Hydrobiologia **00:** 1–8, 2002.

Chemical composition of the sponge *Chondrosia reniformis* from the Canary Islands

Jordan Nechev[1], William W. Christie[2], Rafael Robaina[3], Fernando M. de Diego[3], Albena Ivanova[4], Simeon Popov[1] & Kamen Stefanov[1,*]

[1]*Inst. of Org. Chemistry with Centre of Phytochemistry, Bulg. Acad. Sci., Sofia 1113, Bulgaria*
Tel: 29606135. Fax: 2700225. E-mail: kamen@orgchm.bas.bg
[2]*The Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland*
[3]*University of Las Palmas, 35001 Las Palmas, Gran Canaria, Spain*
[4]*Inst.of Plant Physiology, Bulg. Acad. Sci., Sofia 1113, Bulgaria*
(*Author for correspondence)

Received 15 April 2002; in revised form 24 September 2002; accepted 21 October

Key words: sponges, *Chondrosia reniformis, Demospongia,* lipids, fatty acids, sterols, volatiles, polar compounds

Abstract

The fatty acid composition of the lipids from *Chondrosia reniformis* (Nardo, 1847) was investigated and 57 acids were identified. One of them is new for nature and its structure was elucidated by GC/mass-spectrometry. This acid was identified as 8,10-dimethyl-16:0. The sterol composition was relatively simple and only 12 sterols were present. In the volatile fraction, 21 compounds were identified, mainly fatty acids, their esters and hydrocarbons, while in the n-butanol fraction we found mainly free fatty acids and free amino acids, together with significant amounts of sterols which probably are included in some polar complexes.

Introduction

The marine sponges from Family Chondrosiidae (Class Demospongia) contain fatty acids and sterols with unusual compositions (Sica et al., 1978; Imre & Acikkol, 1981; Lichfield et al., 1980; Carballeira et al., 1993). Nothing is known for the composition of volatiles and for those polar compounds extractable with n-butanol.

Methyl-branched fatty acids originate from the selective incorporation of methylmalonyl-CoA by the fatty acid synthetase. Such enzymes, resulting in the synthesis of methyl-branched fatty acids, were isolated from the harderian gland of the guinea pig (Seyama et al., 1981) Straight- and even-chain fatty acids with 14–18 carbon atoms and methyl branches on carbons 2, 4, 6 or 8 were isolated from the phospholipids of this gland (Seyama et al., 1983). From barley-fed lambs were isolated uncommon 4,8-dimethyl-13:0 and 4,8-dimethyl-15:0 acids (Duncan et al., 1974). In some instances, these methyl-branched acids were typical of bacteria, and for example, in some Mycobacterium species, trimethyl-branched fatty acids such as 2,4,6-trimethyl-22:0 and 2,4,6-trimethyl-24:0 occur.

Recently, from a halophilic Bacillus sp. isolated from the salt pans of Bourgas in Bulgaria, six novel dimethylated iso-anteiso fatty acids with methyl substituents at carbons 2 and 4 were identified (Carballeira et al., 2001). In this paper, we report the identification of three homologous 3-methyl-branched fatty acids with 14, 15 and 16 C-atoms from the sponge *Chondrosia reniformis* from the Canary Islands. Recently another methyl-branched fatty acid was isolated from demosponges and was identified as 9-methyl-14:0 (Thiel et al., 1999). One new acid named 8,10-dimethyl-16:0 was also present.

It is known that the volatiles from plants often contain compounds that are insoluble in water and with defensive functions, attractants, repellents, antifeed-

JS: DISK: CP: PIPS No. 5112553 (hydrkap:bio2fam) v.1.1
1645.tex: 4/12/2002; 15:02; p.1

ants, insecticides, etc (Jiang et al., 1997; Wang et al., 1999). Till now the research concerns almost entirely the volatiles from terrestrial plants, while there is a limited number of publications on marine algae (Mahran et al., 1993; Gally et al., 1993; Kamenarska et al., 2000).

