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REVIEW ARTICLE

Mycosporine-Like Amino Acids for Skin Photoprotection

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Abstract: ***Background:*** Excessive human exposure to solar ultraviolet radiation (UVR) continues to be a major public health concern, with skin cancer rates increasing year on year. The major protective measure is the use of synthetic UVR filters formulated into sunscreens, but there is a growing concern that some of these chemicals cause damage to delicate marine ecosystems. One alternative is the use of biocompatible mycosporine-like amino acids (MAA), which occur naturally in a wide range of marine species. Their role within nature is mainly thought to be photoprotective. However, their potential for human photoprotection is largely understudied.

Objective: To review the role of MAA in nature and assess their potential as natural sunscreens for human skin photoprotection.

Method: A literature review of all relevant papers was conducted.

Conclusion: MAA are natural photostable compounds that are thought to offer photoprotection to marine species. Initially thought of as protective based on their absorption properties in the solar UVR spectrum, it is clear that MAA are multifunctional photoprotective compounds acting as chemical and biological anti-oxidants. This suggests that MAA may offer a novel eco-friendly approach to human skin photoprotection. Most studies have been carried out *in vitro* and current data strongly suggest that MAA have potential for development as natural biocompatible sunscreens that protect against a diverse range of solar UVR induced adverse effects on human health.

Keywords: Photoprotection, mycosporine-like amino acids, skin, natural products, mechanisms, solar radiation.

1. INTRODUCTION

Exposure to terrestrial solar ultraviolet (~295-400nm) and visible (400-700nm) radiation has profound effects on all living systems. Photon energy absorbed by cellular chromophores may have beneficial or detrimental effects. The wavelength dependence (action spectrum) of a given photobiological outcome is primarily dependent on the absorption spectrum of the chromophore. The skin is the major organ exposed to solar radiation. The molecular effects of this exposure are well defined, causing either direct damage to DNA, proteins or lipids, such as in the formation of DNA photoproducts [1], and indirect damage via the generation of reactive oxygen species (ROS) [2, 3] that attack a range of molecular and cellular targets. It was initially thought that direct damage was mainly responsible for the most damage; however in recent years the importance of indirect and oxidative damage has been realised, with the generation of oxidative [4] and photosensitised [5] DNA damage, and the oxidation of proteins leading to inhibition of the DNA repair machinery [6]. The main established beneficial effect of ultraviolet radiation (UVR) exposure is the production of vitamin D in the skin.

The molecular effects of sunlight are summarised in Fig. (**1**). These effects can lead to sunburn in the short term, and skin cancer [7] and photoageing [8, 9] in the long term. Public health advice to prevent this damage mainly advocates the use of sunscreens, along with clothing cover and avoiding the sun at the time when exposure is strongest. Modern sunscreens are formulations that are applied to the skin [10]. They contain a range of different synthetic organic and inorganic UVR

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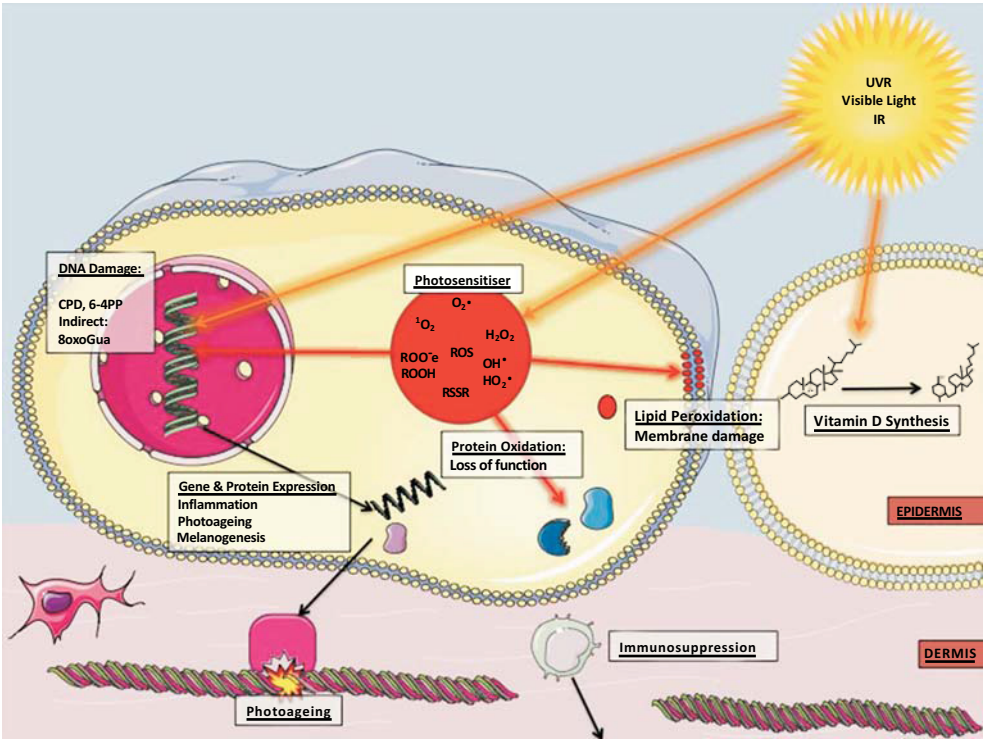


Fig. (1). The effects of solar radiation on the skin. There are numerous effects of solar radiation on the skin. Some of these are positive such as the production of vitamin D, however the vast majority of these is negative. The negative effects include direct damage to molecules such as DNA and the formation of cyclobutane pyrimidine dimers (CPD) and indirect damage through photosensitisation reactions resulting in the production of reactive oxygen species (ROS). Both these routes of damage induction cause secondary effects such as DNA, protein and lipid oxidation (resulting in reduced function), differential gene and protein expression (leading to photoageing, inflammation and melanogenesis) and immunosuppression. Damage to DNA can lead to mutations and eventually cancer [13].

filters with different absorbance profiles to provide broad-spectrum protection across the solar UVR spectrum.

The organic chemical filters contain a chromophore, which is typically an aromatic molecule conjugated to carbonyl groups. In general, increasing the number of conjugated double bonds and number of resonance structures stabilizes the excited state and shifts the absorption spectrum to longer wavelengths and a larger absorption cross section – leading to UVB (280-315nm) absorbers having typically smaller molecular weights compared to UVA (315-400nm) and broad spectrum (UVB + UVA) filters [11, 12].

The energy of UVR photons lies in the same magnitude as the energy of the filters' electrons, and allows for the absorption of the photons. Once absorbed, this causes a photochemical excitation of the electrons to a higher energy (π^*) state (excited singlet state), the electrons then return to the ground state emitting the excess energy, which can occur by a number of different processes shown in Fig. (**2**). The preferred route, to dissipate the energy returning the excited molecule to the ground state, is by internal conversion (IC), leading to dissipation by heat, or reversible isomerisation. When this is not possible, the remaining energy can be converted into photons as fluorescence, corresponding to the difference in energy between the two levels. This energy can be emitted in either the visible, infrared or long wave UVR region. Through intersystem crossing (ISC), the singlet excited state can cross to the triplet state, which can lead to phosphorescence or photosensitisation reactions. These reactions can result in the transfer of the excess energy to oxygen molecules to form ROS, or cause bonds to break [11]. This is highly undesirable for a sunscreen molecule; as the molecules need to be stable enough to prevent further damage rather than enhance damage.

The inorganic filters that are commonly used in sunscreen formulations are titanium dioxide (TiO_2) and zinc oxide (ZnO), and despite popular belief also act by absorption of UVR, with only a minimal effect by reflection or scattering. A recent study demonstrated that the average reflection range of UVR by TiO_2 and ZnO was around 4-5%, equating to a sun protection factor (SPF) of less than 2 [14]. The UVR is absorbed by excitation of the electrons from the valence band to the

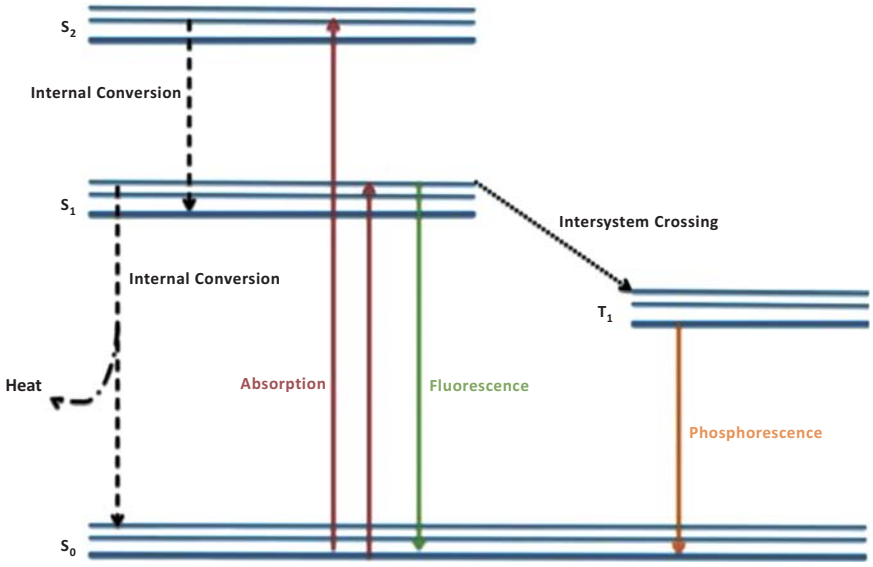


Fig. (2). Routes of excitation and dissipation of photochemical excitation of electrons.

conduction band. These molecules do not absorb in the visible range but their particulate nature results in scattering and reflecting, which accounts for the white appearance on the skin. Clearly this is highly undesirable for a sunscreen, so these molecules are increasingly micronized to improve their cosmetic properties [11].

There is evidence that suggests sunscreens may prevent a range of UVR-induced clinical outcomes including erythema (sunburn), squamous cell carcinoma (SCC), basal cell carcinoma (BCC) [15] and photoageing [16], however this is contested [17] and their ability to prevent malignant melanoma (MM) is yet to be fully established [18, 19].

In recent years there has been a shift in consumer trends to prefer more natural products, rather than their synthetic equivalents, as they are perceived to be safer and better [20]. Additionally, in the case of sunscreens there is a growing body of evidence that suggests that the synthetic UVR filters such as octocrylene (OCT), benzophenone-4 (BP-4), ethyl 4-aminobenzoate (Et-PABA), 3-benzylidene camphor (3BC) and TiO2 nanoparticles may cause damage to the marine environment in which they are widely used. Several negative effects have been reported, including the bioaccumulation of filters in many different species [21, 22], hormonal changes and endocrine disruption in fish [23, 24], production of hydrogen peroxide [25] and the bleaching of corals [26]. The Environmental Effects Assessment Panel (EEAP), that answers to the United Nations Environment Programme (UNEP), recently expressed concern about sunscreen damage to fragile marine ecosystems [27].

