Sterol Composition of the Black Sea Sponges *Hymeniacidon sanguinea* (Grant) and *Halichondria panicea* (Pallas)

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The sterol composition of *Hymeniacidon sanguinea* and *Halichondria panicea* from the Black Sea was investigated. Both sponges contain similar mixtures of stanols and of dietary Δ^5-sterols. Main sterols appeared to be C_{27}-sterols, which could be connected with a common diet for the both sponges. Saturated short side chain sterols have been found in *Hymeniacidon sanguinea*. Three of them were novel for sponges. A possibility for the transformation of some dietary sterols into stanols is discussed.

Introduction

Sterol mixtures isolated from sponges are usually rather complex and often contain unusual compounds. This could be related to the ability of sponges to obtain sterols by different routes: *de novo* biosynthesis, diet (plankton or detritus), modification of dietary sterols or through *de novo* biosynthesis *via* symbionts (Djerassi and Silva, 1991).

In the Black Sea there are 26 Porifera species. Only few of them, including *Hymeniacidon sanguinea* (family Hymeniacidonidae, order Halichondrida) and *Halichondria panicea* (family Halichondriidae, order Halichondrida) produce relatively large biomass. Sponges from both genera contain the same types of sterols. Δ^5-Sterols and stanols are the main representatives in the sterol mixtures, but their ratio varies in different species (Bergquist *et al.*, 1980; Kanazawa *et al.*, 1979; Dmitrenok *et al.*, 1988; Sica *et al.*, 1978; Erdman and Thompson, 1972; Voogt, 1976) (Tables I and II). In some cases unusual sterols have been found, for example A-nor sterols in some *Hymeniacidon* sp. (Kitagawa *et al.*, 1983; Teshima *et al.*, 1980) and sterols with unusual alkylation in the side chain in *Halichondria sp.* (Ravi *et al.*, 1978; Shubina *et al.*, 1984; Zielinski *et al.*, 1981).

Because of the ecological specificity of the Black Sea, we decided to investigate samples from *H. panicea* and *H. sanguinea*, even though there are lots of data about the sterol composition of these species inhabiting other parts of the Ocean. The water in the Black Sea has half the salinity of other seas and there is a high concentration of hydrogen sulphide in areas more than 100 m in depth. These ecological conditions might affect the metabolism and composition of the sponges as well as of their specific symbionts and diet. On the other hand *H. panicea* and *H. sanguinea* are relatively widely distributed in the Black Sea and data about their sterol composition can give valuable information about the food chains in the sea. These data can also be used for taxonomic conclusions, concerning the position of *Hymeniacidon* genus.

Experimental

The sample of *Hymeniacidon sanguinea* (11.5 g dry weight) was collected in May around Kiten (southern Bulgarian Black Sea coast) at 10 m depth. The sample of *Halichondria panicea* was collected in August around Tyulenovo village (northern Bulgarian Black Sea coast) at the same depth. Representative specimens were deposited in the Museum of Natural History in Sofia, Bulgaria and were identified by Dr. Andreev. Sponges were washed with fresh water, dipped in ethanol and transported to the laboratory. Extraction was performed according to Elenkov *et al.* (1994).

Total lipid extracts were subjected to column chromatography on silica gel with mixtures of hex-

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ane and diethyl ether. Fractions containing free sterols were further purified by column chromatography on alumina with the same mobile phase. GC- analyses of the mixtures were performed on a Hewlett Packard 3500 gas chromatograph equipped with a FID (t = 300 °C) and a capillary column HP-5 (25 m × 0.25 mm, 0.52 μm). Temperature regime 300 °C. Gas carrier – nitrogen. GC-MS analyses of the samples were performed on a Hewlett Packard 6890/5972, equipped with MSD, and a HP-5 capillary column (25m × 0.25 mm, 0.25 μm). Temperature regime 220° (2 min.), 10°/min., 280° (15 min.). Helium was used as a carrier gas.

Results and Discussion

Total free sterols were isolated from the lipophilic extracts of the sponges and purified by chromatography on a silica gel column. The fractions obtained were further investigated by GC and GC/MS.

The results obtained for the sterol composition of *H. sanguinea* and *H. panicea* are summarised in Table I and Table II and are compared with the data from previous investigations of species inhabiting other seas. These data show that most of the representatives of the both genera are characterised by the presence of Δ^5- sterols and corresponding stanols, but their ratio varies in different species.

As it is shown in Table I the main component in *H. sanguinea* is **1i**. Typically "animal" sterols were more than 70% of the sterol mixture while C-24 alkylated ones were in lower concentrations. There are no data about *de novo* sterol biosynthesis in sponges from this genus. Such a sterol pattern might arise from a plankton origin (zooplankton or unicellular red algae). The possibility for *de novo* biosynthesis cannot be omitted either. While in all dietary sources in the sea Δ^5-sterols predominate, the stanols are in very low concentrations. The high concentration of stanols in some marine invertebrates is often a product of symbiotic biotransformation of Δ^5-precursors, as was shown earlier (Djerassi and Silva, 1991). Similar transformation we found recently in some Black Sea *Haliclona* species (Comp. Biochem. Physiol. B, in press). According to all existing knowledge about the marine sterols (Djerassi and Silva, 1991) we can propose that in the investigated two sponges the Δ^5-sterols are from a dietary origin

Table I. Sterol composition (%) of investigated *Hymeniacidon* species.

Species	s.c.	C_{26}		C_{27}				C_{28}			C_{29}		C_{30}	
		e	f	g	h	i	j	k	l	m	n	o	p	q
	Nuclei													
H. sanguinea	**1**	1.6	1.3	5.2	–	63.9	7.4	2.8	2.7	–	0.5	0.6	–	–
	2	0.6	–	–	–	1.4	3.5	0.3	–	2.0	0.6	4.1	–	–
H. sanguinea[1)]	**1**	–	–	2.5	–	60.6	3.4	–	2.2	–	–	–	–	–
	2	1.2	–	5.3	–	3.8	8.3	4.9	2.4	2.8	–	4.1	–	–
H. perleve[2)]	**1**	3.0	–	5.0	–	75.0	8.0	7.0	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	2.0	–	–	
H. perleve[3)]	**1**	tr.	–	tr.	–	50.0	4.0	4.0	4.0	–	–	tr.	–	–
	2	tr.	–	tr.	–	8.0	6.0	tr.	tr.	tr.	tr.	6.0	–	–
H. hauraki[3)]	**1**	–	–	–	–	5.0	–	–	–	–	–	10.0	–	–
	2	–	–	4.0	–	21.0	5.0	6.0	8.0	9.0	–	19.0	–	–
	3	–	–	–	–	tr.	–	–	–	–	–	10.0	–	–
H. assimiles[4)]	**1**	3.5	4.0	6.6	–	59.6	9.8	tr.	1.5	0.9	tr.	4.3	0.5	2.2
	3	–	–	–	–	–	–	–	–	6.5	–	–	–	–
H. perlevis[5)]	**1**	–	–	–	–	–	–	0.2	–	–	–	–	–	–
	2	tr.	–	2.4	0.1	32.4	11.5	–	24.5	–	–	–	–	–
	4	–	–	–	–	–	0.2	–	–	14.3	–	9.5	–	–
	3	–	–	–	–	–	0.9	–	–	–	–	–	–	–
	5	–	–	2.8	–	25.7	13.9	–	28.9	7.7	–	15.8	–	–
H. aldis[6)]	**5**	–	–	6.5	–	44.6	11.5	–	26.6	tr.	10.8	–	–	–

[1)] Sica *et al.*, 1978, [2)] Erdman and Thompson, 1972, [3)] Bergquist *et al.*, 1980, [4)] Dmitrenok *et al.*, 1988, [5)] Kanazawa *et al.*, 1979, [6)] Kitagawa *et al.*, 1983. Sterols which amount is less than 0.1% of the total mixture are represented as traces (tr.). For nuclei and side chains see Fig. 1.

and part of them are transformed into stanols. If so, we can expect that the individual composition of stanols and Δ^5-sterols (end product and precursor) must not differ, but as is evident from Table I, there are very big differences. The C_{27}-sterols were transformed almost entirely into stanols, about 80% from the C_{28}-sterols were transformed into stanols and only traces from the C_{29}-sterols suffer the same transformation. Similar differences in the composition of precursors and end products were found in some other *Hymeniacidon* species (Bergquist *et al.*, 1980; Erdman and Thompson, 1972; Sica *et al.*, 1978). We found in other Black Sea sponges that transformations of Δ^5-precursors proceeded at a different rate. In *Dysidea fragilis*, for example, sterols with double bond at C-22 were transformed to $\Delta^{5,7}$-analogues faster than these ones with saturated side chains. The transformation proposed in this case was confirmed by feeding with radiolabeled precursors (Elenkov *et al.*, 1994). Similar phenomena were observed in other investigated sponges and were explained as an enzyme selectivity to the substrate (John *et al.*, 1989).

Sterols with short side chains have been found in some lower invertebrates: gorgonians, sponges (Carlson *et al.*, 1978) and recently in one algae (Elenkov *et al.*, 1995). We found in trace amounts four sterols from this group in *H. sanguinea* and identified them as **1a, 1b, 1c, 1d**. Three of them (**1a, 1c, 1d**) have never been found in any sponges before. We have previously found small amounts of the sterol **1b** in another Black Sea sponge, *Haliclona flavescens* (*Comp. Biochem. Physiol B*, in press). Analogues of all these sterols with C-5 double bond have been found earlier in gorgonians and sponges (Carlson *et al.*, 1978) and probably the sterols identified by us are products of a biotransformation of the above mentioned sterols in *H. sanguinea*.

The sterol pattern in the Black Sea *Halichondria panicea* appeared to be similar to that of the above

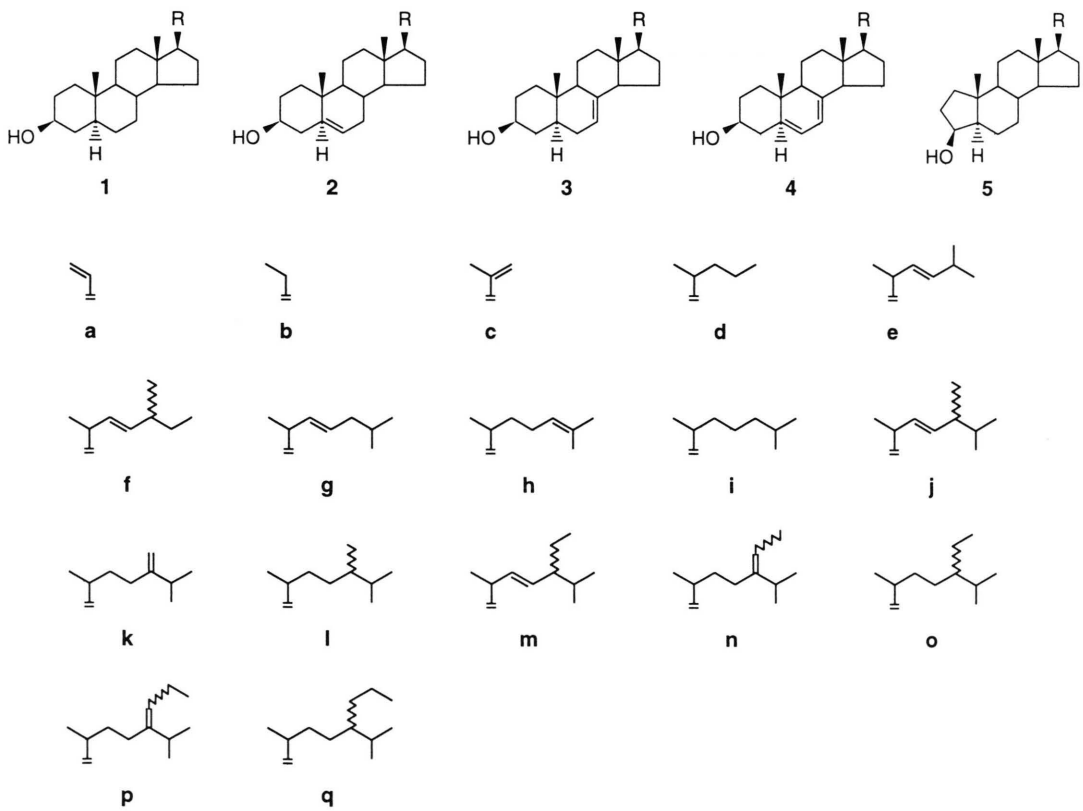

Fig. 1. Formulae: Nuclei and side chains of sterols found in *Hymeniacidon* and *Halichondria* species.

mentioned *Hymeniacidon sanguinea* (Table II). The main sterols were stanols (67% from the total sterols) which indicates an active reduction of the dietary Δ^5-sterols. The reduction of the sterols is also more intense for C_{27}-sterols and is practically absent in C_{29}-sterols.

Two samples of *H. panicea*, collected at different depths at Kurili islands (Dmitrenok *et al.*, 1988) showed significant differences in the ratio of dietary sterols and stanols. In samples collected at depth 10–15 m (the same as the Black Sea sample) stanols were the main sterol components, while in a sponge inhabiting deeper regions (147 m) their concentration was very low. Moreover, instead of Δ^5-sterols in the second sample their Δ^7-analogues were found.

It is evident from Tables I and II that different species from the both genera have different sterol composition. Most of the variances concerning the length and unsaturation of the side chain might be explained with plankton changes in different parts of the Ocean. The sponges of the both genera may transform the dietary Δ^5-sterols into stanols, probably with a participation of some symbiotic bacteria. Microfloral variations at different depths might be an explanation for the differences in the ratio of the end products (stanols and Δ^7-sterols) and precursors (Δ^5-sterols). However, based on the data obtained we can assume that there are several chemotypes in both of these genera. Sponges containing almost entirely Δ^5-sterols or mixtures of them with stanols might be representatives of one group while these ones containing Δ^7-sterols might be included in another group. Species like *Hymeniacidon perlevis* (Teshima *et al.*, 1980; Kanazawa *et al.*, 1979) and *H. aldis* (Kitagawa *et al.*, 1983) which contain the rare group of A-nor stanols might be from a different taxon.

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Table II. Sterol composition (%) of investigated *Halichondria* species.

Species	s.c.	C_{26} e	f	C_{27} g	i	j	C_{28} k	l	m	C_{29} n	o	C_{30}
	Nuclei											
H. panicea	**1**	tr.	–	2.5	57.2	3.8	3.3	–	–	–	–	–
	2	1.3	1.3	1.9	8.0	9.3	5.6	–	1.6	1.7	2.4	–
H. panicea I [1)†]	**1**	0.4	0.4	–	1.9	tr.	1.0	1.6	–	–	0.7	34.9
	3	1.1	0.9	tr.	38.5	9.8	3.4	3.0	–	–	1.9	–
H. panicea II [1)‡]	**1**	0.6	–	1.8	91.2	–	0.2	0.3	–	0.9	0.1	4.8
	2	–	–	–	–	–	–	–	–	–	tr.	–
H. panicea [2)]	**2**	tr.	–	tr.	78.0	5.0	tr.	4.0	tr.	tr.	4.0	–
	3	–	–	–	4.0	–	–	–	–	–	–	–
H. moorei [2)]	**2**	tr.	–	4.0	13.0	11.0	61.0	tr.	tr.	4.0	5.0	–
H. bawerbanki [3)]	**1**	–	–	2.4	38.4	1.0	–	1.6	–	–	–	–
	2	1.4	–	4.4	21.0	19.5	5.0	2.0	–	1.4	1.8	–
H. sp. I [4)]	**2**	2.2	5.1	8.7	26.3	16.6	19.7	3.9	5.9	5.4	3.9	–
H. sp. II [4)]	**1**	0.7	–	8.5	28.9	4.5	–	3.4	1.2	–	16.9	–
	3	–	–	–	7.2	–	–	3.4	2.0	–	23.7	–

[1)] Dmitrenok *et al.*, 1988, [2)] Bergquist *et al.*, 1980, [3)] Sica *et al.*, 1978, [4)] Voogt, 1976, [†]) sponge collected at depths of 147 m, [‡]) sponge collected at depths of 10–15 m. Sterols which amount is less than 0.1% of the total mixture are represented as traces (tr.).

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