Materials and methods

Collection of sponges and separation

of the main groups of constituents Sponges *Chondrosia reniformis* (Nardo, 1847) were collected from the rocks of the Las Palmas shore at low tide in November 1999. The samples were frozen at minus −30 °C for one week. After transportation to the laboratory, the sponges were extracted with a mixture of chloroform and methanol (2:1 v/v), followed by filtration and addition of an equal volume of water. The lower layer, containing the total lipids, was evaporated under reduced pressure. The upper layer was concentrated under vacuum and extracted twice with n-butanol. The butanol extracts were combined and evaporated under vacuum. The yield of lipids was 10.0% of the dry weight of the sponges, and the yield from the butanol extracts was 10.5% of the dry weight of the sponges (from 36 sponges – total dry weight 11.30 g). A voucher specimen was deposited in the University of Las Palmas, Canary Islands, Spain.

Preparation of the fatty acid methyl esters

Part of the total lipid extract (40 mg) was transesterified with 15% acetyl chloride in methanol according Christie (1989). The fatty acid methyl esters (FAME) were purified by preparative thin-layer chromatography (TLC) (plates 20 × 20 cm silica gel Merck 60, layer 0.5 mm, mobile phase hexane - acetone 95:5 v/v).

Catalytic hydrogenation

Fatty acid methyl esters in methanol solution were subjected to catalytic hydrogenation with hydrogen gas and platinum oxide catalyst (Christie, 1989).

Gas Chromatography-Mass Spectrometry of fatty acid derivatives

The lipids were hydrolysed to free fatty acids before conversion to the picolinyl esters (Balazy & Nies, 1989) and to 4,4-dimethyloxazoline (DMOX) derivatives (Fay & Richli, 1991) by the established methods. The derivatives were submitted to Gas Chromatography-Mass Spectrometry (GC-MS), with a Hewlett Packard 5890 Series II plus gas chromatograph attached to an HP model 5989 MS engine. The latter was used in the electron impact mode at 70 eV with a source temperature of 250 °C. The GC was fitted with on-column injection, and equipped with a capillary column of fused silica coated with DB5-MS^{TM} (0.25 mm × 30 m, 0.25 μm film; J. & W. Scientific, Folsom, CA, U.S.A.). After holding the temperature at 80 °C for 3 min, the column was temperature-programmed at 20 °C/min to 160 °C, then at 4 °C/min to 350 °C, where it was held for 20 min. Helium was the carrier gas.

Isolation and analysis of sterols

Part of the lipophylic extract (200 mg) was chromatographed on a silica gel column (20 g) with mixtures of hexane and acetone in ascending polarity. The fractions containing sterols TLC were combined and purified by preparative TLC, with hexane-acetone 9:1 as a mobile phase. The sterols obtained were analysed by gas chromatography and by GC/MS under the same conditions as for the picolinyl esters above.

Isolation and analyses of the volatile compounds

Part from the lipophylic extract (350 mg) was subjected to a 4-h distillation-extraction in a Liken-Nickerson apparatus (Hendriks et al., 1981). The volatiles were extracted from the distillate with diethyl ether and investigated by analytical GC/MS Hewlett Packard 6890 + MS 5973 with a capillary column HP5-MS (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. Helium was used as a carrier gas (linear velocity of 31 cm/s).

Isolation and analyses of the n-butanol fraction

The n-butanol fraction was isolated as described above. Part of it (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 on the system, described above, but equipped 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 of 100 °C – 315 °C at 5 °C/min and a 10-min hold.

