this scheme is *C. verticillata*, a distinctive, loosely whorled, small and delicate epiphytic or mud-collecting species that shows a unique adaptation in the form of variously orientated rhizoids, which allow growth to extend irregularly in a manner preventing burial by mud. We found this radially branched species, from both Bermuda and Florida, to be caulerpin-negative, although Norris and Fenical [5] found specimens from Belize containing caulerpin.

There are a few other contradictions between our results and the literature data. Our collection of *C. racemosa* var. *uvifera* from Skyway South in Tampa Bay contained small amounts of **1**, although Vest *et al.*'s Florida collection [6] and Doty and Aquilar-Santos's Philippine collection [7] were negative for caulerpin. Similarly, we and Vest *et al.* [6] found no caulerpin in *C. ashmeadii* from three locations in Florida, while McConnell *et al.* [8] reported **1** from *C. ashmeadii* from Tarpon Springs. Vest *et al.* [6] found trace quantities of **1** (< 0.1 mg per g dry wt) in *C. paspaloides* and *C. prolifera*; we found none in collections from several sites in Florida. Despite careful checking of algal identifications and

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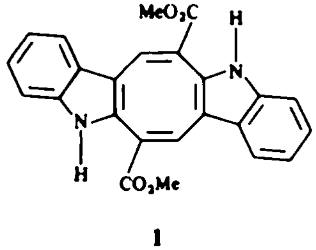

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Table 1. Distribution of caulerpin in *Caulerpa* species from Bermuda

Species	Part	Collection site	Caulerpin*
C. fastigiata var. *conferoides*	Whole	Whiskey Cave Pool	−
C. racemosa	Whole	Deep Blue Pool	+
C. racemosa var. *macrophysa*	Branches	Harbour Pool	+
	Axes, stolons, rhizomes	Harbour Pool	+
C. sertularioides	Whole	Mangrove Lake	+
	Branches	Bathtub Sink	+
	Axes, stolons, rhizomes	Bathtub Sink	+
C. verticillata	Whole	Bathtub Sink	−

* +, Caulerpin present; −, caulerpin absent.

Table 2. Distribution of caulerpin in *Caulerpa* species from Florida

Species	Part	Collection site	Caulerpin*
C. ashmeadii	Whole	Tarpon Springs	−
	Whole	Blackwater Sound Canal	−
	Whole		−†
	Whole	Tarpon Springs	+‡
C. cupressoides	Whole	Florida Keys	+
	Whole	Marathon Shores	+†
	Whole		+
C. lanuginosa	Whole	Key West	−
	Whole		−†
C. mexicana	Branches	Tampa Bay	−
	Axes, stolons, rhizomes	Tampa Bay	−
	Whole		−†
	Whole	Sarasota	−†
C. microphysa	Whole	West Sister Rock, Marathon	+
C. paspaloides	Whole	Florida Keys	−
	Whole	Blackwater Sound	−
	Whole	Boca Chica	−
	Whole	Bahia Honda, Keys	−
	Whole		+†,§
C. peltata	Branches	Tampa Bay	+
	Axes, stolons, rhizomes	Tampa Bay	+
	Whole	Tampa Bay	+
C. prolifera	Branches	Point O'Rocks	−
	Axes, stolons, rhizomes	Point O'Rocks	−
	Whole		trace†,§
C. prolifera var. *zosterifolia*	Whole	Tampa Bay	−
C. racemosa var. *clavifera*	Whole	Tampa Bay	+
	Whole	Knight's Key	+
C. racemosa var. *laetevirens*	Whole	Sarasota	+‡
C. racemosa var. *macrophysa*	Whole	Tampa Bay	+‡
C. racemosa var. *uvifera*	Whole	West Summerland Key	+†
	Whole		−
C. sertularioides	Whole	Tampa Bay	+
	Whole		+†
C. verticillata	Whole	Blackwater Sound	−

* +, Caulerpin present; −, caulerpin absent.
†ref. [6].
‡ref. [8].
§ < 0.10 mg/g dry wt.

Table 3. Distribution of caulerpin in *Caulerpa* species from Tasmania

Species	Collection site(s)	Caulerpin*
C. annulata	Rocky Cape, Waterhouse Island	−
C. brownii	Southport, Little Christmas Island, Waterhouse Island, Sandersons Rocks	−
C. cactoides	Flinders Island	−
C. distichophylla	Waterhouse Island	−
C. flexilis	Little Christmas Island, Maria Island	−
C. geminata	Tinderbox, Smooth Island	−
C. longifolia	Smooth Island, Southport, Coningham, Low Point, Coles Bay, Tinderbox	−
C. longifolia var. *crispata*	Fortesque Bay	−
C. obscura	Waterhouse Island, Flinders Island	−
C. papillosa	Smooth Island, Low Point	−
C. scapelliformis	Maria Island, Windlass Bay, Spring Beach, Lachlan Island	−
C. trifaria	Fortesque Bay, Randells Bay, Tinderbox, Smooth Island	−
C. vesiculifera	Flinders Island	−

* +, Caulerpin present; −, caulerpin absent.

