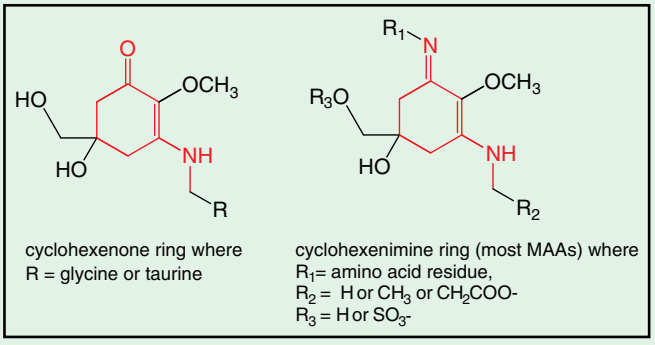
15
Mycosporine-Like Amino Acids (MAAs) in Biological Photosystems

Stéphane La Barre, Catherine Roullier, and Joël Boustie

Abstract

Mycosporine-like amino acids (MAAs) represent a suite of small molecules which have unique ultraviolet-absorbing capacities, based on their common cyclohexenone or cyclohexenimine conjugated arrangements. MAAs also have strong antioxidant properties, and participate in the osmotic equilibrium of the numerous and diverse marine organisms that produce them, especially in photosymbiotic partnerships. In this chapter a historical introduction is provided to the discovery of MAAs, together with details of their primary and secondary functions. Details are also provided of isolation and spectral information, illustrated by pertinent examples, and aspects of biosynthesis and the modulating environmental factors involved in the production and degradation of MAAs. Two contrasting biological models models are described: lichens, which are mostly terrestrial and extremophiles; and shallow-water tropical reef corals, which are marine and stenotolerant; these have one point in common, namely a permanent exposure to direct sunlight. Finally, brief details are provided of the medical, cosmetological and biotechnological applications of these primary metabolites with secondary roles, that were most likely instrumental in the adaptation of early cyanobacterial life forms to surface conditions. Useful practical information for structural chemists is included in an Appendix.



cyclohexenone ring where
R = glycine or taurine

cyclohexenimine ring (most MAAs) where
R_1= amino acid residue,
R_2 = H or CH_3 or CH_2COO^-
R_3 = H or SO_3^-

Water-soluble and photostable
Low-MW compounds (usually less than 400 Da)
No proven toxicity
Strong anti-UV absorption, especially between $\lambda = 310$–340 nm, with molar absorptivities (ε) at around 40 000 l mol^{-1} cm^{-1}.
Widely known as natural sunscreens, they also act as osmolytes and/or antioxidants with secondary metabolite characteristics that are mostly, but not exclusively, aquatic.

15.1
Background

15.1.1
Life in Full Light and its Constraints

Life probably appeared in the depths of a primitive ocean some 3.5 billion years ago, supposedly in the absence of light and under anoxic conditions. Cyanobacteria were the first organisms to use oxygenic photosynthesis, which deeply modified the biogeochemistry of the primitive ocean. Later, the development of an atmosphere created the conditions for ever-more complex life forms to appear, evolve, and colonize new habitats under constantly changing environmental conditions. Today, oxygen redox chemistry and photon energy are indispensable to all aquatic and terrestrial life forms that have not receded to a chemolithotrophic mode of subsistence. This was made possible by the development of biochemical pathways that integrated molecular oxygen, using novel enzymatic reactions, and also by acquiring protection against oxygen toxicity and exposure to harmful solar radiations. The

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Box 15.1: Useful and Harmful Solar Radiations

Ultraviolet radiation (UVR) is probably the single most influential abiotic factor that has shaped the evolution and ecology of the biosphere (Banaszak and Lesser, 2009). Under prebiotic conditions, exposure to unfiltered solar radiations (UVA, UVB and UVC) would have led rapidly to DNA damage and the death of exposed organisms. The gradual O_2 enrichment of the atmosphere by cyanobacteria who invented photosynthesis and molecular sunscreens (most probably MAAs) led to the development of new life forms, both autotrophic and heterotrophic, but it was not until atmospheric O_2 photooxidation into ozone had created an efficient antiradiation shield that terrestrial life was made possible.

Today, the ozone photoprotective layer is being depleted, leaving "holes" above large tropical zones that are now under progressive risk of overexposure to harmful shortwave UVB radiations. Shallow-water (less than 20 m) coral reef communities are at particular risk, especially in areas of high water transparency and under clear skies. Cryptic and soft-bodied coral-associated biota cannot withstand even accidental exposure to such conditions. The indirect effects of UVR is the generation of reactive oxygen species (ROS), including radical species, via the excitation of for example, aromatic intermediates and the downstream production of highly reactive hydroxyl radicals in an iron-catalyzed Fenton reaction. ROS generate oxidative stress, with adverse effects on coral polyps and their symbiotic zooxanthellae, causing the *bleaching* phenomenon.

Perhaps the most dramatic situation has to be faced by lichens, which thrive only on bare rock surfaces, in latitudes that are inhospitable (e.g., exposure to high-UVR regimes, temperature extremes, desiccation and almost no nutrients) to any other macroscopic life form, yet they also break longevity records!

protective strategies employed include enzymes for DNA repair and against aggressive oxygenated and radicalar species, UV avoidance behavior, and the biosynthesis of small molecules acting as natural sunscreens. Sessile and planktonic organisms that live while exposed to sunlight have developed chemical protections that help them to absorb harmful and genotoxic ultraviolet radiation (UVR), especially in the 310–360 nm (more-penetrating) UVA range (see Box 15.1). In addition, oxidative stress due to the production of reactive oxygen species (ROS) can be controlled by the production of antioxidants.

15.1.2 MAAs: To Protect and Serve, Occasionally to Defend

Mycosporine-like amino acids (MAAs) are synthesized (or acquired) by these organisms in response to both challenges. Indeed, MAAs are known to be the strongest UVA-absorbing compounds (in the 320–365 nm range) in Nature, as well as having strong antioxidant properties and occasionally acting as osmoprotectants (Oren and Gunde-Cimerman, 2007).

Interestingly, the existence of UV-protecting molecules had been known for some time before their exact chemical nature was revealed. For example, Wittenburg (1960) in the gas gland of an epipelagic jellyfish, Tsujino and Saito (1961) in red algae, and Shibata (1969) in aqueous coral extracts, had each reported the existence of molecules capable of strongly absorbing UV radiations in the 310–360 nm region, but without characterization. Independently, other research groups working on fungi reproduction mechanisms noticed the production of unidentified substances in the mycelia of several fungi (e.g., *Pyronema omphalodes*, *Alternaria chrysanthemi*, and *Ascochyta pisi*), when sporulation was induced by UV light (Leach, 1965). Molecules having a UV absorption maximum at 310 nm were notionally designated P-310. However, no correlation was made at the time between those studies and previously reported marine organisms observations. Consequently, fungi continued to be explored, and UV-absorbing compounds similar to P310 continued to be observed. Only several years later was the structure of the first P310 finally elucidated, after its isolation from the basidiomycete *Stereum hirsutum*; the compound became known mycosporine I, as its biosynthesis was largely observed in fungi and thought to be related to sporulation and reproduction mechanisms (Favre-Bonvin, Arpin, and Brevard, 1976). Subsequently, Ito and Hirata (1977) established the structure of the first MAA, namely mycosporine-glycine, from the zoanthid *Palythoa tuberculosa*, and this was followed by other similar molecules (Hirata *et al.*, 1979). From 1977 onwards, this type of molecule began to be more largely isolated and identified from many organisms, such as cyanobacteria, algae, phytoplankton and even animals, mostly using reversed-phase high-performance liquid chromatography (Nakamura, Kobayashi, and Hirata, 1982). Many of these molecules were isolated from corals that live symbiotically with dinoflagellates, but also from mollusks and fish. As a result the term "mycosporine-like amino-acid" or MAA emerged, reserving "mycosporine" for molecules that had been isolated exclusively from fungi.

The phylogenomics of the MAAs is still in progress as routes to MAAs appear to be multiple (Balskus and Walsh, 2010). In marine photosymbiotic systems such as reef corals, both the zooxanthellae photosymbionts and the coral host are genetically capable of producing MAAs (Shinzato *et al.*, 2011), involving gene transfer from microbiont to host (Rosic, 2012). In addition, some MAAs can be acquired directly from food sources by the coral hosts, though this adds to the complexity of the biosynthetic origin of MAAs found in these organisms (Rosic and Dove, 2011). Hundreds of taxonomically diverse marine, freshwater and terrestrial organisms have the capacity to synthesize, accumulate and metabolize MAAs in order to address

the direct and indirect damaging effects of UVR (Carreto and Carignan, 2011).

To date, at least 21 MAAs have been described from various marine sources, two with the cyclohexenone ring bearing one amino acid substituent, and 19 with the cyclohexenimine ring bearing up to three substituents (Figure 15.1). The quest is not over, however, as aplysiapalythines A, B and C (Kicklighter *et al.*, 2011) were recently discovered in the sea hare *Aplysia*, which acquires them from the diet and subsequently uses them as alarm cues in defensive secretions (opaline). In the realm of lichens, new structures are continually being found (Nguyen *et al.*, 2013) one example being mycosporine hydroxy-glutamicol from the lichen *Nephroma laevigatum* (Roullier *et al.*, 2011).

Altogether, up to 40 mycosporines and derivatives have been described from both terrestrial and marine sources, some bearing functional groups or being covalently linked with saccharidic units. Fungal mycosporines are closely related to marine mycosporines, and include mycosporine-2, mycosporine-alanine, mycosporine-glutamine, mycosporine-glutamicol, mycosporine-glutaminol, mycosporine-glutamic acid, mycosporine-glutaminol glucoside, mycosporine-serinol, and normycosporine-glutamine (Sinha, Singh, and Hader, 2007). Interestingly, all ten fungal structures feature a maximum absorbance at a wavelength of 310 nm; this is in contrast to MAAs, some of which have a λ_{max} of 360 nm. The accumulation of MAAs in the conidia is reported to increase the tolerance of the fungi against adverse environmental conditions, among other roles in their developmental biology, but this is beyond the scope of this chapter. Suffice to mention is the interesting parallel that Klisch and Häder (2008) established between MAAs and toxins in their overlapping phyletic distributions, and possibly in their similar biogenetic pathways, namely the putative involvement of non-ribosomal polyketide synthase (NRPSs) in both, albeit with totally distinct and complementary functions: the MAAs are there to protect, while toxins are there to defend.

15.2 Chemistry

15.2.1 Physico-Chemical Characteristics of MAAs

The MAAs are low-molecular-weight (most are under 400 Da), generally colorless, water-soluble compounds that are also resistant to thermodegradation and photodegradation under environmental conditions. Yet, they are differentially susceptible to acidic and oxygenating conditions. The truly outstanding characteristic, however, is their high UV-absorbing capability, with a unique strong peak in the 305–360 nm (λ_{max}) range (harmful UVA and shortwave UVB radiations) with molecular absorptivities (ε) of about 40 000 $l\,mol^{-1}\,cm^{-1}$. This makes them the strongest UVA-absorbing compounds in Nature, and they are also effective against shortwave UVB radiations. The physico-chemical properties of individual MAAs have been reviewed in detail (Carreto and Carignan, 2011).

Together, these features are essential in the roles of MAAs as photoprotectants in the outermost tissue layers of marine metazoans, and also as antioxidants and osmoprotectants in cell or body fluids, or in external mucus layers. Especially surprising is the ease by which the MAAs can be ferried from one biological compartment to another.

15.2.2 MAAs and Related Molecules

15.2.2.1 MAAs in the Marine World

Their high water solubility enables MAAs to be easily dispersed in the cytoplasm of microalgae and diatoms, and also to be rapidly accumulated in the superficial sun-exposed cell layers of numerous marine invertebrates. Some organisms acquire the MAAs directly from food by assimilation, without transformation (like free amino acids); examples include echinoderms such as starfish, sea-cucumbers and sea-urchins (for a review, see Dunlap and Shick, 1998). Cnidarians and ascidians acquire their MAA precursors from their photomicrobionts (cyanobacteria, prochloron or dinoflagellates); as most are colorless this allows "filtered" sunlight to reach the photosynthetic zooxanthallae that are sequestered in the endodermal layers of the diploblastic photosymbionts. The presence of a suite of 10 MAAs in *Stylophora pistillata* (Shick *et al.*, 1999) is most likely due to a need for photoprotection over an extended range of potentially harmful wavelengths, especially in photosymbiotic systems which may have different and specific sensitivities to sun radiations. This corresponds to MAA production being stimulated primarily by UV radiation of different wavelength ranges (Klish and Hader, 2008).