Results and discussion

Investigation of lipids

The GC analysis of the FAME indicated that at least 57 different fatty acids were present in *Chondrosia reniformis* (Table 1). The main fatty acid was the demospongic acid 5,9–26:2, a typical one for the sponges from Class Demospongia. Most of the remaining fatty acids have been isolated previously from other marine sponges (Litchfield et al., 1980; Walkup et al., 1981; Carballeira & Reyes, 1990; Carballeira et al., 1993), sea anemones (Carballeira and Medina, 1994) and from a fresh water sponge (Dembitsky et al., 1994). It was interesting to note that there were significant differences between the fatty acid compositions of our sample and a sample of *Chondrosia remiformis* from the Caribbean Sea (Carballeira et al., 1993). The latter contained 5,9,23-30:3 and 3,7,11,15-tetramethyl-20:0 acids as major components, while in our sample 5,9-26:2 predominated. The novel 27-methyl-5,9-28:2, 26-methyl-5,9-28:2, and 15-methyl-5,9-16:2 acids found in the Caribbean sample were absent in that from the Canary Islands. This difference could be due to the fact that we analysed the fatty acids from the total lipid extract, while in the Caribbean sample the fatty acids were analysed from a phospholipid fraction. On the other hand, our sponge contained new acids, namely 8,10-dimethyl-16:0.

Samples were analysed by GC-MS in the form of various derivatives before and after catalytic hydrogenation in order to simplify the chromatograms and make it easier to locate the branch points.

Figure 1 represents the mass spectra of the picolinyl ester (above) and DMOX derivative (below) of the novel acid, 8,10-dimethyl-16:0. It was easy to locate the methyl groups in the spectrum of the picolinyl ester from the gaps of 28 amu between m/z = 220 and 248/9, and m/z = 262 to 290. Similarly, for the DMOX derivative the corresponding gaps were between m/z = 182 and 210, and m/z = 224 to 252.

Table 1. Fatty acid composition (wt% of the total) from the sponge *Chondrosia reniformis*

Fatty acid[a]	Retention Time (min)	Wt%[b]
i-methyl-13:0	37.38	0.2
14:0	38.95	0.8
3-methyl-14:0	39.79	0.2
9-methyl-14:0	40.66	1.0
i-methyl-14:0	41.58	4.7
ai-methyl-14:0	41.86	2.0
3-methyl-15:0	42.19	0.3
15:0	42.82	1.3
5,9-16:2	44.95	3.4
i-methyl-15:0	45.29	0.8
9-16:1	45.96	2.7
16:0	46.94	6.7
5,9-17:2 ?	47.25	2.5
3-methyl-16:0	47.48	0.3
8,10-diMe-16:0 [c]	48.63	1.1
i-methyl-16:0	49.01	1.3
ai-methyl-16:0	49.33	1.5
9,10-cyclopropyl-16:0	49.77	1.0
17:0	50.23	0.8
5,9-18:2	51.98	0.9
9-18:1	52.81	1.3
11-18:1	53.14	3.0
18:0	53.93	2.5
11-19:1	54.09	0.5
11-methyl-18:0	55.08	3.8
11,12-cyclopropyl-18:0	56.99	1.8
19:0	57.29	0.7
5,8,11,14-20:4	57.82	0.9
5,8,11,14,17-20:5	58.01	0.4
5,9-20:2	58.30	0.2
11,14-20:2	59.48	0.4
20:0	60.75	3.5
methyl-20:0	61.76	1.0
i-methyl-20:0	62.56	0.3
ai-methyl-20:0	62.89	0.3
21:0	63.85	1.2
5,9-22:2	65.10	1.2
22:0	67.00	1.1
5,9-23:2	67.34	0.1
methyl-22:0	68.12	1.7
i-methyl-22:0	68.86	0.4
23:1	69.81	0.3
23:0	69.98	0.1
17-methyl-5,9-24:2	72.36	1.4
24:0	73.01	0.5
5,9-25:2	74.23	3.2
5,9,19-26:3	75.93	0.5
5,9-26:2	78.07	24.4
21-methyl-5,9-26:2	78.74	4.6
5,9,?-27:3	79.41	0.2
5,9,23-27:3	79.59	0.2
5,9-27:2	80.13	2.6
5,9,?-28:3	81.97	0.3
5,9,?-28:3	82.21	0.2
5,9-28:2	82.58	0.8
5,9-29:2	83.52	0.4
5,9,?-30:3	87.40	0.2

[a] A shorthand designation is used, e.g. 5,9–26:2 means Δ5,9-hexadocosadienoic acid.

