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# Two new UV-absorbing mycosporine-like amino acids from the sea anemone *Anthopleura elegantissima* and the effects of zooxanthellae and spectral irradiance on chemical composition and content

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**Abstract.** Many tropical cnidarians living in shallow water contain a class of ultraviolet-A (UV-A, 320 to 400 nm) and ultraviolet-B (UV-B, 280 to 320 nm) absorbing compounds known as mycosporine-like amino acids (MAAs). These compounds may provide protection from the deleterious effects of solar UV radiation. Using a novel application of reverse-phase high performance liquid chromatography, we find that the temperate sea anemone *Anthopleura elegantissima* (collected in 1988 from Bodega Bay, California, and in 1991 from Santa Barbara, California) contains four major MAAs: shinorine, porphyra-334, and two new compounds, mycosporine-taurine and mycosporine-2 glycine. Analysis of zooxanthellate (containing zooxanthellae) and naturally apozooxanthellate (lacking zooxanthellae) specimens acclimated in the presence and absence of UV for 28 d in the spring of 1988 suggests that this anemone, unlike some other anthozoans, does not regulate the concentration of its MAAs in response to UV radiation. The presence of similar concentrations of MAAs in apozooxanthellate and zooxanthellate specimens indicates that symbiosis with algae is not required for these compounds to be present in the anemone. The total concentration of MAAs in the zooxanthellae is only about 12% of that in their host's tissues.

## Introduction

The relative transparency of tropical ocean waters (Smith and Baker 1979, Fleischmann 1989) results in the routine exposure of reef-dwelling invertebrates from shallow water to high levels of solar UV radiation. Such radiation is a recognized biological hazard affecting the survival, growth, and physiology of marine invertebrates and algae (Jokiel 1980, Jokiel and York 1982). The effects include damage to DNA and proteins, oxidation of membrane lipids, and inhibition of algal photosynthesis and growth (reviewed by Harm 1980, Worrest 1982, Renger et al. 1986, Kyle 1987), which may involve toxic forms of active oxygen (Dykens et al. 1992, Shick 1993).

One defense against the damaging effects of UV radiation involves filtering harmful wavelengths with UV-absorbing compounds. One family of such compounds comprises the mycosporine-like amino acids (MAAs), which are characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid, and absorption maxima ranging from 310 to 360 nm (Hirata et al. 1979, Tsujino et al. 1980, Nakamura et al. 1982). MAAs have been found in taxonomically varied symbiotic and non-symbiotic marine invertebrates (Dunlap and Chalker 1986, Dunlap et al. 1991, Karentz et al. 1991, Shick et al. 1991, 1992), fishes (Dunlap et al. 1989), and algae (Takano et al. 1979, Carreto et al. 1990, Karentz et al. 1991).

A protective function for these compounds has been indicated by several studies. Jokiel and York (1982) showed that the concentrations of UV-B-absorbing compounds (280 to 320 nm) in *Pocillopora damicornis* increase in response to UV exposure. Shallow-water corals appear to be acclimatized to such potentially stressful conditions (Siebeck 1981, 1988) and contain higher concentrations of UV-absorbing compounds than do conspecifics living at greater depths (Maragos 1972, Dunlap et al. 1986, Scelfo 1986, Stochaj et al. 1989). Transplantation of corals to shallower depths may increase the tissue concentrations of these compounds (Scelfo 1986), although not all cnidarians respond in this manner. After an initial increase, attributed to the mechanical disturbance of transplantation, Scelfo (1985) found no significant differences in the levels of UV-B absorbing compounds in the zoanthid, *Zoanthus pacificus*, acclimatized for 56 d in the presence and absence of UV at different fluxes of visible radiation. Similarly, no significant increase in the concentration of UV-B-absorbing compounds was found in *Stylophora pistillata* colonies 4 wk after tansplantation from 30 to 5 m (Gattuso 1987). Shick et al. (1991) found no significant difference in MAA con-

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centrations over a depth gradient of 4 to 17 m in the octocoral *Clavularia* sp., and only a small (but significant) response of individual MAAs when colonies were acclimatized with and without UV for 208 d, suggesting that the MAAs may by constitutive in this symbiosis. The apparent lack of effect of UV radiation in *Z. pacificus* and *S. pistillata* may be attributable to the relatively short duration of the acclimations. However, Scelfo (1986) found significantly higher concentrations of UV-B absorbing compounds in specimens of the coral *Montipora verrucosa* acclimatized in the presence of UV than in those acclimatized without UV after only 13 d.

With the exception of studies by Scelfo (1985, 1988 a, b) and Shick et al. (1991), most research on cnidarians concerning the effects of UV exposure on the levels of these compounds involves scleractinian corals. Some actiniarians (sea anemones) commonly found in shallow water or intertidally are also exposed to high levels of UV radiation and therefore require some means of protection. Shick and Dykens (1984) reported that aqueous extracts of the temperate-zone sea anemone *Anthopleura elegantissima* exhibit a high absorbance at 320 nm, indicating the presence of UV-absorbing compounds. The present study describes an improved chromatographic method for separating the major UV-A- and UV-B-absorbing compounds in *A. elegantissima* and identifies them as MAAs. The effects of exposure to UV radiation, and the presence or absence of algal endosymbionts (zooxanthellae) on the concentration of these compounds, were also examined.

## Materials and methods

### Maintenance of sea anemones

Zooxanthellate and apozooxanthellate specimens (terminology in keeping with Schumacher and Zibrowius 1985) of *Anthopleura elegantissima* (Brandt) were collected in the spring of 1988 from two distinct groups in the vicinity of Bodega Marine Laboratory, Bodega Bay, California, USA and shipped live to Orono, Maine, USA. Zooxanthellate specimens (harboring dinoflagellates, *Symbiodinium* sp.) from a fully illuminated site were maintained in a 250-liter aquarium receiving filtred sunlight (the UV component of the natural solar spectrum being filtered out by the window and aquarium glass). Apozooxanthellate (zooxanthella-free) specimens collected from dark recesses within the jetty at Bodega Harbor were maintained in aquaria in a temperature-controlled incubator under constant darkness except during feeding periods. Water temperature was maintained at $15 \pm 2$ °C for both groups. Anemones were fed minced squid once per week.

### Identification of UV-B-absorbing compounds

Freshly collected specimens of *Anthopleura elegantissima* were lyophilized and shipped to the Australian Institute of Marine Science (AIMS) for identification of UV-absorbing compounds. The extraction and separation procedures used in the initial acclimation experiments (see next section) provided insufficient material for the identification of new UV-absorbing compounds, so these procedures were modified accordingly. Dry specimens (5 g) were extracted successively in three volumes of 80% aqueous methanol (20 ml) with sonication on ice. The extracts were centrifuged and the supernatants combined and then filtered (Whatman GF/C glass fiber) and passed through a $C_{18}$ Sep-Pak cartridge (Waters, Milford, Massachusetts, USA) to remove chromatographically intractable materials.

The organic solvent was removed under reduced pressure and the residue diluted to approximately 20 ml with double distilled water. This diluted extract was filtered and passed through a second $C_{18}$ Sep-Pak cartridge to remove non-polar and pigmented components, lyophilized, and reconstituted in 2 ml of high performance liquid chromatography (HPLC) mobile phase (75:24.9:0.1 methanol:water:acetic acid) for fractionation.

The UV-absorbing compounds were fractionated by HPLC using a 25 cm Brownlee RP-8 column and guard connected to a Waters Model 440 dual-wavelength detector (340 and 313 nm) at a flow rate of 0.8 ml $min^{-1}$. Fractions corresponding to individual UV-absorbing compounds were concentrated and further purified by HPLC on a 25-cm Brownlee silica column using the same mobile phase as a above. Final purification was achieved on a 25-cm Brownlee amino column using a mobile phase consisting of 40 m*M* ammonium acetate and 17.5 m*M* acetic acid in 80% aqueous methanol.

Aliquots (100 µl) of each purified fraction were hydrolyzed at room temperature for 6 h with 10 µl of 10 *N* NaOH and then neutralized with 10 µl of 10 *N* HCl. The hydrolyzed amino acids were identified by both the *o*-phthaldialdehyde (OPA) derivatization and HPLC method of Gardner and Miller (1980) and by the fluorenylmethyl chloroformate (FMOC-Cl) derivatization and HPLC method of Einarsson et al. (1983). Hydrolyzed amino acids were confirmed by co-chromatography with amino acid standards (Sigma) dissolved in 0.1 *N* HCl and treated with OPA and FMOC-Cl reagents as above.

Mass analyses of purified fractions were performed on a Perkin-Elmer SciEx API III LC/MS/MS mass spectrometer with atmospheric pressure, electrospray ionization. Positive ion, parent ion (MS), and daughter ion (MS/MS) spectra were recorded.

### Effects of algal symbiosis and exposure to UV radiation

Zooxanthellate ($n = 5$) and apozooxanthellate ($n = 5$) specimens of *Anthopleura elegantissima* were acclimated in 20 cm diameter bowls of 30‰ S seawater at 15 °C for 28 d on a 8 h light:16 h dark photoperiod in the presence of UV (measured visible irradiance 600 to 620 µmol photons $m^{-2}$ $s^{-1}$) and in the absence of UV (590 to 620 µmol photons $m^{-2}$ $s^{-1}$). Irradiance was measured with a LiCor LI-185B quantum photometer fitted with a cosine corrected sensor, Model LI-1905B, for photosynthetically active radiation (PAR, 400 to 700 nm). A Kratos 1 kW xenon arc solar simulator (Model SS1000X) equipped with an airmass 1 filter provided illumination having a spectral qualitiy approximating natural sunlight at the earth's surface at midday. UV wavelengths below approximately 380 nm were absorbed by 1-cm thick acrylic Plexiglas (Rohm and Haas) (see Jokiel and York 1982) located above the culture bowls.

At the end of the acclimation period, tentacle squashes from both zooxanthellate and apozooxanthellate specimens were examined for the presence of zooxanthellae using visible light and UV microscopy. Each individual was then frozen and lyophilized. A 50 to 120 mg subsample was ground in 2 ml of deionized, double distilled water in a Brinkman Polytron tissue grinder, sonicated for 5 min on ice, and extracted for 16 h at 4 °C (Shibata 1969, Jokiel and York 1982).

Samples were centrifuged ($270 \times g$ for 10 min) and 25 µl aliquots of the supernatant were injected onto a 25-cm Brownlee RP-8 reverse phase HPLC column protected with a 3-cm Brownlee RP-8 guard column connected to a Hewlett-Packard diode array detector (Model 1040M). The mobile phase consisted of 90% 5 µ*M* acetate buffer (pH 3.0) and 10% methanol at a flow rate of 0.7 ml $min^{-1}$. Detection was by UV absorbance at 313 nm. Chromatographic peaks of known MAAs were identified by UV-spectral comparison and by co-chromatography with authenticated standards. New MAAs were isolated by chromatography and were chemically identified (see preceding section).

The chromatographic peak area for each UV-absorbing compound was converted to molar concentration using purified fraction (see preceding section). Purified fractions were quantified using published extinction coefficients at the wavelength of maximum absorbance (porphyra-334, $\varepsilon_{334}=43\,300$: Takano et al. 1979; shinorine, $\varepsilon_{334}=44\,668$: Tsjuino et al. 1980) and corrected for extraction efficiency using the method of Dunlap and Chalker (1986). Because extinction coefficients for the new compounds, mycosporine-2 glycine and mycosporine-taurine (see below), have not yet been determined, the values for the structurally similar compounds shinorine (see above) and mycosporine-glycine methyl ester ($\varepsilon_{310}=28\,000$; Ito and Hirata 1977) were used, respectively, to quantify the novel compounds.

All data on UV-absorbing compounds were evaluated for treatment effect by two-way ANOVA ($P=0.05$; Sokal and Rohlf 1981; StatView 512$^+$, Brainpower Inc., Calabasas, California, USA), with the fixed effects being UV (present or absent) and type of anemone (zooxanthellate or apozooxanthellate). Concentrations of individual compounds in the various groups were compared using the Student-Neuman-Keuls multiple comparison test (Zar 1984).

### Partitioning of mycosporine-like amino acids between host and zooxanthellae

Zooxanthellate specimens of *Anthopleura elegantissima* were collected from Santa Barbara harbor, California, in January 1991 and immediately carried to Orono, Maine. An entire large anemone was bisected longitudinally. One-half of the anemone was immediately frozen and lyophilized, and the other half was homogenized in calcium-free artificial seawater (MBL formula 2: Cavanaugh 1975) to yield its contained zooxanthellae, which were cleaned and recovered quantitatively as described in Lesser and Shick (1989), then frozen and lyophilized. This method of cleaning and concentrating zoxanthellae yields intact algal cells. An aliquot of the crude homogenate was removed for determination of total symbiosis (animal host + zooxanthella) protein. Both the first half of the anemone and the zooxanthellae isolated from the other half were sonicated and thrice extracted in 80% methanol on ice, and the clarified extracts were analyzed by HPLC using a mobile phase containing 55 or 75% methanol, as described in "Materials and methods – Identification of UV-B-absorbing compounds". The protein contents of the extracted anemone and of the zooxanthella sample were measured by the method of Bradford (1976) using Coomassie Brilliant Blue and bovine gamma globulin standards (Bio-Rad Laboratories). Protein content, and hence MAA concentrations (nmol mg$^{-1}$ protein), in the host animal tissue were calculated by difference (see Dykens et al. 1992).

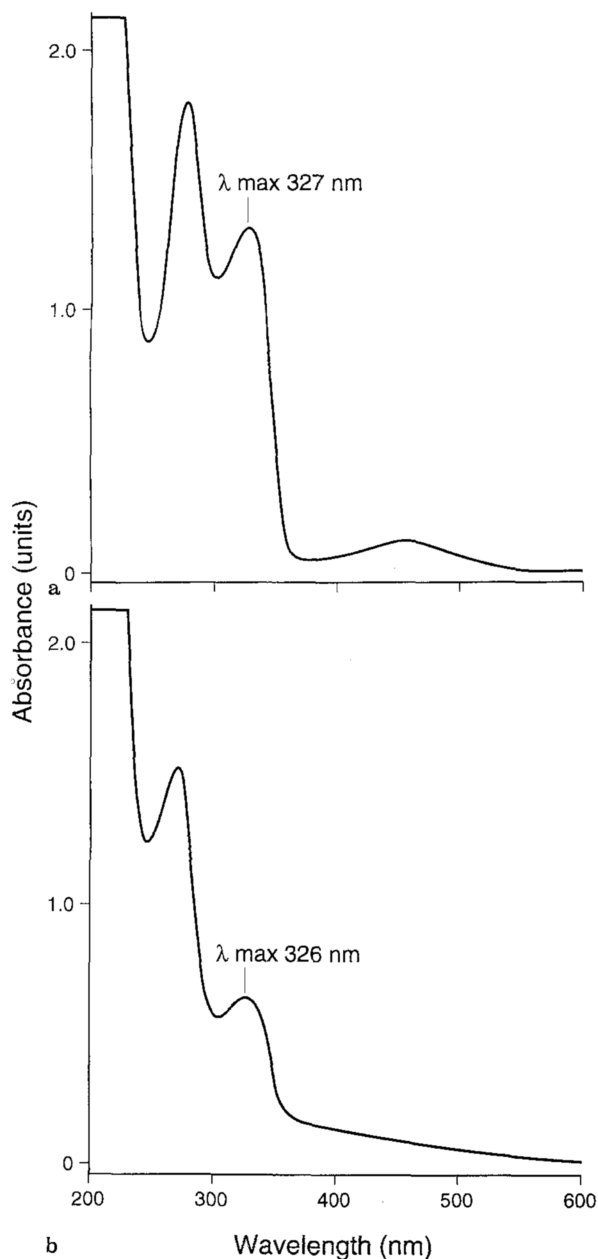


**Fig. 1.** *Anthopleura elegantissima.* (a) UV-visible light absorption spectrum of crude methanolic extract of a lyophilized zooxanthellate specimen. (b) UV-visible absorption spectrum of crude aqueous extract of a lyophilized zooxanthellate specimen

## Results

### Identification of UV-absorbing compounds

Crude methanolic extracts of *Anthopleura elegantissima* showed absorption maxima at 270 and 327 nm (Fig. 1 a), the latter of which suggested the presence of MAAs. Initial separation of cleaned methanolic extracts of *A. elegantissima* resulted in the isolation of four major compounds (peaks 1, 2, 3, and 4; Fig. 2a). Two MAAs from *A. elegantissima* were identified by co-chromatography with authenticated samples and two new MAAs were confirmed by mass spectrometry. Extraction efficiencies for MAAs in aqueous methanol were 95% or higher.

Compound 1 had an absorption maximum of 309 nm and the sulfonic amino acid taurine was the sole amino acid to hydrolyze from the mycosporine chromophore. This new compound therefore was suggested to have structure 1 (Fig. 3) and was named mycosporine-taurine. The structure of mycosporine-taurine [$MH^+=296$] was confirmed by positive ion mass spectrometry.

Compound 2 had an absorption maximum at 333 to 334 nm and contained serine and glycine as the amino acids attached to the mycosporine chromophore. These characteristics matched those of shinorine (structure 2, Fig. 3) isolated from the red alga *Chondrus yendoi* (Tsujino et al. 1980).

Compound 3 had an absorption maximum of 334 nm and co-chromatographed with a standard of porphyra-

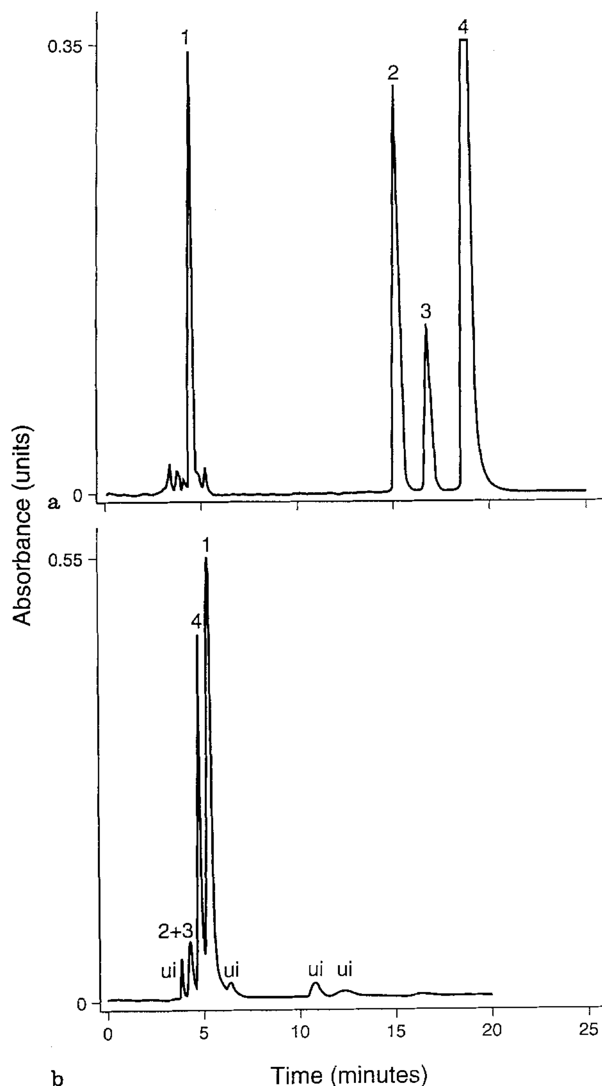


**Fig. 2.** *Anthopleura elegantissima.* (a) High performance liquid chromatography (HPLC) chromatogram of mycosporine-like amino acids from an 80% methanolic extract of a lyophilized zooxanthellate specimen. Brownlee Spheri-5, RP-8 column and guard; mobile phase 75% methanol and 0.1% acetic acid (V:V); flow rate 0.8 ml $min^{-1}$; detection by absorbance at 340 nm. (b) HPLC chromatogram of mycosporine-like amino acids from an aqueous extract of a lyophilized zooxanthellate specimen. Brownlee Spheri-5, RP-8 column and guard; mobile phase 10% methanol and 0.1% acetic acid (V:V); flow rate 0.7 ml $min^{-1}$; detection by absorbance at 313 nm. 1: mycosporine-taurine; 2: shinorine; 3: porphyra-334; 4: mycosporine-2 glycine; ui: unidentified

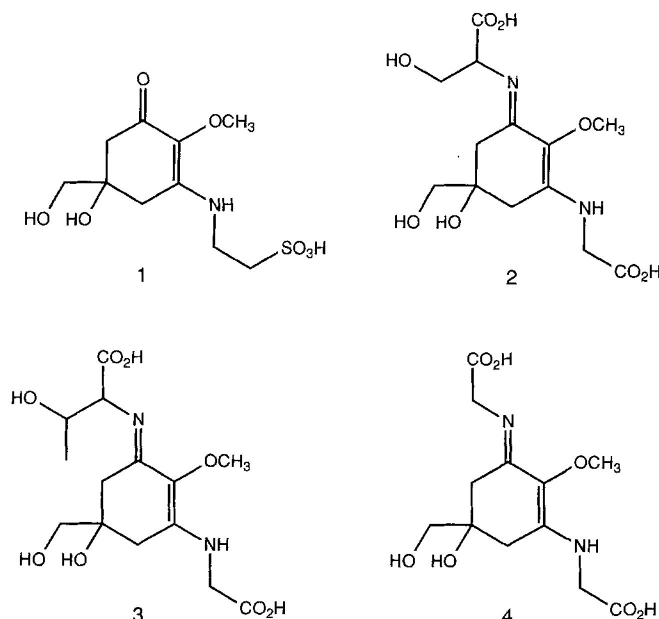


**Fig. 3.** *Anthopleura elegantissima.* Structures of mycosporine-like amino acids isolated from methanolic extract of lyophilized zooxanthellate specimens. 1: mycosporine-taurine; 2: shinorine; 3: porphyra-334; 4: mycosporine-2 glycine

334 (extracted from the alga *Porphyra tenera*, "nori"; Takano et al. 1979), which structure was assigned to compound 3 in *Anthopleura elegantissima* (structure 3 in Fig. 3). The absence of porphyra-334 from samples extracted with distilled water in the acclimation experiment (see next section) was attributed to the different chromatographic method used in the acclimation experiment, which was conducted before the improved method was discovered. Separation with only 10% methanol in the mobile phase caused porphyra-334 to co-elute with shinorine (Fig. 2b).

Compound 4 had an absorption maximum of 331 nm, indiciative of an N-substituted iminomycosporine. Since fractionation and alkaline hydrolysis of compound 4 yielded only glycine (in approximately twice the molar yields as that from mycosporine-glycine), this new compound was named mycosporine-2 glycine and suggested to have structure 4 (Fig. 3). The structure of mycosporine-2 glycine [$MH^+ = 303$] was confirmed by positive ion, electrospray mass spectrometry.

## Effects of algal symbiosis and exposure to UV radiation

Zooxanthellate specimens of *Anthopleura elegantissima* had dense populations of algal endosymbionts, whereas apozooxanthellate anemones had none. Since no "blooming" of any vegetative zooxanthellae that might have been present in apozooxanthellate specimens of *A. elegantissima* occurred during their acclimation to visible light and UV, we concluded that these anemones were functionally free of zooxanthellae.

Aqueous extracts of both zooxanthellate (Fig. 1b) and apozooxanthellate (data not shown) specimens acclimated in the laboratory had an absorption maximum at 326 nm and showed seven UV-absorbing peaks (Fig. 2b): compounds 1, 2+3, 4 ($\lambda_{max}$ 310, 334, and 332 nm, respectively) and four unidentified peaks as minor constituents. Based on UV spectral analysis and co-chromatography with authenticated samples (see "Results – Identification of UV-absorbing compounds"), compounds 1, 2 (+3) and 4 were identified as mycosporine-taurine, shinorine (+porphyra-334) and mycosporine-2 glycine. Extraction efficiencies in double distilled water for these compounds (including porphyra-334, which was determined in subse-

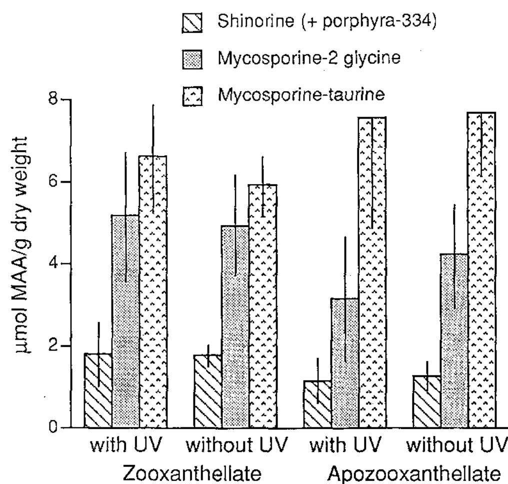


**Fig. 4.** *Anthopleura elegantissima.* Concentrations of major mycosporine-like amino acids (MAAs) (mean ± SD; $n=5$) in specimen from Bodega Bay, California, acclimated for 28 d in light with and without a UV component. Zoöxanthellate specimen have significantly higher concentrations of shinorine (+porphyra-334) and mycosporine-2 glycine than do apozooxanthellate anemones ($P=0.010$ and 0.023, respectively)

**Table 1.** *Anthopleura elegantissima.* Concentrations of mycosporine-like amino acids (MAAs) in the symbiosis (host + zooxanthellae) and in zooxanthellae quantitatively isolated from one-half of the same individual from Santa Barbara, California. Concentrations in the animal tissue were calculated by difference, with the information that animal tissue constitutes 88.8%, and zooxanthellae 11.2%, of the total symbiosis protein in this specimen

| MAA | Concentration in symbiosis (nmol mg$^{-1}$ total protein) | Concentration in zooxanthellae (nmol mg$^{-1}$ algal protein) | Concentration in host (nmol mg$^{-1}$ animal protein) |
|---|---|---|---|
| Mycosporine-taurine | 12.54 | 0.98 | 14.12 |
| Shinorine | 1.70 | 0.24 | 1.91 |
| Porphyra-334 | 0.62 | trace | 0.70 |
| Mycosporine-2 glycine | 6.73 | 1.70 | 7.58 |
| Total | 21.59 | 2.92 | 24.31 |

quent analyses) were 99 to 100%. The remaining four compounds were in concentrations too low to allow identification.

There was no significant effect of UV exposure on the concentration of shinorine (+porphyra-334), mycosporine-2 glycine or mycosporine-taurine (Fig. 4). Shinorine (+porphyra-334) and mycosporine-2 glycine had significantly higher concentrations in zooxanthellate than in apozooxanthellate specimens ($P=0.010$ and 0.023, respectively), while mycosporine-taurine showed a non-significant trend toward higher concentrations in apozooxanthellate specimens (Fig. 4).

### Partitioning of MAAs between host and zooxanthellae

Concentrations of individual MAAs differed in the animal and algal moieties of *Anthopleura elegantissima* (Table 1). All four MAAs identified in this species at Bodega Bay were found in the Santa Barbara specimens in approximately the same concentrations and ratios. In the animal tissue, mycosporine-taurine was the most concentrated, followed in order of decreasing concentration by mycosporine-2 glycine, shinorine, and porphyra-334. In the zooxanthellae, mycosporine-2 glycine replaced mycosporine-taurine as the most abundant MAA, and porphyra-334 was present only in trace amounts. Total MAA concentration in the zooxanthellae was only about 12% of that in the animal tissue (Table 1); since zooxanthellae in this specimen made up 11.2% of the protein biomass of the whole anemone, it follows that the zooxanthellae contained only about 1.5% of the total MAAs present in the intact symbiosis, based on tissue protein weights. Subsequent trials on zooxanthellae freshly isolated from the coral *Acropora formosa* and cleaned and concentrated in the same manner as those from *A. elegantissima* showed that MAA concentrations in the coral zooxanthellae did not change for at least 2 h post-cleaning (Shick and Dunlap unpublished data), so the MAA concentrations reported for zooxanthellae probably are representative of those of zooxanthellae *in hospite*.

## Discussion

We have used mixed-mode, reverse-phase and ion-exchange chromatography to isolate the major mycosporine-like amino acids present in *Anthopleura elegantissima*, which were identified as mycosporine-taurine, shinorine, porphyra-334, and mycosporine-2 glycine. Of these, mycosporine-taurine and mycosporine-2 glycine have not been previously described.

When the methanol concentration of the mobile phase was increased to greater than 40%, the polar MAAs interacted with the weak anion exchange properties of silica (Secreast 1991), or the silica bed of a reverse phase column, to give an improved chromatographic separation of MAAs. Increasing the methanol content of the mobile phase increases the retention of the highly polar mycosporine-like amino acids. This technique allows the isolation of compounds that are not separated using previously published methods (see Nakamura et al. 1982, Dunlap et al. 1986) and should allow further investigations of other MAAs that cannot otherwise be clearly resolved.

The association of taurine with a mycosporine is noteworthy. Taurine constitutes more than 90% of the free amino acid pool in *Anthopleura elegantissima* (Stochaj unpublished data) and in the congeneric *A. xanthogrammica* (Male and Storey 1983). Thus, in addition to being an important osmolyte (see Shick 1976, 1991, Kasschau et al. 1984), this sulfonic amino acid is also incorporated into the predominant MAA in *A. elegantissima*. By incorporating taurine into a UV-absorbing compound, the anemone may be exploiting the ready availability of the most concentrated component of its free amino acid pool.

There was no significant effect of UV on the concentration of MAAs in *Anthopleura elegantissima* after 28 d

of acclimation (Fig. 4). Similarly, Scelfo (1988 a, b) found no significant effect of visible (full solar spectrum vs complete darkness) or UV radiation on the levels of unidentified UV-absorbing compounds in this species following 6 mo of acclimation. Thus, neither visible light nor UV radiation regulated the levels of MAAs in *A. elegantissima*. Moreover, we found no significant difference in the concentration of mycosporine-taurine (the most concentrated MAA in this species) between zooxanthellate specimens from exposed areas and apozooxanthellate anemones that normally inhabit deeply shaded habitats, and similar concentrations of shinorine (+porphyra-334) and mycosporine-2 glycine in these groups.

In absolute terms, the concentrations of the individual MAAs in the animal tissues and in the zooxanthellae of *Anthopleura elegantissima* are somewhat lower than in the tropical sea anemone, *Phyllodiscus semoni* (cf. Shick et al. 1991). *A. elegantissima* lives at higher latitudes and in relatively turbid waters, and therefore experiences less UV exposure than do tropical cnidarians (see Frederick et al. 1991). Although some tropical cnidarians vary their MAA concentrations according to UV exposure, not all do so (see references in "Introduction"), and the response of *A. elegantissima* is more like that of *P. semoni*, which shows only slight increases in MAA concentration during long-term acclimation to solar UV, the increase being more pronounced in the zooxanthellae than in the animal tissue (Shick et al. 1991). Further studies on the UV photophysiology of cnidarians are necessary to determine whether latitudinal differences in solar UV exposure can affect tissue concentrations of these UV-absorbing compounds and to clarify the ability of the organisms to regulate them. Rather than regulating the concentrations of its UV-absorbing compounds, *A. elegantissima* seemingly maintains them at a given level, relying on behavioral mechanisms to protect against short-term increases in UV radiation. This anemone retracts its tentacles in response to peak irradiances, moves to less stressful photic conditions (i.e., shaded areas) and attaches debris to its column, possibly to shield exposed surfaces (Clark and Kimeldorf 1971, Pearse 1974, Shick and Dykens 1984, Shick 1991).

The source of MAAs in cnidarians has yet to be identified. Favré-Bonvin et al. (1987) report that the closely related mycosporines in fungi are synthesized via the shikimic acid pathway, which occurs only in higher plants, algae, bacteria, and fungi (Yoshida 1969, Towers and Subba Rao 1972, Floss 1979). Thus, Dunlap and Chalker (1986) infer that the MAAs in cnidarians may be produced by the endosymbiotic algae and transferred to the host. The identification in *Anthopleura elegantissima* and *Phyllodiscus semoni* (Shick et al. 1991) of the same MAAs in the zooxanthellae as in their respective hosts' tissues is consistent with this hypothesis.

The present study also suggests that symbiosis with algae is not a requirement for the presence of MAAs in cnidarians. All of the MAAs isolated from zooxanthellate specimens of *Anthopleura elegantissima* are also found in naturally apozooxanthellate individuals. MAAs likewise are present in the apozooxanthellate actiniid anemones, *Actinia bermudensis* (Stochaj 1989) and *A. tenebrosa* (Shick unpublished data). Therefore, these compounds, or their precursors, might be of dietary origins, be synthesized by the anemones themselves, or come from bacteria harbored in the anemone's coelenteron (see Herndl and Velimirov 1985) or ectodermal tissue (see Palincsar et al. 1989).

The diet of *Anthopleura elegantissima* in the laboratory consisted solely of squid, which contained UV-absorbing compounds, two of which showed chromatographic retention times similar to MAAs present in *A. elegantissima* (data not shown). Thus, a dietary source of MAAs or related compounds in *A. elegantissima* in the acclimation experiments was possible. Dunlap et al. (1991) presented evidence that the MAAs present in a variety of tropical holothuroid echinoderms are of dietary origin. Grant et al. (1985) suggested that the brine shrimp, *Artemia* sp., is capable of synthesizing gadusol (structurally similar to MAAs), perhaps via an acetate pathway (Grant et al. 1980, Favré-Bonvin et al. 1987). It is unknown whether cnidarians possess a similar pathway for the synthesis of MAAs.

Our data on naturally apozooxanthellate specimens indicate that the presence of MAAs in the animal tissue is not absolutely linked to the presence of zooxanthellae, but the data do not rule out a dietary or bacterial source of MAAs. The finding of the same complement of MAAs in the zooxanthellae as in the animal tissue from which the zooxanthellae were isolated suggests some exchange of these compounds between host and symbionts, although the direction of such transfer is unknown. The much higher concentration of MAAs in the animal than in the zooxanthellae suggests that, in this symbiosis, the host tissue is the first line of defense against solar UV.

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