Table 4. Literature survey on the distribution of caulerpin in *Caulerpa* species

Species	Collection site	Caulerpin*	Reference
C. brownii	Wellington, New Zealand	−	2
C. cupressoides	Belize	+	5
C. lamourouxii	Philippines	+	7
C. lentillifera	Philippines	+	7
C. mexicana	Belize	−	5
C. peltata	Western Australia	+	14
C. racemosa	Philippines	+	1
	Sri Lanka	+	2
	Perth, Australia	+	2
	Belize	+	5
	Western Australia	+	14
C. racemosa var. *uvifera*	Philippines	−	7
C. sedoides	Wellington, New Zealand	−	2
C. serrulata	Philippines	+	1
	Belize	+	5
C. sertularioides	Philippines	+	7
	Gulf of California	+	2†
	Belize	+	5
C. taxifolia	Sri Lanka	+	2
C. verticillata	Belize	+	5

* +, Caulerpin present; −, caulerpin absent.
†Footnote 2 in ref. [2].

detailed analyses for caulerpin in each case, these few discrepancies, all involving very low concentrations of caulerpin, remain. It is noteworthy that *C. paspaloides* and *C. racemosa* var. *uvifera* are two species difficult to identify, since each exhibits considerable morphological variation from one sample to another.

Seasonal fluxes in caulerpin content would not seem to be a significant factor. Collections of *C. sertularioides* made in February, July, August and October all contained high concentrations of 1. Similarly, *C. peltata*, whether collected in June or August, tested positive for caulerpin. On the other hand, *C. verticillata* contained no caulerpin in either February or July collections, nor did *C. mexicana* or *C. cupressoides* collected in March–May [5] and August.

On the basis of pyrenoid and chloroplast morphology, Calvert *et al.* [9] have presented evidence that radial forms of *Caulerpa* represent phylogenetically earlier levels of development, with increasing specialization of carbohydrate-storage structures occurring together with a trend towards flattened bilateral growth forms. It would thus appear that the disappearance of caulerpin production correlates with this trend in specialization, which, in turn, accompanies the transition to deeper water habitats. Judging from light conditions prevailing at our sample sites, however, it appear unlikely that caulerpin production is generated or controlled by high intensity illumination alone.

In an effort to determine whether caulerpin was localized in certain parts of the alga, several collections of caulerpin-positive species were carefully cut and examined by sections branches in one analysis, and axes, stolons and rhizomes together in another. *C. sertularioides*, *C. racemosa* var. *macrophysa* and *C. peltata* were tested in this fashion; caulerpin was present in roughly equal quantities in all parts. Two caulerpin-negative species, *C. mexicana* and *C. prolifera*, were treated in the same manner as a check for trace quantities of **1** localized in algae seemingly devoid of **1**. All parts proved to be lacking in caulerpin.

However, the occurrence of an auxin-like substance promoting abundant branching together with vertical extension of axes, under genetic control, would be consistent with our observations. Caulerpin, in fact, is a dimer of indole-3-acrylic acid, a known auxin [10], and may serve as a precursor to a growth promoter, much as hypaphorine is a precursor of indoleacrylic acid in lentils [11]. We have recently found that caulerpin exhibits growth regulatory activity in a standard lettuce seedling assay [12]; caulerpin itself then might serve as a growth regulator in the alga.

An interesting inverse correlation between the presence of caulerpin and peroxidase is suggested from a comparison of our survey and Menzel and Grant's analysis [13] of peroxidase activity in 17 species of *Caulerpa*. Thirteen of those species were examined by us or others; in each case, the presence of peroxidase was marked by the absence of caulerpin.

Caulerpin has not been reported from any source other than the Caulerpales. We found none in the green algae *Cymopolia barbata*, *Valonia macrophysa*, *Penicillus capitatus*, *Bryopsis pennata*, *Palmogloea protuberans*, *Udotea sublittoralis*, *Neomeris annulata*, *Acetabularia crenulata*, *Avrainvillea longicaulis* or *Batophora oerstedii*. Caulerpin would seem to be a valid chemotaxonomic marker in *Caulerpa* species and may function as a growth hormone or auxin precursor in the alga.

EXPERIMENTAL

^1H NMR: Bruker WM-250 FT NMR spectrometer using $CDCl_3$ as solvent and internal standard or a Jeol JNM-1H 100 MHz NMR spectrometer using $CDCl_3$ as solvent and TMS as internal standard. TLC: 0.25 mm Macherey-Nagel Polygram Sil G/UV_{254} precoated plastic sheets (developed with Et_2O) or Merck Silica Gel 60 F254 precoated glass plates (developed with CH_2Cl_2 EtOAc, 4:1). The crude extracts were co-chromatographed with purified caulerpin; caulerpin was detected under UV light or as a bright yellow spot after spraying with H_2SO_4.

Extraction. The various algae from Bermuda and Florida were collected and stored in either Me_2CO or 95% EtOH at 5° prior to extraction. In each case the solvent was decanted and concentrated to an aq. suspension. The alga was then chopped by hand or homogenized in a Waring blender, extracted with Me_2CO (24 hr), and then CH_2Cl_2 (24 hr, twice). The Me_2CO-EtOH extracts were reduced to aq. suspensions which were equilibrated with the CH_2Cl_2 extracts. The organic phase was then reduced, *in vacuo*, to a dark green gum.

The freshly collected Tasmanian specimens were macerated and extracted with CH_2Cl_2–MeOH (1:1) at 0° (24 h, twice). The lower (organic) phase was then reduced, *in vacuo*, to a dark green gum.

Isolation of caulerpin. Caulerpin was isolated from the crude extract (14.6 g, 6.1% of dry wt) of *C. sertularioides* (240 g dry wt) by two permeations through Sephadex LH-20 with CH_2Cl_2–MeOH (1:1); 341 mg of caulerpin, **1**, a red solid whose ^1H NMR spectrum was identical with that reported previously [2], was obtained.

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