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Role of secondary metabolites and pigments in the epidermal tissues, ripe ovaries, viscera, gut contents and diet of the sea cucumber *Holothuria atra*

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Abstract The epidermal tissues, ovaries, viscera, gut contents, and the diet of *Holothuria atra* (Jaeger), collected from the Great Barrier Reef (GBR), Australia, just prior to spawning, contained carotenoid pigments, mycosporine-like amino acids (MAAs) and three other metabolites in varying proportions. Mycosporine-glycine (mycosporine-gly), palythine, asterina-330, shinorine, mycosporine-2-gly, porphyra-334, and palythinol were present in the epidermal tissues. Mycosporine-gly was the major MAA in the epidermal tissues, gut contents and the diet, and the only MAA present in the ripe ovaries and the viscera. Gadusol, a metabolite with antioxidant properties, which co-occurs with MAAs, and in certain instances with trace amounts of 6-deoxygadusol in unfertilised and fertilised eggs and developing larvae of some marine invertebrates and vertebrates, was absent from *H. atra*. However, 6-deoxygadusol, with similar physical and chemical properties to gadusol, and the proposed biosynthetic precursor of MAAs, was present, but only in the ovaries. This is first report of the presence of this proposed biogenetic precursor of MAAs in significant amounts in a marine invertebrate. Approximately 90% of the total carotenoids of the epidermal tissues, ovaries and viscera of *H. atra* are highly oxidised, the main component being astaxanthin followed by canthaxanthin. These were the major carotenoids present in the ovaries and the total carotenoid content was highest in the ovaries. β-carotene, a common egg carotenoid, was present in trace quantities in the ovaries but, along with the xanthophylls lutein and zeaxanthin, it occurred in significant amounts in the gut contents and the diet of *H. atra*. Carotenoid patterns in the epidermal tissues and viscera were strikingly similar, containing β-carotene, canthaxanthin, astaxanthin, zeaxanthin, lutein, phoenicoxanthin and echinenone. The alkaloids homarine and isomeric trigonelline, (structurally unrelated to MAAs) and 6-deoxygadusol were present only in the ovaries and the viscera. It is suggested that these metabolites and pigments are either involved with photoprotection or reproduction, or associated with both processes. The origin, biogenetic relationships and the roles of these metabolites and pigments in *H. atra* are discussed.

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Introduction

The black sea cucumber *Holothuria atra* (Jaeger) is one of the most widely distributed of the lower invertebrates in the Indo-West Pacific, and is the most common aspidochirotid holothurian on tropical reef flats (Conand and De Ridder 1990) and on the Great Barrier Reef (GBR) Australia. The organism is ubiquitous, except on outer reef slopes; it varies from 2 to 60 cm in length and weighs 10 to 2000 g. Tropical holothurians often feed on an array of food items such as organic contents of sediment, coral rubble, living microorganisms, filamentous blue-green and red algae, diatoms, foraminiferans, and detritus, or by filtration of sea water (Bakus 1973; Feral and Cherbonnier 1986). Only a small fraction of the holothurians, such as *H. atra* reproduce both asexually and develop mature gonads (Chao et al. 1993). *H. atra* divides by fission, mainly in the cooler months, and spawns in warmer months as well as in winter (Conand and De Ridder 1990; Uthicke 1997). In the Northern Hemisphere, however, fission occurs mainly in summer (Chao et al. 1993). At several low-latitude sites in the tropical Indo-Pacific, mature gonads of *H. atra* have been found through out the year (Pearse 1968). Knowledge of the biochemistry of tropical holothurians is limited, except for research on toxins (Bakus 1973;

James 1986). It has been demonstrated that poisonous toxins can eradicate undesirable organisms from fish farms (James 1986). Antifungal triterpenoid oligoglycosides (Kobayashi et al. 1991; Anjaneyulu and Raju 1996) phospholipids and fatty acids (Svetashev et al. 1991) have been characterized from holothurians. Here we report the occurrence of metabolites and pigments and suggest the significance of their presence in the dorsal skin, gut contents, viscera, and mature eggs of *H. atra*, and in the feeding sediment of its natural habitat.

Materials and methods

Biological material

Three specimens of *Holothuria atra* (Jaeger), 20 to 25 cm long and weighing ≃650 to 800 g (gonad index values of 38, 41 and 43) were collected by SCUBA diving at a depth of ∼2 m from Palfrey Island in the Lizard Island group, GBR, Australia, in December 1995, a week before a mass spawning phenomenon was observed. The sea cucumbers were kept alive in a well-aerated 1000-litre tank with running seawater for 14 d. The bottom of the tank contained sand, coral rubble and sediment from their natural habitat, and five lots of sediment (each 1000 g) was collected for chemical analysis. The sea cucumbers were frozen (−20 °C) for 6 h and the "spaghetti-like" ovaries (dark orange/red in colour), black epidermal tissues, viscera and gut contents were removed. Handling was kept to a minimum to minimize the release of a burgundy-coloured pigment into the surrounding water (release of this pigment is postulated to be a defence mechanism and a response to injury).

Extraction

Wherever possible, all operations were carried out at 5 to 10 °C, in subdued light and under nitrogen. Rotary evaporation was in vacuo at 35 to 40 °C. In a typical experiment, epidermal tissues (20 g), ovaries (150 g), and viscera (40 g) obtained from a single individual were homogenized in methanol. The homogenized organs, gut contents (10 g), and sediment (5 × 500 g) were soaked in methanol (500 ml) for 3 d to extract the mycosporines, 6-deoxygadosol, homarine, and trigonelline, and the remaining samples were extracted with acetone (500 ml) to separate the carotenoids. What remained of each sample was re-extracted with the respective solvents until the final extraction yielded ≤2% of the total metabolites or pigments, as determined by high-pressure liquid chromatography (HPLC) and/or UV spectroscopy. The combined supernatants were evaporated to dryness, suspended in 50 ml methanol or 50 ml acetone, filtered to remove salt, and concentrated to 25 ml.

Separation and identification

Analytical, semi-preparative HPLC and column chromatography, and the separation of MAAs and 6-deoxygadusol from the extracts of the ovaries were accomplished by methods described previously (Shick et al. 1992; Bandaranayake et al. 1996b, 1997); 6-deoxygadusol and gadusol have similar chromatographic behavior. Semi-preparative HPLC fractionation was also accomplished with a Waters pump Model 6000 A, and Waters Model 455 variable-wavelength UV detector. UV-absorption spectra were recorded with a GBC Model 918 or Philips Model PU 8720 UV/VIS spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer, using residual solvent as the internal standard. The mass spectra, MS (FAB) were recorded on a VIGI A otospec spectrometer, using nitrobenzyl alcohol as the matrix.

Trigonelline (which co-eluted with homarine) was separated by repeated column chromatography on Dowex AG 50W 8H forms (100 to 200-mesh and 200 to 400-mesh) followed by AG X8 acetate form (200 to 400-mesh, Bio-Rad). The columns were eluted with H_2O, 0.1 *M* HCl and 0.5 *M* acetic acid, respectively, to yield these two compounds as colourless amorphous solids. Extraction and isolation of the carotenoids were carried out according to the modified method of Matsuno and Tsushima (1995). The acetone extracts were transferred to 50 ml ether/*n*-hexane (1:1), with water, and the cooled solution (5 °C) was concentrated to 25 ml by bubbling nitrogen. Carotenoid-pigment analysis was accomplished by thin-layer chromatography (TLC) on Polygram SIL G/UV 254 (20 cm × 20 cm × 0.25 mm) silica gel sheets. Separation of pigments by preparatory thin-layer chromatography (PLC), was on 230 to 400-mesh Kieselgel silica gel plates, 20 × 20 cm × 1 mm. The developing solvents were acetone/benzene (1:9), dichloromethane/ethylacetate (4:1) (Miki et al. 1982), and acetone/n-hexane (1:2), (Matsuno and Tsushima 1995). Palythine, asterina-330, and palythinol were identified by comparison of their UV characteristics and co-chromatography (HPLC) with authentic samples. Shinorine, mycosporine-2-glycine and porphyra-334 were identified by comparison with reported UV-absorption characteristics (Bandaranayake 1998) and HPLC retention times (Shick et al. 1992). Mycosporine-gly, 6-deoxygadusol (Chioccara et al. 1980), homarine and trigonelline were characterized by their UV, ^{13}C, and/or ^{1}H, NMR, mass spectral (MS) data and comparison with authentic samples. An authentic sample of 6-deoxygadusol was obtained by acid hydrolysis of mycosporine-gly isolated from *Holothuria atra*.

Carotenoids were identified by comparison of visible spectra in different solvents (Miki et al. 1982; Grung et al. 1993), partition characteristics of the carotenoids, comparison by thin-layer chromatography of reported R_f values (Matsuno and Tsushima 1995), and with authentic samples (Hoffman-La Roche, Basle-Switzerland); and by the iodine isomerisation test and reduction with $NaBH_4$, treatment with alcoholic potassium hydroxide, the epoxide test, and the allylic OH-activity test.

The concentrations of MAAs were determined from their molar extinction coefficients (ε) at the wavelengths of maximum absorption. The ε values used were: $\varepsilon_{330} = 43\,900$ (mycosporine-2-glycine and shinorine) (Shick et al. 1992) $\varepsilon_{320} = 36\,200$ (palythine), $\varepsilon_{330} = 43\,900$ (asterina-330), $\varepsilon_{310} = 28\,800$ (mycosporine-gly), $\varepsilon_{334} = 42\,300$ (porphyra-334) $\varepsilon_{332} = 43\,500$ (palythinol) (Bandaranayake 1998). The corresponding value for 6-deoxygadusol was $\varepsilon_{268} = 12\,800$, and $\varepsilon_{270} = 8\,200$ for homarine and trigonelline. The results reported for estimation of the MAAs by HPLC are averages of three injections for different samples from each individual. 6-deoxygadusol, homarine and trigonelline had similar retention times on HPLC and co-eluted with several other metabolites of similar UV absorption; hence, they could not be quantified accurately by HPLC area-integration. Estimates of these three compounds were obtained by quantitative column-chromatography. Quantitative determination of total carotenoids was based on the extinction coefficient at maximum absorbance of astaxanthin. The concentration of known pigments was measured using extinction coefficients reported in the literature (Foppen 1971).

Results

Quantitative analysis of the organs and gut content of the three individuals of *Holothuria atra* examined revealed very little variation in the content of secondary metabolites and pigments. HPLC separation of the methanolic extracts of epidermal tissues indicated the presence of several MAAs in quantifiable amounts. Mycosporine-gly, and palythine, the two most common

MAAs among marine organisms, were the most predominant in the epidermis of *H. atra*, followed by asterina-330, and shinorine. Mycosporine-2-glycine, porphyra 334 and palythinol were minor components (see Bandaranayake et al. 1996b, 1997, and Bandaranayake 1998 for structures). Although HPLC analysis of the gut content and the diet suggested the presence of several mycosporines, only mycosporine-gly and palythine could be quantified. In contrast, mycosporine-gly was the only MAA in the ripe ovaries and viscera. The UV-absorption maximum of the ovaries indicated the presence of metabolites with strong UV absorption at 310 nm and a secondary maximum at 270 nm. The extract was purified on ion-exchange columns to produce two major fractions with UV-absorption maxima at 268 and 310 nm. The UV-absorbing pigment absorbing at λ_{max} 310 nm was purified to homogeneity as described in Bandaranayake et al. 1996b, and was found to be a single component identified as mycosporine-gly from its UV spectrum, ^{1}H, ^{13}C NMR and MS analysis and comparison with an authentic sample. No other MAAs were detected in the extracts. When subjected to ion-exchange chromatography (Bandaranayake et al. 1997), the fraction with an absorption maximum at 268 nm yielded three pure metabolites, two of which were crystalline solids with maximum absorption at 270 nm. The third, an oil of λ_{max} 268 nm exhibited the same pH-dependent chromophore as gadusol (3,5,6-trihydroxy-5-hydroxymethyl-2-methoxycyclohex-2-ene-1-one), a component of the ovaries and larvae of some invertebrates and vertebrates (Plack et al. 1981; Bandaranayake 1997). The chromatographic behaviour (HPLC), UV, ^{13}C and/or ^{1}H NMR and MS indicated that the solids were homarine (*N*-methylpyridine-2-carboxylic acid) and trigonelline (*N*-methylpyridine-3-carboxylic acid), and the oil was 6-deoxygadusol (3,5-dihydroxy-5-hydroxymethyl-2-methoxycyclohex-2-ene-1-one). Homarine and trigonelline were present only in the viscera and the ovaries, and 6-deoxygadusol was present only in the ovaries. Gadusol was absent from all the samples (Table 1). The presence of these three metabolites was confirmed by comparison with authentic samples. Hydrolysis of mycosporine-gly with 0.01 *M* HCl at 50 °C yielded an intermediate (unidentified), which in turn yielded 6-deoxygadusol; the reaction was complete in $\simeq$12 h.

The concentrations of carotenoids present in the epidermal tissues, ovaries, viscera, gut contents and the diet of *Holothuria atra* are shown in Table 2, together with literature values for other holothurians. β-carotene, a common egg carotenoid, was absent from the ovaries, but was the most abundant carotenoid in the gut contents and sediments. Many other carotenoids present in the diet and gut contents could not be characterized due to their low concentrations. The carotenoid patterns in the epidermal tissues and viscera were strikingly similar, but differed from those in the ovaries. The hydrocarbon β-carotene, the xanthophylls zeaxanthin and lutein and the ketocarotenoids canthaxanthin, astaxanthin, phoenicoxanthin and echinenone were present in the epidermal tissues and viscera while the ovaries were rich in canthaxanthin and astaxanthin. Astaxanthin was the most abundant pigment in all the organs, and the total carotenoid content was highest in the ovaries.

Discussion

The epidermal tissues of *Holothuria atra* contained several MAAs, many in significant amounts (Table 1). Mycosporine-gly, palythine, asterina-330, and shinorine were the major MAAs, while mycosporine-2-glycine, porphyra-334 and palythinol were minor components. The total MAA content was similar in the epidermal tissues and the ovaries but differed in the individual MAAs. Mycosporine-gly is the most widespread MAA among the holothuroids, both taxonomically and in tissue distribution (Shick et al. 1992), and is also the major MAA in *H. atra*. Asterina-330, first characterised from the starfish *Asterina pectinifera*, appears to be common among echinoderms (Bandaranayake 1998). *H. atra*, which has no endosymbiotic algae, obtains food by ingestion of marine sediments and detritus. Mycosporine-gly and palythine were the only quantifiable MAAs present in the gut contents and sediment. Most significantly, the only MAA present in the mature ovaries and the viscera was mycosporine-gly. Immature ovaries of *H. atra* contain mycosporine-gly in very low concentrations, and its concentration in the ovaries increased with maturity (Bandaranayake unpublished results). It appears that the MAAs were accumulated by the organism in the epidermal tissues and the iminomycosporines were transformed into mycosporine-gly, most probably in the digestive tract. Alternatively, while rejecting the imonomycosporines, mycosporne-gly could be selectively accumulated and stored in the viscera and later "transferred" to the ovaries. Shick et al. (1992) and Carroll and Shick (1996) presented indirect evidence for the former hypothesis. The presence of intact bacteria directly associated with the holothurian gut lining has been demonstrated (Deming and Colwell 1982; Ward-Rainey et al. 1996). Several reports have suggested that the gut bacteria metabolises organic matter ingested by *H. atra* (Deming and Colwell 1982; Phillips 1984) and that they are capable of de novo synthesis of MAA precursors (Favre-Bonvin et al. 1987; Haslam 1993) or MAAs (Arai et al. 1994). For example, bacteria could metabolise organic matter ingested by *H. atra*, releasing specific essential amino acids. It is therefore reasonable to predict that while *H. atra* and other marine organisms in non-symbiotic associations obtain their MAAs from their diet, the aerobic bacterial flora in the gut may also be the provider of at least some MAAs, and may be responsible for the interconversion of MAAs such as the transformation of iminomycosporines to mycosporine-gly as observed in *H. atra*.

There seem to be few major taxonomic boundaries to the presence of MAAs. They are present in a broad

Table 1 *Holothuria atra*. Concentration (μmol g^{-1} dry wt) of individual and total mycosporine-like amino acids (*MAAs*) and other metabolites in organs, gut content and diet (– not detected). Concentrations in parentheses were obtained by column chromatography; efficiency of recovery from this process was estimated to be between 55 and 65%: results are corrected for this low efficiency. Determinations by high-performance liquid chromatography (HPLC) are average values from three HPLC analysis

MAAs	Epidermal tissues	Ovaries	Viscera	Gut contents	Diet
Total	4.93 ± .14	4.38 ± 0.10	0.95 ± 0.04	0.18 ± 0.02	0.06 ± 0.02
Mycosporine-glycine	1.57 ± .02	4.38 ± 0.10	0.95 ± .04	0.10 ± 0.02	0.04 ± .02
Palythine	1.03 ± .02	–	–	0.08 ± 0.02	0.03 ± 0.01
Asterina-330	0.58 ± .01	–	–	–	–
Shinorine	0.22 ± .04	–	–	–	–
Mycosporine-2-glycine	0.19 ± .02	–	–	–	–
Porphyra-334	0.18 ± .02	–	–	–	–
Palythinol	0.16 ± .02	–	–	–	–
6-deoxygadusol	–	(4.61)	–	–	–
Homarine	–	(3.93)	(8.20)	–	–
Trigonelline	–	(0.75)	(1.08)	–	–

array of marine species and have been implicated in many biochemical processes (Bandaranayake 1998). However, the two major functions attributed to MAAs are their capacity to act as photo-protective UV filters in various marine and terrestrial organisms (Bandaranayake 1998) and/or to exercise a regulatory effect on sporulation (Trione et al. 1966; Arpin and Bouillant 1981). Whilst evidence supporting the photo-protective defensive role in marine organisms has not been rigorously established (Garcia-Pichel et al. 1993; Bandaranayake 1998), and is limited in the fungi (Brook 1981; Young and Patterson 1982), it appears that sporogenic activity has been well demonstrated for fungal mycosporines (Arpin and Bouillant 1981; Bandaranayake 1998). There is very limited reference to their role in reproduction among marine organisms. Researchers have suggested that the symbionts are responsible for the synthesis of MAAs in symbiotic associations (Bandaranayake 1998). Some holothurians are exceptions (Stochaj et al. 1994); the origin of MAAs in non-symbiotic species is proposed to be dietary (Shick et al. 1992; Gleeson and Wellington 1993; Stochaj et al. 1994; Banaszak and Trench 1995; Carroll and Shick 1996).

While some holothurians are cryptic or nocturnally active, others (e.g. *Holothuria atra*) are active foragers in shallow water in daylight (Hammond 1982; Feral and Cherbonnier 1986) and may require protection from UV radiation. MAAs in the epidermal tissues of *H. atra*, covering ≃310 to 340 nm, certainly could function as broad-spectrum UV-absorbers (Carreto et al. 1990; Gleeson and Wellington 1993; Carroll and Shick 1996). This may well be their function in the epidermal tissues of *H. atra*. A photoprotective role of MAAs in sea urchin eggs has been demonstrated (Adams and Shick 1996; Carroll and Shick 1996). Accumulation of MAAs in the eggs may protect them from UV damage during their planktonic development. The sperm of sea urchin and other invertebrates need remain viable in seawater only long enough to fertilise the eggs, so the duration of their exposure to UV is far less than that of eggs and embryos. This was considered to be the reason for the lack of accumulation of MAAs in sperm of sea urchins (Adams and Shick 1996) and other invertebrates (Chioccara et al. 1980; Karentz et al. 1991; Shick et al. 1992; Carroll and Shick 1996).

If the MAAs were to perform a photoprotective role in the ovaries of *Holothuria atra* and other organisms, then the MAAs present in the epidermis or the diet which cover the UV absorption range of 310 to 334 nm could easily fulfil this role. There would be no need for the organism to use its energy in order to produce mycosporine-gly, with a lower absorption maximum (λ_{max} 310 nm) and a narrower absorption band, to perform the same photoprotective function. The metabolic "cost" associated with this transformation could be high. Our results from *H. atra* and existing data (Chioccara et al. 1980; Grant et al. 1985; Karentz et al. 1991; Shick et al. 1992; Stochaj et al. 1994; Banaszak and Trench 1995; Bandaranayake et al. 1996a; Carroll and Shick 1996; Bandaranayake unpublished results) indicate that UV exposure is not the sole determinant of MAA concentration, and that these compounds may have another function, at least in some organisms.

Most marine organisms contain a complex mixture of MAAs, and some MAAs increase in concentration during exposure to UV radiation. It has been proposed that the concentration of some MAAs does not increase in direct response to UV radiation, but accumulates as a result of seasonal changes in reproductive state or diet (Stochaj et al. 1994). Evidence is available to suggest that at least some MAAs may play a role in reproduction (Chioccara et al. 1980; Grant et al. 1985; Post and Larkum 1993; Adams and Shick 1996; Bandaranayake et al. 1996a, 1997; Bandaranayake unpublished results). Our observations on *Holothuria atra* strongly support the view that while some MAAs have a photoprotective role, others are involved with the reproductive process of this organism. The viscera do not require UV protection. It appears that the viscera serve as "temporary storage depots" until the MAAs are translocated to the reproductive organs. It is significant that mycosporine-gly, one of the most abundant MAAs found amongst marine organisms (Bandaranayake 1998) is the predominant MAA present in the tissues and ovaries of *H. atra*.

Table 2 *Holothuria* spp. Total content (mg/100 g, above columns; *nd* no data) and % composition of individual carotenoids in organs or whole body of various holothurians, and in organs, gut contents, and diet of *H. atra*. Extinction coefficient for astaxanthin was used for all compounds, hence concentrations are approximate (*tr* trace; – not detected)

Carotenoids	*H. leucospilota*[a]*					*H. moebi*[b]	*H. pervicas*[b]	*H. tibulosa*[c]		*H. atra*			
	Viscera (9.4 mg)	Gonad (20.1 mg)	Body wall (1.2 mg)	Testes (nd)	Ovaries (nd)	Whole body (0.30 mg)	Whole body (nd)	Whole body (1.0 mg)	Epidermal tissue (10.08 mg)	Viscera (8.0 mg)	Gonad (16.6 mg)	Gut contents (4.9 mg)	Diet (2.0 mg)
β-carotene	15.6	tr	0.9	5.2	1.5	tr	tr	–	3.1	11.0	tr	50.9	62.5
Canthaxanthin	7.0	6.2	24.8	34.2	18.0	39.0	31.2	–	26.0	24	28	1.2	–
Astaxanthin	37.8	73.6	47.9	21.2	55.4	29.0	64.1	–	48.0	46	57	3.0	–
Zeaxanthin	2.7	1.2	2.2	17.3	3.9	23.0	–	–	1.7	2.0	1.5	14.5	9.9
Isozeaxanthin	–	–	–	–	–	–	–	31.5	–	–	–	–	–
Lutein	2.7	1.2	0.3	–	–	–	–	13.6	3.3	–	–	12.8	14.1
Lutein epoxide	–	–	–	–	–	–	–	8.3	–	–	–	–	–
β-cryptoxanthin	–	–	–	–	–	–	–	13.8	–	–	–	–	–
Phoenicoxanthin	2.0	2.4	8.0	–	–	9.0	4.7	–	4.1	8.0	4.0	1.1	–
Astacene	–	–	–	10.4	18.2	–	–	–	–	–	–	–	–
Mutachrome	–	–	–	–	–	–	–	25.5	–	–	–	–	–
Echinenone	1.5	–	1.5	4.9	0.4	–	–	–	2.2	4.0	1.9	3.6	tr
Unidentified	6.0	8.2	11.1	6.5	2.6	–	–	7.3	9.2	5.5	3.2	9.6	8.3

[a] Matsuno and Tsushima (1995); [b] Matsuno et al. (1971); [c] Czeczuga (1984)
* Diatoxanthin, alloxanthin, ketozeaxanthin, pectenolene, 7,8,7^1,8^1-tetrahydroastaxanthin, 7,8-didehydroastaxanthin, fucoxanthin, fucoxanthinol, and iodoxanthin are present in varying quantities in all tissues of *H. leucospilota*

The metabolite gadusol co-exists with MAAs and, in certain instances, with 6-deoxygadusol, in the ovaries and developing larvae of some marine organisms (Chioccara et al. 1980, 1986). With the exception of fish eye-lenses (Bandaranayake et. al. 1994), neither gadusol nor 6-deoxygadusol has been identified in other organs including testes and sperm (Chioccara et al. 1980, 1986; Grant et al. 1985; Bandaranayake et al. 1996a). 6-deoxygadsol and not gadusol was present in the ovaries of *Holothuria atra*, and was absent from the other organs, the gut contents and the sediment. This is the first report of the presence of 6-deoxygadusol, the proposed biosynthetic precursor of MAAs (Favre-Bonvin et al. 1987) in significant quantities in the gonads of a marine invertebrate. Specific assays have shown that gadusol has antioxidant properties similar to the natural antioxidant ascorbic acid (Plack et al. 1981). 6-deoxygadusol, with physical and chemical properties similar to gadusol (Bandaranayake unpublished results), therefore has the potential to act as a biological antioxidant. It may be that 6-deoxygadusol is biosynthesised by the heterotrophic bacterial flora present in the organism, or it may be a product of a metabolic process involving MAAs. The bacteria in the gut of *H. atra* are capable of metabolising ingested organic matter, releasing essential free amino acids. It is therefore reasonable to assume that the aerobic bacterial flora in the gut lining of *H. atra* has the potential to release free amino acids and 6-deoxygadusol from MAAs. Alternatively, enzymes could bring about these transformations. For example, the rapid reaction of MAAs catalysed by an iodide peroxidase that has been purified from the cysts and nauplii of *Artemia* sp. yielded 6-deoxygadusol and free amino acids. Early embryological development in *Artemia* sp. (Grant et. al. 1985) sea urchins (Chioccara et al. 1986) and *Acanthaster planci* (Bandaranayake et. al. 1996a) results in the utilisation or the "decomposition" of MAAs, with subsequent formation of 6-deoxygadusol and/or gadusol. The concentrations of 6-deoxygadusol and/or gadusol increase in passing from unfertilised to fertilised eggs, and decrease in the gastrulas, suggesting the existence of a biological relationship between MAAs and 6-deoxygadusol and or gadusol. The function of the enzymic reaction may be to provide an alternative antioxidant but at the same time increase the pool of free amino acids available to the embryo (Clegg and Lovallo 1977). Gadusol and 6-deoxygadusol with an UV maximum of 264 and 268 nm, respectively, are unlikely to have an UV-protective function.

Approximately 90% of the total carotenoids of the body wall, ovaries and viscera of *Holothuria atra* are highly oxidised, the main components being astaxanthin followed by canthaxanthin; this is very much in agreement with the carotenoid profile among other members of Aspidochirotida investigated (Matsuno et al. 1969, 1971; Bullock and Dawson 1970; Matsuno and Tsushima 1995; present Table 2). The xanthophylls zeaxanthin and lutein and the ketocarotenoids phoenicoxanthin and echinenone were present to a lesser

extent. With a few exceptions (Miki et al. 1982), β-carotene is considered to be a universal constituent of egg carotenoids, especially of fishes (Czeczuga 1975). However, only a trace quantity of β-carotene was detected in the eggs of *H. atra*. The allenic carotenoids fucoxanthin and peridinin, two carotenoids present in diatoms, dinoflagelltes and phytoplankton, which are encountered in the marine food chain, were absent from our samples. The gut-content results indicate that the carotenoids in the diet of *H. atra* are mainly β-carotene and xanthophylls. Carotenoids other than β-carotene were present in the sediments, but were below detection limits. *H. atra* may provide specific carotenoids to the ovaries either by concentrating minute amounts of oxygenated carotenoids from the diet or, more probably, by metabolising β-carotene and/or xanthophylls to the ketocarotenoids canthaxanthin and astaxanthin. The presence of significant amounts of β-carotene in the gut contents and diet and minute quantities in the epidermal tissues and ovaries could be taken as evidence for this. It appears that the carotenoid profile changes as a function of season, and even differs in the same species at different geographical locations (Bandaranayake unpublished results). For example, the carotenoid content of *H. leucospilota* in tropical and sub-tropical regions are similar, but, these differ significantly from those for *H. atra* in the Adriatic Sea (Table 2).

Although ubiquitous (Chioccara et al. 1986), little is known of the functions of homarine, which is found in the viscera and gonads of most marine organisms, or of trigonelline, which is mostly reported from terrestrial plant sources (Bandaranayake 1998).

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