[b] Triplicate values for methyl esters in standard mixture by gas chromatographic analyses varied within 12% for minor components (<5% content) and within 5% for the others.

[c] Not previously recognised in nature.

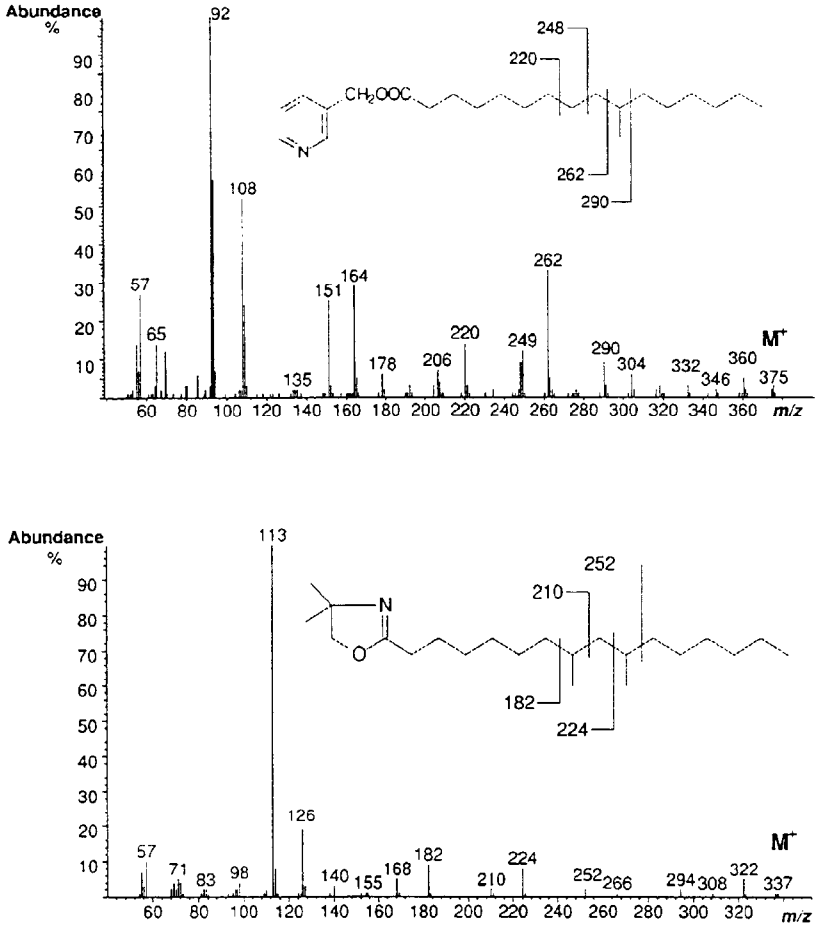

Figure 1. Mass-spectrum of 8,10-dimethyl-16:0 acid as picolinyl ester (top) and DMOX-derivative (bottom).

Table 2. Composition of total sterols isolated from *Chondrosia reniformis*

Sterol	wt-%[a]
(22Z)-cholesta-5,22-dien-3β-ol	0.5 ± 0.04
(22E)-cholesta-5,22-dien-3β-ol	2.2 ± 0.21
cholesterol	71.0 ± 5.7
24-methyl-cholesta-5,22-dien-3β-ol	5.0 ± 0.4
cholest-7-en-3β-ol	0.6 ± 0.5
24-methyl-cholesta-5,24(28)-dien-3β-ol	2.0 ± 0.2
24-methyl-cholest-5-en-3β-ol	11.0 ± 0.9
24-ethyl-cholesta-5,22-dien-3β-ol	1.0 ± 0.1
24-ethyl-cholesta-7,22-dien-3β-ol	0.1 ± 0.01
24-ethyl-cholest-5-en-3β-ol	5.3 ± 0.4
24-ethyl-cholesta-5,24(28)-dien-3β-ol	6.2 ± 0.5
24-ethyl-cholestan-3β-ol	<0.1

[a] wt-% of total sterols ± SD from three parallel GC-analyses.