15.2.2.2 MAAs and Related Molecules in Lichens

Lichens associate with different partners, typically a saprotroph (fungus) and a phototroph (unicellular green alga or cyanobacterium, sometimes both), where the eubacterial consortia play an important role in nutrient cycling. Not surprisingly, cyanolichens (which comprise 10% of all lichen species) have been shown to possess both mycosporines and MAAs, and possibly also some unique structures (Figure 15.2), according to the environmentally "favored" partner – that is, to its prevailing metabolic expression.

15.2.3 Extraction, Separation, Purification, and Detection

15.2.3.1 Extraction, Separation, and Purification

As the semi-purification of mycosporines from other polar compounds is crucial, the protocol must be optimized, taking into account the possible hydrolysis and conversion of some of these compounds. Glycosylated mycosporines can be hydrolyzed under acidic conditions (0.5 M HCl, 100 °C), and conversion from an aliphatic amino acid chain to a cyclized amide form is likely to occur on open-column resins in the H^+ form used for separation. Cleavage of the amino acid moiety is readily achieved by alkaline hydrolysis (Pittet *et al.*, 1983).

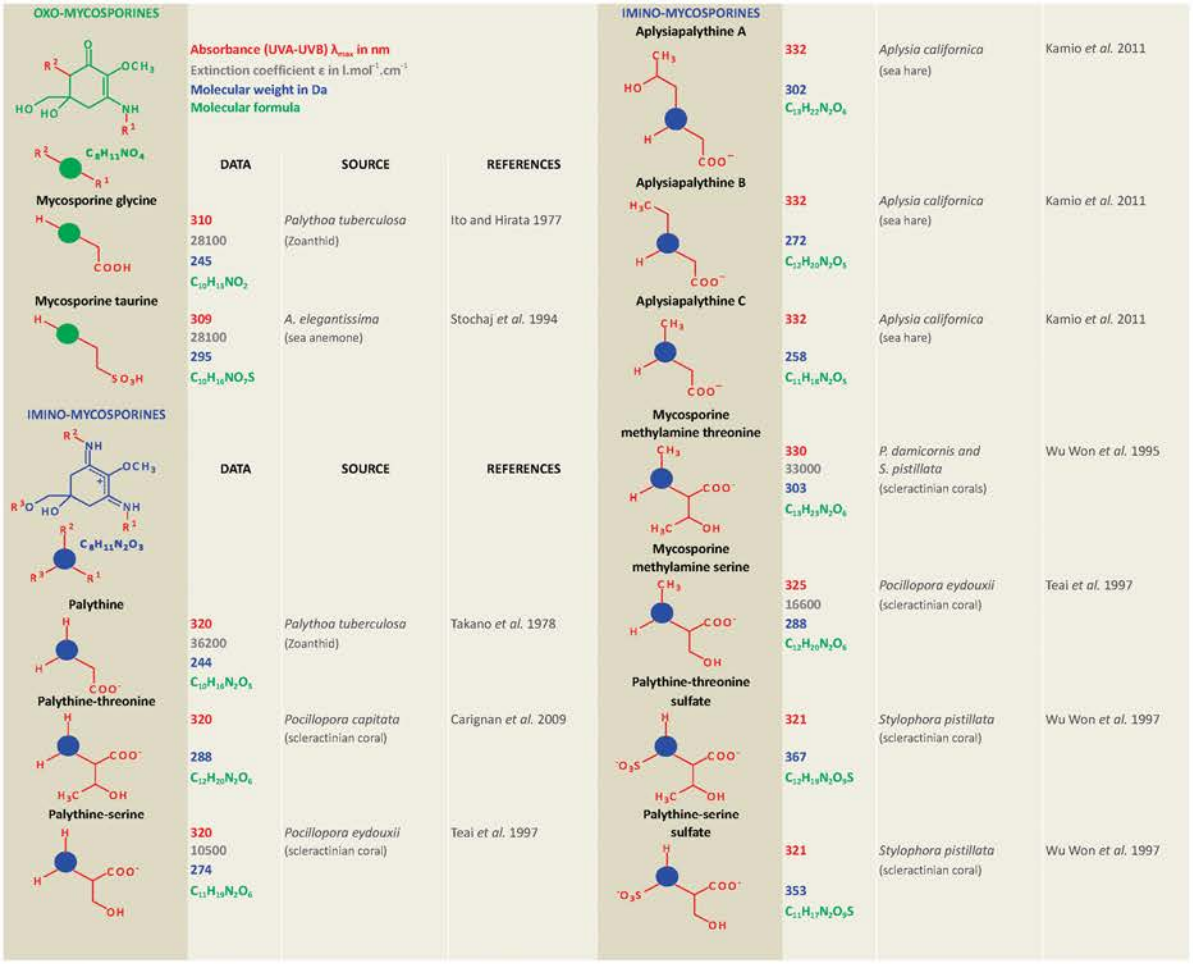


Figure 15.1 Mycosporine-like amino acids commonly encountered in marine organisms, from cyanobacteria to complex photosymbiotic systems. Oxo-mycosporines, imino-mycosporines and related products are indicated, with their structures, chemical and spectroscopic characteristics, their original biological source, and the corresponding references of their original characterization.

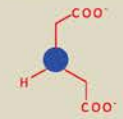
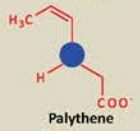
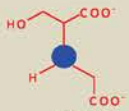
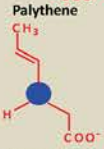
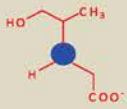
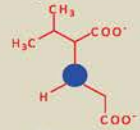
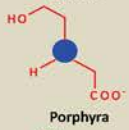
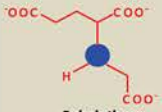
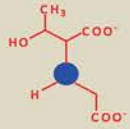
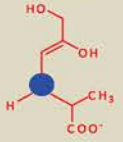
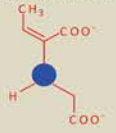
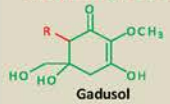
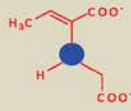
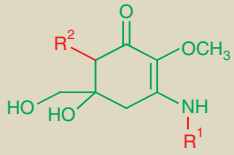
IMINO-MYCOSPORINES				**IMINO-MYCOSPORINES**			
Mycosporine-2-glycine				**Usujirene**			
	334 302 $C_{12}H_{18}N_2O_7$	*A. elegantissima* (sea anemone)	Stochaj *et al.* 1994		357 284 $C_{13}H_{20}N_2O_7$	*Palmaria palmata* (Rhodophyte)	Tsujino *et al.* 1979
Shinorine				**Palythene**			
	334 44668 332 $C_{13}H_{20}N_2O_8$	*Chondrus yendoi* (Rhodophyte)	Tsujino *et al.* 1980		360 50000 284 $C_{13}H_{20}N_2O_7$	*Palythoa tuberculosa* (Zoanthid)	Takano *et al.* 1978
Palythinol				**Mycosporine glycine-valine**			
	332 43500 302 $C_{13}H_{22}N_2O_6$	*Palythoa tuberculosa* (Zoanthid)	Takano *et al.* 1978		335 344 $C_{15}H_{24}N_2O_7$	Antarctic fish and invertebrate survey	Karentz *et al.* 1991
Asterina				**Mycosporine glutamic acid-glycine**			
	330 43800 288 $C_{12}H_{20}N_2O_6$	*Asterina pectinifera* (starfish)	Nakamura *et al.* 1981		330 43900 374 $C_{15}H_{22}N_2O_9$	*Dysidea herbacea* (marine sponge)	Bandaranayake *et al.* 1996
Porphyra				**Euhalothece**			
	334 42300 346 $C_{14}H_{22}N_2O_8$	*Porphyra tenera* (Rhodophyte)	Takano *et al.* 1979		362 330 $C_{14}H_{23}N_2O_7$	*Euhalothece* sp. (cyanobacteria)	Volkmann *et al.* 2006
***(Z)*-palythenic acid**							
	337 29200 328 $C_{14}H_{20}N_2O_7$	*Halocynthia roretzi* (ascidian)	Kobayashi *et al.* 1981	**RELATED COMLPOUNDS**	**DATA**	**SOURCE**	**REFERENCES**
***(E)*-palythenic acid**							
	337 29200 328 $C_{14}H_{20}N_2O_7$	*Halocynthia roretzi* (ascidian)	Kobayashi *et al.* 1981	**Gadusol** R = OH	268 21800 (pH>7) 204 $C_8H_{12}O_6$	eye lens of cod and other teleost fish	Plack *et al.* 1981
				4-deoxygadusol R = H	294 25100 (pH>7) 188 $C_8H_{12}O_5$	*Palythoa tuberculosa* (Zoanthid) biosynthetic intermediate	Ito and Hirata 1977

Figure 15.1 (*Continued*)

OXO-MYCOSPORINES



Absorbance (UVA-UVB)λ_{max} in nm
Extinction coefficient ε in l.mol^{-1}.cm^{-1}
Molecular weight in Da
Molecular formula

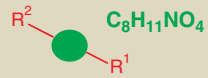
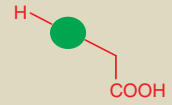
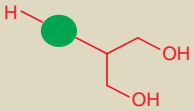
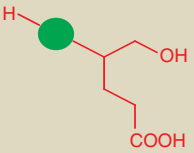
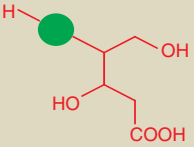
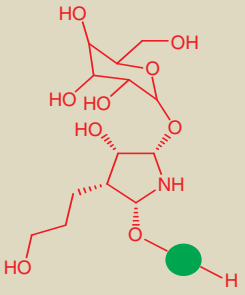
	DATA	SOURCE	REFERENCES
 **$C_8H_{11}NO_4$**			
Mycosporine glycine 	**310** 28100 **245** **$C_{10}H_{15}NO_2$**	*Collema* sp. *Peltula* spp. *Gonohymenia* sp. *Lichina pygmaea*	Büdel *et al.* 1997 de la Coba *et al.* 2009
Mycosporine serinol 	**310** 25516 **261** **$C_{11}H_{19}NO_6$**	*Collema cristatum* *Lichina pygmaea* *Peltigera horizontalis*	Torres *et al.* 2004 Roullier *et al.* 2009
Mycosporine glutamicol 	**310** **303** **$C_{13}H_{21}NO_7$**	*Gnomonia leptostyla* *Degelia plumbea*	Fayret *et al.* 1981 Roullier *et al.* 2011
Mycosporine hydroxy-glutamicol 	**310** **319** **$C_{13}H_{21}NO_8$**	*Nephroma laevigatum*	Roullier *et al.* 2011
Collemine A 	**310** 34000 **496** **$C_{19}H_{32}N_2O_{13}$**	*Collema cristatum*	Torres *et al.* 2004

Figure 15.2 Mycosporine-like compounds more typically found in lichens.

The usual protocols start by extracting the crude material with water, alcohols (ethanol, methanol) and mixtures thereof (water/methanol (75/25) ± 0.1% acetic acid), performed on fresh or freeze-dried samples with great variations in the temperature range (from cool to boiling). However, in order to prevent artifact generation or hydrolysis, it is recommended that temperatures higher than 45 °C are avoided.

Two methods are available to perform a simultaneous extraction and characterization (by HPLC) of mycosporines and MAAs:

Cyanobacteria/Phytoplankton/ Macroalgae Gröniger *et al.*, 2000	**Cyanobacteria/Fungi/Red algae** Volkmann and Gorbushina (2006), (according to Whitehead *et al.*, 2001a)
Homogenization 2.5 h in MeOH 20%, 45 °C	100 mg moist sample/Eppendorf
Centrifugation + lyophilization/ evaporation of the supernatant	Lyophilization
Redissolve the residue in 100% MeOH 2–3 min	Pulverization in liquid nitrogen
Centrifugation	20 mg crushed, suspended in 1 ml water
Supernatant evaporated to dryness, 45 °C	(+0.2% acetic acid, +0.5% MeOH)
Redissolve the residue in 0.2% acetic acid/double-distilled water	Vortex, 4 °C, 12 h
Filtrate passed through a 0.2 µm pore size	Centrifugation 14 000 × *g*, 5 min
	Filtrate passed through a 0.2 µm pore size

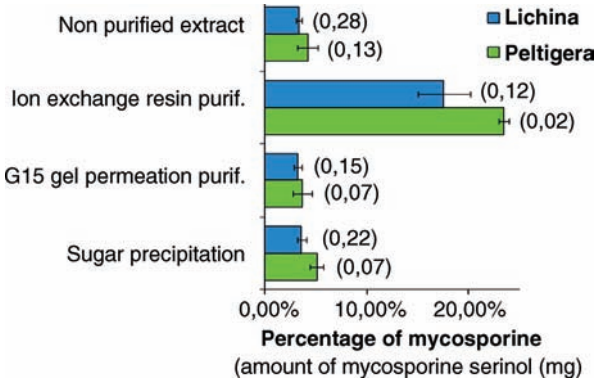


Figure 15.3 Influence of different purification techniques on the content of mycosporine serinol from *L. pygmaea* and *P. horizontalis* extracts.