2. MYCOSPORINE-LIKE AMINO ACIDS

One class of such natural compounds is the mycosporines and mycosporine-like amino acids (MAA), which are thought to provide photoprotection to marine species and terrestrial fungi. Many marine species are exposed to very high levels of solar radiation, particularly in shallow clear waters. Solar UVR can penetrate to depths of between 0.5-47m depending on the water clarity. DNA is very susceptible to UVR-induced damage and wavelengths that readily damage DNA can reach depths of 16.4m [28]. Organisms of all classes have evolved complex DNA repair mechanisms, but some have also evolved other strategies, one of which is by the biosynthesis and accumulation of UVR absorbing molecules such as MAA. These are compounds that are synthesized or acquired by the diet in a taxonomically diverse range of marine organisms, including protozoa, algae, seaweed, corals, other invertebrates and fish [29]. The first MAA were discovered in fungi species in 1965 [30] and a recent study found a terrestrial alga containing MAA [31].

The cellular concentration and type of MAA vary with species, geographical location and environment (*e.g.* nitrate concentration) in which they are found. There are also ways to increase the MAA content such as with irradiation with different visible light/UVR sources, and treatment with nitrate compounds. Table **1** shows some examples of the different MAA and their concentrations produced by different species, and some treatments used to increase the yield of MAA [32-39].

Table 1. The reported MAA and total content of different species and treatments to induce production of MAA. (DW= Dry weight, PAR= Photosynthetically active radiation).

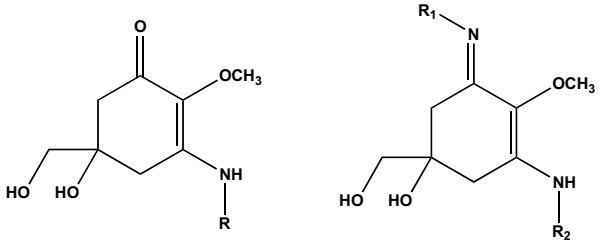
Species	Reported MAA	Total MAA DW Content [ref]	MAA DW Content after Treatment
Ulva lactuca	Porphyra-334	0.005 mg/g [39]	-
Gymnogongrus antatcticus	Shinorine	0.1 mg/g [38]	PAR + UVA + UVB > 0.9 mg/g
Pilayella Littoralis	Porphyra-334	0.177 mg/g [39]	-
Kallymenia antartica	Shinorine, Palythine, Asterina-330	0.3 mg/g [38]	PAR + UVA + UVB > 1.6 mg/g
Palmaria decipiens	Shinorine, Porphyra-334 Palythine, Asterina-330	0.4 mg/g [38]	PAR + UVA + UVB > 0.9 mg/g
Chondrus crispus	Palythine, Palythinol	0.470 mg/g [39]	-
Gracilaria tenuis-tipitata	Information not available	0.6 mg/g [33, 34]	NO_3^- 0.5mM + PAR + UVR > 4.29 mg/g
Hydropuntia cornea	Shinorine, Porphyra-334, Palythine	1.3 mg/g [32]	Increase in nitrate availability > 2.25 mg/g
Porphyra leucosticta	Shinorine, Palythine, Porphyra-334, Asterina	6.99 mg/g [35]	300uM ammonium > 9.67 mg/g
Devaleraea ramen-tacea	Mycosporine-glycine, Shinorine, Porphyra-334, Palythine, Asterina, Palythinol, Palythene	3.552mg/g [39]	-
Porphyra endiviifo-lium	Shinorine, Porphyra-334	4.2 mg/g [38]	PAR + UVA > 8 mg/g
Pophyra columbina	Mycosporine-glycine, Shinorine, Porphyra-334, Palythine, Asterina,	5.5 mg/ g [36] (7.2 - 10.6 mg/g [37])	PAR + UVA + UVB + NH_4Cl > 0.9 mg/g
Gelidium pusillum	Shinorine, Porphyra-334, Palythine, Asterina, Palythinol	6.506 mg/g [39]	-
Gymnogongrus griffithsiae	Shinorine, Porphyra-334, Palythine, Asterina,	7.786 mg/g [39]	-

Research on the photoprotective potential of these compounds has largely been focused on the species in which they are produced or found, but more recently there has been more work investigating their possible role as photoprotective agents in human skin models.

In addition to their UVR absorption characteristics, MAA appear to have other properties, such as antioxidant activity. This review reports on the mechanisms involved with the photoprotective role of MAA in nature and potential application to skin photoprotection.

2.1. MAA Structure and Biosynthesis

MAA are typically small (<400 Da) colourless, water-soluble compounds, of which over 20 are currently known. They have a similar general structure based on 4-deoxygadusol, containing cyclohexenone or cyclohexenimine rings conjugated to the nitrogen substituent of an amino acid or imino alcohol. These can undergo further carboxylation or demethylation, which changes their UVR absorption properties [40]. The general structures for mycosporines and MAA are displayed in Fig. (**3**).



Mycosporine **Mycosporine-like Amino Acid**

Fig. (3). The general structures of mycosporines and MAA.

The route of MAA biosynthesis is a contentious area. Historically it was believed that MAA were synthesised via the shikimate pathway. This pathway is found in many microorganisms including bacteria, al-

gae, fungi and plants and is responsible for the biosynthesis of the essential aromatic amino acids phenylalanine, tyrosine and tryptophan. This pathway is not found in animals and these amino acids must be acquired by diet. It was first implicated because the addition of the radiolabeled precursor [U-^{14}C]3-dehydroquinate to the fungus *Trichothecium roseu* produced labelled glutamicol. Furthermore, the *cyanobacterium* species *Chlorogloeopsis* successfully synthesised the MAA shinorine and mycosporine-glycine when ^{14}C-pyruvate was added to the culture [41, 42]. The use of the shikimate pathway inhibitors glyphosate and tyrosine has also demonstrated the ability to inhibit the production of MAA in the cyanobacteria *Nostoc commune* and the coral *Stylophora pistillata* [43, 44].

Further investigation has also implicated the pentose phosphate pathway in MAA biosynthesis. A four-gene cluster, linked to the pentose phosphate pathway in *Anabaena variabilis* ATCC 29413, was able to produce the MAA shinorine when inserted in to the heterologous host *E.coli* [45]. The genes in this cluster have been identified as shown in Table **2**.

Table 2. The four-gene cluster found in *Anabaena variabilis* ATCC 29413 linked to the pentose phosphate pathway synthesis of MAA.

Designation	Name	Product
Ava_3858	2-epi-5-epi-valiolone synthase (EVS)	2-epi-5-epi-valiolone
Ava_3857	*0*-methyltransferase (OMT)	4-deoxygadusol
Ava_3856	ATP-grasp amino acid ligase	Mycosporine-glycine
Ava_3855	NRP-like synthase	Shinorine

This finding has also been confirmed in another cyanobacterium *N. punctiforme*, which shares homologues of the first three genes found in *A. variabilis* (NpR5600, NpR5599 and NpR5598), and produced mycosporine-glycine after treatment with the 2-epi-5-epi-valiolone precursor sedoheptulose 7-phosphate (SH-7P) [46]. The incubation of the first two proteins of this cluster (NpR5600 and NpR5599) with SH-7P has also demonstrated the production of 4-deoxygadusol [46]. Typically the EVS gene is found in genome mining of species that produce MAA and is absent in those without this ability [47, 48]. There is however one known exception, in *Synechocystis sp.* PCC6803, which lacks EVS but produces three novel MAA after exposure to UVR. These are mycosporine-tau, dehydroxylusujirene and M-343 [49] and suggests an alternative pathway to MAA in this cyanobacterium.

Despite experimental data that support the pentose phosphate pathway, there is an evidence that this is not the major route of MAA synthesis. *A. variabillis* ATCC 29413 still produced shinorine, at levels equivalent to the wild type exposed to UVR, after a deletion of the gene encoding the enzyme EVS. This eliminates the role of the pentose phosphate pathway as the only mechanism for MAA synthesis [50]. Another proteomic study of the same cyanobacterium found that UVA exposure led to an increase in expression of the enzymes DAHPS and DHQS (part of the shikimate pathway) after irradiation. There was no increase in any enzymes associated with the pentose phosphate pathway, and when shikimate inhibitors were used there was only minimal shinorine produced, suggesting any activity from the pentose phosphate pathways was minimal. Overall, this implies the shikimate route of production as the most predominant for MAA synthesis in sufficient quantities to provide photoprotection. This is confirmed in studies with shikimate inhibitors, which found expression of shinorine after UVR exposure was very low, at levels equivalent to no exposure [51]. Quantities of MAA produced by the pentose phosphate pathway are likely to have other biological functions, for example in *Anabaena* there is evidence of a possible phycobillosome trimming role [50].

There are however clear links between the pentose phosphate and shikimate pathways. SH-7P, an intermediate of the pentose phosphate pathway is easily converted to the shikimate intermediate erythrose-4-phosphate by a transaldolase enzyme. The enzymes 3-dehydroquinate synthase (DHQS), from the shikimate pathway, and EVS, from the pentose phosphate pathway, are both part of the sugar phosphate cyclase family of enzymes. These enzymes have also remarkably similar amino acid sequences and carry out very similar reactions [52]. A knockout of the gene encoding the enzyme O-methyltransferase (OMT) (linked to the pentose phosphate pathway) in *A. variabillis* ATCC 29413 completely prevented shinorine synthesis [51] implying that both pathways must be linked at this point.