Investigation of sterols

Sterols were analysed by gas chromatography (qualitative and quantitative analyses) and by GC/MS. The results obtained are summarized in Table 2. It is evident that in contrast to most of the marine sponges investigated till now (Djerassi & Silva, 1991), the sterol composition of this sample is very simple, in accordance with the earlier results of Sica et al. (1978). Cholesterol is the main sterol in the sample investigated by us, accompanied by low concentrations of sterols typical of marine sponges. Some of the minor sterols are characteristic for phytoplankton, while cholesterol and 22-dehydrocholesterol could be produced mainly by the zooplankton, but participitation of some phytoplankton species cannot be excluded. In contrast to most marine organisms, we found no stanols in

Table 3. Volatile components in *Chondrosia reniformis*

Component	%[a]
acids	
nonanoic acid	0.06
tetradecanoic acid (14:0)	0.52
methyl tetradecanoic acid (15:0 isomer)	1.08
pentadecanoic acid (15:0)	2.21
hexadecanoic acid (16:0)	1.63
hydrocarbons	
hexadecane (16:0)	1.71
heptadecane (17:0)	0.20
octadecane (18:0)	0.09
alcohols	
phenol	0.07
2-octanol	0.04
benzyl alcohol	0.05
2-methyl phenol	0.03
β-phenyl ethyl alcohol	0.485
esters	
tetradecanoic acid (Me ester)	0.20
tetradecanoic acid (Et ester)	0.12
pentadecanoic acid (Et ester)	0.22
hexadecanoic acid (Me ester)	2.59
hexadecanoic acid (Et ester)	1.57
octadecenoic acid (Me ester)	1.12
aldehydes	
benzaldehyde	<0.1
N-containing	
formamide	0.05

[a]The determination is semiquantitative. Only identified compounds are reported as percent of total volatile compounds.

this sterol mixture with the exception of sitostanol. Probably it comes from another dietary source.

The ^{1}H-NMR spectrum of the total sterol mixture confirmed the absence of stanols – there were peaks for H-6 and H-3 with equal intensity. The peaks for H-22 and H-23 protons showed that in the sterol mixture the (22E) and (22Z) double bonds were presented in an aproximate ratio 1:2.

The concentration of the sterol esters was very low and no investigations on this group of compounds was performed.

Investigation of the volatile substances

In contrast to the algae, which are an important dietary source for the filter-feeders, including sponges, the composition of volatiles in *Chondrosia reniformis* appeared to be relatively simple (Table 3). Analogously to the algae (Kamenarska et al., 2000), free fatty acids and their esters predominated in the volatile fraction of this sponge, followed by hydrocarbons and alcohols. Free fatty acids have different functions in the organism compared to those included in the lipids. They serve as energy substrates and allelopathic agents (Yasumoto et al., 2000). It is also reported in literature that the antibiotic activity of some algae 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). Most of the fatty acids we identified were common for marine organisms, and are normally found as glycerol esters in different groups of lipids. The presence of free acids is sometimes accepted as a result of hydrolysis during the isolation procedure. The distillation-extraction is a relatively mild process and we do not expect degradation of the lipids, so the identified fatty acids could exist in a free state in *C. reniformis*. This is in agreement with the identification of the same acids in the polar fractions extracted with n-butanol (discussed below), because during their extraction and silylation there was no possibility for hydrolysis. The identification of one branched fatty acid and two fatty acids with an odd number of carbon atoms is an indication for the presence of associated bacteria, which are characteristic for some sponges.

Esters of fatty acids with methanol and ethanol are often considered as artifacts, formed by an alcoholysis of lipids through the extraction and separation procedures. In *C. reniformis* we found a mixture of methyl and ethyl esters, when only methanol was used in our experiments. This confirms our statement that the esters obtained are not artefacts. Analogous is the situation in algae investigated recently by us (Kamenarska et al., unpublished results).

