Chemical Composition of *Cystoseira crinita* Bory from the Eastern Mediterranean

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_Cystoseira crinita_, Lipids, Secondary Metabolites

The chemical composition of the brown alga *Cystoseira crinita* Bory from the Eastern Mediterranean was investigated. Fourteen sterols have been identified, five of them for the first time in algae. The structure of one new sterol was established. The origin of seven sterols with short side chains was discussed. In the volatile fraction 19 compounds and in the polar fraction 15 compounds were identified. The main lipid classes were isolated and their fatty acid composition was established.

**Introduction**

There are more than 265 genera of brown algae (Chromophycota, Phaeophyceae), grouped in 15 orders (South and Whittick, 1987), widely spread all over the world. Although there are many investigations on their chemical composition, the information, concerning their taxonomy is still incomplete. Lipids (Johns et al., 1979), sterols (Al Easa et al., 1995; Stefanov et al., 2000), terpenes (Valls et al., 1993) and polysaccharides (Usov et al., 1985) have been used for taxonomic conclusions.

Recently it was demonstrated that the composition of the volatiles, obtained by distillation-extraction, could give some extra information concerning the taxonomic problems in algae (Kamenarska et al., 2000). The algae from the genus *Cystoseira* have been extensively investigated. Polysaccharides (Khafaji, 1986; Pelivan and Lutkic, 1994), terpenes (Amico et al., 1989; Amico et al., 1990; Valls et al., 1993), sterols (Al Easa et al., 1995) and lipids (Heiba et al., 1997; Moreno et al., 1998) have been analyzed. In *C. crinita* Bory from the Black Sea, which is one of the main brown alga in this sea, were identified seven sterols (Milkova et al., 1997). The main one appeared to be fucosterol (61.2% of the total sterols), which is characteristic for the brown algae. High concentration of the unusual for the brown algae 24-ethyl-cholest-5-en-3β-ol (25.4%) was also found. A preliminary investigation on the volatile compounds of the same sample of *C. crinita* was also performed. In the complex mixture was shown the presence of some monoterpenes, from which only dihydroactinidiolide was identified (Milkova et al., 1997). The volatiles of *C. barbata*, collected at the same time and location, contained mainly chlorinated ethanes, while the volatiles of *C. crinita* possessed no halogenated compounds (Milkova et al., 1997). This shows that in the different species, growing at identical conditions, the volatiles differ very much and probably these differences could be used for taxonomic conclusions.

Another problem is to what extent the differences in the ecological conditions could change the chemical composition of algae. Here we present an investigation on sterols, volatiles, lipids and polar compounds from *Cystoseira crinita* from the Eastern Mediterranean where the ecological conditions (salinity, temperature, etc.) are different from these in the Black Sea. Moreover, the chemical composition of the algae from the Eastern Mediterranean has not been deeply investigated.

**Materials and Methods**

**Algal material**

The sample of *Cystoseira crinita* was collected by hand using scuba from Mediterranean Sea in Kaş (Turkey) in May 1999 at a depth of 2–8 m. Voucher-specimen HUEF-M99–001 was deter-
mined by Biologist Murat Bilecenoplu and Professor Veyes Aysel and deposited in Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara, Turkey.

**Preparation of the extracts**

The lyophilized sample of *C. crinita* (300 g) was homogenized in methanol (3:1). The extract obtained was filtered and the alga was consecutively extracted with 2:1 methanol-chloroform (1:1 v/v) and with chloroform (2:1). The total extracts were combined, diluted with 500 ml water and the chloroform fraction was separated. The methanol fraction was diluted with water and further extracted with 2 × 500 ml n-butanol.

**Isolation and analysis of sterols**

The chloroform extract was evaporated under reduced pressure at a temperature of 40 °C and a part of the dry residue (1.3 g) was subjected to column chromatography on 40 g of silica gel (1:30). Petroleum ether, followed by petroleum ether-aceton mixtures, chloroform and chloroform–methanol mixtures in ascending polarity were used as eluents. The fractions containing sterols (eluted with petroleum ether–acetone (10:1 v/v), chloroform and chloroform–methanol (99:1 v/v) were combined and purified by prep. TLC on silica gel G with petroleum ether–acetone (10:1 v/v). The sterol fraction was investigated by gas chromatography/mass spectrometry (GC/MS) and gas chromatography (GC).

GC/MS: Hewlett Packard 6890 + MS 5973 with a capillary column HP-5 (23 m × 0.2 mm, 0.5 µm film thickness). Carrier gas: Helium. Temperature programme: 100 °C–315 °C at 5 °C·min⁻¹ and a 10-min hold at 315 °C.

**Isolation and analysis of lipids**

Part of the chloroform extract (153 mg dry residue) was used for the lipid analysis. The lipid classes were separated by preparative TLC on 20 × 20 cm silica gel G (Merck) plates (layer thickness 0.5 mm). Chloroform–methanol–acetone–acetic acid (70:14:24:0.4 v/v/v/v) was used as a mobile phase. Three main lipid classes – triacylglycer-ols (TAG), glycolipids (GL) and phospholipids (PL) were isolated. The fatty acids of each lipid class were converted to methyl esters with 5 ml of 15% acetyl chloride in absolute methanol. After three h at 55 °C, the samples were diluted with water and extracted with petroleum ether. The methyl esters produced were purified by TLC on silica gel G with petroleum ether–acetone 95:5 (v/v) and analyzed by GC.

GC: Hewlett Packard gas chromatograph equipped with FID and 30 m × 0.25 mm i.d. fused silica capillary column SP-2340. Carrier gas: Helium. Temperature programme: 150–210 °C at 4 °C·min⁻¹, and a 10-min hold at 210 °C.

**Isolation and analysis of volatiles**

Part of the chloroform extract (dry residue 480 mg) was subjected to a four h distillation in a Lickens-Nickerson apparatus (Hendriks *et al.*, 1981). The volatiles were extracted from the distilate with diethyl ether (yield: 30 mg, 6.3% of the extract). They were investigated by analytical GC/MS on the same system as described above. HP5-MS capillary column was used (30 m × 0.25 mm, 0.25 µm film thickness). The temperature was programmed from 40 °C to 280 °C at a rate of 6 °C·min⁻¹. Helium was used as a carrier gas.

**Isolation and analysis of polar compounds**

Part of the n-butanol extract (5 mg) was dissolved in 50 µl pyridine and 75 µl of bis-(trimethylsilyl)trifluoroacetamide (BSTFA) was added. The mixture was heated at 80 °C for 30 min and analyzed by GC/MS.

GC/MS: Hewlett Packard 6890 + MS 5973 with a capillary column HP-5 (23 m × 0.2 mm, 0.5 µm film thickness). Carrier gas: Helium. Temperature programme 100 °C–315 °C at 5 °C·min⁻¹ and a 10-min hold at 315 °C.
silyl)-trifluoroacetamide (BSTFA) was added. The mixture was heated at 80 °C for 30 min and analyzed by GC/MS.

The silylated extract was investigated by GC/MS on the system, described above with a capillary column HP-5 (23 m × 0.2 mm, 0.5 µm film thickness). As a carrier gas Helium was used with a temperature programme 100–315 °C at 5 °C·min⁻¹ and a 10-min hold at 315 °C.

Identification of the constituents by GC/MS

The GC/MS investigation was based on the interpretation of the mass spectral fragmentation followed by comparisons of the obtained spectra with those of authentic samples. Computer searches in a HP Mass Spectral Library NIST98 were also applied. In the cases when the spectra of some isomers were very similar and these compounds could not be identified unambiguously, comparisons of the GLC retention times, obtained under the same conditions, were used. When there were no suitable authentic samples and spectra for comparison, no identification was made. Only the unambiguously identified compounds were reported in the Tables.

Results and Discussion

Sterol composition

The composition of the main sterols in the Eastern Mediterranean sample (Table I, Part A) appears to be close to this of C. crinita from the Black Sea (Milkova et al., 1997). Fucosterol is the main sterol, analogously to other brown algae. Its concentration (58.5%) is similar to that in the Black Sea C. crinita. The difference between the two samples is in the concentration of 24-ethyl-cholest-5-en-3β-ol. It is one of the main sterols in the Black Sea sample (25.4% from total sterols), however, in C. crinita from the Eastern Mediterranean it is only in traces. Instead of 24-ethyl-cholest-5-en-3β-ol, significant concentrations of cholesterol and 24-methyl-cholesta-5,24(28)-dien-3β-ol were found in the sample from the Eastern Mediterranean. The observed differences in the concentration of 24-ethyl-cholest-5-en-3β-ol in the two samples evidently will change the properties of the cell membranes and this is probably connected with the differences in the ecological conditions in the Black Sea and the Mediterranean.

<table>
<thead>
<tr>
<th>Sterols</th>
<th>Part A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androst-5-en-3β-ol</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>Pregn-5-en-3β-ol</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>23,24-Dinor-cholesterol-5,20-dien-3β-ol</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>23,24-Dinor-cholesterol-5-en-3β-ol</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>24-Nor-cholesterol-5,22-dien-3β-ol</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>24-Nor-cholesterol-5-en-3β-ol</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>24-Methylcholesterol-5,22-dien-3β-ol</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>24-Methylcholesterol-5,24(28)-dien-3β-ol</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>24-Ethyl-cholesterol-5-en-3β-ol</td>
<td>&lt;0.1</td>
<td></td>
</tr>
<tr>
<td>24-Ethyl-cholesterol-5,24(28)E-dien-3β-ol</td>
<td>58.5</td>
<td></td>
</tr>
<tr>
<td>(Fucosterol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Ethyl-cholesterol-5,24(28)Z-dien-3β-ol</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>(Isofucosterol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Isopropylcholesterol-5,22-dien-3β-ol</td>
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Fatty acids                     | Part B |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Triacylglycerols (TAG)</td>
<td></td>
</tr>
<tr>
<td>Glycolipids (GL)</td>
<td></td>
</tr>
<tr>
<td>Phospholipids (PL)</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>13.1</td>
</tr>
<tr>
<td>15:0</td>
<td>1.5</td>
</tr>
<tr>
<td>16:0</td>
<td>63.0</td>
</tr>
<tr>
<td>16:1</td>
<td>2.1</td>
</tr>
<tr>
<td>16:3</td>
<td>--</td>
</tr>
<tr>
<td>16:4</td>
<td>4.6</td>
</tr>
<tr>
<td>18:0</td>
<td>8.6</td>
</tr>
<tr>
<td>18:1</td>
<td>2.9</td>
</tr>
<tr>
<td>18:3</td>
<td>0.9</td>
</tr>
<tr>
<td>18:4</td>
<td>0.4</td>
</tr>
<tr>
<td>20:1</td>
<td>2.9</td>
</tr>
<tr>
<td>22:5</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Values obtained from three parallel measurements. The standard deviations (related to peak proportions on the chromatogram) are as follows: ±0.3 for 16:0, ±0.2 for 14:1 and 18:1 and ±0.1 for the others.

the silylated fraction eluted after the sterols was identified for the first time in C. crinita from the Black Sea. C. crinita. The sterol precursor squalene. Also for the first time in C. crinita 24-keto-cholesterol was identified.

Some minor constituents, identified in the sterol mixture, appeared to be of special interest. One of them, 24-isopropyl-cholesta-5,22-dien-3β-ol belongs to the rare group of C₃₀ sterols. It was earlier found in marine invertebrates (Elyakov and Stonic, 1988) but to the best of our knowledge, never in algae. Probably some algae, containing this sterol, can be its dietary source for the invertebrates.

Table I. Sterol composition (in % of the total sterol mixture) (Part A) and fatty acid composition of lipid classes (in %* of the total fatty acids mixture) (Part B) of Cystoseira crinita.
Another rare group of sterols are these with unusually short hydrocarbon side chains. They were discovered for the first time in some lower invertebrates (sponges and corals) (Carlson et al., 1978; Popov et al., 1976). Only twice such sterols were found in algae: 23,24-dinor-chol-5,20-dien-3β-ol was found in the Black Sea alga Cladophora vagabunda (Elenkov et al., 1995) and 24-nor-chol-5-en-3β-ol was found in the Black Sea sample of Zanardinia prototypus (Stefanov et al., 2000). Now we identified some of the known, as well as one new short side chain sterol and this allows us to propose that short side chain sterols might originate from algae and through the food chains are transferred to invertebrates. Evidently these sterols are not cell membrane constituents, because the cell membrane sterols must have a side chain, consistent of at least five carbon atoms (Carlson et al., 1978). Moreover their low concentration in tissues makes their structural role disputable.

One possibility for the biosynthesis of these sterols is by terpene cyclization, but it would require the unlikely and unprecedented existence of a whole series of nonsqualene nonpolyrenoid precursors. Another process, which is known to result in shortening of sterol side chains, is autoxidation. It is known that such autoxidation could produce sterols with short side chains (Carlson et al., 1978) as these, identified in this study. The processes of autoxidation in algae would be stimulated by the considerable quantities of oxygen, produced by the algae and by the large amounts of chlorophyll in them, which is an excellent photosensitizer for production of singlet oxygen.

We identified six known short side chain sterols: androst-5-en-3β-ol, pregn-5-en-3β-ol, 23,24-dinor-chol-5,20-dien-3β-ol, 23,24-dinor-chol-5-en-3β-ol, 24-nor-chol-5-en-3β-ol and chol-5-en-3β-ol. The seventh sterol appeared to be a new one. Its molecular ion peak appeared at m/z 328 (100%), which corresponds to a sterol possessing 23 carbon atoms and two double bonds. The elimination of water from the molecular ion (m/z 310, 48%) was more intensive than the elimination of the methyl group (m/z 313, 40%). This, together with the intensive peak at m/z 295 (73%, elimination of water plus methyl group) is an indication for the presence of a double bond at C-5. This was confirmed by the intense peaks at m/z 217 (54%) and 243 (65%) which are due to ions, containing the side chain, rings C and D and parts from ring B. These ions are formed only from sterols, containing a double bond at C-5. The other double bond, according to the intense peaks at m/z 213 (42%) and 255 (54%), is in the side chain. The side chain contains four carbon and seven hydrogen atoms. The position of the double bond must be between C-22 and C-23. If it is in another position, the observed intensive fragments at m/z 255 (54%) and 273 (40%), due to the cleavage of the side chain from the ring system, would not be formed. The fragments at m/z 213 (42%) and 231 (20%), produced by the elimination of ring D together with the side chain were also present. Evidently, the new sterol is 24-nor-chol-5,22-dien-3β-ol.

**Lipid composition**

Three main lipid classes were isolated from the total lipophylic extract by preparative TLC. GL predominated (6.1 mg in 1 g of the lipophylic extract), followed by TAG (5.2 mg), while PL were in a low concentration (2.6 mg). The concentrations of the fatty acids in the different lipid classes are presented in Table I, Part B. It is evident that the main fatty acid is palmitic acid, which is characteristic for most of the algae, followed by myristic and oleic acids. There are no significant differences in the fatty acid composition of the three lipid classes isolated. In the sample investigated the content of 16:3 and 16:4 acids is very low in the PL and practically absent in TAG and GL. In the sample of Cystoseira abies-marina from Gran Canaria, there were high concentrations of 16:3 and 16:4 acids, concentrated in MGDG, while arachidonic and 20:5 acids predominated in the PL and TAG (Moreno et al., 1998). In our sample, we found high concentrations of saturated acids (about 80% from the total acids). Such high concentration of saturated fatty acids was earlier reported for the lipids of C. myrica from the Red Sea (Karawya et al., 1987). 16:0, 14:0, and 18:0 acids also predominated in the lipids of C. trinodis from the coastal zones of Qatar (Heiba et al., 1997).

**Volatile compounds**

It is known that the volatiles from plants often contain water-insoluble compounds with defensive functions, attractants, repellents, antifeedants, in-
sesticides, etc. (Jiang et al., 1997, Wang et al., 1999). Until now the research concerns almost entirely the volatiles from terrestrial plants, while there is a very limited number of publications on algae (Dembitsky et al., 1999; Gally et al., 1993; Kamenarska et al., 2000; Mahran et al., 1993). For this reason we performed an investigation on the volatiles, isolated by distillation of the total chloroform extracts. The results obtained are summarized in Table II, Part A.

The main components of the volatiles appeared to be chlorine-containing compounds. Earlier it was found that the main volatiles in the Black Sea Cystoseira barbata were chlorinated ethanes, but no chlorinated compounds were identified in the volatiles from the Black Sea C. crinita, collected at the same place (Milkova et al., 1997). However, in the present study, the chlorinated compounds in C. crinita samples from the Eastern Mediterranean were about 27% of the total volatiles. The main ones appeared to be ethanes, substituted with chlorine and ethoxy groups. These compounds, especially chloroacetic acid and its ethyl ester possess antibacterial and antifungal activities and might have defensive functions in the alga. Probably, different ethane derivatives, containing chlorine atoms, could be characteristic for Cystoseira sp. The detection of chlorine-containing compounds in algae is an indication for the presence of the enzyme chloroperoxidase. The biological halogenation by algal haloperoxidases is considered to lead to the emission of the volatile halogenated compounds (Collén et al., 1994) and these compounds are recognized as substrates that destroy the ozone layer. The halogenated compounds are a type of allelochemicals produced by algae. They have antibacterial and antifungal effects, suppress the development of other algae, induce larval settlement (Oshihiro et al. 1999). The presence of thioacetic acid as an O-ethyl ester is also of interest.

In the volatiles of the Black Sea Cystoseira crinita terpenoids predominated (Milkova et al., 1997). In the Mediterranean C. crinita sample we identified dihydroactinidiolide, hexahydrofarnesylacetone and 3,7-dimethyl-1,6-octadiene-3-ol-2-aminobenzoate.

### Table II. Composition of the volatiles (in %* from the total volatiles) (Part A) and polar compounds (in %* of the total silylated fraction) (Part B) from Cystoseira crinita.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Part A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Hexadecane</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Heptadecane</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Octadecane</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Nonadecane</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Alcohols</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>2,3-Butanediol</td>
<td></td>
<td>6.5</td>
</tr>
<tr>
<td>Free acids</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Nonanoic acid</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Decanoic acid</td>
<td></td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>Terpenes</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Dihydroactinidiolide</td>
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<td>0.2</td>
</tr>
<tr>
<td>Hexahydrofarnesylacetone</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>3,7-Dimethyl-1,6-octadiene-3-ol-2-aminobenzoate</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Cl-containing compounds</td>
<td></td>
<td>27.6</td>
</tr>
<tr>
<td>1-Chloro-2-ethoxy-2-methoxyethane</td>
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<td>0.2</td>
</tr>
<tr>
<td>1-Chloro-2,2-diethoxyethane</td>
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<td>21.5</td>
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<tr>
<td>1,1-Dichloro-2,2-diethoxyethane</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Chloroacetic acid</td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td>Chloroacetic acid, ethyl ester</td>
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<td>0.2</td>
</tr>
<tr>
<td>S-containing compounds</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td></td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Thioacetic acid-O-ethyl ester</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Dihydro-3-hydroxy-3-hydroxymethyl-2(3H)-furanone</td>
<td></td>
<td>0.2</td>
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<table>
<thead>
<tr>
<th>Polar compounds</th>
<th>Part B</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aliphatic acids</td>
<td></td>
<td>34.9</td>
</tr>
<tr>
<td>2-Hydroxypropanoic acid</td>
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<td>&lt;0.1</td>
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<td>4-Hydroxy pentanoic acid</td>
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<tr>
<td>Myristic acid</td>
<td></td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Pentadecanoic acid</td>
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<td>&lt;0.1</td>
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<tr>
<td>Palmitoleic acid</td>
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<td>&lt;0.1</td>
</tr>
<tr>
<td>Palmitic acid</td>
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<td>32.8</td>
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<tr>
<td>Linoleic acid</td>
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<td>&lt;0.1</td>
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<td>Oleic acid</td>
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<tr>
<td>Stearic acid</td>
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<td>&lt;0.1</td>
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<tr>
<td>Aromatic acids</td>
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</tr>
<tr>
<td>Benzoic acid</td>
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<td>2.3</td>
</tr>
<tr>
<td>2-Methylbenzoic acid</td>
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<td>1.2</td>
</tr>
<tr>
<td>4-Methylbenzoic acid</td>
<td></td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Esters</td>
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<td>&lt;0.1</td>
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<tr>
<td>2,3-Dihydroxypalmitic acid, propyl ester</td>
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<td>&lt;0.1</td>
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<tr>
<td>Poliols</td>
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<td>2.6</td>
</tr>
<tr>
<td>1,3-Butanediol</td>
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<td>2.6</td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

* The ion current generated depends on the characteristics of the compound and is not a true quantification.
**Polar compounds**

In all living organisms the polar compounds, soluble in alcohol and water, predominate. Furthermore, compared to the lipophylic substances, they more often possess a biological activity (Kubo et al., 1990). Despite that, especially in marine organisms, the investigations of polar constituents are not frequent, mostly because of the difficult separation and purification.

For these reason GC/MS can be considered as a suitable method for the investigation of polar compounds, after a derivatization (most often silylation), which increases the volatility of the polar compounds. The results obtained in this study are given in Table II, Part B.

The composition of the polar fraction of the Mediterranean *C. crinita* sample turned out to be a simple one. Saturated fatty acids (palmitic and stearic) predominated in it, accompanied by a lower concentration of oleic acid. It is reported in literature that the antibiotic activity of some algal species could be attributed to the presence of a mixture of organic acids such as: capric, lauric, linoleic, myristic, oleic, palmitic, stearic (Kanias et al., 1992). The free fatty acids also serve as energetic substrates and allelopathic agents (Yasumoto et al., 2000; Ramsewak et al., 2001). Low concentrations of benzoic acid as well as 2-methyl- and 4-methylbenzoic acids were also identified. These compounds could have defensive functions (Cowan, 1999). We did not find any free monosaccharides and mannitol in the Mediterranean Sea *C crinita*, while mannitol was found in large amounts in the Mediterranean *C. stricta* (Amico et al., 1976). Contrary to *C. stricta*, we did not find any free amino acids either.

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