Evaluating this evidence, one proposed scheme for MAA synthesis is that SH-7P of the pentose phosphate pathway is fed into the shikimate pathway to form erythrose-4-phosphate (which is also formed from the earlier stages of the shikimate pathway). This then reacts with phosphoenolpyruvate (PEP) to form 3-deoxy-D-arabinoheptulosinate phosphate (DAHP) and 3-deyhdroquinate (DHQ). This explains the upregulation

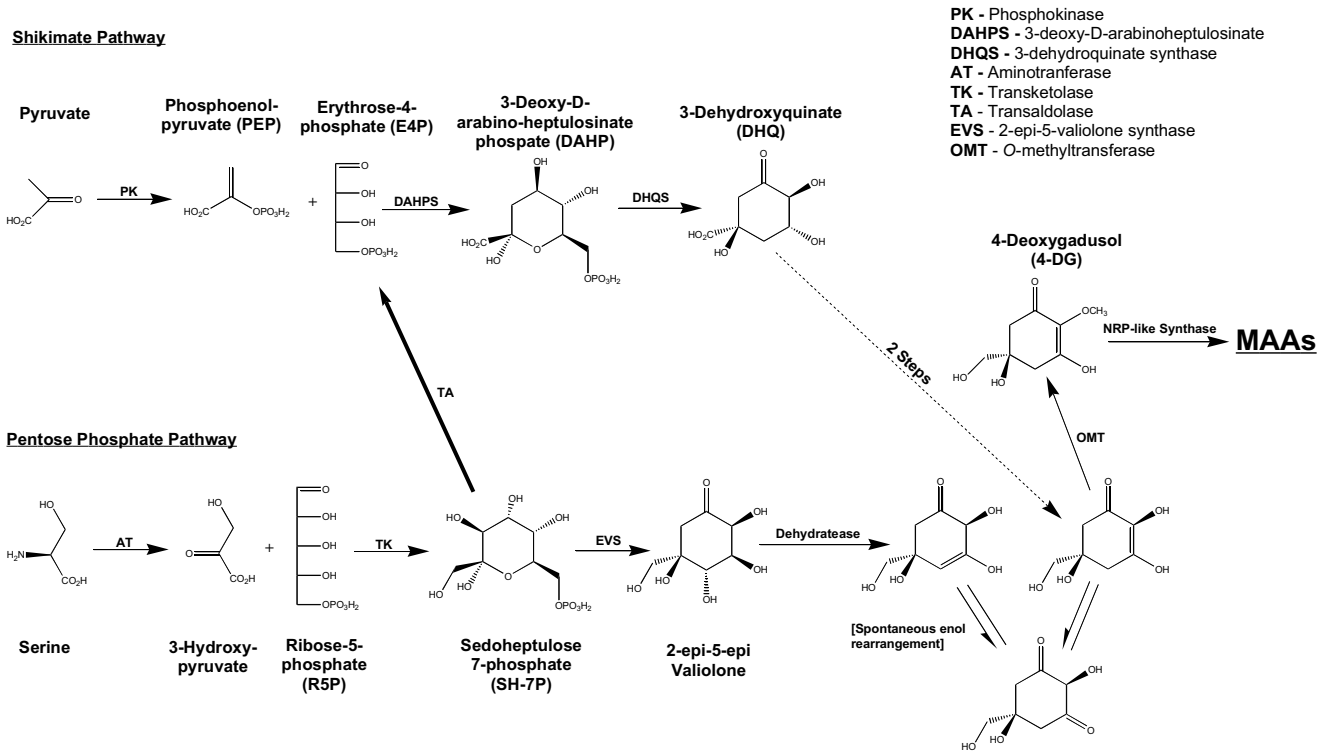


Fig. (4). The proposed route of MAA biosynthesis.

of the enzymes DAHPS and DHQS. The involvement of OMT implies that DHQ cannot be the direct precursor of 4-deoxygadusol, and it must feed back into the pentose phosphate pathway and undergo conversion to an intermediate (by an unidentified enzyme(s)), which is then converted by OMT into 4-deoxygadusol. A suggested biosynthesis scheme is depicted in (Fig. **4**), which incorporates both pathways.

The synthesis of mycosporine-glycine from 4–deoxygadusol has been shown to occur via ligase, and shinorine through NRP-like synthase of mycosporine-glycine. The biosynthesis routes of many other MAA are yet to be established [53]. However, a new five-gene cluster has recently been discovered in the soil dwelling cyanobacteria *Cylindrospermum stagnale sp*. PCC 7417, which when cloned into *E. coli* produced mycosporine-ornithine and mycosporine-lysine, giving insight into the synthesis of other MAA and a possible route to large scale production [54].

3. PHOTOPROTECTIVE ROLE OF MAA IN NATURE

3.1. Structural Evidence for Photoprotection

The UVR protective properties of MAA are largely inferred from their absorption spectra and high molar extinction coefficients. Typically they have a peak absorption wavelength (λ_{max}) between 268-362nm, covering much of the spectral range of UVR (~295-400nm) that reaches the earth's surface [29].

The photochemistry of MAA is poorly understood, with only a select few compounds having been investigated. The MAA that have been most studied are porphyra-334, shinorine and mycosporine-glycine. The photo-excited states of these molecules have been shown to relax by intersystem crossing from the singlet excited state to the triplet excited state and by subsequent non-radiative decay, resulting in a controlled dissipation of the energy as heat without the production of ROS [55]. Porphyra-334 and shinorine dissipate 96-98% of absorbed energy in this way [56, 57]. This pathway is consistent with the strong photostability of MAA. Palythine in particular has been shown to be extremely photostable in a saturated aqueous solutions [55], as well in the presence of seawater and the strong photosensitising agents riboflavin and rose Bengal [58]. The increased photostability of palythine over other MAA (such as shinorine and asterina-330) has been attributed to the substitution of the nitrogen atom R group (R_1=H), in relation to the geometrical isomerisation around the C=N double bond [55]. Displayed in (Fig. **5**) are data generated demonstrating the photostability of MAA as (a) a mixture and (b) palythine as a single molecule. Both examples demonstrate excellent photostability, with palythine as a single molecule demonstrating only a 3% degradation after an exposure

of 40 standard erythema doses (SED) of solar simulated radiation (SSR), equivalent to around a full day of UK summer sun on an unshaded surface (a dose of around 60J/cm^2). Many synthetic filters rely upon additional filters to provide photostability; this demonstrates that this is not necessary with MAA.

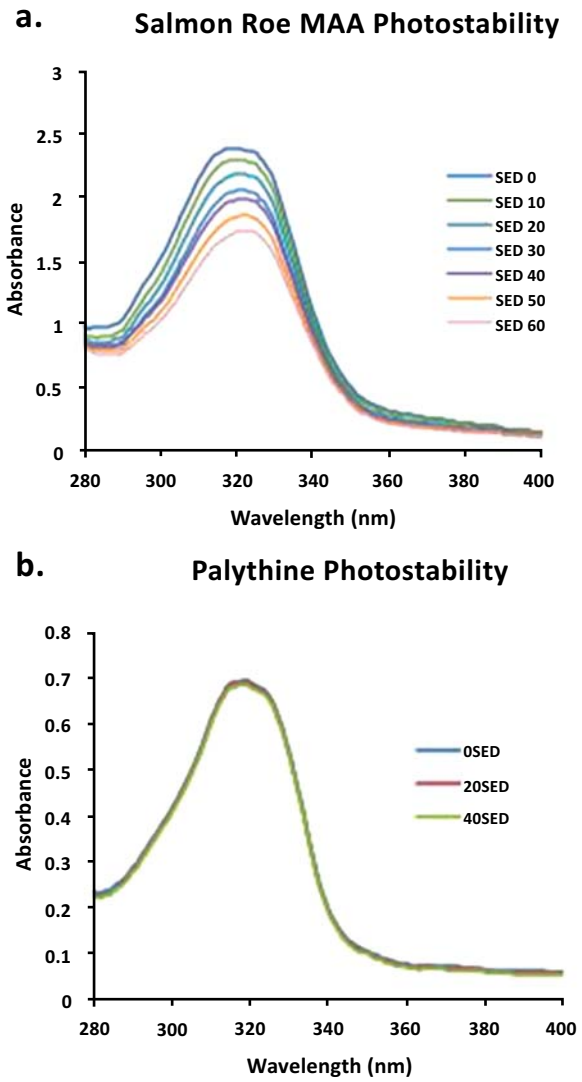


Fig. (5). The photostability of MAA. (a) MAA extracted from salmon roe (0.06%w/v and of (b) of palythine as a single molecule at a concentration of 0.001% w/v in PBS was exposed to increasing doses of SSR and the absorbance subsequently measured by UV spectrometry between 280-400nm.

3.2. Circumstantial Evidence for Photoprotection

In addition to the optical properties of MAA, there is also a large body of circumstantial evidence to suggest a photprotective role in their natural environments. This has been extensively reviewed by Shick and Dunlap [29], but the key conclusions are summarised below.

MAA appear to be preferentially accumulated in tissues that receive the greatest UVR exposure – the epidermis of coral reef holothuroids, sea urchin eggs and the eggs and lenses of freshwater and marine teleosts [29, 59, 60]. In other circumstances, such as with corals, the MAA are transferred through symbiosis with algae (*e.g.* zooanthellae). In this relationship, the host also receives organic carbon in the form of carbohydrates, lipids and amino acids and the symbiont is nourished by the host's waste products such as nitrogen, phosphorus, sulphur and carbon dioxide for photosynthesis [61, 62].

The MAA concentration of a species is directly related with its UVR exposure level that is dependent on latitude and altitude. Zooplankton sampled from lakes at different altitudes showed increasing MAA concentration with increasing altitude [63]. Species found in tropical waters have a greater concentration of MAA than those found in cooler climates. High levels of MAA are also found in species in the Antarctic Ocean, possibly due to high irradiances from the ozone layer hole [64]. Species show seasonal variation in MAA concentration. In winter months, they have a lower concentration compared to summer months when UVR exposure is highest as demonstrated in plankton growing in lake environments [65, 66]. There is also a strong negative correlation between the coral depth and MAA concentration that reflects the attenuation of UVR by water. This has been demonstrated experimentally by Dunlap *et al*, who relocated corals, with low MAA concentration, from deep to shallow waters [67]. This resulted in an increased in MAA content that supports a role in photoprotection. An increase in extracellular nitrogen concentration has also demonstrated an increase in MAA production, suggesting a potential role in intracellular nitrogen storage [68].

3.3. Biological Evidence for Photoprotection

The role of MAA in protecting marine species from UVR damage is a widely researched area. One study has shown an inverse relationship between the production of DNA photolesions, especially the cyclobutane pyrimidine dimer (CPD), and total MAA concentration in the coral *Montipora verrucosa* (found in Kaneohe Bay, Oahu, Hawaii) which contains mycosporine-glycine, shinorine and porpyra-334 [69]. Reduction of CPD and 6,4 pyrimidine-pyrimidone photoproducts by MAA has also been demonstrated, acting by attenuating the UVR before it reaches critical cellular targets in addition to their quenching effects (discussed in DNA Damage section) the photo-excited state thymine base

[70]. Photoprotection has also been demonstrated in green sea urchin embryos by preventing UVB induced abnormalities [71, 72]. There are numerous studies investigating the effect of increased MAA content on UVR resistance for a range of species and environmental stressors such as UVR and desiccation [73-77].

Initially it was thought that MAA acted solely by absorbing UVR before it could reach the critical cellular targets, but they also appear to have antioxidant properties. This is an extremely desirable characteristic for a photoprotective molecule, as much of the damaging effect of UVR is due to ROS. This has been demonstrated with different MAA from a large variety of species [78-81]. MAA have also been shown to block specific consequences of oxidative damage preventing lipid peroxidation and superoxide radicals [82]. An extensive review of MAA antioxidant abilities has been carried out by Wada *et al* [83].

3.4 Additional Protective Roles of MAA in Nature

Apart from their photoprotective properties, MAA exhibit additional protective effects, particularly to other environmental stressors. These roles are summarised below and are reviewed in more detail by Oren and Gunde-Cimerman [77].

3.4.1. Osmotic Stress

One such stressor the MAA appear to have an action against is osmotic stress, where a change in the solute concentration surrounding an organism, causes a loss or gain of cellular solvents. One halotolerant unicellular cyanobacterium, inhabiting in a gypsum crust in a hypersaline saltern pond, has an extremely high concentration of MAA (≥98mM), accounting for >3% of its mass. A reduction of the salinity of its surroundings was accompanied with a rapid expulsion of MAA, suggesting a role in osmotic stabilisation [84]. This hypothesis has since been investigated and the role of MAA in prevention from osmotic stress supported [77, 85-88].

3.4.2. Desiccation Stress

There is also evidence that MAA can protect against desiccation. Cyanobacteria under desiccation stress contain high concentrations of water stress protein (WSpA) and MAA in a 1:1 ratio (around 4-5% of dry mass each), along with other compounds including scytonemin (another UVR filter), superoxide dismutase and glycan. This group of compounds is thought to act by modifying the structure of the extracellular matrix. Upon rehydration there is an expulsion of MAA. Overall, this supports a role for MAA comparable to that for osmotic stress [76]. Another study found that cyanobacteria experimentally stressed by desiccation increased their total MAA content. When these pre-stressed cells were placed under desiccation conditions, they had better viability compared to control cyanobacteria (with a lower MAA content) [73]. Many different bacteria have shown this property in a range of environments [77].

3.4.3. Thermal Stress

In the above mentioned desiccation study, cyanobacterium survival was also measured under different temperatures. Pre-stressed cells had a higher survival rate than the controls between -20^{o}C and 40^{o}C but this difference was lost at 50^{o}C [73]. There are also other examples of thermal stress protection by an increase in MAA production in a range of species [77].

3.4.4. Photosynthesis Accessory Pigments

There are other reported properties of MAA but these are much less researched. There is evidence that porphyra-334 may act as a photosynthetic accessory pigment due its UVA absorption and subsequent production of small amounts of fluorescence in the Soret band of chlorophyll. This has been debated due to the relatively low amount of fluorescence that is produced in this way and that MAA are produced in environments of significant irradiance, suggesting photosynthetic wavelengths are in abundance [77, 89].

4. PHOTOPROTECTION OF THE SKIN

Despite the evidence that MAA are prime candidates for use as biocompatible photoprotective molecules for human use, there has been surprisingly little work carried out in skin models to demonstrate potential for human use. The reported effects in skin models are described below.

4.1. Cell Viability/Proliferation

Cell viability and toxicity are critical endpoints for MAA assessment. One study, done according to the International Organization for Standardization (ISO) 'extracted media' recommended short-term toxicity assay (ISO 10993-12), showed no toxicity of MAA including shinorine, porphyra-334 and mycosporine-glycine in murine fibroblasts. This was confirmed in a second longer-term direct incubation assay in the same cell line. After 14 and 21 days of incubation, with the different MAA, there was no significant toxicity and only minor effects on cell morphology for some of the

MAA tested [90]. The same three MAA were shown to be non-toxic in human TIG-114 lung fibroblast cells at concentrations between 0-100μM at 48 hours, and actually increased cell proliferation [91]; an effect confirmed in by Kim *et al* studying cell viability [92]. Porphyra-334 has also shown have no effect on cell viability of human skin fibroblasts at concentrations up to 200μM [93]. In contrast with these findings, is work by Choi *et al* who found shinorine, porphyra-334 and mycosporine-glycine all significantly reduced cell viability in HaCaT keratinocytes, to differing extents, at concentrations from 0.1-mg/ml (around 0.301mM for shinorine) and above [94]. As mentioned MAA have demonstrated cell proliferation properties and have potential wound healing applications [94].

There are several studies that show that MAA prevent UVR induced toxicity. This protective effect has also been demonstrated in other MAA such as collemin A (a compound with a structure related to MAA), where UVB exposure of HaCat keratinocytes through a collemin A coated quartz plate produced an increase in cell viability, demonstrating a filtering effect [95]. Post 20J/cm^2 UVA exposure, application of poryhyra-334 at concentrations 10-40μM to skin fibroblasts also prevented reduction in cell viability and induction of senescence [93], confirmed with UVB irradiation with a greater effect with increasing MAA concentration [91]. Application of MAA post exposure contributing to increased cell viability compared to control suggests a significant effect outside of UVR filtering.

4.2. Oxidative Stress

Oxidative stress is a major consequence of UVR exposure [2, 3]. This results from photosensitization reactions, which can produce highly reactive molecules (such ROS). UVR-induced ROS may also be generated post-UVR exposure [96]. As previously mentioned, oxidative DNA damage can lead to mutations, and recently oxidative damage to proteins has shown to inhibit DNA repair, exacerbating the effect of UVR induced DNA damage [6].

Many studies using non-biological chemical assays have shown that MAA are antioxidants [78, 81, 97, 98]. A DPPH radical scavenging assay demonstrated that mycosporine-glycine had significant, dose dependent radical scavenging ability, but that porphyra-334 and shinorine had no effect. The authors concluded that this was because mycosporine-glycine has an oxocarbonyl structure whereas porphyra-334 and shinorine have an imino structure [99]. However, many other chemical and biological studies have reported that porphyra-334 and shinorine have antioxidant properties, and it is possible that MAA act in multiple ways.

Studies have also been carried out in biological models. Porphyra-334, the most widely studied MAA, has also demonstrated a dose dependent reduction in oxidative stress in skin fibroblasts, when added post exposure, again suggesting antioxidant capability [93]. These studies measured oxidative stress immediately post irradiation suggesting a ROS quenching role of MAA. The results from the biological and chemical assays are not always in agreement suggesting the further investigation is required to elucidate the mechanism of the anti-oxidant effects. Catalase and superoxide dismutase (SOD) showed reduced post-irradiation activity over time in unprotected mouse skin. However, the application of a reference sunscreen, or a porphyra-334 and shinorine formulation offered complete protection, along with a decrease in the expression of 70 kilodalton heat shock (stress) protein (Hsp70) [100]. For the most part, studies demonstrate that MAA efficiently prevent oxidative stress though filtering, direct and indirect quenching mechanisms, however the exact mechanism are yet to be elucidated.

4.3. NRF-2 Activation

Closely linked to prevention of environmental damage to the skin is the Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid-2-related factor 2 (Nrf2) complex. Under conditions of stress (particularly oxidative stress), this complex dissociates to release Nrf-2 which subsequently binds to the antioxidant response element (ARE), leading to the transcription of over 200 cytoprotective genes linked to DNA repair, inflammation anti-oxidant response (among others). This is an area of emerging interest for photoprotection, using Nrf2 activators to boost the skin's natural responses to UVR damage [101, 102].

Recently, a bioinformatics based protein modelling and virtual screening approach has been applied to investigate potential compounds that interact with Keap1-Nrf2 complex. This approach identified 75 promising compounds that activated Nrf2, of which 25 were experimentally known to be potent activators. Eleven of these compounds were known to have antioxidant activities but had not been previously linked to Nrf2 activation, of which three were MAA: mycosporine-glycine, mycosporine-glycine-valine and porphyra-334 [103]. This *in silico* approach has been confirmed experimentally with porphyra-334, which demonstrated potent Nrf2 activation activity through the prevention of UVA induced markers of inflammation

and cell death. Skin fibroblasts were incubated with increasing concentrations of porphyra-334 (0-40μM) after UVA irradiation. This resulted in a significant reduction of gene and protein expression of IL-6, IL-1β, TNF-α and nuclear expression of NF-κB. In addition there was sustained Nrf2 activation, leading to the expression of a number of cytoprotective genes such as (HMOX-1), glutathione (GSH) and NAD(P)H dehydrogenase [quinone] 1 (NQO1) and the direct scavenging of reactive oxidative entities and their conversion to less harmful and inert products [104].

4.4. Accumulation in Human Skin Models

A key property of MAA is their accumulation in the food chain in marine species. This is a poorly researched area in non-marine species. In one study, investigators fed SKH-1 hairless mice a standard daily diet or the same diet with a freeze-dried red alga that contained a mixture of MAA that was known to accumulate in medaka fish. They found no MAA accumulation in the eyes, skin or liver after 14-130 days, apart from small amounts in the small intestine, suggesting no route for accumulation in mammals [60]. As part of the same study, the uptake of the MAA shinorine by human skin cancer A431 cells was also investigated. A dose dependent increase in shinorine (1-1.5mM) was observed after 48 hours of incubation, but saturation occurred at concentrations from 1.5-2.5mM. Raman confocal spectroscopy has shown that MAA incorporated into polymer gels applied to the skin *in vivo* penetrate and accumulate at depths of 2μm in the *Stratum corneum* at a concentration 103.4% higher than at the surface. These results suggest that MAA may accumulate in the skin, if not through the diet but further research is needed [105].

4.5. DNA Damage/Erythema

It is generally accepted that the most damaging consequence of UVR exposure to the skin is the formation of DNA photoproducts, which can subsequently lead to genomic mutations [106]. The CPD is the predominant and most important photoproduct induced by both UVA and UVB radiation, however oxidative photoproducts such as 8-oxo-7,8-dihydroguanine (8-oxoGua) are proving to be of increasing importance [107]. Closely related to the formation of DNA photoproducts, particularly the CPD, is the development of erythema in the skin, with DNA absorption and erythema sharing very similar action spectra [1].

In terms of photoprotection, the most widely used metric of the efficacy of sunscreen products is the is the SPF, which is a measure of their ability to prevent erythema (and presumed causal DNA damage). Despite this, the investigation of MAA to prevent DNA damage and/or erythema in human models is hugely under researched.

Collemin A significantly reduced UVB-induced CPD in HaCat human keratinocytes cells *in vitro* compared to an irradiated control [95]. In the same study, a crude formation of collemin A was made by mixing with olive oil and then applied to the skin ($6\mu g/cm^2$) of one volunteer. This formulation was estimated to have an SPF of at least 4. Little can be concluded from this pilot study other than it requires confirmation [95]. A more robust study in SKH-1 hairless mice, with a galenic formulation of 2% porphyra-334 and shinorine (ratio of 88:12) applied to the dorsal skin, prevented solar simulated UVR induced erythema, *stratum corneum* thickening, edema and sun burn cell formation (apoptosis) comparable with a reference sunscreen (the reference sunscreen is the standard used in sunscreen testing according to COLIPA guidelines). The calculated SPF was 3.71±0.78 [100]. One criticism of this study is the formulation was applied at a thickness of $4mg/cm^2$, double the thickness at which sunscreens are tested suggesting that the real SPF would be at least half of this value, and used at a concentration significantly thicker than sunscreens are typically applied in real life situations [108].

Studies in a chemical model have shown that UVR-induced CPD can be inhibited by an MAA extract containing porphyra-334, shinorine and palythine. Thymine monomers were irradiated through the MAA extract with, no direct contact (in manner similar to a sunscreen application to the surface of the skin), and also irradiated with the extract and monomers mixed together. There was a greater protective effect in the mixed samples than those with no contact, suggesting an effect beyond the absorption properties of MAA. Further investigation established this was through quenching of the triplet state of UVR-excited thymine [70]. This shows that MAA may have even greater potential for photoprotection over current filters, especially with the recent discovery of delayed (also know as 'dark') CPD formation, which suggests that CPD can form for hours after exposure through a triplet photoexcitation mechanism [5].

4.6. Inflammation

The ability of MAA to inhibit biomarkers of skin inflammation is poorly studied. Over expression of these markers is linked to a range of inflammatory skin con-

ditions such as psoriasis. Expression of cyclooxygenase-2 (COX-2) mRNA, widely associated with inflammation, was also prevented by topical application of mycosporine-glycine to HaCat keratinocytes at the highest concentration tested (0.3mM) and with only at the lowest concentration of shinorine (0.03mM) having a statistically significant effect (questioning the validity of the result), and with porphyra-334 having no effect [99]. This used a broad-spectrum UVR source and the results could possibly be explained by the peak absorbance of each of the MAA, with mycosporine-glycine (λmax =310nm) and shinorine (λmax =334nm) absorbing in shorter wavelengths and porphyra-334 (λmax =344nm) absorbing at slightly longer wavelengths, suggesting COX-2 expression is linked to shorter wave UVR, however the lack of dose-response relationship is unclear.

4.7. Photoageing

Skin photoageing is a consequence of long-term solar UVR exposure. This is different from chronological skin ageing and is associated with deep wrinkles and sagging. It is generally accepted that photoageing is the consequence of UVR induced activation of a group of proteins known as the matrix metalloproteinases (MMPs), which degrade the structural extracellular matrix proteins of the dermis such as elastins and collagens [9].

The incubation of fibroblasts with porphyra-334 (0-40μM) after UVA exposure inhibited MMP-1 and MMP-8 gene expression, but had no effect on MMP-13. Elastase activity was dose dependently reduced by porphyra-334, with an increase in collagen and elastin mRNA and protein expression, and procollagen secretion [93]. Shinorine, porphyra-334, and palythine significantly inhibited MMP-2 activity in an *in vitro* fluorogenic assay, which was hypothesised to be due to competitive inhibition by binding to the active site determined using computer modelling [109].

In addition to their photoprotective properties, mycosporine-glycine, porphyra-334 and shinorine have been shown to be procollagen C proteinase enhancers (PCOLCE) and induced elastin mRNA upregulation in a largely dose dependent manner after UVA exposure, whereas only porphyra-334 showed an upregulation of involucrin, another skin protein [99].

Overall, relatively limited data suggest that MAA have multiple actions in the prevention of photoageing.

4.8. Potential Human Use of MAA

The evidence reviewed above demonstrates that MAA have huge photoprotection potential in traditional optical ways as well as in with new photomolecular strategies. Many studies have suggested the widespread use of MAA as sunscreens [110-113], however they have yet to been be exploited on a large scale, with only a few products currently available. One MAA product currently available called Helioguard 365, which contains MAA porphyra-334 and shinorine (11.5:1 ratio) extracted from the red alga P*orphyra umbilicalis* [114]. This product however mainly provides protection in UVA region with minimal protection in the more damaging UVB range, and the final concentration of MAA in the product is extremely small when compared to the concentration of UVR filters in most sunscreen products. One product contains a final MAA concentration of 0.0005%, whereas most sunscreen formulations contain filters at 0.5-10% w/v. This suggests the addition of a very low MAA concentration to a formulation will have a negligible influence on the SPF claims of the product.

There are numerous reasons for the lack of widespread use of MAA, one of which is the poor understanding of biosynthesis pathways involved to make specific MAA in an industrially economic manner. This makes the production process more complex, for example the need to farm vast amounts of seaweed. Further understanding of these pathways could lead to easier large-scale commercial biosynthesis, for example in a heterologous bacterial host *e.g. E. coli,* which is easier to cultivate. The chiral centres of MAA compounds make them highly difficult to synthesis chemically; again meaning large-scale synthesis is difficult, with unrealistic costs associated to production. One way that has been proposed to overcome this issue is via the synthesis of 'MAA-like' compounds, which are structurally similar and retain the chromophore of MAAs, but are simpler and cheaper to synthesize [115].

MAAs are highly water soluble, which makes it more difficult to formulate sunscreens intended for beach use. Water-soluble filters would however be much better for day-care products, because aqueous formulations have better sensorial properties than those based on oils. These properties improve compliance and therefore photoprotection. It is important to note MAA photostability would also have to be assessed in sunscreen formulations because photostability may vary with solvent.

Finally, the European Chemicals Agency (ECHA) has recently responded to environmental and human health concerns about some sunscreen filters by adding them to its Community Rolling Action Plan (CoRAP)

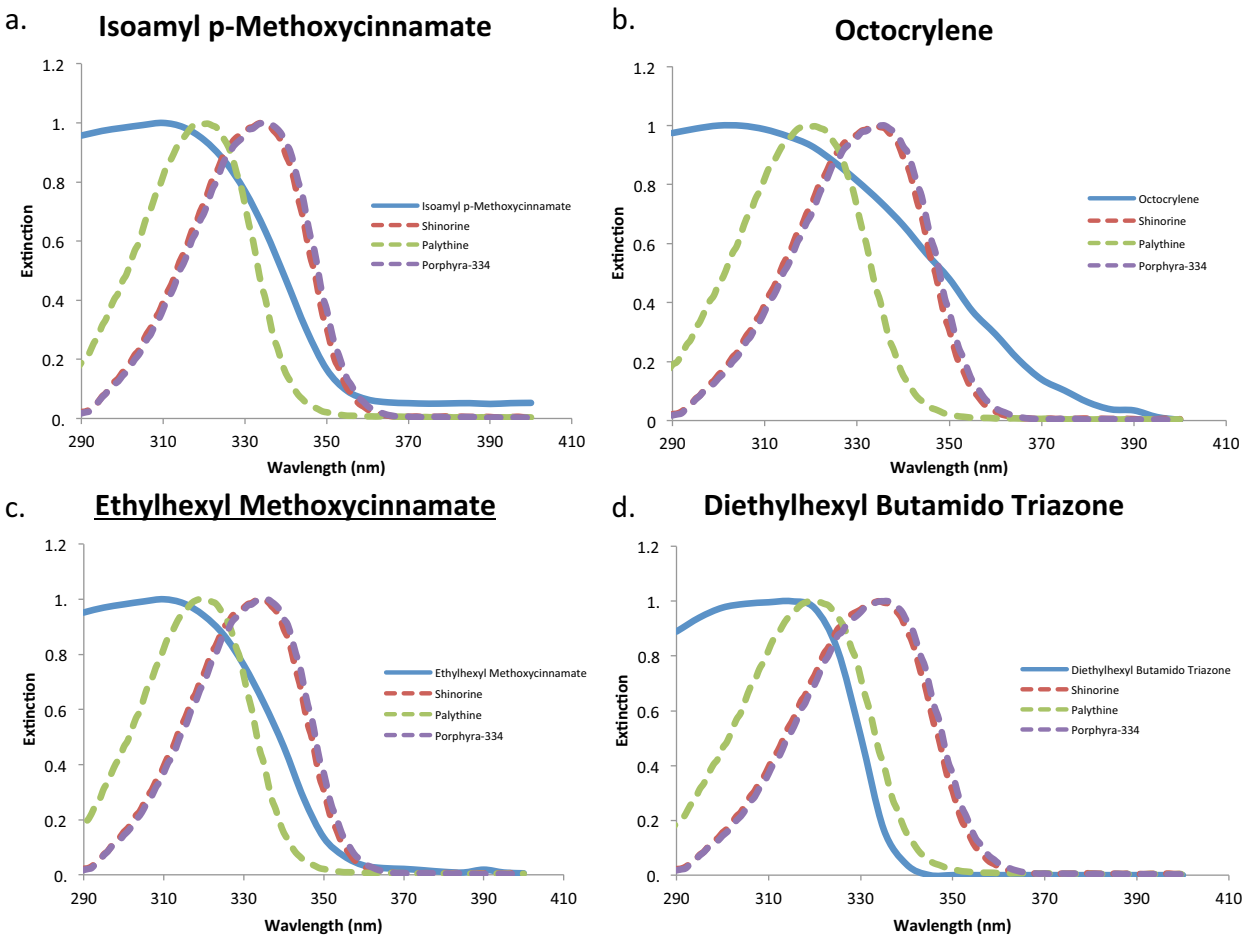


Fig. (6). The UVR absorbance spectra of CoRAP listed filters compared to MAAs. The relative UVR spectra of four CoRAP listers filters (a) isoamyl p-methoxycinnimate, (b) octocrylene, (c) ethylhexyl methoxycinnamate and (d) diethylhexyl butamido triazone were generated using the BASF sunscreen calculator [117] and compared to the spectra of the MAAs palythine, shinorine and porphyra-334.

list that includes 8/16 UVR filters that are commonly used in European sunscreen formulations [116]. These concerns, especially for marine environments, support the development of spectrally equivalent MAA as alternative biocompatable sunscreens. Fig. (**6**) shows the similarity of the relative UVR absorbance spectra of 4 four CoRAP filters: (a) isoamyl p-methoxycinnimate, (b) octocrylene, (c) ethylhexyl methoxycinnamate and (d) diethylhexyl butamido triazone with those of palythine, shinorine and porphyra-334.

CONCLUSION

MAA are natural compounds that are thought to offer photoprotection to marine species. Initially thought of as protective based on their absorption properties in the solar UVR region, it is clear that they have additional activities, some of which may be also useful after exposure to UVR, such as anti-oxidant capacity and Nrf2 activation. This suggests that MAA offer a novel approach to photoprotection, which is usually focused on attenuation of UVR before it reaches cellular targets. Most MAA studies have been *in vitro* and concentrated on porphyra-334, shinorine and mycosporine-glycine, as these are some of the most abundant in nature, but systematic *in silico* and *in vitro* screening of all MAA may identify other compounds. Current *in vitro* data strongly suggest that MAA have potential for the protection of human skin from a diverse range of adverse effects of solar UVR.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare the following interests: The results of our research into MAA compounds are subject of a patent application by King's College London, UK (PCT/GB2016/052227).

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REFERENCES

[1] Young, A.R.; Chadwick, C.A.; Harrison, G.I.; Nikaido, O.; Ramsden, J.; Potten, C.S. The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J. Invest. Dermatol.,* **1998**, *111*(6), 982-988.

[2] Bickers, D.R.; Athar, M. Oxidative stress in the pathogenesis of skin disease. *J. Invest. Dermatol.,* **2006**, *126*(12), 2565-2575.

[3] Wölfle, U.; Seelinger, G.; Bauer, G.; Meinke, M.C.; Lademann, J.; Schempp, C.M. Reactive molecule species and antioxidative mechanisms in normal skin and skin aging. *Skin Pharmacol. Physiol.,* **2014**, *27*(6), 316-332.

[4] Courdavault, S.; Baudouin, C.; Charveron, M.; Favier, A.; Cadet, J.; Douki, T. Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. *Mutat. Res.,* **2004**, *556*(1-2), 135-142.

[5] Premi, S.; Wallisch, S.; Mano, C.M.; Weiner, A.B.; Bacchiocchi, A.; Wakamatsu, K.; Bechara, E.J.; Halaban, R.; Douki, T.; Brash, D.E. Photochemistry. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science,* **2015**, *347*(6224), 842-847.

[6] McAdam, E.; Brem, R.; Karran, P. Oxidative stress-induced protein damage inhibits DNA repair and determines mutation risk and therapeutic efficacy. *Mol. Cancer Res.,* **2016**, *14*(7), 612-22.

[7] Armstrong, B.K.; Kricker, A. The epidemiology of UV induced skin cancer. *J. Photochem. Photobiol. B,* **2001**, *63*(1-3), 8-18.

[8] Fisher, G.J.; Datta, S.C.; Talwar, H.S.; Wang, Z.Q.; Varani, J.; Kang, S.; Voorhees, J.J. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature,* **1996**, *379*(6563), 335-339.

[9] Quan, T.; Qin, Z.; Xia, W.; Shao, Y.; Voorhees, J.J.; Fisher, G.J. **2009**.

[10] Young, A.R.; Claveau, J.; Rossi, A.B. Ultraviolet radiation and the skin: Photobiology and sunscreen photoprotection. *J. Am. Acad. Dermatol.,* **2017**, *76*(3S1), S100-S109.

[11] Herzog, B. In Photochemistry: Volume 40. Royal Society of Chemistry, **2012**, 40, 245-273.

[12] Osterwalder, U.; Sohn, M.; Herzog, B. Global state of sunscreens. *Photodermatol. Photoimmunol. Photomed.,* **2014**, *30*(2-3), 62-80.

[13] Servier Medical Art (**2016**) Powerpoint Image Bank. Servier Medical Art. (https://smart.servier.com/) (Accessed on April 2016).

[14] Cole, C.; Shyr, T.; Ou-Yang, H. Metal Oxide Sunscreens Protect Skin by Absorption, Not by Reflection or Scattering. *Photodermatol. Photoimmunol. Photomed.,* **2016**, *32*(1), 510.

[15] Green, A.C.; Williams, G.M. Point: sunscreen use is a safe and effective approach to skin cancer prevention. *Cancer Epidemiol. Biomarkers Prev.,* **2007**, *16*(10), 1921-1922.

[16] Iannacone, M.R.; Hughes, M.C.; Green, A.C. Effects of sunscreen on skin cancer and photoaging. *Photodermatol. Photoimmunol. Photomed.,* **2014**, *30*(2-3), 55-61.

[17] Sánchez, G.; Nova, J.; Rodriguez-Hernandez, A.E.; Medina, R.D.; Solorzano-Restrepo, C.; Gonzalez, J.; Olmos, M.; Godfrey, K.; Arevalo-Rodriguez, I. Sun protection for preventing basal cell and squamous cell skin cancers. *Cochrane Database Syst. Rev.,* **2016**, *7*, CD011161.

[18] Green, A.C.; Williams, G.M.; Logan, V.; Strutton, G.M. Reduced melanoma after regular sunscreen use: randomized trial follow-up. *J. Clin. Oncol.,* **2011**, *29*(3), 257-263.

[19] Ghiasvand, R.; Weiderpass, E.; Green, A.C.; Lund, E.; Veierød, M.B. Sunscreen Use and Subsequent Melanoma Risk: A Population-Based Cohort Study. *J. Clin. Oncol.,* **2016**, JCO675934.

[20] Kim, S.; Seock, Y-K. Impacts of health and environmental consciousness on young female consumers' attitude towards and purchase of natural beauty products. *Int. J. Consum. Stud.,* **2009**, *33*(6), 627-638.

[21] Gago-Ferrero, P.; Alonso, M.B.; Bertozzi, C.P.; Marigo, J.; Barbosa, L.; Cremer, M.; Secchi, E.R.; Domit, C.; Azevedo, A.; Lailson-Brito, J., Jr; Torres, J.P.; Malm, O.; Eljarrat, E.; Díaz-Cruz, M.S.; Barceló, D. First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. *Environ. Sci. Technol.,* **2013**, *47*(11), 5619-5625.

[22] Gago-Ferrero, P.; Díaz-Cruz, M.S.; Barceló, D. An overview of UV-absorbing compounds (organic UV filters) in aquatic biota. *Anal. Bioanal. Chem.,* **2012**, *404*(9), 2597-2610.

[23] Kunz, P.Y.; Gries, T.; Fent, K. The ultraviolet filter 3-benzylidene camphor adversely affects reproduction in fathead minnow (Pimephales promelas). *Toxicol. Sci.,* **2006**, *93*(2), 311-321.

[24] Weisbrod, C.J.; Kunz, P.Y.; Zenker, A.K.; Fent, K. Effects of the UV filter benzophenone-2 on reproduction in fish. *Toxicol. Appl. Pharmacol.,* **2007**, *225*(3), 255-266.

[25] Sánchez-Quiles, D.; Tovar-Sánchez, A. Sunscreens as a source of hydrogen peroxide production in coastal waters. *Environ. Sci. Technol.,* **2014**, *48*(16), 9037-9042.

[26] Danovaro, R.; Bongiorni, L.; Corinaldesi, C.; Giovannelli, D.; Damiani, E.; Astolfi, P.; Greci, L.; Pusceddu, A. Sunscreens cause coral bleaching by promoting viral infections. *Environ. Health Perspect.,* **2008**, *116*(4), 441-447.

[27] E.E.A.P., Environmental effects of ozone depletion and its interactions with climate change: Progress report, 10 2016. *Photochem. Photobiol. Sci.,* **2017**, 10.1039/C7PP90001E.

[28] Tedetti, M.; Sempéré, R. Penetration of ultraviolet radiation in the marine environment. A review. *Photochem. Photobiol.,* **2006**, *82*(2), 389-397.

[29] Shick, J.M.; Dunlap, W.C. Mycosporine-like amino acids and related Gadusols: biosynthesis, acumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.,* **2002**, *64*, 223-262.

[30] Leach, C.M. Ultraviolet-absorbing substances associated with light-induced sporulation in fungi. *Can. J. Bot.,* **1965**, *43*(2), 185-200.

[31] Hartmann, A.; Holzinger, A.; Ganzera, M.; Karsten, U. Prasiolin, a new UV-sunscreen compound in the terrestrial green macroalga Prasiola calophylla (Carmichael ex Greville) Kutzing (Trebouxiophyceae, Chlorophyta). *Planta,* **2015**.

[32] Figueroa, F.L.; Korbee, N.; Abdala, R.; Jerez, C.G.; López-de la Torre, M.; Güenaga, L.; Larrubia, M.A.; Gómez-

Pinchetti, J.L. Biofiltration of fishpond effluents and accumulation of N-compounds (phycobiliproteins and mycosporine-like amino acids) versus C-compounds (polysaccharides) in Hydropuntia cornea (Rhodophyta). *Mar. Pollut. Bull.,* **2012**, *64*(2), 310-318.

[33] Barufi, J.B.; Mata, M.T.; Oliveira, M.C.; Figueroa, F.L. Nitrate reduces the negative effect of UV radiation on photosynthesis and pigmentation in Gracilaria tenuistipitata (Rhodophyta): the photoprotection role of mycosporine-like amino acids. *Phycologia,* **2012**, *51*(6), 636-648.

[34] Barufi, J.B.; Korbee, N.; Oliveira, M.C.; Figueroa, F.L. Effects of N supply on the accumulation of photosynthetic pigments and photoprotectors in Gracilaria tenuistipitata (Rhodophyta) cultured under UV radiation. *J. Appl. Phycol.,* **2011**, *23*(3), 457-466.

[35] Korbee, N.; Huovinen, P.; Figueroa, F.L.; Aguilera, J.; Karsten, U. Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two Porphyra species (Bangiales, Rhodophyta). *Mar. Biol.,* **2005**, *146*(4), 645-654.

[36] Peinado, N.K.; Diaz, R.T.; Figueroa, F.L.; Helbling, E.W. Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in Porphyra columbina (Rhodophyta) from Patagonia, Argentina. *J. Phycol.,* **2004**, *40*(2), 248-259.

[37] Huovinen, P.; Gomez, I.; Figueroa, F.L.; Ulloa, N.; Morales, V.; Lovengreen, C. Ultraviolet-absorbing mycosporine-like amino acids in red macroalgae from Chile. *Bot. Mar.,* **2004**, *47*(1), 21-29.

[38] Hoyer, K.; Karsten, U.; Wiencke, C. Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. *Mar. Biol.,* **2002**, *141*(4), 619-627.

[39] Karsten, U.; Sawall, T.; Hanelt, D.; Bischof, K.; Figueroa, F.L.; Flores-Moya, A.; Wiencke, C. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot. Mar.,* **1998**, *41*(5), 443-453.