The main part of the identified hydrocarbons contains an even number of carbon atoms, which confirms our suggestions for the presence of associated bacteria in this sponge.

Alcohols often possess important allelopathic functions. We identified two phenols, which have antibacterial and antimicotic functions and might possess defensive functions in the sponge. Such phenols have been found in volatiles from scallop (Chung et al., 2001) and algae (Kamenarska et al., 2000) and probably are widely distributed in the sea.

β-Phenylethyl alcohol was found in significant concentrations in the investigated sponge which is an indication that it might possess some important functions in this organism. β-Phenylethyl alcohol is often

found in plants and algae and it is known that this compound has allelopathic functions as an aggregation pheromone of some insects and a repellent to others (Wang et al.,*1999*). Probably it can take part in the interactions between sponges and marine Arthropoda, which are one of the main predators on sessile organisms. Benzyl alcohol and benzaldehyde are biogenetically related and probably also have defensive functions.

Investigation of the n-butanol fractions:

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

GC/MS is a suitable method for the investigation of polar compounds, after a derivatization (most often silylation), which increases the volatility. The results obtained by this method are summarized in Table 4.

In the polar fraction from *C. reniformis* the main groups of compounds appeared to be free fatty acids and sterols, but in contrast to algae there were only traces of one oxidized acid. Further experiments will show if this is valid for other sponges and invertebrates. Evidently the enzymes which oxidize fatty acids in this sponge are less active than the corresponding enzymes in algae. In the same fraction from Black Sea algae, the main components were free fatty acids and their oxidized derivatives (Kamenarska et al., unpublished results).

We discussed above the possibility that free fatty acids might be artifacts, and their identification in the polar fraction obtained by a gentle method (extraction and silylation), which excludes hydrolysis. It is evident that the free fatty acids and amino acids obtained in the sponge investigated are not artifacts and probably have some functions in the organism. The presence of significant amounts of fatty acids with an odd number of carbon atoms confirms our above suggestion for the presence of significant amount of associated bacteria in *C. reniformis*.

Analogously to the Black sea algae investigated by us (Kamenarska et al., unpublished results), the rare amino acid 5-oxo-proline appeared to be one of the main free amino acids. Probably this can be characteristic for more marine organisms. For the first time in marine organisms, we identified the rare aminomalonic acid.

In contrast to the algae, we identified in *C. reniformis* some nitrogen containing compounds

Table 4. Components in n-butanol fraction of *Chondrosia reniformis*

Component	%[a]
acids	
benzoic acid	0.07
2-hydroxy-hexanoic acid	0.07
butanedioic acid	0.47
tetradecanoic acid (14:0)	0.33
pentadecanoic acid (15:0)	1.64
pentadecanoic acid (15:0)	0.75
palmitelaidic acid	0.79
hexadecanoic acid (16:0)	1.57
heptadecenoic acid (17:1)	0.97
heptadecanoic acid (17:0)	1.44
3,7,11,15-tetramethyl hexadecanoic acid	0.52
oleic acid (18:1)	0.97
octadecanoic acid (18:0)	0.54
nonadecanoic acid (19:0)	1.49
arachidonic acid	0.13
eicosanoic acid (20:0)	0.52
H_3PO_4-propyl ester	0.57
alcohols	
glycerol	1.27
sterols	
cholesterol	16.12
24-methyl-cholesta-5,22-dien-3β-ol	1.42
Δ7-cholesterol	0.14
24-methyl-cholest-5-en-3β-ol	1.15
24-ethyl-cholest-5-en-3β-ol	1.23
24-ethyl-cholesta-5,24(28)-dien-3β-ol	1.18
N-containing	
alanine	0.83
valine	1.06
leucine	1.12
proline	0.32
isoleucine	0.81
threonine	0.15
5-oxo-proline	0.96
phenylalanine	0.38
tyrosine	0.26
2,4-dihydroxy-pyrimidine	1.85
2,4-dihydroxy-5-CH_3-pyrimidine	1.59
(9H)purine-2,6-dihydroxy	1.39
hypoxantine-diTMS	1.30
aminomalonic acid	0.1

[a]See footnote in Table 3.

(pyrimidines, purines, hypoxanthine), which are characteristic for some marine invertebrates. Different esters of phosphoric acid are often found in marine algae and now we find one of them, monopropyl ester of phosphoric acid, in a sponge. We can assume that such compounds have a defensive role.

Recently, we found that macroalgae from the Black Sea contain significant concentrations of free fatty acids, amino acids, monosaccharides and hydrocarbons, while some algae from the Mediterranean do not contain such compounds and we tried to explain this difference with the differences in the ecological conditions – temperature, salinity, predators, etc. Now we investigate another type of organism inhabiting a sea with ecological conditions similar to these of the Mediterranean. It contains free fatty acids, amino acids and hydrocarbons analogously to the Black Sea algae, but no free monosaccharides were found.

The identification of significant amounts of sterols in the polar fraction is of considerable interest. According to the extraction procedure that we followed, sterols should be completely extracted with chloroform from the total extract. However, significant amounts of sterols with same relative concentrations as those from the chloroform extract were identified in the n-butanol extract. Probably they form unstable complexes with some polar compounds (sugars, amino acids, etc.) which are not soluble in chloroform. They remained in the polar fraction, isolated by n-butanol extraction and probably are decomposed during the silylation procedure, liberating TMS-ethers of sterols.

Acknowledgements

Partial support by the National Foundation for Scientific Research of Bulgaria (Contract X-1101) and by the Scottish Executive Rural Affairs Department are gratefully acknowledged.

References

Balazy, M. &A. S. Nies, 1989. Characterization of Epoxides of Polyunsaturated Fatty and GC-Mass Spectrometry in the Analysis of Fatty Acids. In Cambie, R.C. (ed.), Fats for the Future. Ellis Horwood Ltd, Chichester: 335–344.

Carballeira, N. M. &E. D. Reyes, 1990. Identification of the New 23-Methyl-5,9-pentacosadienoic Acid in the Sponge Cribrochalina vasculum. Lipids 25: 69–71.

Carballeira, N. M., E. D. Reyes &F. Shalabi, 1993. Identification of Novel iso/anteiso Nonacosadienoic Acids from the Phospholipids of the Sponges *Chondrosia reniformis* and *Myrmecioderma styx*. J. Nat. Prod. 56: 1850–1855.

Carballeira, N. M. &J. R. Medina, 1994. New delta-5,9 Fatty Acids in the Phospholipids of the Sea Anemone *Stoichactis helianthus*. J. Nat. Prod. 57: 1688–1695.

Carballeira, N. M., C. Miranda, C. M. Lozano, J. T. Nechev, A. Ivanova, M. Ilieva, I. Tzvetkova &K. Stefanov, 2001. Characterization of Novel Methyl-Branched Chain Fatty Acids from a Halophilic *Bacillus* Species. J. Nat. Prod. 64: 256–259.

Christie, W. W., 1989. Gas Chromatography and Lipids, Oily Press, Ayr.

Chung, H. Y., I. K. S. Yung &J-S. Kim, 2001. Comparison of volatile compounds in dried scallops (*Chlamys farreri* and *Patinopecten yessoensis*) prepared by boiling and steaming methods. J. Agric. Chem. 49: 192–202.

Dembitsky, V. M., T. Rezanka &A. G. Kashin, 1994. Comparative study of the endemic freshwater fauna of Lake Baikal-VI. Unusual fatty acids and lipid composition of the endemic sponge *Lubomirskia baicalensis* and its amphipod crustacean parasite *Brandtia* (*Spinacanthus*) *parasitica*. Comp. Biochem. Physiol. 109B: 415–426.

Djerassi, C. &C. J. Silva, 1991. Sponge sterols: origin and biosynthesis. Acc. Chem. Res. 24: 371–378

Duncan, W. R. H., A. K. Lough, G. A. Garton &P. Brooks, 1974. