Chapter

CHEMICAL ECOLOGY OF ECHINODERMS: IMPACT OF ENVIRONMENT AND DIET IN METABOLOMIC PROFILE

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ABSTRACT

The phylum Echinodermata constitutes a successful and widespread group comprising Asteroidea, Ophiuroidea, Echinoidea, Holothuroidea and Crinodeia. Nowadays, marine organisms are being given a lot of attention in drug discovery pipelines. In these studies, sponges and nudibranchs are frequently addressed, however an increasing number of

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works focus their attention in echinoderms. Given the fact that many of the bioactive molecules found in echinoderms are diet-derived, different feeding behavior and surrounding environment plays a critical role in the chemical composition of echinoderms.

In this work, the most relevant chemical classes of small molecules present in echinoderms, such as fatty acids, carotenoids and sterols will be addressed. When data is available, the influence of the environment on the chemical profile of these organisms will be discussed.

Keywords: Echinoderms; fatty acids; carotenoids; sterols.

INTRODUCTION

Marine environment remains, nowadays, the most diversified ecosystem on Earth as well as the least studied one. Marine organisms have to cope with the several challenges that marine life represents, which include high pressures, low temperatures and light availability. Furthermore, many organisms have primitive immune systems and soft bodies and, for this reason, what they lack in physical defences is frequently balanced with remarkable chemical defences. Thus, it is not by coincidence that organisms with lower physical defences, such as sponges and molluscs, are usually the ones with higher amounts of bioactive molecules.

Marine natural products have already shown to be an amazing source of new chemical entities (NCE), with many sea-derived molecules exhibiting a number of different pharmacological activities, many of which are already being marketed or under clinical trials to treat pathological conditions such as cancer (dolastatins, cytarabine), inflammation (manoalide, avarol) and pain (conotoxins) [1-4].

In this chapter we will review some classes of natural products described in echinoderms, such as carotenoids, fatty acids and sterols, with emphasis on its variation according to ecological conditions and diet.

CAROTENOIDS

Carotenoids are, typically, C_{40} terpenes that constitute one of the most widespread classes of natural pigments and, simultaneously, the one with more information available in scientific bibliography [5]. They can be found in Nature mainly in lower trophic levels due to their tight connection with photosynthesis, although they can occur in non-photosynthetic organisms as result of predation and dietary uptake.

Two major groups of carotenoids exist: carotenes, which are hydrocarbons, and xanthophylls, which are oxygenated derivatives of the former. By using a different criteria, we can group carotenoids as primary carotenoids, required for photosynthesis, and secondary carotenoids, which are present in fruits and flowers [6].

As it happens with most classes of marine pigments, high performance liquid chromatography-diode array detection (HPLC-DAD) is a remarkable tool for the analysis of carotenoids, mainly due to their characteristic chromophores, which in turn are translated into complex UV-vis profiles. In fact, the position of higher absorbance bands is a function of the number of conjugated double bonds. An increase in this number is thus accompanied by an increase in the wavelength of maximal absorption [8]. Similarly, addition of a carbonyl group in a cyclic end group, if in conjugation with the polyene chain, will result in two effects: loss of fine structure, originating a rounded shape, and secondly a bathochromic shift.

Major carotenoids in echinoderms are β-carotene, canthaxanthin, echinenone, astaxanthin, 7,8-didehydroastaxanthin, fucoxanthin and fucoxanthinol [7] (Figure 1). Given the biosynthetic pathway of carotenoids, which takes place in chloroplasts, their presence in animals is a consequence of predation.

Asteroidea

Asteroidea, commonly known as sea-stars or starfishes, is the largest class of Echinodermata [8]. They are described as voracious predators and, for this reason, the impact of the environment, namely diet, on the chemical composition of these echinoderms is particularly relevant.

Marthasterias glacialis L., also known as the spiny sea-star, is an echinoderm which has been shown to play one of the most determinant roles in benthic ecosystems on a variety of scales [4].

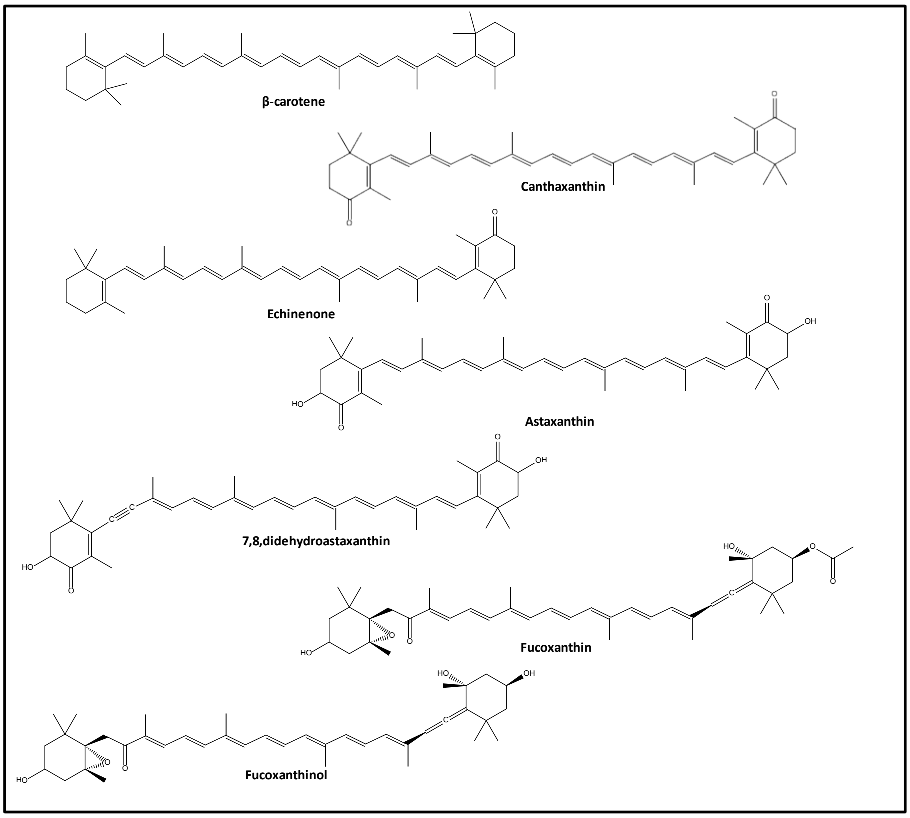

Figure 1. Major carotenoids described in echinoderms.

In 1977 this organism was investigated for its carotenoid composition [9]. At the time, thin layer chromatography (TLC) was used and several carotenoids were identified in specimens from the Adriatic Sea, namely β-echinenone, cryptoxanthin, β-carotene, lutein 5,6-epoxide, among others. In a more recent work, high performance liquid chromatography-diode array detection-mass spectrometry (HPLC-DAD-MS) was used to revisit this organism's carotenoid profile in samples from the Portuguese Coast, Atlantic Ocean. Three compounds were initially identified: astaxanthin, lutein and zeaxanthin [10], all of which had already been found by Czeczuga et al. [9]. Several previously described carotenoids such as lutein-5,6-epoxide or 3,4-didehydro-α-carotene could not be found, which might be the result either of misidentified compounds in TLC or the distinct origin of the specimens used. Other compounds with spectra compatible with carotenoids were also noted, however at the time they could not be identified. Subsequent studies using a

C_{30} column instead of a C_{18} allowed extracting further information regarding the structure of the unknown compounds. In the atmospheric pressure chemical ionization (APCI) process, most compounds lost water, thus originating the ion $[M+H-18]^+$, while the protonated molecule was frequently absent. Other typical ions of the MS fragmentation of carotenoids were detected, namely those resulting from the loss of 56 mass units (u) and/or 56+18 u and 80+18 u [11]. A total of 20 compounds were identified, eight of them being reported in this species for the first time. These differences are likely to be a consequence of the distinct geographical origins of the samples used in those works.

The crown-of-thorns starfish, *Acanthaster planci L.*, is a predator that is known for its extreme detrimental effects upon coral reefs [12].

Earlier studies showed the presence of an astaxanthin derivative, 7,8-didehydroastaxanthin, as the main carotenoid in this species in individuals collected in Okinawa, Japan [13]. In subsequent studies, other major carotenoids, peridininol and astaxanthin, were identified [14]. Minor carotenoids displaying either an allenic or acetylenic group were also reported in a sample from another location, Ootsuki coast, Kochi Prefecture, Japan, namely 7,8,7′,8′-tetrahydroastaxanthin, diadinoxanthin, diatoxanthin and alloxanthin [15].

In the species *Asterias rubens* L., the common starfish, β-carotene, free and esterified alloxanthin and esterified α-ketols have been described [16]. Differently, in another species of the same genus, *Asteria amurensis*, five acetylenic carotenoids have been described, namely 4-hydroxy-mytiloxanthin, 4,4′-dihydroxy-diatoxanthin, 4,4′-dihydroxy-alloxanthin, 4-keto-4′-hydroxy-diatoxanthin and 4-keto-4′-hydroxy-alloxanthin [14].

Holothurians

Sea cucumbers are a diversified group of soft-bodied echinoderms with elongated and worm-like gelatinous body that resemble common cucumbers and are of great nutritional importance, especially in Asian countries [17]. Their diversity is nowadays estimated to be around 1250 species, although it is generally accepted that this number is underestimated.

As it has been reviewed recently [18], many classes of natural products have been identified in sea cucumbers, namely triterpenic saponins, sulphated polysaccharides, sterols, cerebrosides, among others. In order to allow direct comparison with other classes of echinoderms discussed herein, we will focus in their carotenoid, fatty acid and sterol content.

Carotenoid pigments were determined from the gut sediments of seven species of abyssal holothurian, sampled from the Porcupine Abyssal Plain [19], namely *Amperima rosea* R. Perrier, *Oneirophanta mutabilis* Théel, *Psychropotes longicauda* Théel, *Pseudostichopus villosus* Théel, *Pseudostichopus* sp. *and Molpadia blakei* Théel. *A. rosea* had a gut profile dominated by zeaxanthin, echinenone and β-carotene, which were also present in other species albeit in lower amounts. A similar carotenoid profile was also found in the ovarian [19].

In another study, seven species of sea sucumber *(Stichopus japonicas* Selenka, *Holothuria leucospilota* Brandt, *H. moebi* Ludwig and *H. pervicax* Selenka of the order Aspidochirotida, *Cucumaria japonica* Gunnerus, *C. echinata* Von Marenzeller and *Pentacta australis* Ludwig of the order Dendrochirotida) collected in Ise Bay and Tanabe Bay, Japan, were evaluated with all specimens containing β-carotene, β-echinenone, canthaxanthin, phoenicoxanthin and astaxanthin. Furthermore, the novel carotenoids (cucumariaxanthin A, B and C) were obtained from the sea cucumbers of the order Dendrochirotida [20].

Echinoids

Echinoids, commonly known as sea urchins due to their characteristic morphology, are another class of echinoderms. Over 950 species are known, their distribution being global and present in all oceans. In many countries, notably Japan, sea urchin roe or gonads are a delicacy whose price can reach considerable values, the colour constituting one of the most important organoleptic characteristics to infer its quality. *Paracentrotus lividus* Lamarck is probably the best studied echinoid due to its nutritional value and presence in several dietarian regiments across the world.

The most widespread carotenoids in sea urchins are β-carotene, α-/β-echinenone, fucoxanthin and its metabolite fucoxanthinol. Minor compounds, such as the allenic carotenoid paracentrenone can also be found. The carotenoid profile of sea urchins depends directly of both the diet and the metabolic capacity of the organism [21,22]. For this reason, it is a widespread practice, in aquaculture, to modulate the diet of sea urchins in what regards carotenoids in order to obtain specimens with gonads that exhibit the coloration necessary to be marketed at higher prices.

In a report by Symonds et al., β-echinenone was the major compound in *P. lividus*, although it was absent from diet, thus arising from the metabolism of other compounds [23].While all-*trans* β-echinenone can probably be formed by conversion of the all-*trans* β-carotene in diet with a isocryptoxanthin intermediate, the precursor of 9'-*cis* β-echinenone is not as straightforward to predict [23]. Apart from their organoleptic properties, these compounds have also been shown to play important roles in the reproductive biology of these organisms [21].

In the ovaries and eggs of *Strongylocentrotus dröbachiensis* Müller, β-echinenone, β-carotene, zeaxanthin and isocryptoxanthin were found to be the most relevant compounds, trace amounts of a fucoxanthin isomer being found [24]. The authors also report seasonal variation in ovarian carotenoids, with echinenone constituting between 79 and 85% of the total carotenoids, except in the case of spawned out ovaries where it fell to 50%, an effect probably arising for incorporation of β-echinenone into eggs [24]. In other study, using *Heliocidaris erythrogramma* Valenciennes and *H. tuberculata* Lamarck, β-echinenone was also the major carotenoid, although marked quantitative differences were reported [25]. Differently, in *Peronella japonica* Mortensen, astaxanthin was the carotenoid present in higher amounts [26].

FATTY ACIDS

Fatty acids are a class of lipophilic molecules that can be found in all living organisms. They can occur in their free form or, alternatively, integrate more complex lipids, such as triglycerides, phospholipids or glycolipids [27]. In addition, fatty acids can be part of lipoproteins, lipopolysaccharides and alkaloids.

The most frequent fatty acids exhibit 16-18 carbons, with higher-chain compounds being named very long chain fatty acids (VLCFA). These last cannot be synthesized using "standard" fatty acid synthases [28], thus being frequently acquired through diet of vegetables/cyanobacteria [29, 30].

Asteroidea

A work is available regarding the influence of the geographical origin and month of collection upon the fatty acid composition of *M. glacialis*.

Samples collected in February in Peniche, Portugal, presented ca. 40% higher amounts of fatty acids when compared to those collected in the same month but at Baleal, Portugal. In both cases, unsaturated fatty acids were predominant compounds.

Samples from September displayed a decrease in total amounts of around 35%, when compared with samples obtained in February. In the case of sea stars, palmitic acid is frequently reported as the compound present in higher amounts [31]. In the specific case of this study, this is true for samples collected in July and September, with samples collected in February displaying arachidonic acid as the major compound.

In a study by Howell et al., the fatty acid profile of 9 deep-sea stars (*Hyphalaster inermis* Sladen, *Styracaster chuni* Ludwig, *Dytaster grandis grandis* Verril, *Bathybiaster vexillifer* W. Thomson, *Hymenaster membranaceus* W. Thomson, *Freyella elegans* Verril, *Hymenodiscus coronata* Sars G.O. [formerly *Brisingella coronate*], *Brisinga endecacnemos* Asbjørnsen and *Zoroaster fulgens* W. Thomson [formerly *Zoroaster longicauda*] were evaluated [32]. Overall, polyunsaturated fatty acids (PUFAs) were the most abundant categories of fatty acid in the total lipids of all species, with 20:5 (n-3) and 20:4 (n-6) being the most expressive compounds from a quantitative point of view. Monounsaturated fatty acids (MUFAs) in particular 20:1 (n-13 or n-9) were also found, in addition to odd-numbered, branched-chain fatty acids and non-methylene interrupted dienes (NMIDs), which are frequent in marine organisms [27], were present in relatively high levels in all species. In addition, the authors show that the fatty acid profile of these species could be clustered taking into account their feeding behavior as suspension feeders, predators/scavengers and mud ingesters [32].

Holothurians

Several species of sea cucumbers have been investigated for their fatty acid profile, with results pointing to palmitic, eicosenoic and arachidonic acids as the predominant saturated, monounsaturated and polyunsaturated fatty acids, respectively, regardless of the geographical origin, although marked quantitative differences are known to occur [18,33-35]. This is probably a consequence of the fact these compounds are synthesized *de novo via* the acetate pathway, hence being independent of exogenous sources such as diet. Nevertheless, a case can be made regarding the relative proportion of such fatty acids as a variation of the origin of the species. For example, species from abyssal and tropical regions display higher proportion of PUFA when compared to 8 species collected in temperate regions [34,36,37]. In a recent work using specimens from the Atlantic Ocean, it was shown that ω-6 fatty acids were in higher amounts than their ω-3 homologues.

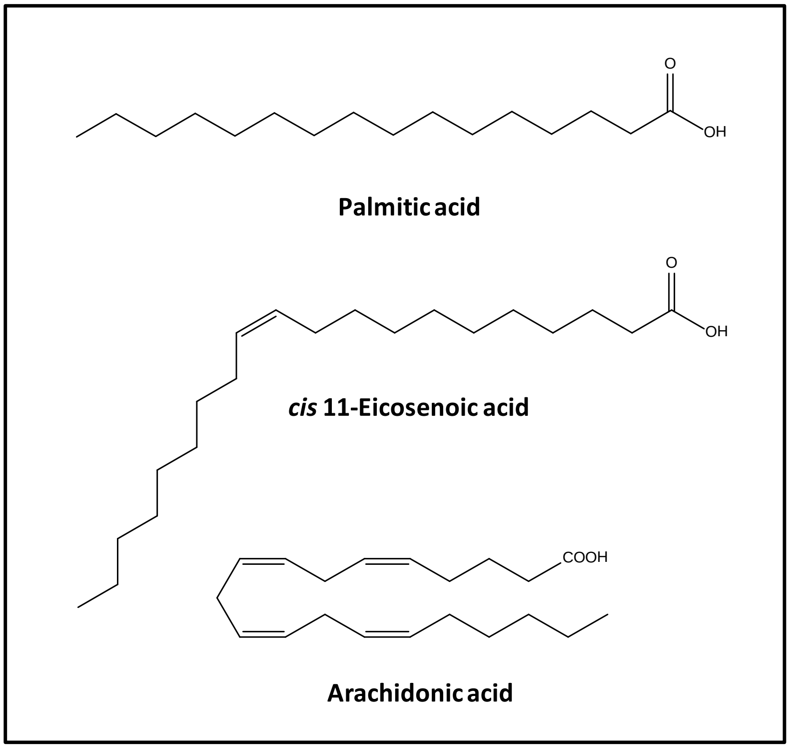

Figure 2. Most relevant saturated and unsaturated fatty acids in echinoderms.

Aydin et al., (2011) evaluated the fatty acid composition of several *Holothuria* species, namely *H. tubulosa* Gmelin, *H. poli* Delle Chiaje and *H. mammata* Grube, having concluded that palmitic and stearic acids were the saturated fatty acids present in higher levels [33], a trend also found for *H. forskali* Delle Chiaje. Unsaturated fatty acids were also studied, with arachidonic and *cis* 11-eicosaenoic acids being predominant compounds in *H. forskali*, in opposition to species such as *H. tubulosa*, *H. poli* and *H. mammata* [35].

In a work by Hudson et al., 3 species of sea cucumbers were collected along Porcupine Seabight and Porcupine Abyssal Plain, NE Atlantic in

Summer 2001, Spring 2002 and Autumn October 2002 [38]. This work successfully showed that, although season-dependent changes in fatty acid profile are frequent, some species do not follow this trend. As so, *A. rosea* and *Bathyplotes natans* M. Sars had higher levels of PUFA in summer *vs* spring, being replaced by MUFA. In the species *Deima validum* Théel, the fatty acid profile remained largely unchanged throughout the year [38].

Echinoids

Previous works addressing the fatty acid composition of *P. lividus* from distinct geographical origins identified palmitic, stearic and tetradecanoic acids as predominant saturated fatty acids, while major unsaturated fatty acids include arachidonic acid and eicosapentaenoic acid [39-41].

The influence of season was also investigated, results suggesting that although the proportion of unsaturated:saturated fatty acids varies during the year, the most relevant compounds from a quantitative point of view are fairly stable [42,43]. It is interesting to realize that in a recent work conducted *P. lividus* from the Atlantic Ocean, the same trend was found, thus suggesting that, in what regards saturated and unsaturated fatty acid, and the composition is location-independent. In what concerns to unsaturated fatty acids, arachidonic, eicosapentaenoic acid and *cis* 11,14-eicosadienoic acids were described as predominant compounds in *P. lividus* [34].

In the species *S. dröebachiensis* harvested in subtidal water near Halifax, and in deep water in St. Margaret's Bay an eicosenoic acid with 5,6-ethylenic unsaturation and two non-methylene-interrupted eicosadienoic acids with one 5,6 bond and a second in either the 11,12 or 13,14 positions were found [44]. The authors suggest that the consistent distribution of the fatty acids with 5,6 unsaturation, irrespective of the nutritional status of the animal, may be the result of a pivotal biological role in membrane function [44]. Reports addressing the fatty acid profile as a result of distinct algal [45] or salmon [46] diets are available in literature.

STEROLS

Sterols are typically, albeit not always, C_{27} steroid alcohols synthesized by higher plants, algae, nearly all fungi and also most animals. Although they can be found in their free form, the presence of the C_3 hydroxyl group turns esterification a possibility and hence sterols can be esterified with fatty acids, hydroxycinnamic acids, hexoses or 6-fatty acyl hexoses.

Chemically, sterols comprise a 4 ring system constituted by a cyclopenta[a]phenanthrene in *trans* junctures, methyl groups at C_{18} and C_{19} with β-stereochemistry and a carbon side chain at C_{17}. Saturated derivatives of sterols are known as stanols and they usually occur in trace amounts [47,48].

These natural products are of pivotal biological importance either as components of membranes or as chemical defences against predation and environmental conditions [49-51].

In the past few years the field of sterol chemistry has grown steadily, with the identification of many compounds with remarkable chemical diversity, notably sponge metabolites [52].

Sterols in echinoderms can generally be sorted in the class of C_{26}-C_{30} molecules. When it comes to sterol biosynthesis in Asteroideia, feeding experiments employing radioactive acetate precursors confirm that these organisms are able to conduct *de novo* synthesis of sterols to some extent [53]. In particular, results point that all echinoderms have the biochemical machinery to conduct the biosynthesis of, at least, cholest-Δ^7-en-3β-ol from the acetate or mevalonate pathway involving squalene and lanosterol intermediates. Ergosterol and β-sitosterol (Figure 3) are also compounds frequently present. While feeding experiments show that C_{27} sterols can be derived both from biosynthesis and diet, the same is not true for C_{26} and C_{28}-C_{30} sterols, which are exclusively diet-derived [53].

A work from 1968 stated that “Sterols of asteroids (sea stars) and holothurians (sea cucumbers) are Δ^7-sterols, while sterols of echinoids (sea urchins), ophiuroids (brittle stars) and crinoids (sea lilies) are Δ^5-sterols” [54]. While some trends can, indeed, be drawn regarding the class-related chemistry of sterols, we know nowadays that this statement is not completely true.

An interesting example from the metabolism of sterols can be drawn from the above-mentioned *A. planci*, which feeds upon Δ^5-gorgosterol-containing corals, subsequently metabolizing it into Δ^0-gorgostanol and Δ^7-acanthasterol (Figure 4) [53,55].

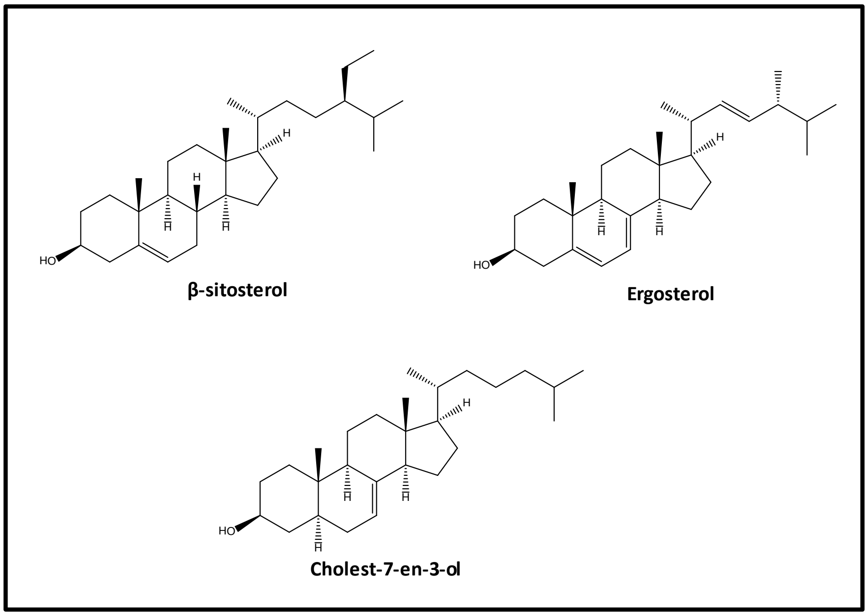

Figure 3. Some sterols frequently described in echinoderms.

Asteroidea

The variation of the sterol composition of *M. glacialis* from the Portuguese coast in different months was studied. Samples collected in February were significantly different to those from July and September, with the former having both higher diversity and amounts of sterols. Some unidentified sterols were found only in organisms collected in February. Ergosterol was the only compound that could be identified in all samples, with β-sitosterol and fucosterol being absent in all cases.

In the species *A. rubens*, 4,4-dimethyl-5α-cholesta-8,24-dien-3β-acetate, 4,4-dimethyl-5α-cholesta-7,24-dien-3β-acetate and 5α-cholest-7-en-3β-ol were major compounds present [56].

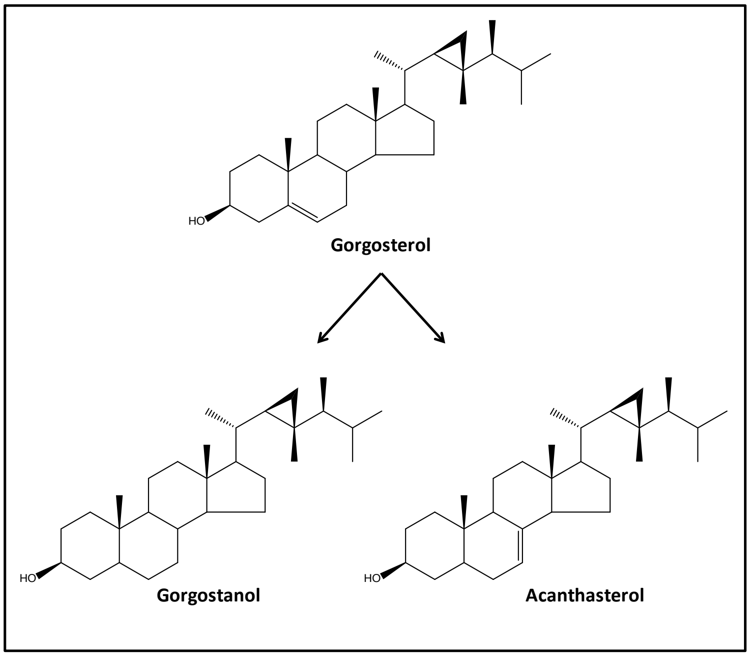

Figure 4. Proposed biosynthetic route for acanthasterol in *A. planci*.

Holothurians

Sterols in sea cucumbers are, typically, Δ^7 molecules [57,58], although Δ^5 and stanols are known to occur in low amounts. Another subset of molecules, 14α-methyl- and 4α,14α-dimethyl- $\Delta^{9,11}$ -sterols is known to occur. In fact, in some species such as *Bohadschia argus* Jaeger, *Holothuria mexicana* Ludwig, *Holothuria arenicola* Semper and *Stichopus californicus* Stimpson radiolabelling experiments show that lanost-$\Delta^{9,11}$-dien-3β-ol, 4α-14α-dimethyl-5α-cholest-$\Delta^{9,11}$-dien-3β-ol and 14α-methylcholest-$\Delta^{9,11}$-dien-3β-ol are the only sterols synthesized *de novo*. From a biosynthetic point of view, these compounds are obtained by cyclization of squalene and sequential $\Delta^{24,25}$ bond reduction, loss of the 4α methyl group and loss of the remaining 4-methyl group. Interestingly, saponins were not labeled [59].

In a very elegant and detailed study, Stonik et al., evaluated the free sterols from the sea cucumbers *Pseudostichopus trachus* Sluiter, *Holothuria nobilis* Selenka, *Holothuria scabra* Jaeger, *B. natans* and *Trochostoma* *orientale* Savel'eva. About 80 sterols were found, with about 70 having been identified [60].

The high number of compounds indentified hinders its detailed discussion here, however some conclusions could be drawn. Differently from what is found in echinoid species, presented later in this chapter, in the case of Holothurians the different species had markedly distinct sterol profiles, which can be a consequence of either distinct biosynthetic capacity or different diets [60].

In a recent study using *H. forskalis* collected along the Portuguese coast (Atlantic Ocean), only cholesterol, β-sitosterol, choleste-Δ^7-ene-3β-ol and one unidentified cholesterol derivative could be identified.

Echinoids

In *P. lividus* collected in the Adriatic sea, cholesterol accounted for about 75% of all sterols, with other Δ^5-sterols such as cholesta-$\Delta^{5,24}$-dien-3β-ol being minor compounds [61]. The same major compound was found in *P. lividus* from Spain [62] and Portugal [34], as well as other species such as *Echinus esculentus* L. [63]. Thus, results suggest that cholesterol is the main sterol in sea urchins, regardless of species or location, although minor compounds, notably 4-demethyl sterols can be species-specific.

CONCLUSION

There is an undeniable increasing interest in the study of marine organisms from a chemical point of view. While this trend has been mainly driven by biomedical research, which intends to use these organisms as a source of new drugs and applications for human health and therapeutics, this knowledge can also be used and developed from the point of view of chemical ecology. Thus, the increasing body of data regarding the metabolomic profile of marine organisms can be used to track the fate of several molecules through trophic nets. As so, we know, nowadays, that many classes of natural products found in an organism are, in fact, diet-derived and suffer further biotransformation. In the next few years this area is expected to grow steadily, with the biosynthetic origin of many molecules being elucidated.

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REFERENCES

[1] Albericio F, Álvarez M, Cuevas C, Francesch A, Pla D, et al. (2010) The sea as a source of new drugs. Molecular imaging for integrated medical therapy and drug development: Springer. pp. 237-249.

[2] Jha RK, Zi-rong X (2004) Biomedical compounds from marine organisms. *Marine drugs* 2: 123-146.

[3] Pereira DM, Correia-da-Silva G, Valentão P, Teixeira N, Andrade PB (2011) Marine metabolomics in cancer chemotherapy. In: Barh D, editor. OMICS: Biomedical Perspectives and Applications CRC Press.

[4] Pereira DM, Valentão P, Andrade PB (2013) Lessons from the sea: distribution, SAR and molecular mechanisms of anti-inflammatory drugs from marine organisms. In: Atta-ur-Rahman, editor. Studies in Natural Products Chemistry (Bioactive Natural Products). The Netherlands: Elsevier Science Publishers.

[5] Goodwin TW (1980) Biochemistry of the carotenoids. New York: Chapman and Hall.

[6] Lichtenthaler HK (1987) Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. *Methods in Enzymology* 148: 350-382.

[7] Matsuno T (2001) Aquatic animal carotenoids. *Fisheries Science* 67: 771-783.

[8] Knott E (2004) Asteroidea. Sea stars and starfishes.: The Tree of Life Web Project.

[9] Czeczuga B (1977) Investigations of carotenoids in some animals of the Adriatic Sea V. Echinodermata. *Hydrobiologia* 54: 177-180.

[10] Ferreres F, Pereira DM, Gil-Izquierdo A, Valentão P, Botelho J, et al. (2010) HPLC-PAD-atmospheric pressure chemical ionization-MS metabolite profiling of cytotoxic carotenoids from the echinoderm

Marthasterias glacialis (spiny sea-star). *Journal of separation science* 33: 2250-2257.

[11] Mariutti LR, Pereira DM, Mercadante AZ, Valentão P, Teixeira N, et al. (2012) Further insights on the carotenoid profile of the echinoderm *Marthasterias glacialis* L. *Marine drugs* 10: 1498-1510.

[12] Moran PJ (1988) The Acanthaster phenomenon: Australian Institute of Marine Science.

[13] Tanaka Y, Katayama T (1976) Biochemical studies on the carotenoids in Echinoderamata. The structure of an astaxanthin-like pigment (7,8-didehydro-astaxanthin) and the carotenoids in starfish. *Bull Jap Soc Sci Fish* 42: 807-812.

[14] Maoka T, Tsushima M, Matsuno T (1989) New acetylenic carotenoids from the starfishes *Asterina pectinifera* and *Asterias amurensis*. Comparative Biochemistry and Physiology Part B: *Comparative Biochemistry* 93: 829-834.

[15] Maoka T, Akimoto N, Terada Y, Komemushi S, Harada R, et al. (2010) Structure of Minor Carotenoids from the Crown-of-Thorns Starfish, *Acanthaster planci*. *Journal of natural products* 73: 675-678.

[16] Elgsaeter A, Tauber JD, Liaaen-Jensen S (1978) Animal carotenoids 15: Carotenoid distribution and carotenoprotein of *Asterias rubens*. Biochimica et Biophysica Acta (BBA)-*Lipids and Lipid Metabolism* 530: 402-411.

[17] Conand C (1990) The Fishery Resources of Pacific Island Countries: Holothurians: Food & Agriculture Org.

[18] Bordbar S, Anwar F, Saari N (2011) High-value components and bioactives from sea cucumbers for functional foods—A review. *Marine drugs* 9: 1761-1805.

[19] Wigham BD, Hudson IR, Billett DS, Wolff GA (2003) Is long-term change in the abyssal Northeast Atlantic driven by qualitative changes in export flux? Evidence from selective feeding in deep-sea holothurians. *Progress in Oceanography* 59: 409-441.

[20] Matsuno T, Tsushima M (1995) Comparative biochemical studies of carotenoids in sea cucumbers. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology* 111: 597-605.

[21] Plank LR, Lawrence JM, Lawrence AL, Olvera RM (2002) The effect of dietary carotenoids on gonad production and carotenoid profiles in the sea urchin *Lytechinus variegatus*. *Journal of the World Aquaculture Society* 33: 127-137.

[22] Shpigel M, Schlosser SC, Ben-Amotz A, Lawrence AL, Lawrence JM (2006) Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. *Aquaculture* 261: 1269-1280.

[23] Symonds RC, Kelly MS, Caris-Veyrat C, Young AJ (2007) Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9′-*cis*-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology* 148: 432-444.

[24] Griffiths M, Perrott P (1976) Seasonal changes in the carotenoids of the sea urchin *Strongylocentrotus dröbachiensis.* Comparative Biochemistry and Physiology Part B: *Comparative Biochemistry* 55: 435-441.

[25] Tsushima M, Byrne M, Amemiya S, Matsuno T (1995) Comparative biochemical studies of carotenoids in sea urchins—III. Relationship between developmental mode and carotenoids in the Australian echinoids *Heliocidaris erythrogramma*, and *H. tuberculata* and a comparison with Japanese species. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology* 110: 719-723.

[26] Tsushima M, Matsuno T (1990) Comparative biochemical studies of carotenoids in sea-urchins—I. Comparative Biochemistry and Physiology Part B: *Comparative Biochemistry* 96: 801-810.

[27] M Pereira D, Vinholes J, Correia-da-Silva G, Valentao P, Teixeira N, et al. (2011) Fatty Acids in Marine Organisms: In the Pursuit of Bioactive Agents. *Current Pharmaceutical Analysis* 7: 108-119.

[28] Poulos A (1995) Very Long-Chain Fatty-acids in higher animals - a review. *Lipids* 30: 1-14.

[29] Drazen JC, Phleger CF, Guest MA, Nichols PD (2008) Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications. *Comp Biochem Phys B* 151: 79-87.

[30] Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty-Acid and Lipid-Composition of 10 Species of Microalgae Used in Mariculture. *J Exp Mar Biol Ecol* 128: 219-240.

[31] Guenther J, Wright AD, Burns K, De Nys R (2009) Chemical antifouling defences of sea stars: Effects of the natural products hexadecanoic acid, cholesterol, lathosterol and sitosterol. *Mar Ecol - Prog Ser* 385: 137-149.

[32] Howell KL, Pond DW, Billett DS, Tyler PA (2003) Feeding ecology of deep-sea seastars (Echinodermata: Asteroidea): a fatty-acid biomarker approach. *Marine Ecology Progress Series* 255: 193-206.

[33] Aydin M, Sevgili H, Tufan B, Emre Y, Kose S (2011) Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science & Technology* 46: 500-508.

[34] Pereira DM, Valentão P, Teixeira N, Andrade PB (2013) Amino acids, fatty acids and sterols profile of some marine organisms from Portuguese waters. *Food chemistry* 141: 2412-2417.

[35] Wen J, Hu C, Fan S (2010) Chemical composition and nutritional quality of sea cucumbers. *Journal of the Science of Food and Agriculture* 90: 2469-2474.

[36] Drazen JC, Phleger CF, Guest MA, Nichols PD (2008) Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: food web implications. *Comp Biochem Physiol B Biochem Mol Biol* 151: 79-87.

[37] Svetashev V, Levin V, Lam CN (1991) Lipid and fatty acid composition of holothurians from tropical and temperate waters. Comparative Biochemistry and Physiology Part B: *Comparative Biochemistry* 98: 489-494.

[38] Hudson IR, Pond DW, Billett DS, Tyler PA, Lampitt RS, et al. (2004) Temporal variations in fatty acid composition of deep-sea holothurians: evidence of bentho-pelagic coupling. *Marine Ecology Progress Series* 281: 109-120.

[39] Kalogeropoulos N, Mikellidi A, Nomikos T, Chiou A (2012) Screening of macro- and bioactive microconstituents of commercial finfish and sea urchin eggs. *LWT - Food Science and Technology* 46: 525-531.

[40] la Cruz-García D, López-Hernández J, González-Castro MJ, Rodríguez-Bernaldo De Quirós AI, Simal-Lozano J (2000) Protein, amino acid and fatty acid contents in raw and canned sea urchin (*Paracentrotus lividus*) harvested in Galicia (NW Spain). *Journal of the Science of Food and Agriculture* 80: 1189-1192.

[41] Zlatanos S, Laskaridis K, Sagredos A (2009) Determination of proximate composition, fatty acid content and amino acid profile of five lesser-common sea organisms from the Mediterranean sea. *Int J Food Sci Tech* 44: 1590-1594.

[42] Arafa S, Chouaibi M, Sadok S, El Abed A (2012) The influence of season on the gonad index and biochemical composition of the sea

urchin *Paracentrotus lividus* from the golf of Tunis. *The Scientific World Journal* 2012.

[43] Mol S, Baygar T, Varlik C, Tosun ŞY (2008) Seasonal variations in yield, fatty acids, amino acids and proximate compositions of sea urchin (*Paracentrotus lividus*) Roe. *Journal of Food and Drug Analysis* 16: 68-74.

[44] Takagi T, Eaton CA, Ackman RG (1980) Distribution of fatty acids in lipids of the common Atlantic sea urchin *Strongylocentrotus droebachiensis. Canadian Journal of Fisheries and Aquatic Sciences* 37: 195-202.

[45] Kelly JR, Scheibling RE, Iverson SJ, Gagnon P (2008) Fatty acid profiles in the gonads of the sea urchin *Strongylocentrotus droebachiensis* on natural algal diets. *Mar Ecol Prog Ser* 373: 1-9.

[46] Cook EJ, Bell MV, Black KD, Kelly MS (2000) Fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliaris*: trophic and nutritional implications. *Journal of Experimental Marine Biology and Ecology* 255: 261-274.

[47] Bruneton J (2001) Farmacognosia: Fitoquímica, Plantas Medicinales: Acribia, Editorial, S.A. .

[48] Dewick PM (2009) Medicinal Natural Products: A Biosynthetic Approach: John Wiley & Sons Ltd.

[49] Bernsdorff C, Winter R (2003) Differential properties of the sterols cholesterol, ergosterol, β-sitosterol, trans-7-dehydrocholesterol, stigmasterol and lanosterol on DPPC Bilayer Order. *Journal of Physical Chemistry B* 107: 10658-10664.

[50] Chen C, Tripp CP (2012) A comparison of the behavior of cholesterol, 7-dehydrocholesterol and ergosterol in phospholipid membranes. *Biochimica et Biophysica Acta* 1818: 1673-1681.

[51] Edidin M (2003) The state of lipid rafts: From model membranes to cells. *Annual Review of Biophysics and Biomolecular Structure* 32: 257-283.

[52] Sarma NS, Sri Rama Krishna M, Ramakrishna Rao S (2005) Sterol ring system oxidation pattern in marine sponges. *Marine Drugs* 3: 84-111.

[53] Kanazawa A (2001) Sterols in marine invertebrates. *Fisheries Science* 67: 997-1007.

[54] Gupta K, Scheuer P (1968) Echinoderm sterols. *Tetrahedron* 24: 5831-5837.

[55] Kanazawa A, Teshima S, Tomita S, Ando T (1974) Gorgostanol, a novel C30 sterol from an asteroid, *Acanthaster planci*. *Bulletin of the Japanese Society of Scientific Fisheries* 40.

[56] Smith A, Goad L (1971) Sterol biosynthesis in the starfish Asterias rubens and Henricia sanguinolenta. *Biochemical Journal* 123: 671.

[57] Ballantine J, Lavis A, Morris R (1981) Marine sterols. XV. Sterols of some oceanic holothurians. *Journal of Experimental Marine Biology and Ecology* 53: 89-103.

[58] Goad LJ (1978) The sterols of marine invertebrates: composition, biosynthesis and metabolites. In: Scheuer PJ, editor. Marine Natural Products: Chemical and Biological Perspectives. New York: Academic Press. pp. 76-172.

[59] Cordeiro ML, Djerassi C (1990) Biosynthetic studies of marine lipids. 25. Biosynthesis of .DELTA.9(11)- and .DELTA.7-sterols and saponins in sea cucumbers. *The Journal of Organic Chemistry* 55: 2806-2813.

[60] Stonik VA, Ponomarenko LP, Makarieva TN, Boguslavsky VM, Dmitrenok AS, et al. (1998) Free sterol compositions from the sea cucumbers *Pseudostichopus trachus*, *Holothuria nobilis*, *Holothuria scabra*, *Trochostoma orientale* and *Bathyplotes natans*. Comparative Biochemistry and Physiology Part B: B*iochemistry and Molecular Biology* 120: 337-347.

[61] Serrazanetti G, Pagnucco C, Conte L, Cattani O (1995) Hydrocarbons, sterols and fatty acids in sea urchin *Paracentrotus lividus* of the adriatic sea. *Chemosphere* 30: 1453-1461.

[62] Lopez-Hernandez J, Gonzalez-Castro M, Pineiro-Sotelo M (1999) Determination of sterols in sea urchin gonads by high-performance liquid chromatography with ultraviolet detection. *Journal of chromatographic science* 37: 237-239.

[63] Smith AG, Goad LJ (1974) Sterol biosynthesis by the sea urchin *Echinus esculentus*. *Biochem J* 142: 421-427.