[40] Singh, S.P.; Kumari, S.; Rastogi, R.P.; Singh, K.L.; Sinha, R.P. Mycosporine-like amino acids (MAAs): chemical structure, biosynthesis and significance as UV-absorbing/screening compounds. *Indian J. Exp. Biol.,* **2008**, *46*(1), 7-17.

[41] Portwich, A.; Garcia-Pichel, F. Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium Chlorogloeopsis sp. strain PCC 6912. *Phycologia,* **2003**, *42*(4), 384-392.

[42] Favre-Bonvin, J.; Bernillon, J.; Salin, N.; Arpin, N. Biosynthesis of mycosporines: Mycosporine glutaminol in Trichothecium roseum. *Phytochemistry,* **1987**, *26*(9), 2509-2514.

[43] Sinha, R.P.; Ambasht, N.K.; Sinha, J.P.; Häder, D-P. Wavelength-dependent induction of a mycosporine-like amino acid in a rice-field cyanobacterium, Nostoc commune: role of inhibitors and salt stress. *Photochem. Photobiol. Sci.,* **2003**, *2*(2), 171-176.

[44] Shick, J.M.; Romaine-Lioud, S.; Romaine-Lioud, S.; Ferrier-Pagès, C.; Gattuso, J.P. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral Stylophora pistillata despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.,* **1999**, *44*(7), 1667-1682.

[45] Balskus, E.P.; Walsh, C.T. The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science,* **2010**, *329*(5999), 1653-1656.

[46] Gao, Q.; Garcia-Pichel, F. An ATP-grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in Nostoc punctiforme ATCC 29133. *J. Bacteriol.,* **2011**, *193*(21), 5923-5928.

[47] Singh, S.P.; Klisch, M.; Sinha, R.P.; Häder, D.P. Genome mining of mycosporine-like amino acid (MAA) synthesizing and non-synthesizing cyanobacteria: A bioinformatics study. *Genomics,* **2010**, *95*(2), 120-128.

[48] Rosic, N.N. Phylogenetic analysis of genes involved in mycosporine-like amino acid biosynthesis in symbiotic dinoflagellates. *Appl. Microbiol. Biotechnol.,* **2012**, *94*(1), 29-37.

[49] Zhang, L.; Li, L.; Wu, Q. Protective effects of mycosporine-like amino acids of Synechocystis sp. PCC 6803 and their partial characterization. *J. Photochem. Photobiol. B,* **2007**, *86*(3), 240-245.

[50] Spence, E.; Dunlap, W.C.; Shick, J.M.; Long, P.F. Redundant pathways of sunscreen biosynthesis in a cyanobacterium. *ChemBioChem,* **2012**, *13*(4), 531-533.

[51] Pope, M.A.; Spence, E.; Seralvo, V.; Gacesa, R.; Heidelberger, S.; Weston, A.J.; Dunlap, W.C.; Shick, J.M.; Long, P.F. O-Methyltransferase is shared between the pentose phosphate and shikimate pathways and is essential for mycosporine-like amino acid biosynthesis in Anabaena variabilis ATCC 29413. *ChemBioChem,* **2015**, *16*(2), 320-327.

[52] Asamizu, S.; Xie, P.; Brumsted, C.J.; Flatt, P.M.; Mahmud, T. Evolutionary divergence of sedoheptulose 7-phosphate cyclases leads to several distinct cyclic products. *J. Am. Chem. Soc.,* **2012**, *134*(29), 12219-12229.

[53] D'Agostino, P.M.; Javalkote, V.S.; Mazmouz, R.; Pickford, R.; Puranik, P.R.; Neilan, B.A. Comparative profiling and discovery of novel glycosylated mycosporine-like amino acids in two strains of the cyanobacterium Scytonema cf. crispum. *Appl. Environ. Microbiol.,* **2016**, *82*(19), 5951-5959.

[54] Katoch, M.; Mazmouz, R.; Chau, R.; Pearson, L.A.; Pickford, R.; Neilan, B.A. Heterologous production of cyanobacterial mycosporine-like amino acids mycosporine-ornithine and mycosporine-lysine in E. coli. *Appl. Environ. Microbiol.,* **2016**, *82*(20), 6167-6173.

[55] Conde, F.R.; Churio, M.S.; Previtali, C.M. Experimental study of the excited-state properties and photostability of the mycosporine-like amino acid palythine in aqueous solution. *Photochem. Photobiol. Sci.,* **2007**, *6*(6), 669-674.

[56] Conde, F.R.; Churio, M.S.; Previtali, C.M. The deactivation pathways of the excited-states of the mycosporine-like amino acids shinorine and porphyra-334 in aqueous solution. *Photochem. Photobiol. Sci.,* **2004**, *3*(10), 960-967.

[57] Conde, F.R.; Churio, M.S.; Previtali, C.M. The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. *J. Photochem. Photobiol. B,* **2000**, *56*(2-3), 139-144.

[58] Whitehead, K.; Hedges, J.I. Photodegradation and photosensitization of mycosporine-like amino acids. *J. Photochem. Photobiol. B,* **2005**, *80*(2), 115-121.

[59] Chalker, B.E.; Dunlap, W.C.; Oliver, J.K. Bathymetric adaptations of reef-building corals at davies reef, great barrier reef, Australia. II. Light saturation curves for photosynthesis and respiration. *J. Exp. Mar. Biol. Ecol.,* **1983**, *73*(1), 37-56.

[60] Mason, D.S.; Schafer, F.; Shick, J.M.; Dunlap, W.C. Ultraviolet radiation-absorbing mycosporine-like amino acids (MAAs) are acquired from their diet by medaka fish (Oryzias latipes) but not by SKH-1 hairless mice. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.,* **1998**, *120*(4), 587-598.

[61] Starcevic, A.; Akthar, S.; Dunlap, W.C.; Shick, J.M.; Hranueli, D.; Cullum, J.; Long, P.F. Enzymes of the shikimic acid pathway encoded in the genome of a basal metazoan, Nematostella vectensis, have microbial origins. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(7), 2533-2537.

[62] Starcevic, A.; Dunlap, W.C.; Cullum, J.; Shick, J.M.; Hranueli, D.; Long, P.F. Gene expression in the scleractinian Acropora microphthalma exposed to high solar irradiance reveals elements of photoprotection and coral bleaching. *PLoS One,* **2010**, *5*(11), e13975.

[63] Tartarotti, B.; Laurion, I.; Sommaruga, R. Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient. *Limnology and Oceanography,* 2001 Sep;*46*(6), 1546-1552.

[64] Dunlap, W.C.; Shick, J.M. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef oragnisms: a biochemical and environmental perspective. *J. Phycol.,* **1998**, *34*(3), 418-430.

[65] Tartarotti, B.; Sommaruga, R. Seasonal and ontogenetic changes of mycosporine-like amino acids in planktonic organisms from an alpine lake. *Limnol. Oceanogr.,* **2006**, *51*(3), 1530-1541.

[66] Ha, S-Y.; Lee, Y.; Kim, M-S.; Kumar, K.S.; Shin, K-H. Seasonal Changes in Mycosporine-Like Amino Acid Production Rate with Respect to Natural Phytoplankton Species Composition. *Mar. Drugs,* **2015**, *13*(11), 6740-6758.

[67] Dunlap, W.C.; Chalker, B.E.; Oliver, J.K. Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. *J. Exp. Mar. Biol. Ecol.,* **1986**, *104*(1), 239-248.

[68] Peinado, N.K.; Abdala Díaz, R.T.; Figueroa, F.L.; Helbling, E.W. Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in porphyra Columbina (rhodophta) from Patagonia Argentina. *J. Phycol.,* **2004**, *40*(2), 248-259.

[69] Torregiani, J.H.; Lesser, M.P. The effects of short-term exposures to ultraviolet radiation in the Hawaiian Coral Montipora verrucosa. *J. Exp. Mar. Biol. Ecol.,* **2007**, *340*(2), 194-203.

[70] Misonou, T.; Saitoh, J.; Oshiba, S.; Tokitomo, Y.; Maegawa, M.; Inoue, Y.; Hori, H.; Sakurai, T. UV-absorbing substance in the red alga Porphyra yezoensis (Bangiales, Rhodophyta) block thymine photodimer production. *Mar. Biotechnol. (NY),* **2003**, *5*(2), 194-200.

[71] Adams, N.L.; Shick, J.M. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin Strongylocentrotus droebachiensis. *Mar. Biol.,* **2001**, *138*(2), 267-280.

[72] Adams, N.L.; Shick, J.M. Mycosporine-like Amino Acids Provide Protection Against Ultraviolet Radiation in Eggs of the Green Sea Urchin Strongylocentrotus droebachiensis. *Photochem. Photobiol.,* **1996**, *64*(1), 149-158.

[73] Olsson-Francis, K.; Watson, J.S.; Cockell, C.S. Cyanobacteria isolated from the high-intertidal zone: a model for studying the physiological prerequisites for survival in low Earth orbit. *Int. J. Astrobiol.,* **2013**, *12*(4), 292-303.

[74] Feng, Y.N.; Zhang, Z.C.; Feng, J.L.; Qiu, B.S. Effects of UV-B radiation and periodic desiccation on the morphogenesis of the edible terrestrial cyanobacterium Nostoc flagelliforme. *Appl. Environ. Microbiol.,* **2012**, *78*(19), 7075-7081.

[75] Bhatia, S.; Garg, A.; Sharma, K.; Kumar, S.; Sharma, A.; Purohit, A.P. Mycosporine and mycosporine-like amino acids: A paramount tool against ultra violet irradiation. *Pharmacogn. Rev.,* **2011**, *5*(10), 138-146.

[76] Wright, D.J.; Smith, S.C.; Joardar, V.; Scherer, S.; Jervis, J.; Warren, A.; Helm, R.F.; Potts, M. UV irradiation and desiccation modulate the three-dimensional extracellular matrix of Nostoc commune (Cyanobacteria). *J. Biol. Chem.,* **2005**, *280*(48), 40271-40281.

[77] Oren, A.; Gunde-Cimerman, N. Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? *FEMS Microbiol. Lett.,* **2007**, *269*(1), 1-10.

[78] Rastogi, R.P.; Incharoensakdi, A. Characterization of UV-screening compounds, mycosporine-like amino acids, and scytonemin in the cyanobacterium Lyngbya sp. CU2555. *FEMS Microbiol. Ecol.,* **2013**.

[79] Takamatsu, S.; Hodges, T.W.; Rajbhandari, I.; Gerwick, W.H.; Hamann, M.T.; Nagle, D.G. Marine natural products as novel antioxidant prototypes. *J. Nat. Prod.,* **2003**, *66*(5), 605-608.

[80] Matsui, K.; Nazifi, E.; Hirai, Y.; Wada, N.; Matsugo, S.; Sakamoto, T. The cyanobacterial UV-absorbing pigment scytonemin displays radical-scavenging activity. *J. Gen. Appl. Microbiol.,* **2012**, *58*(2), 137-144.

[81] Nazifi, E.; Wada, N.; Yamaba, M.; Asano, T.; Nishiuchi, T.; Matsugo, S.; Sakamoto, T. Glycosylated porphyra-334 and palythine-threonine from the terrestrial cyanobacterium Nostoc commune. *Mar. Drugs,* **2013**, *11*(9), 3124-3154.

[82] de la Coba, F.; Aguilera, J.; Figueroa, F.L.; de Galvez, M.V.; Herrera, E. Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. *J. Appl. Phycol.,* **2009**, *21*(2), 161-169.

[83] Wada, N.; Sakamoto, T.; Matsugo, S. Mycosporine-Like Amino Acids and Their Derivatives as Natural Antioxidants. *Antioxidants,* **2015**, *4*(3), 603-646.

[84] Oren, A. Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiol. J.,* **1997**, *14*(3), 231-240.

[85] Portwich, A.; Garcia-Pichel, F. Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium chlorogloeopsis PCC 6912. *Arch. Microbiol.,* **1999**, *172*(4), 187-192.

[86] Kogej, T.; Gostinčar, C.; Volkmann, M.; Gorbushina, A.A.; Gunde-Cimerman, N. Mycosporines in Extremophilic Fungi—Novel Complementary Osmolytes? *Environ. Chem.,* **2006**, *3*(2), 105-110.

[87] Singh, S.P.; Klisch, M.; Sinha, R.P.; Häder, D.P. Effects of abiotic stressors on synthesis of the mycosporine-like amino acid shinorine in the Cyanobacterium Anabaena variabilis PCC 7937. *Photochem. Photobiol.,* **2008**, *84*(6), 1500-1505.

[88] Waditee-Sirisattha, R.; Kageyama, H.; Sopun, W.; Tanaka, Y.; Takabe, T. Identification and upregulation of biosynthetic genes required for accumulation of Mycosporine-2-glycine under salt stress conditions in the halotolerant cyanobacterium Aphanothece halophytica. *Appl. Environ. Microbiol.,* **2014**, *80*(5), 1763-1769.

[89] Sivalingam, P.M.; Ikawa, T.; Nisizawa, K. Physiological roles of a substance 334 in algae. *Bot. Mar.,* **1976**, *19*, 9-21.

[90] Fernandes, S.C.; Alonso-Varona, A.; Palomares, T.; Zubillaga, V.; Labidi, J.; Bulone, V. Exploiting Mycosporines as Natural Molecular Sunscreens for the Fabrication of UV-Absorbing Green Materials. *ACS Appl. Mater. Interfaces,* **2015**, *7*(30), 16558-16564.

[91] Oyamada, C.; Kaneniwa, M.; Ebitani, K.; Murata, M.; Ishihara, K. Mycosporine-like amino acids extracted from scallop (Patinopecten yessoensis) ovaries: UV protection and growth stimulation activities on human cells. *Mar. Biotechnol. (NY),* **2008**, *10*(2), 141-150.

[92] Kim, S.; You, D.H.; Han, T.; Choi, E.M. Modulation of viability and apoptosis of UVB-exposed human keratinocyte HaCaT cells by aqueous methanol extract of laver (Porphyra yezoensis). *J. Photochem. Photobiol. B,* **2014**, *141*, 301-307.

[93] Ryu, J.; Park, S.J.; Kim, I.H.; Choi, Y.H.; Nam, T.J. Protective effect of porphyra-334 on UVA-induced photoaging in human skin fibroblasts. *Int. J. Mol. Med.,* **2014**, *34*(3), 796-803.

[94] Choi, Y-H.; Yang, D.J.; Kulkarni, A.; Moh, S.H.; Kim, K.W. Mycosporine-Like Amino Acids Promote Wound Healing through Focal Adhesion Kinase (FAK) and Mitogen-Activated Protein Kinases (MAP Kinases) Signaling Pathway in Keratinocytes. *Mar. Drugs,* **2015**, *13*(12), 7055-7066.

[95] Torres, A.; Hochberg, M.; Pergament, I.; Smoum, R.; Niddam, V.; Dembitsky, V.M.; Temina, M.; Dor, I.; Lev, O.; Srebnik, M.; Enk, C.D. A new UV-B absorbing mycosporine with photo protective activity from the lichenized ascomycete Collema cristatum. *European Journal of Biochemistry / FEBS,* **2004**, *271*(4), 780-784.

[96] Valencia, A.; Kochevar, I.E. Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. *J. Invest. Dermatol.,* **2008**, *128*(1), 214-222.

[97] Rastogi, R.P.; Madamwar, D.; Incharoensakdi, A. Sunscreening bioactive compounds mycosporine-like amino acids in naturally occurring cyanobacterial biofilms: role in photoprotection. *J. Appl. Microbiol.,* **2015**, *119*(3), 753-762.

[98] Andreguetti, D.; Stein, E.M.; Pereira, C.M.; Pinto, E.; Colepicolo, P. Antioxidant properties and UV absorbance pattern of mycosporine-like amino acids analogs synthesized in an environmentally friendly manner. *J. Biochem. Mol. Toxicol.,* **2013**, *27*(6), 305-312.

[99] Suh, S-S.; Hwang, J.; Park, M.; Seo, H.H.; Kim, H-S.; Lee, J.H.; Moh, S.H.; Lee, T-K. Anti-inflammation activities of mycosporine-like amino acids (MAAs) in response to UV radiation suggest potential anti-skin aging activity. *Mar. Drugs,* **2014**, *12*(10), 5174-5187.

[100] de la Coba, F.; Aguilera, J.; de Gálvez, M.V.; Alvarez, M.; Gallego, E.; Figueroa, F.L.; Herrera, E. Prevention of the ultraviolet effects on clinical and histopathological changes, as well as the heat shock protein-70 expression in mouse skin by topical application of algal UV-absorbing compounds. *J. Dermatol. Sci.,* **2009**, *55*(3), 161-169.

[101] Saw, C.L.; Huang, M.T.; Liu, Y.; Khor, T.O.; Conney, A.H.; Kong, A.N. Impact of Nrf2 on UVB-induced skin inflammation/photoprotection and photoprotective effect of sulforaphane. *Mol. Carcinog.,* **2011**, *50*(6), 479-486.

[102] Tao, S.; Justiniano, R.; Zhang, D.D.; Wondrak, G.T. The Nrf2-inducers tanshinone I and dihydrotanshinone protect human skin cells and reconstructed human skin against solar simulated UV. *Redox Biol.,* **2013**, *1*, 532-541.

[103] Gacesa, R.; Dunlap, W.C.; Long, P.F. Bioinformatics analyses provide insight into distant homology of the Keap1-Nrf2 pathway. *Redox Biol.,* **2015**, *88*(Pt B), 373-380.

[104] Ryu, J.; Kwon, M.J.; Nam, T.J. Nrf2 and NF-κB Signaling Pathways Contribute to Porphyra-334-Mediated Inhibition of UVA-Induced Inflammation in Skin Fibroblasts. *Mar. Drugs,* **2015**, *13*(8), 4721-4732.

[105] Tosato, M.G.; Orallo, D.E.; Churio, M.S.; Martin, A.A.; Soto, C.A.; Dicelio, L.E. Influence of mycosporine-like amino acids and gadusol on the rheology and Raman spectroscopy of polymer gels. *Biorheology,* **2014**, *51*(4-5), 315-328.

[106] Pfeifer, G.P.; Besaratinia, A. UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. *Photochem. Photobiol. Sci*., **2012**, 11(1), 90-97.

[107] Huang, X.X.; Scolyer, R.A.; Abubakar, A.; Halliday, G.M. Human 8-oxoguanine-DNA glycosylase-1 is downregulated in human basal cell carcinoma. *Mol. Genet. Metab.,* **2012**, *106*(1), 127-130.

[108] Petersen, B.; Datta, P.; Philipsen, P.A.; Wulf, H.C. Sunscreen use and failures--on site observations on a sunholiday. *Photochem. Photobiol. Sci.,* **2013**, *12*(1), 190-196.

[109] Hartmann, A.; Gostner, J.; Fuchs, J.E.; Chaita, E.; Aligiannis, N.; Skaltsounis, L.; Ganzera, M. Inhibition of Collagenase by Mycosporine-like Amino Acids from Marine Sources. *Planta Med.,* **2015**, *81*(10), 813-820.

[110] Bandaranayake, W.M. Mycosporines: are they nature's sunscreens? *Nat. Prod. Rep.,* **1998**, *15*(2), 159-172.

[111] Cardozo, K.H.; Guaratini, T.; Barros, M.P.; Falcão, V.R.; Tonon, A.P.; Lopes, N.P.; Campos, S.; Torres, M.A.; Souza, A.O.; Colepicolo, P.; Pinto, E. Metabolites from algae with economical impact. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.,* **2007**, *146*(1-2), 60-78.

[112] Torres, A.; Enk, C.D.; Hochberg, M.; Srebnik, M. Porphyra-334, a potential natural source for UVA protective sunscreens. *Photochem. Photobiol. Sci.,* **2006**, *5*(4), 432-435.

[113] Scheuer, P.J. Some marine ecological phenomena: chemical basis and biomedical potential. *Science,* **1990**, *248*(4952), 173-177.

[114] Schmid, D.; Schürch, C.; Zülli, F. *UV-A sunscreen from red algae for protection against premature skin aging*; Cosmetics and Toiletries Manufacture Worldwide, **2003**.

[115] Losantos, R.; Funes-Ardoiz, I.; Aguilera, J.; Herrera-Ceballos, E.; Garcia-Iriepa, C.; Campos, P.J.; Sampedro, D. *Rational Design and Synthesis of Efficient Sunscreens To Boost the Solar Protection Factor,* International ed.; Angewandte Chemie, **2017**. in English

[116] Agency, E.C. Community Rolling Action Plan (CoRAP) List. Available at: https://echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/coraptable [Accessed date: 10th May 2017].

[117] BASF BASF Sunscreen Simulator. Available at: https://www.sunscreensimulator.basf.com/Sunscreen_Simulator/Login_show.action [Accessed date: 10th May 2017],