The HPLC quantification of mycosporine-serinol from the lichen *Lichina pygmea* was performed following a variety of protocols, including varying parameters such as the percentage of methanol in water, the temperature (4 °C or 40 °C), and the presence or absence of mechanical crushing of the raw material. The mycosporine ratio was about 2–3% of the extracted polar compounds (50 mg raw material per ml solvent, 3 h). For this lichen, water was found to be the best extraction solvent and, somewhat surprisingly, the greatest quantity of mycosporines to be extracted (0.26 mg) was achieved with pure water at +4 °C, without crushing the lichen material.

Metabolite screening on cyanolichens revealed the complexity of the extracts, which occasionally was too high to allow any conclusive characterization of the detected compounds. In most reports, the crude extract is semi-purified by chromatographic techniques involving gel permeation, activated charcoal and/or ion-exchange resins or precipitation techniques. Three different methods, including sugar precipitation, gel permeation and ion-exchange chromatography have been compared. The relative efficiency of these methods was assessed on 50 mg of two cyanolichens, *Lichina pygmaea* and *Peltigera horizontalis*, for which a thin-layer chromatography migration had suggested mycosporine-serinol to be present (Figure 15.3). In this case, the cation-exchange resin Dowex 50W-X8 (H^+ form) was confirmed as the most efficient stationary phase for concentrating the mycosporines (elution was achieved with a solution of 200 mg ml^{-1} NaCl after the cleaning of nonadsorbed compounds). After evaporating to dryness, salt removal was achieved by the solubilization of mycosporines in ethanol. Ultimately, the mycosporine proportion of the purified extract was about 20%, compared to 2.5% in the crude extract.

15.2.3.2 Detection, Quantification, and Monitoring in Live Samples

Because of the lack of commercially available MAA standards, much of the characterization has been accomplished using the distinctive nature of their absorption spectra. Rezanka *et al.* (2004) have proposed an approach adapted to the aquatic environment, based on liquid chromatography (LC) coupled with electron ionization mass spectrometry (ESI-MS-MS), which allows all known MAAs to be distinguished based on individual retention times, wavelength maxima and molar masses. A further advantage is that the fragmentation patterns of selected ions can be examined.

15.2.4 Structure Determination

15.2.4.1 Ultraviolet (UV) Spectroscopy

As mentioned above, one of the most remarkable characteristic of mycosporines and MAAs is the presence of a unique strong absorption peak in the UV region (Figures 15.1 and 15.2) (Arpin, Curt, and Favre-Bonvin, 1979). The λ_{max} values range from 310 to 360 nm, and are correlated to high molar extinction coefficients (i.e., $\varepsilon = 25\,000$–$60\,000\,l\ mol^{-1}\ cm^{-1}$; Bandaranayake, 1998), which explains their potential role in photoprotection.

Based on this specific UV profile (symmetrical peak centered on ≈310 nm for aminocyclohexenone rings, ≈320 nm for nor-aminocyclohexenone rings, and ≈320–360 nm for iminocyclo-heximines), most can be detected and characterized as MAAs through diode-array detection (DAD), following separation on HP-TLC plates or HPLC/UPLC columns in order to perform the required spectroscopic analysis (Figure 15.4).

The characterization and quantification of MAAs is sometimes performed using reverse-phased columns with a TFA/ammonium mobile phase to enhance the polarity separation of MAA mixtures, and UV-DAD (Cardozo *et al.*, 2008) (Box 15.2).

15.2.4.2 Mass Spectrometry (MS)

Early mass spectroscopy studies on MAAs only enabled estimations of their molecular mass to be made. Recently, however, several MS techniques have been applied to the structural elucidation of MAAs (Whitehead and Hedges, 2003), especially since the analysis of the fragmentation patterns has become more reliable. For example, by using ESI-MS on

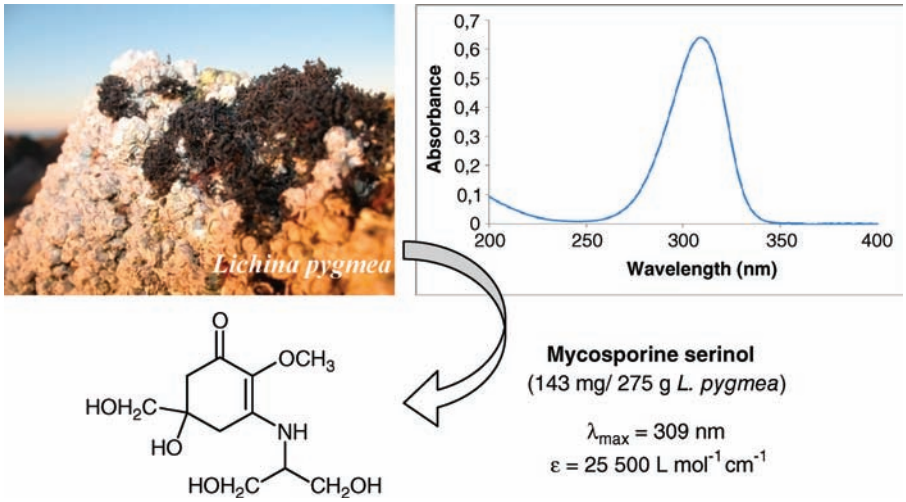


Figure 15.4 Characteristic UV absorption spectrum of a mycosporine; example of, mycosporine serinol isolated from the marine lichen *Lichina pygmaea*.

positive mode, Cardozo *et al.* (2008) proposed a complete analysis of the fragmentation pattern of palythine, palythinol and asterina MAAs. In general, MS^n with accurate HR-mass measurement enable known MAAs recognition and an anticipation of structures. However, the identification of new derivatives can only be ascertained through the isolation of suitable quantities.

15.2.4.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR remains the method of choice for the scalar determination of MAA structures. As previously described, mycosporines and MAAs present a rather well-conserved scaffold, which consists of a cyclohexenone or cyclohexenimine ring that is substituted with amino alcohols or amino acids (Figures 15.1 and 15.2). Their NMR spectroscopic data are then comparable and show characteristic patterns (as indicated in Appendices 15A.1 and 15A.2).

For example:

i) For ^{1}H NMR data, the hydroxymethyl group at C-5 usually appears around 3.60 ppm as a singlet (ranging from 3.50 to 4.04 ppm), together with the methoxyl group at C-2, which is usually represented by another singlet around the same region (3.55–3.93 ppm). In the same way, the four protons on C-4 and C-6 are often noted on ^{1}H NMR spectra as four doublets, with a geminal coupling constant around 17 Hz. In many cases, the authors did not assign these.
ii) The ^{13}C NMR spectroscopic data are very well conserved among mycosporines and MAAs. One clear difference

Box 15.2: Bathochromic Shift

The degree of electron delocalization varies with the nature of the different substituents on the oxygen, or on the nitrogen borne by the C-1 carbon. The greater it is, the higher the wavelength of the maximum absorbance peak (λ_{max}). This partly explains the λ_{max} differences observed between different MAAs (Figure 15.5).

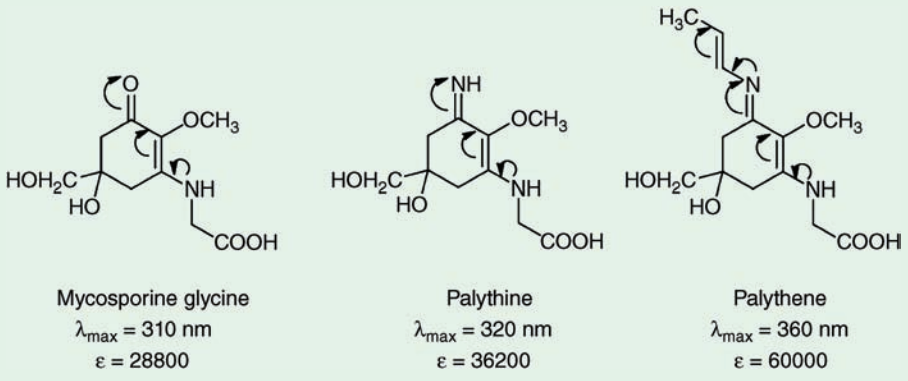


Figure 15.5 UV absorption and bathochromic effect observed in mycosporine-like amino acids.

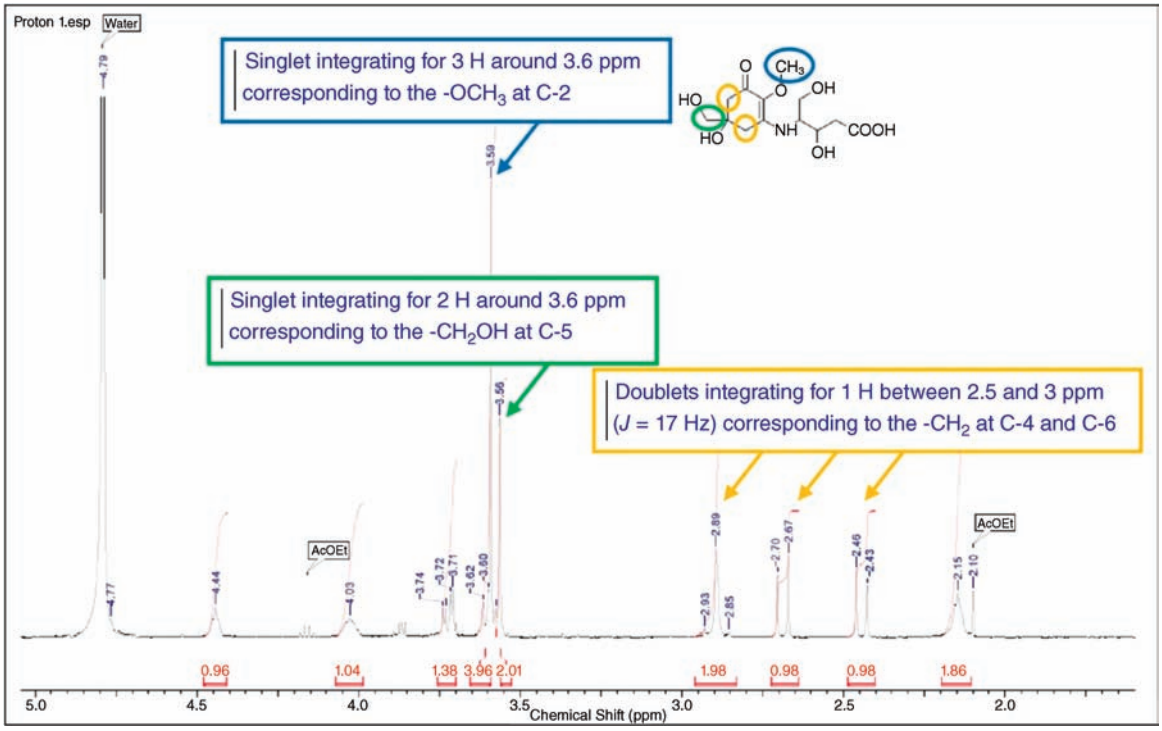


Figure 15.6 ^{1}H NMR spectrum of mycosporine hydroxyl-glutamicol (500 MHz, D_2O).

between oxomycosporines and iminomycosporines is the chemical shift of C-1, which is more around 180 ppm for oxo-mycosporines and 160 ppm for imino-mycosporines. C-4 and C-6 are usually located very close, with chemical shifts around 30–40 ppm.

The proton (Figure 15.6) and the ^{13}C NMR (Figure 15.7) data from mycosporine hydroxy-glutamicol are presented as an example of NMR patterns typical of mycosporines (Roullier *et al.*, 2011).

15.2.5 Synthesis

Early MAA syntheses are reviewed by Bandaranayake (1998). The exceptional efficiency of MAAs as sunscreens has prompted synthetic chemists to not only reproduce key natural structures but also devise synthetic analogs with enhanced activities, without any loss of molecular stability for their intended applications. Recent patents have been filed in relation to extraction methodology and formulation, more than to structural novelties. Today, most efforts are directed towards the production of MAAs by genetically modified microorganisms.

15.2.6 Biosynthesis: Labeled Precursor Investigations

15.2.6.1 The Shikimic Acid Pathway

The common precursor to MAAs is 4-deoxygadusol (4-DG), as evidenced from genome-mining and biochemical investigations on referenced strains of cyanobacteria. These serve as models to other MAA-producing marine organisms, as gene transfer (LTG) between cyanobacteria and host have occurred – and still occur – as a common *evo-devo* (evolutionary and developmental) feature.

Multiple investigations have demonstrated the role of the shikimic acid pathway in the biosynthesis of 4-DG, a precursor common to both fungal mycosporines and cyanobacterial MAAs. In fungi, evidence was initially derived from the metabolic incorporation of a ^{14}C-labeled 3-dehydroquinate intermediate (DHQ) by the fungal parasite *Trichothecium roseum*, which subsequently transformed DHQ into structurally related mycosporines (Favre-Bonvin *et al.*, 1987). Radiolabeling experiments on the cyanobacterium *Chlorogloeopsis* sp. PCC 6912 later confirmed that the shikimic acid pathway is involved in MAA biosynthesis, through the incorporation of ^{14}C-labeled pyruvate, an early and obligate precursor of this pathway, and its selective transformation into radiolabeled MAAs (Portwich and Garcia-Pichel, 2003). In the same study, it was also shown that the polyketide pathway was not involved, as ^{14}C-labeled acetate was not traceable in the MAA cytoplasmic pool of metabolites. Additional evidence was obtained via the use of exogenous tyrosine (a shikimic acid pathway repressor), while the use of glyphosate (Roundup®) as a shikimic acid pathway inhibitor was shown to block MAA biosynthesis in the cyanobacterium *Nostoc commune* (Sinha *et al.*, 2003), and also in the scleractinian coral *Stylophora pistillata* (Shick *et al.*, 1999).

The suite of MAAs that are synthesized from 4-DG (itself a strong antioxidant) as a common precursor, is initiated with mycosporine-glycine (inclusion of glycine residue at C-3) as a

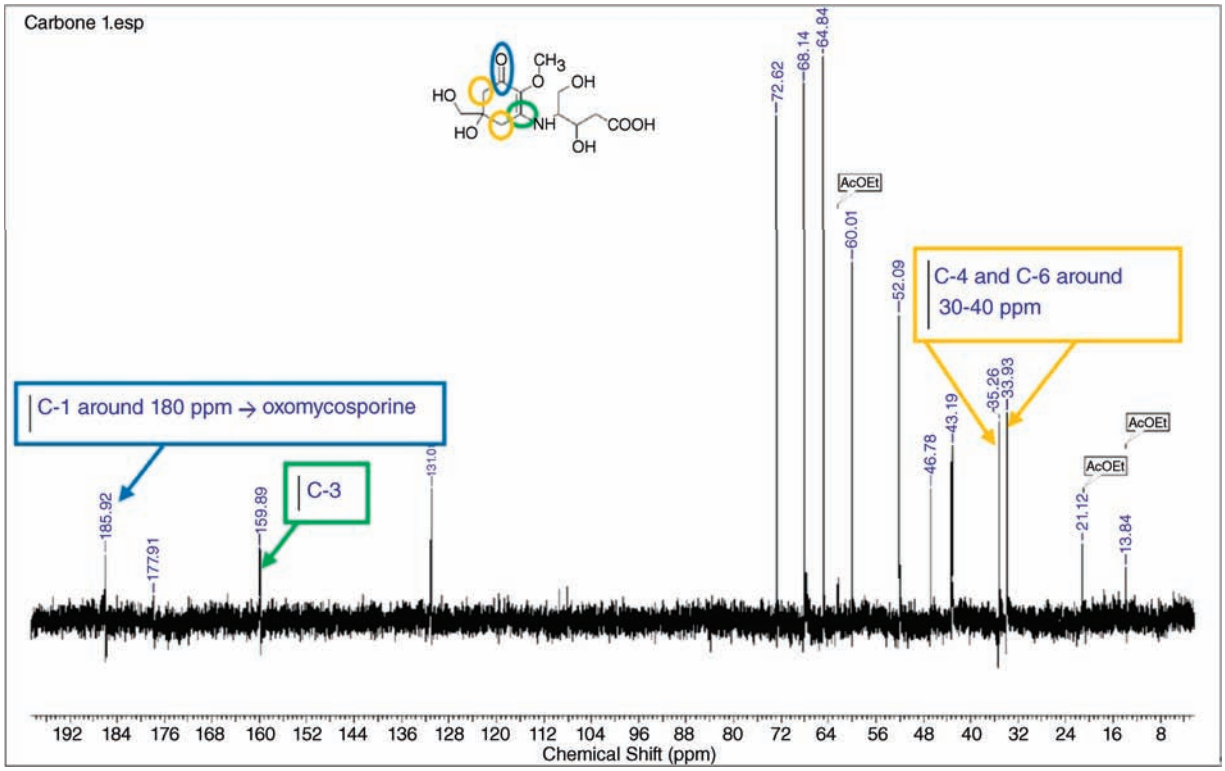


Figure 15.7 ^{13}C NMR spectrum of mycosporine hydroxyl-glutamicol (125 MHz, D_2O).

primary product, which is itself transformed into secondary structures through chemical and/or biochemical conversions into secondary products (Singh *et al.*, 2008; Rosic and Dove, 2011). Through gene-mining and bioinformatics studies, Singh *et al.* (2010a) have determined the gene system which is responsible for the expression of the putative enzymes producing a 4-DG precursor of shinorine in *Anabaena variabilis* strain PCC 7937 (Figure 15.8). In this figure the enzymes are indicated in red, the putative genes loci from the gene cluster which express these enzymes are indicated in black, and databank accession numbers in blue (see Singh *et al.*, 2010a for details).

15.2.6.2 The Pentose Phosphate Pathway

Shinorine is a secondary product of mycosporine-glycine in which a serine residue is linked at the imino site of the cycle. Its biosynthesis in *Anabaena variabilis* (ATCC 29413) – via the intermediates 4-DG and shinorine – was recently proved to proceed via an alternative route to that of the shikimic acid pathway (Figure 15.9). Instead of DHQ (a branchpoint intermediate in the shikimic acid pathway), 2-*epi*-5-*epi*-valiolone (EV, a product of the pentose phosphate pathway) was found to be the precursor of 4-DG in this cyanobacterium. Besides structural similarities between the two precursors EV and DHQ, the enzymes involved in their conversion into 4-DG – respectively EV synthase (EVS) and DHQ synthase (DHQS) – have strikingly similar three-dimensional structures, and both belong to the same superfamily of enzymes. Genome mining studies have shown that the EVS gene is present only in cyanobacterial strains that produce MAAs. It appears that 4-DG can be produced in a convergent manner as the general MAA precursor, via two different biosynthetic pathways (Spence *et al.*, 2012).

Gene-mining investigations in the cyanobacterial model strain *Anabaena variabilis* ATCC 29413 showed that four enzymes are involved in the synthesis of the specific MAA shinorine: (i) a dehydroquinase synthase homolog (DHQS); (ii) an *O*-methyl-transferase (O-MT); (iii) an ATP grasp ligase; and (iv) an NRPS-like enzyme. Cloning of the entire gene cluster in *Escherichia coli* led to the production of shinorine. The production of the intermediate 4-deoxygadusol by DHQS and O-MT could be reproduced *in vitro* (Balskus and Walsh, 2010).

15.2.7 Regulation of MAA Production: Light and Nutrients

15.2.7.1 Light

Exposure to UVB radiation has a definite positive effect on the production of MAAs in a variety of micro- and macro-organisms. Wängberg, Persson, and Karlson (1997) have shown that cultures of the dinoflagellate *Heterocapsa triquetra* exposed to artificial UVB, when compared to growth in absence of UVB, showed an increase in MAA production, in synchrony with cell cycle, and changes in the absorbance spectra during UVB exposure indicated that the composition of the MAAs varied accordingly. Sinha *et al.* (2001) showed that the cyanobacterium *Scytonema* sp. increased its MAA production during the light phases of its circadian cycle. Torres *et al.* (2007) found that *Acropora cervicornis* (stagshorn coral), when transplanted from 20 m to 1 m depth, reacted rapidly to the increase in UVR levels

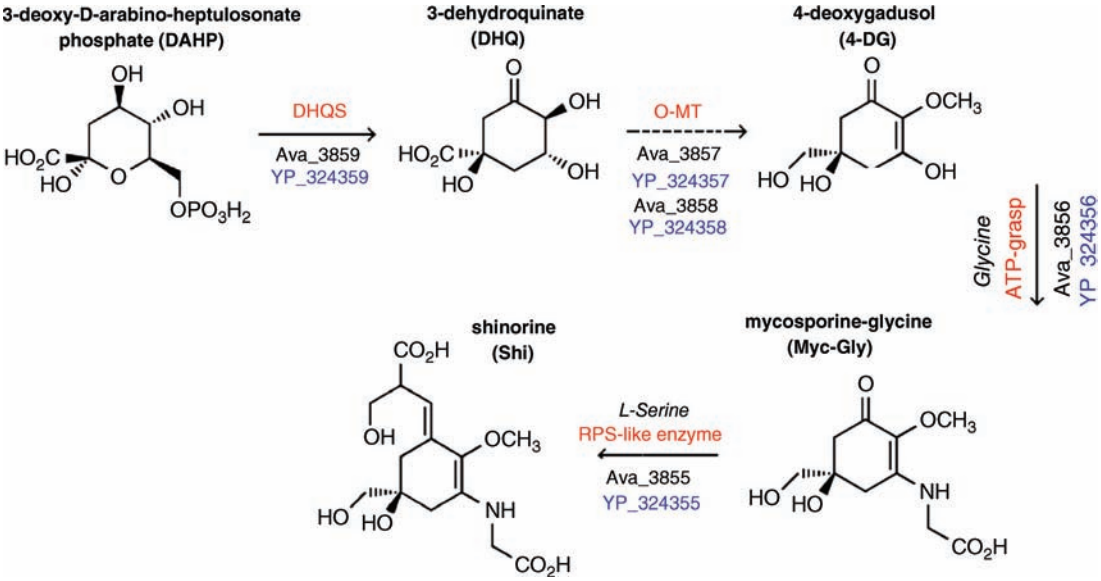


Figure 15.8 Proposed biosynthetic pathway of shinorine in the cyanobacterium *Anabaena variabilis*.

by a general 40-fold increase in MAA production, including both polyp hosts and photosymbionts. In the latter case, activation of the shikimic acid pathway (itself dependent on photosynthesis) with the production of MAA precursors is expectedly dependent on the amount of sunlight available. Shick *et al*. (1999) found that the bleaching process (loss of zooxanthellae) of UVB-exposed *Stylophora pistillata* corals did not prevent MAA accumulation by the remaining microbionts.

15.2.7.2 Nutrients

The effects of nutrient limitation on MAA production is less clear. Nitrogen limitation may lead to a quantitative decrease in MAA production (Karsten, Lembcke, and Schumann, 2007), while a sulfur deficiency may affect the qualitative bioconversion of a primary MAA to a secondary MAA (Singh *et al*., 2010b).

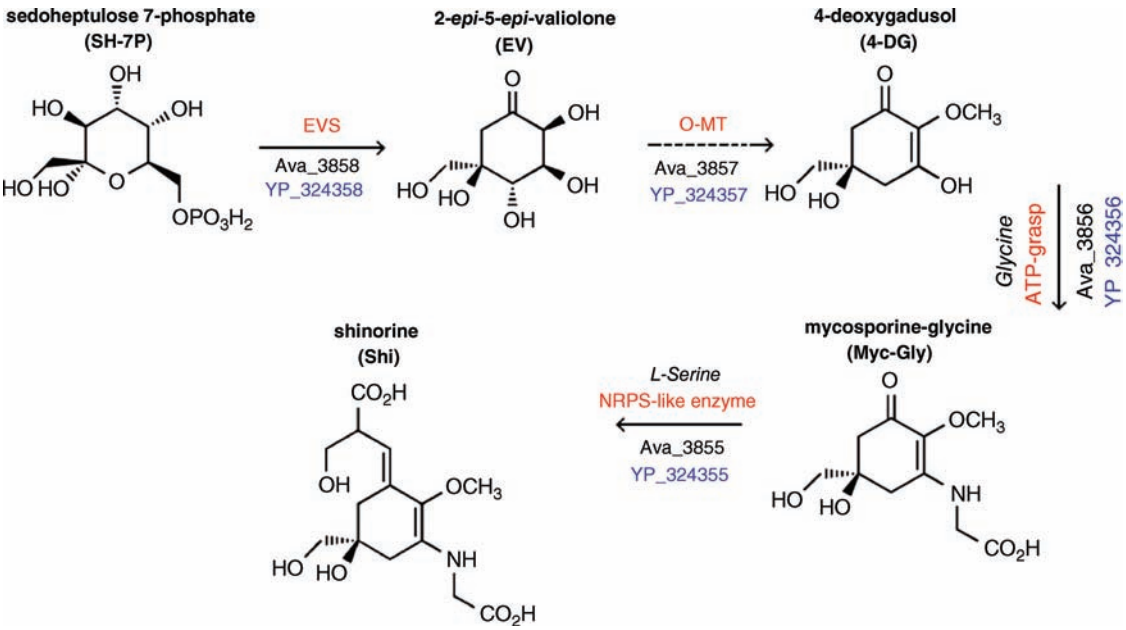


Figure 15.9 Proposed alternative biosynthetic pathway of shinorine in the cyanobacterium *Anabaena variabilis*. The enzymes are indicated in red, the putative genes loci from the gene cluster which express these enzymes are indicated in black, and databank accession numbers in blue. See Singh *et al*., 2010a for details.

15.2.8 Degradation

The photoprotective role of MAAs has been demonstrated for all study models, from cyanobacteria to photosymbiotic systems, against UVA (315–400 nm) and UVB (280–315 nm) radiations. Since MAAs are considered to be highly photostable, very few studies have examined the molecular degradations aspects. Photosensitization by an external agent is required for indirect and temperature-dependent photodegradation. This was confirmed by Bernillon *et al.* (1990), who degraded mycosporine-glutamine (an oxo-MAA) into aminocyclohexenone and 2-hydroxyglutaric acid, in presence of flavin, oxygen and light. Later, Whitehead and Hedges (2005) confirmed this with imino-MAAs by testing different inducers (riboflavin, rose Bengal, natural seawater) on palythine, shinorine and porphyra-334, three early products of the putative MAA biosynthesis suite proposed by Sinha and Häder (2008), and in the presence of light and oxygen. In the ocean, MAAs are resistant to photodegradation, a process that requires the presence of photoreactive solutes that have the potential to create radicals necessary to initiate the photooxidation process.

15.3 MAA-Producing Organisms

15.3.1 Chemical Protection Against Abiotic Stress

Aquatic life forms that derive their metabolic energy directly from capturing and transforming sunlight by photosynthesis are the primary producers of the organic matter upon which all food chains are based. In the marine world, these autotrophs range from cyanobacteria to phytoplankton (Whitehead *et al.*, 2001b) and to large macrophytic algae, and are present from superficial oceanic waters worldwide to small tide pools.

When sharing the same habitats, other life forms depend on the "goods and services" of these primary producers, whether by predation (i.e., eating and digesting them) or by symbiosis (i.e., using them as associates). The first category includes heterotrophic consumers, ranging from browsing herbivores to carnivorous predators, while the second category lacks true mesoderm-derived organs and performs basic metabolic functions with help from microbionts that live in close association with them. This includes the synthesis of structural and energy-yielding primary compounds, and the various catalytic processes that enable their transformation into secondary metabolites through environmentally mediated regulatory networks.

15.3.1.1 Symbiont-Assisted Metabolism

Many reef corals and sponges shelter dinoflagellates and/or cyanobacteria and exploit an alternative energy source from photosynthates produced during light hours, in addition to being night-time heterotrophs. Nutrient cycling in such photosystems is achieved with bacterial and archaeal functional consortia. Photosymbioses between a eukaryotic host and its dedicated protistan and/or bacterial microbionts represent elaborate adaptive processes that allow whole living systems to function as a biological entity, termed the *holobiont* (see Box 15.3). Some specialized herbivores transiently sequester plastids or even whole dinoflagellates in diverticula as part of their digestion process, and become "solar-powered" or, more precisely, "hybrid-powered." This *kleptoplasty* is regarded by some as part of an evolutionary process towards acquiring a fully integrated organelle if the genes coding for essential photosynthesis proteins are acquired by horizontal gene transfer (e.g., Rumpho *et al.*, 2008).

15.3.1.2 The "*ménage à trois*" Solution

Symbiotic photosystems have also colonized terrestrial habitats, some to the point of becoming extremophiles, in a totally different direction to that taken by chemoautotrophy-dependent inhabitants of hydrothermal vents in oceanic depths. Lichens are able to colonize and even monopolize arid and extremely inhospitable habitats, possibly to escape predation pressures and access new resources without competition by contending life forms. Lichens are a composite cluster resulting from a three-way symbiotic association

Box 15.3: The Holobiont, the Hologenome, the Metagenome

The original and primary definition of the term holobiont (one host and its dedicated symbiont) by Mindell (1992), was later expanded by Rohwer *et al.* (2002) and Rosenberg *et al.* (2007) to include the functional microbiome of corals. From a functional genomics perspective, Rosenberg and Zilber-Rosenberg (2008) coined the term *hologenome*, which defines the sum of the genetic information of the host and its symbiotic microorganisms, the host being any nucleated organism from dinoflagellate to human. In practice, the *metagenome* obtained by environmental (or clinical) microbial sampling of the host's sphere may not coincide with the *hologenome*. The former may include neutral and pathological microbiota, whereas the latter refers to functional microflora (at least under no-stress conditions).

In evolutionary terms, a major challenge of modern *environmental genomics* is to study precisely how the environment influences the holobiont's performance. *Systems biology* thus encompasses the transcriptomic responses of a model holobiont facing specific stresses, and their downstream proteomic and metabolic consequences on the host organisms, and the metagenomic diversity reduction and composition shifts of their microbiomes. Knowledge of the host's genome is always advantageous, in order to correlate the observed changes to specific genes or gene clusters. Some holobionts are stenotolerant – that is, they are not tolerant to environmental fluctuations which would allow more resistant competitors to thrive and displace them.

between a *photobiont* (green alga or cyanobacteria), a *mycobiont* (fungus), and a dedicated bacterial pool (*microbiont*) which helps maintain the stability of this assemblage (Grube *et al.*, 2009). The fungal partner supplies water and minerals as well as mechanical protection, while the green alga and/or cyanobacteria provides photosynthates; in some cases the fixation of atmospheric nitrogen occurs via specialized cyanobacterial structures, the cephalodia.

15.3.1.3 The Chemical Answer to an Exposed Mode of Life

Some of the challenges to which sessile, slow-moving or free-floating marine life forms exposed to sunlight must to face include: (i) protection against genotoxic radiations; (ii) oxidative stress; and (iii) a maintenance of osmotic balance under fluctuating physico-chemical environmental parameters. The main survival strategy that soft-bodied or unprotected organisms frequently employ is chemical, whether *toxins* for protection and defense against biotic threats or *sunscreens* against harmful radiations and oxidative stress (i.e., abiotic damages). The co-occurrence of these two lines of defense may also be explained by features that many share in common: (i) they are formed by amino acid building blocks for toxic cyclic peptides and mycosporin-like amino acids; and (ii) their possible transmission along food chains and reuse by secondary consumers after further transformation (Klisch and Häder, 2008). However, of all the MAAs known to date none has shown any sign of toxicity, and their biosynthesis is not linked to the regulation of the same genes as defense compounds.

Lichens are geared to live in extreme conditions that do not fluctuate as rapidly as shallow-water marine organisms, and are much less exposed to the intense predation pressures typical of biodiverse ecosystems, such as tropical coral reefs. This is reflected in their chemical secondary repertoire, which is mainly composed of UV-absorbing compounds, whether of polyphenolic nature (depsides and depsidones) or oxo-carbonyl MAAs, depending on the photosymbiont type (algal for the former, cyanobacterial for the latter). In addition, the fungal "host" contributes fungal mycosporines of its own (Roullier *et al.*, 2011), with some unique glycoconjugated examples (Torres *et al.*, 2004).

15.3.1.4 Simple, Effective, and Ubiquitous: Why Change a Winning Recipe?

The wide distribution – both phyletic (some 380 marine species identified to date) and geographic (from tropical to polar regions) – of MAAs is a measure of their longstanding evolutionary success as sunscreens and antioxidants. This is partly explained by their exceptional UVB and short-wave UVA absorbance characteristics, partly by their solubility (i.e., their homogenous cytoplasmic distribution within single cells and superficial localization of exposed tissues in multicellular organisms), in addition to being chemically "simple" (not requiring complex and metabolically expensive biosynthesis). General reviews on this topic have been produced by Shick and Dunlap (2002), and more recently by Careto and Carignan (2011); the multifaceted roles of MAAs are discussed by Bandaranayake (1998) and Oren and Gunde-Cimerman (2007).

In addition to being typically found in cyanobacteria, MAAs are present in most microalgae and in light-exposed phytoplankton (Jeffrey *et al.*, 1999; Llewellyn and Airs, 2010). Diatoms combine the optical anti-UV protection afforded by a siliceous skeleton with the chemical protection of MAAs (Ingalls *et al.*, 2010). Bloom-forming dinoflagellates are able to respond rapidly to changing light regimes by regulating the amount and composition of these molecules. The earlier discovered and greatest source of MAAs is in the Rhodophyta (red algae), both in terms of structural diversity and concentration (Karsten *et al.*, 1998), and more so than in the green algae. This is in contrast to the Phaeophyta (brown algae), which rely on phototannins to achieve equivalent anti-UV (Karentz, 2001), antioxidant, and antiradicalar roles (Karsten *et al.*, 1998; Pavia and Toth, 2000; La Barre *et al.*, 2004).

15.4 Hermatypic Corals: Living Under Tight Constraints

15.4.1 Coral Reefs are Monumental Bioconstructions

Coral reefs occupy 0.1% of the projected surface of the world's oceans, but are confined to sunlit and warm waters in tropical and subtropical latitudes. Coral reef ecosystems shelter an estimated 30% of the total observable marine species diversity, typically organized into complex assemblages around limestone scaffolds made of coral skeletons and debris cemented by coralline algae. Hermatypic (shallow-water, reef-forming) corals require sunlight to gather the photosynthetic energy necessary to achieve biomineralization; that is, the accretion of calcium carbonate from dissolved carbonates and cementation into a most often colonial skeleton. In these diploblastic invertebrates (without mesoderm), the crossmembrane trafficking of ions (mineral and organic) is intense between the immediate hydrosphere and their tissues.

Reef-building corals provide the limestone scaffold inside and around which innumerable communities of marine life forms coexist. Biodiversity and trophic chains are maintained if nutrient cycling is optimal, and without interference at any of the trophic levels (Rohwer, 2010). For example, the nutrient enrichment of oligotrophic waters induces drastic metagenomic shifts from diverse and functional microbiomes to reduced and pathogen-dominated forms (Mouchka, Hewson, and Harvell, 2010). However, climatic changes are likely to modify the degradation scenario even more than direct human interference, with global warming and increased exposure to harmful solar radiations being the most significant features, along with ocean acidification (La Barre, 2013).

15.4.2 Corals are Highly Efficient Photosynthesizers

The symbiosis between scleractinian corals and their indwelling zooxanthellae microalgae (*Symbiodinium*) is an ancient feature

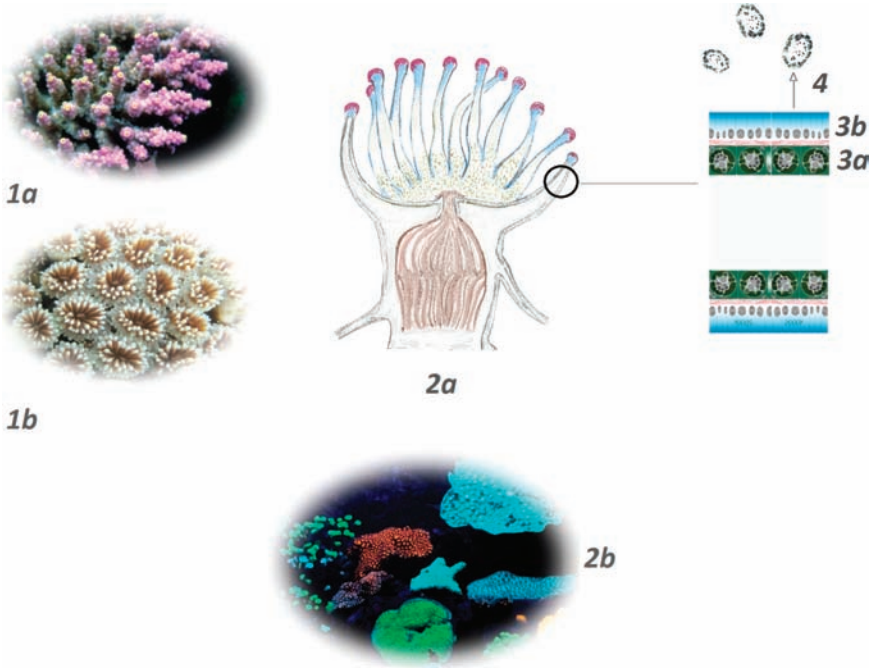


Figure 15.10 Chemical photoprotection of the reef coral holobiont. Coral colonies can be either branched (1a) or massive (1b), and are made of polyps (2a) which are often highly colored in daylight due to phycobilin pigments of the tiny zooxanthellae microalgal symbionts. Fluorescent GFP-like proteins glow under UV light in many coral species (2b). Under stress, the zooxanthellae (4) leave the endodermal layer of the polyp host tissues (3a), causing discoloration. Even when bleached, polyps are still partly protected by the MAAs concentrated in the epidermal tissue layer (3b).

that has required modifications on behalf of both parties to become a light-harvesting system, (Stambler and Dubinsky, 2005), up to sixfold as efficient as that of land plants (Enríquez, Eugenio, and Iglesias-Prieto, 2005), and involving a combination of interfacial and skeletal photon-scattering systems (Reef, Kaniewska, and Hoegh-Guldberg, 2009).

15.4.3 High Temperatures and UV Exposures Induce Oxidative Stress and Bleaching in Corals

Corals are sensitive to seawater temperature elevations above 32 °C (Berkelmans and Willis, 1999), with some species more sensitive than others (Barshis *et al.*, 2013). They are also sensitive to excess exposure to photosynthesis active radiation (PAR) (Banaszak and Lesser, 2009), and to any ensuing photooxidative stress (Lesser, 2006) involving physiological and genotoxic damage. A particularly crucial phase of corals undergoing temperature-induced bleaching is the accelerating negative effects of short-wave UVA and UVB on exposed tissues. Some corals produce green fluorescent protein (GFP)-like materials that afford some protection by quenching excess high-energy PAR and re-emitting it as fluorescent light (Catala-Stucki, 1959). Coral skeletons also have an intrinsic yellow luminescence that helps to dampen high-energy PAR while increasing its harvesting efficiency (Reef, Kaniewska, and Hoegh-Guldberg, 2009). However, MAAs are by and large the most efficient protection against the combined effects of temperature, PAR and oxidative stress (Figure 15.10).

15.4.4 The Chemical Acclimation of Scleractinian Corals to an Exposed Lifestyle

While characterizing the highly colorful pigments of several Acroporid and Pocilloporid corals from the surface waters of the Great Barrier Reef in Queensland, Shibata (1969) was surprised to find elevated concentrations of highly UV-absorbing compounds at and around 320 nm, as well as in a cyanobacterium in the vicinity of these corals. Collectively termed S-320, these compounds were subsequently found in massive *Porites* corals in concentration varying inversely with depth, thereby accrediting their role as anti-UV screens (Maragos, 1972). It was later shown that the production of S-320 in Pocilloporid corals is directly linked to the level and duration of exposure to shortwave UVB radiations (Jokiel and York, 1982). The first S-320 characterization came from the incidental discovery of mycosporine-glycine from the zoanthid coelenterate *Palythoa tuberculosa* (Ito and Hirata, 1977), before a whole suite of similar molecules was described from various algal and invertebrate sources, revealing MAAs as a widespread class of compounds mainly involved in protection against the radicalar and oxidative damages caused by excess exposure to solar radiations.

While the types and concentrations of MAA may differ among coral reef invertebrates, their range in hermatypic (photosymbiotic) corals and of giant clams is quite large (encompassing 15 structures in all, included in Figure 15.1), and seems consistently related to the presence of clades of *Symbiodinium* microalgal symbionts in these hosts. The single model species

Stylophora pistillata can possess up to 12 MAA structures. Various observations reported in Shick and Dunlap (2002) indicate that the MAAs are concentrated in the ectodermal cell layers of coral tissues, rather than in the endodermal layers where the microalgae are sequestered. Also, directly exposed regions (e.g., apical and central zones of the colonies) concentrate more MAAs than the shaded or lateral regions, which again suggests a photoadaptive relocalization from the biogenic site. In branched corals, the co-occurrence of higher concentrations of both MAAs and colorful phycobilin pigments at actively growing apical zones may afford a more general photo- and thermo-protective cover against potentially genotoxic and heat stress conditions. The relocation and accumulation to exposed tissue surfaces can be regarded as temporarily advantageous to coral polyps during bleaching episodes, in the absence of the symbiotic algae which produce these compounds.

15.4.5
Biogenic Sources of MAAs in Scleractinian Corals

In hermatypic (shallow-water and photosymbiotic) scleractinian corals, the microalgal symbionts generate much of the chemical photoprotection for the whole colony, except for the fluorescent GFP-like pigments introduced earlier, which are synthesized by the cnidarian host. This includes those MAAs which are transferred from the dinoflagellates to the host, and the colorful phycobilin pigments which are lost during bleaching episodes. However, some coral genomes possess the genes that would allow them to synthesize UV-absorbing compounds by themselves (Shinzato *et al.*, 2011), and this is a possible further adaptation to extended bleaching periods. Finally, some MAA complements can be acquired by food and transformed, which may explain why a single coral species such as the model *Stylophora pistillata* may possess up to 15 MAA structures, while no more than five different MAA structures can be isolated from *Symbiodinium* microalgae freshly isolated from a coral holobiont, or from the culture medium (see Rosic and Dove, 2011).

Apart from exposure-dependent, ectodermal accumulation and distribution and inversely depth-dependent considerations of the presence of MAAs in coral holobionts, the regulation of their biosynthesis and their expressed repertoire responds to rapid, as well as seasonal, fluctuations in PAR and UVB levels (see Shick and Dunlap, 2002).

15.4.6
The Phylogenomics of MAAs in Scleractinian Corals

The shikimic acid pathway is a major feature of the metabolisms of plants and microorganisms, but not of animals. However, the possibility of gene transfer involved in the shikimate pathway between symbiotic dinoflagellates and/or bacterial symbionts and the coral host is now seriously considered (Rosic and Dove, 2011). Indeed, in photosymbiotic systems such as reef-building coral holobionts, the complete MAA biosynthesis is predicted to be a "shared metabolic adaptation" of symbiosis, with the required biochemical intermediates from the shikimic acid pathway emanating from the dinoflagellate partner and final steps of the biosynthesis occurring in the host (Starcevic *et al.*, 2010). Therefore, gene-mining approaches must take the coral host and its transient microbionts compartments into consideration. Singh *et al.* (2010a) have undertaken a bioinformatics approach on several strains of cyanobacteria, and hypothesized that genes YP_324358 (coding for DHQ synthase) and YP_324357 (coding for *O*-methyl transferase) are transferred from a cyanobacterial donor to dinoflagellates and finally to a metazoan via lateral gene transfer (LTG) events.

A biochemical and phylogenetic study of the genes involved in the biosynthesis of MAAs in symbiotic dinoflagellates was recently undertaken (Rosic, 2012). This was in response, first, to the growing concern for thinning of the high-altitude ozone layer that acts as a protective shield against harmful solar radiations, and which is especially critical in the Great Barrier Reef provinces in Australia. A second concern was the need to produce protective and harmless skincare agents (e.g., Cardozo *et al.*, 2007; de la Coba *et al.*, 2009b) through biomimetic approaches. The putative MAA biosynthetic genes from symbiotic dinoflagellates were found to show a monophyletic clustering with their algal and bacterial homologs, confirming their microbial origin. The cluster of the four genes of the shikimic acid pathway involved in shinorine biosynthesis in cyanobacteria are found not only in the zooxanthellae (Balskus and Walsh, 2010), but also in cnidarians, which suggests that some metazoans may synthesize the UV-absorbing compounds by themselves (Shinzato *et al.*, 2011). However, the precise origin and the regulation transcriptomics of MAAs in coral holobionts will be undertaken in several model species (see Chapter 20), and should be investigated within the context of early stress responses of whole coral holobionts in the face of climatic changes, and integrated into evaluation studies of the fitness of impacted communities (La Barre, 2013).

15.5
Lichenic Systems: Living in the Extremes

Some specialized fungi (mainly Ascomycota) have the ability to combine with algae and/or cyanobacteria, and this results in self-supporting symbiotic associations with extremophilic capabilities. The lichenous lifestyle is maintained by about 18 800 known species (Feuerer and Hawksworth, 2007), most of which are microscopic lichens. Whatever the shape of the thallus, most visible lichens expose their vegetative parts at the substrate surface, enabling the photobiont to harvest energy from solar radiation. For at least 600 million years, lichens have developed highly extremotolerant systems which allow them to withstand warm deserts, polar or alpine areas as pioneer organisms. In these preferred colder and humid habitats, a number of the lichens are cryptoendolithic and are hidden in rocks, while the thalli are associated with a variety of other organisms (e.g., black fungi, bacteria). Some alpine epilithic lichens, when taken as models of complex living organisms, have survived long-term (up to 1.5 years) exposures to cosmic radiation on the outer surface of the International Space Station (Onofri *et al.*, 2012).

A variety of compound classes is associated with these long-living and slow-growing organisms, and the abundance of some of these protective compounds is frequently correlated to environmental exposure (Heber *et al.*, 2000). Their resistance to extreme temperatures, desiccation and UV radiation stress is sustained by various biochemical processes and the synthesis of protective molecules, but above all to their capacity to survive in the stage of anhydrobiosis (Beckett, Kranner, and Minibayeva, 2008). Lichens must cope with drastic and rapid balances of environmental conditions, often counterbalancing reactive oxygen species (ROS) production and deleterious UV lights (Barták *et al.*, 2004). This is well illustrated in polar, alpine or intertidal ecosystems, were lichens frequently constitute the highest represented flora. Chlorolichens are known to produce major phenol metabolites, and most of them have UV-absorbing properties; the cyanolichens are generally devoid of these compounds, although some species accumulate more polar materials, including mycosporines.

Until now, these compounds have been reported in a very limited number of lichens having a cyanobacterial photobiont (Roullier *et al.*, 2011). The first convincing report was made by Büdel *et al.* (1997) from cyanolichens, most of which were black melanized lichens, standing on highly sun-exposed rocks in tropical and subtropical regions from various continents. Except for one of the species studied, near-UV-B absorbing MAAs co-occurred with the near-UVA range-absorbing scytonemin. The MAA concentration was found to be about 0.1–0.8% dry weight (concentrations were higher in *Peltula euploca* collected from shaded habitats), and mycosporine-glycine was characterized as the major compound.

In contrast to scytonemin, which has been proven to be produced by cyanobacteria, the production of MAAs is not clearly defined, as both partners could contribute to the process. The glycosylated collemin A, isolated and identified from the gel-hydrated *Collema cristatum*, was also recognized as being produced by a culture of the mycobiont exhibiting a characteristic UV 311 nm-absorbing shape (Torres *et al.*, 2004) (see Box 15.4).

From the marine lichen, *Lichina pygmea*, which formed dark cushions in the intertidal zone and contained a *Calothrix* cyanobacterium, the antioxidant and photoprotective properties were reported as related to mycosporine-glycine, characterized on the basis of HPLC behavior with a standard source supposed to contain this compound (de la Coba *et al.*, 2009a). After isolation via a dual-mode Centrifugal Partition Chromatography experiment (Roullier *et al.*, 2009), mycosporine-serinol was fully identified as the major MAA from *Lichina pygmea* gathered from the Brittany coast.

In a screening carried out on 15 lichen species, mycosporine-serinol and mycosporine-glutamicol were identified for the first time in five cyanolichens, while six unknown mycosporine-like compounds were characterized in five other cyanolichens from various habitats, some of which were mostly found in shaded and humid habitats (Roullier *et al.*, 2011). Thus, it is questionable MAAs are produced as a response to UV only. While optimizing the extraction process of mycosporines, the highest quantity of mycosporine-serinol (ca. 0.6% dry weight) was obtained with a 3 h period of passive diffusion in 4 °C distilled water from the uncrushed thallus of *Lichina pygmea*. Thus, a dynamic production and diffusion of these highly soluble compounds in an aqueous environment should be considered as they are mixed with other hydrophilic compounds such as polyols, sugars, and amino acids; hence, a suitable protocol (*vide supra*) must be specifically applied to recognize and isolate these MAAs. Following such a procedure, about 8 mg of the newly described mycosporine-hydroxyglutamicol was isolated from 3 g of the squamulose cyanolichen *Nephroma laevigatum* (Roullier *et al.*, 2011).

15.6 Modes of Action and Applications to Human Welfare

Oxidative stress refers to a situation where ROS such as hydrogen peroxide and oxygen-derived free radicals are produced and initiate a chain reaction, with resulting damage to the cellular systems. ROS are produced when photosystems absorb more energy than can either be transferred by the electron transport chain to an electron acceptor, or dissipated as heat. Corals and other fixed invertebrate photosystems usually combine pigments, sunscreens and proteins that optimize protection in a species-specific fashion, and these molecules have an interest-

Box 15.4: Lichens: Looking into the Future

As lichens are composed of organisms that possibly are producing their own mycosporines or MAAs, they represent an interesting challenge for investigating mycosporine biogenesis and distribution within their thalli. So far, cyanobacteria seem to be involved in the presence of such compounds in lichens, as they can absorb atmospheric nitrogen and incorporate it into their metabolism. However, all of the mycosporines described to date in lichens have the characteristic carbonyl moiety of fungal mycosporines. The fungal partner generally constitutes more than 80% of the lichen biomass, and it will be especially interesting to investigate the production and distribution of these compounds in tripartite lichens, where the cyanobacteria are confined to specific tissues termed *cephalodia*. Preliminary results obtained with LC-HRMS analyses of the cephalodia compared to the whole thallus have suggested the presence of 330 nm-absorbing iminomycosporines in the cephalodia, while 310 nm-absorbing oxomycosporines are typically found in the whole thallus (Roullier *et al.*, 2011).

ing biotechnological potential. Large-scale microalgal cultivation represents an important source of food additives – for example, carotenoids, vitamins C and E, unsaturated lipids and many others – the production of which can be boosted by an appropriate combination of stressors (Skjanes, Rebours, and Lindblad (2013). The production of MAAs in microscopic green algae (Sinha, Singh, and Hader, 2007) and marine organisms is directly linked to exposure to short-wave UVR (in terms of both intensity and duration), and inversely correlated with nitrogen levels in some investigations (Karsten *et al.*, 2007).

15.6.1 Skin Care and Cosmetics

Thus, MAAs and synthetic derivatives have inspired applications as skin care products (Bandaranayake, 1998), corresponding to a strong demand in the cosmetics market for chemically stable, potent, and broad-spectrum molecules. As noted by Bhatia *et al.* (2011), many earlier formulations contained carcinogenic agents, some with estrogen-like effects, or generated harmful radical species. The sun-protection factor (SPF) is defined as the ratio of the time of UV exposure necessary to produce minimally detectable erythema in sunscreen-protected skin, to that of the time taken to produce the same effect for unprotected skin (Bhatia *et al.*, 2010). Although useful, the SPF is insufficient to evaluate the carcinogenic potential of a substance, and must be complemented by more specific tests. As an example, Helioguard 365® is a commercial formulation based on the mycosporine-like amino acids shinorine and Porphyra-334 from the red alga *Porphyra umbilicalis*; these compounds are encapsulated into liposomes in order to increase their uptake by the skin.

15.6.2 Biotechnological Applications

Nonmedical applications of MAAs include photostabilizing additives in plastics, paint, and varnish (Torres *et al.*, 2006; Sampedro, 2011). A typical example is that of automobile paints, which are subjected to intense and prolonged exposures to sunlight.

15.7 Conclusions

Mycosporine-like amino acids and their natural analogs are life-essential molecules for life forms that live directly exposed to sunlight, especially if they are fixed on a substratum. As well as being efficient sunscreens, most MAAs are also efficient antioxidants and heat dissipators, and may also contribute to the osmotic balance under ionic stress. In this way, the MAAs achieve both primary and secondary roles in the metabolism of the organism or the holobiont that produces them. Whilst the true origin of these molecules remains uncertain, their future is intricately linked with the survival of essential taxonomic groups, both marine and terrestrial.

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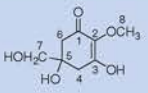
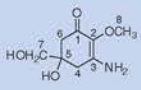
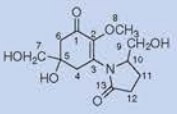
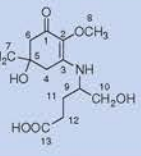
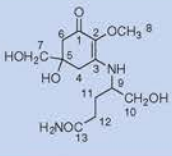
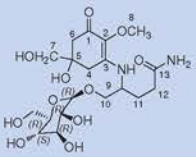
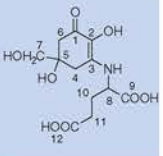
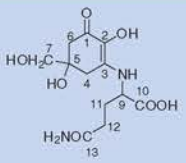
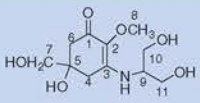
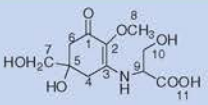
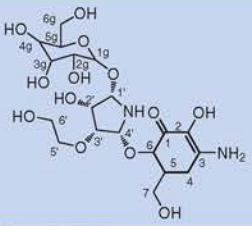
15.A

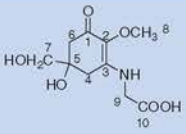
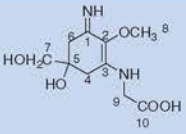
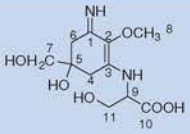
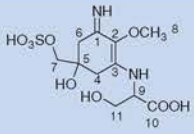
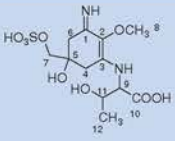
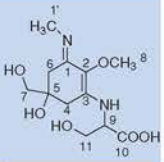
Appendix 15A.1 Proton NMR data of Mycosporines and Mycosporine-like Amino Acids (MAAs)

	Precursors and degradation products		Mycosporines				
Name	**Cyclohexenone**	**Amino-cyclohexenone**	**Mycosporine-2 (cyclic form)**	**Mycosporine-2 (open ring) = Mycosporine glutamicol**	**Mycosporine-glutaminol**	**Mycosporine-glutaminol-glucoside**	**Mycosporine-glutamic acid**
Structure							
	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)	δ_{H} ppm (m, *J* Hz)
Solvent	D_2O	pyridine-d_5	D_2O	pyridine-d_5	pyridine-d_5	pyridine-d_5	D_2O
Position							
1							
2							
3							
4	2.68 (d, 17)[a]	3.07 (d, 16.5)[a] 3.11 (d, 16.5)[a]	2.51 (d, 18) 2.80 (d, 18)	3.00 (d, 17)[a] 3.20 (d, 17)[a]	2.99 (d, 16) 3.19 (d, 16)	2.97 (d, 15.9) 3.16 (d, 15.9)	2.38 (d, 17) 2.65 (d, 17)
5							
6	3.00 (d, 17)[a]	3.28 (d, 16.5)[a] 3.46 (d, 16.5)[a]	2.83 (d, 18) 2.92 (d, 18)	3.20 (d, 17)[a] 3.50 (d, 17)[a]	3.19 (d, 16.8) 3.43 (d, 16.8)	3.13 (d, 16.5) 3.51 (d, 16.8)	2.68 (d, 17.5) 2.72 (d, 17.5)
7	3.67 (s) (3.57)*	3.99 (s)	3.54 (s)	3.90–4.20 (m)	3.90–4.10 (m)	3.93 (d, 10.8) 4.00 (d, 10.8)	3.51 (s)
8	3.76 (s) (3.64)*	3.90 (s)	3.63 (s)	3.80 (s)	3.82 (s)	3.89 (s)	3.59 (s)
9			3.58 (m) 3.73 (dd; 11.5, 4)	3.90–4.20 (m)	3.90–4.10 (m)	4.15–4.60 (m)	4.07 (m)
10			3.92 (m)	3.90–4.20 (m)	3.90–4.10 (m)	4.15–4.60 (m)	
11			1.93 (m)	2.73 (m)	2.23 (m) 2.40 (m)	2.16 (m) 2.25 (m)	2.09 (m) 2.17 (m)
12			2.48 (t, 7)	2.30 (m)	2.67 (t, 7)	2.59 (t, 6.9)	2.39 (t, 7.5)
-OH					7.77 (br. s)	7.70 (br. s)	
-OH					8.34 (br. s)	8.26 (br. s)	
-OH					6.68 (br. s)		
3-NH					6.80 (d, 9.5)	6.92 (d, 9.5)	
13-NH_2					6.84 (m)		
-glucosyl						3.69 (m) 5.32 (d, 3.4) 4.15–4.60 (m)	
References	Lemoyne *et al.*, 1985* (Ito and Hirata, 1977)	Bernillon *et al.*, 1990	Arpin, Favre-Bonvin, and Thivend, 1977	Fayret *et al.*, 1981	Pittet *et al.*, 1983	Pittet *et al.*, 1983	Young and Patterson, 1982

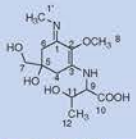
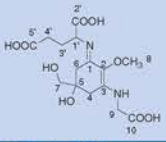
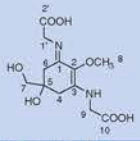
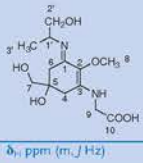
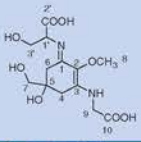
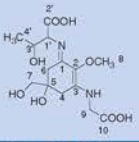
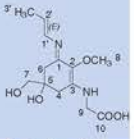
a) Signals were not assigned and can be interchanged.

Name	Mycosporines			
	Normycosporine-glutamine[b]	Mycosporine-serinol = Mycosporine 1 = P310	Mycosporine-serine	Collemine A
Structure				
Solvent	δ_H, ppm (m, J Hz) pyridine-d_5	δ_H, ppm (m, J Hz) pyridine-d_5	δ_H, ppm (m, J Hz) pyridine-d_5 [D_2O]	δ_H, ppm (m, J Hz) CD_3OD
Position				
1				
2				
3				
4	3.04 (d, 17) 3.23 (d, 17)	2.97 (d, 17)[a] 3.18 (d, 17)[a]	3.02 (d, 16) [2.50 (d, 17)] 3.23 (d, 16) [2.77 (d, 17)]	3.62 3.81
5				
6	3.23 (d, 17) 3.44 (d, 17)	3.25 (d, 17)[a] 3.46 (d, 17)[a]	3.31 (d, 16) [2.82 (s)] 3.49 (d, 16)	3.72
7	3.99 (s)	4.00 (s)	4.04 (s) [3.63 (s)]	3.74
8		3.82 (s)	3.93 (s) [3.71 (s)]	
9	4.92 (m)	4.06–4.30 (m)		
10		4.06–4.30 (m)	4.37 (dd, 11.0, 3.5) [4.04 (s)] 4.64 (dd, 11.0, 3.5)	
11	2.54 (m) 2.78 (m)	4.06–4.30 (m)		
12	2.78 (m)			
-OH		6.76 (br. s)		
-OH		6.76 (br. s)		
-OH		5.06 (br. s)		
-OH		6.76 (br. s)		
3-NH	6.70 (d, 10)	7.02 (d)	7.17 (d, 9)	
13-NH_2				
-glucosyl				4.45 (CH en 1 g); 3.24 (CH en 2 g); 3.37 (CH en 3 g); 3.19 (CH en 4 g); 3.35 (CH en 5 g); 3.57 et 3.92 (CH_2 en 6 g) 4.02 (CH en 1'); 3.84 (CH en 2'); 3.86 (CH en 3'); 3.71 (CH en 4'); 3.63 et 3.82 (CH_2 en 5'); 3.6 et 3.76 (CH_2 en 6')
6-group				
References	Lunel, Arpin, and Favre-Bonvin, 1980	Favre-Bonvin, Arpin, and Brevard, 1976	Arpin, Curt, and Favre-Bonvin, 1979	Enk *et al.*, 2003

a) Signals were not assigned and can be interchanged.
b) Atom numbers were changed in order to make easier comparisons of the same groups.

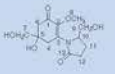
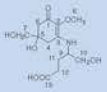
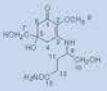
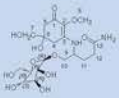
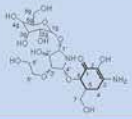
	Mycosporine-like amino acids (MAAs)					
Name	**Mycosporine-glycine**	**Palythine**	**Palythine-serine[b]**	**Palythine-serine-sulfate**	**Palythine-threonine-sulfate[b]**	**Mycosporine-methylamine-serine[b]**
Structure						
	δ_H, ppm (m, J Hz)	δ_H, ppm (m, J Hz)	δ_H, ppm (m, J Hz)	δ_H, ppm (m, J Hz)	δ_H, ppm (m, J Hz)	δ_H, ppm (m, J Hz)
Solvent	D_2O	D_2O	D_2O	D_2O	D_2O	D_2O
Position						
1						
2						
3						
4	2.50 (d, 17)[a] 2.73 (d, 17)[a]	2.69 (d, 15.5)[a] 2.95 (d, 15.5)[a] (3.00 (d, 17))*	2.85 (d, 17)[a]	2.78 (d, 17)[a]	2.77 (d, 17)[a]	2.83 (d, 17.5)
5						
6	2.72 (d, 17)[a] (2.62 (d, 17))* 2.83 (d, 17)[a]	2.78 (s)[a] (2.73 (d, 17))* (2.92 (d, 17))*	2.89 (d, 17)[a]	2.83 (d, 17)[a]	2.81 (d, 17)[a]	2.88 (s)
7	3.57 (s) (3.62)*	3.58 (s) (3.60)*	3.60 (s)	3.90 (s)	3.90 (s)	3.57 (s)
8	3.64 (s) (3.69)*	3.65 (s) (3.67)*	3.70 (s)	3.55 (s)	3.55 (s)	3.64 (s)
9	4.24 (s) (4.36)*	4.02 (s) (4.06)*	4.37 (dd, 6.7, 3.7)	4.25 (dd, 6.6, 4.0)	3.98 (d, 4.5)	4.30 (dd, 6.6, 3.8)
10					4.20 (dq, 6.0, 4.5)	3.90 (dd, 8.8, 6.7)
11			3.95 (dd, 11.6, 7.2) 4.03 (dd, 11.9, 3.7)	3.80 (dd, 11.0, 7.0) 3.88 (dd, 11.0, 4.0)		3.99 (dd, 8.8, 6.7)
12					1.12 (d, 6.0)	
1′						3.09 (s)
2′						
3′						
4′						
5′						
References	Ito and Hirata, 1977 *(Bandaranayake, Bernis, and Bourne, 1996)	Tsujino *et al.*, 1978 *(Takano, Uemura, and Hirata, 1978)	Teai *et al.*, 1997	Wu Won, Chalker, and Rideout, 1997	Wu Won, Chalker, and Rideout, 1997	Teai *et al.*, 1997

a) Signals were not assigned and can be interchanged.
b) Atom numbers were changed in order to make easier comparisons of the same groups.

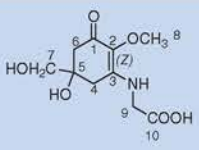
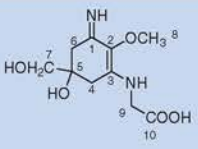
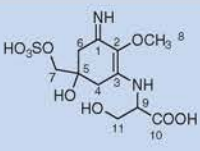
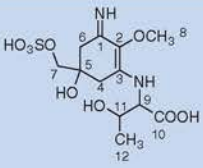
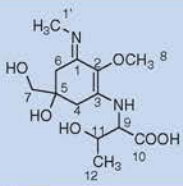
	Mycosporine-like amino acids (MAAs)						
Name	**Mycosporine-methylamine-threonine**[b]	**Mycosporine-glutamic-acid-glycine**[b]	**Mycosporine-2-glycine**[b]	**Palythinol**[b]	**Shinorine**[b]	**Porphyra-334**[b]	**Palythene**[b]
Structure							
	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)	δ_H, ppm (m, *J* Hz)
Solvent	D_2O	D_2O	D_2O	D_2O	D_2O	D_2O	D_2O
Position							
1							
2							
3							
4	2.75 (d, 17.5)	2.72 (d, 17)[a] 2.81 (d, 17)[a]	2.77 (d, 17)[a]	2.82 (d, 17)[a]	2.70 (d, 10.5)[a] 2.88 (d, 10.5)[a]	2.83 (m)	2.88 (d, 17)[a]
5							
6	2.80 (s)	2.90 (s)[a]	2.86 (d, 17)[a]	2.82 (d, 17)[a]	2.75 (br. s)[a]	2.83 (m)	2.96 (d, 17)[a]
7	3.50 (s)	3.58 (s)	3.60 (s)	3.62 (s)	3.48 (s)	3.58 (s)	3.66 (s)
8	3.60 (s)	3.62 (s)	3.68 (s)	3.66 (s)	3.60 (s)	3.70 (s)	3.71 (s)
9	3.95 (d, 4.5)	4.02 (s)	4.06 (s)	4.06 (m)	4.11 (s)	4.09 (s)	4.11 (s)
10							
11	4.22 (dq, 6.0, 4.5)						
12	1.19 (d, 6.0)						
1′	3.00 (s)	3.77 (t, 8.6)	4.06 (s)	4.06 (m) 3.46–3.52 (m)	4.41 (t, 5)	4.11 (d, 5)	6.58 (br. d, 13) 5.75 (dq, 13, 6)
2′							
3′		2.16 (dt, 7.4, 8.6) 2.17 (dt, 7.4, 8.6) 2.58 (dt, 7.3, 7.4)		1.26 (d, 5)	3.90 (d, 5)	4.33 (dq, 6.4, 5) 1.25 (d, 6.4)	1.88 (dd, 6, 2)
4′							
5′							
References	Wu Won, Rideout, and Chalker, 1995	Bandaranayake, Bernis, and Bourne, 1996	Kedar, Kashman, and Oren, 2002	Takano, Uemura, and Hirata, 1978	Tsujino, Yabe, and Sekikawa, 1980	Takano *et al.*, 1979	Takano, Uemura, and Hirata, 1978

a) Signals were not assigned and can be interchanged.
b) Atom numbers were changed in order to make easier comparisons of the same groups.

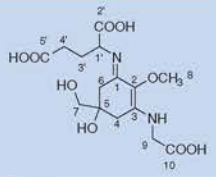
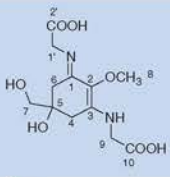
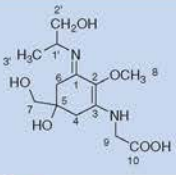
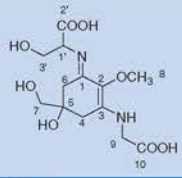
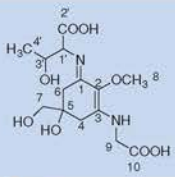
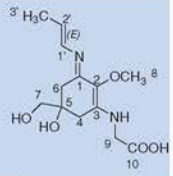
Appendix 15A.2 Carbon thirteen data of Mycosporines and Mycosporine-like Amino Acids

	Precursors	Mycosporines						
Name	Cyclohexenone	Mycosporine-2 (cyclic form)	Mycosporine-2 (open ring) = Mycosporine glutamicol	Mycosporine-glutaminol	Mycosporine-glutaminol-glucoside	Mycosporine-glutamic acid	Mycosporine-serinol = Mycosporine 1 = P310	Collemine A
Structure								
Solvent	δ_C ppm D_2O	δ_C ppm D_2O	δ_C ppm pyridine-d_5	δ_C ppm pyridine-d_5	δ_C ppm pyridine-d_5	δ_C ppm D_2O	δ_C ppm pyridine-d_5 (D_2O)	δ_C ppm CD_3OD
Position								
1	181.0	181.0[a]	186.4	186.4	186.9	188.4[a]	186.2 (187.9)	
2	134.7	134.0	131.1	131.1	131.3	134.0	131.5 (132.5)	
3	181.0	171.0	159.9	159.8	159.4	160.3	153.4 (160.5)	
4	41.4	38.5	31.5	32.5	32.5	35.6	34.9 (35.9)	65.4
5	73.1	75.9	73.1	73.1	73.0	74.6	72.7 (74.5)	73.3
6	41.4	43.1	43.9	43.9	43.9	45.6	46.3 (45.4)	71.7
7	68;5	71.5	68.6	68.6	71.7	70.1	69.4 (70.0)	64.1
8	60.9	64.0	60.3	60.2	60.4	61.9	58.8 (61.5)	
9		67.5	55.8	55.9	53.7	60.2	56.5 (55.8)	
10		60.8	65.3	65.3	68.9	180.5[a]	62.1 (63.7)	
11		29.3	27.1	27.6	27.6	30.9	62.3 (63.7)	
12		34.1	34.6	34.6	34.9	33.9		
13		179.0[a]	178.9	179.0	179.3	179.9[a]		
glucosyl 6-group					99.1; 74.3; 73.0; 72.2; 70.3; 61.5			104.9 (C-1 g); 75.5 (C-2 g); 78.3 (C-3 g); 72.7 (C-4 g); 78.1 (C-5 g); 63.6 (C-6 g) 79.1 (C-1′); 72.8 (C-2′); 72.2 (C-3′); 71.9 (C-4′); 65.4 (C-5′); 65.4 (C-6′)
References	Ito *et al.*, 1977	Arpin, Favre-Bonvin, and Thivend, 1977	Pittet *et al.*, 1983	Pittet *et al.*, 1983	Pittet *et al.*, 1983	Young and Patterson, 1982	Favre-Bonvin, Arpin, and Brevard, 1976	Enk *et al.*, 2003

a) Signals were not assigned and can be interchanged.

	Mycosporine-like amino acids (MAAs)				
Name	**Mycosporine-glycine**	**Palythine**	**Palythine-serine-sulfate**[b]	**Palythine-threonine-sulfate**[b]	**Mycosporine-methylamine-threonine**[b]
Structure					
	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm
Solvent	D_2O	D_2O	D_2O	D_2O	D_2O
Position					
1	187.2	162.0 (162.5[a])*	160.4[a]	160.6	161.4
2	130.4	125.8 (125.4)*	124.9	125.0	125.3
3	159.7	160.4 (160.9[a])*	160.6[a]	160.6	157.6
4	33.8	34.6 (34.2)*	34.0	34.0	33.1
5	72.9	72.4 (72.0)*	69.9	69.8	71.0
6	45.4	37.2 (36.6)*	36.0	36.0	32.8
7	68.4	68.5 (68.2)*	72.7	72.5	67.5
8	60.2	60.2 (59.7)*	59.2	59.3	59.3
9	43.7	48.1 (47.5)*	60.7	64.5	64.3
10	174.5	174.8 (177.5)*	174.3	174.9	175.6
11			62.7	68.2	68.1
12				19.5	19.4
1′					29.8
2′					
3′					
4′					
5′					
References	Ito and Hirata, 1977	Tsujino *et al.*, 1978 *Takano, Uemura, and Hirata, 1978	Wu Won, Chalker, and Rideout, 1997	Wu Won, Chalker, and Rideout, 1997	Wu Won *et al.*, 1995; Wu Won, Rideout, and Chalker, 1995

a) Signals were not assigned and can be interchanged.
b) Atom numbers were changed in order to make easier comparisons of the same groups.

	Mycosporine-like amino acids (MAAs)					
Name	**Mycosporine-glutamic-acid-glycine[b)]**	**Mycosporine-2-glycine[b)]**	**Palythinol[b)]**	**Shinorine[b)]**	**Porphyra-334[b)]**	**Palythene[b)]**
Structure						
	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm	δ_C ppm
Solvent	**D_2O**	**D_2O**	**D_2O**	**D_2O**	**D_2O**	**D_2O**
Position						
1	160.4[a)]	159.7	160.4[a)]	161.8[a)]	161.2[a)]	161.5[a)]
2	125.6	125.2	126.1	128.1	126.4	126.4
3	159.7[a)]	159.7	160.9[a)]	162.9[a)]	157.7[a)]	154.2[a)]
4	33.1	32.7	33.6	35.3	33.7	33.8
5	71.2	70.9	71.9	73.4	71.8	71.8
6	32.9	32.7	33.9	35.8	34.1	33.8
7	67.6	67.3	68.2	69.7	68.2	68.4
8	59.1	59.0	59.9	61.7	60.2	60.3
9	46.7	46.5	47.4	47.9	47.5	47.6
10	175.1	175.0	175.9	175.3	175.5	175.4
11						
12						
1′	54.1	46.5	67.4	61.8	65.2	124.5
2′	174.0	175.0	50.7	175.3	175.5	117.9
3′	25.1		20.2	64.4	69.0	15.2
4′	29.9				20.2	
5′	175.4					
References	Bandaranayake, Bernis, and Bourne, 1996	Kedar, Kashman, and Oren, 2002	Hirata *et al.*, 1979	Tsujino, Yabe, and Sekikawa, 1980	Hirata *et al.*, 1979	Hirata *et al.*, 1979

a) Signals were not assigned and can be interchanged.
b) Atom numbers were changed in order to make easier comparisons of the same groups.

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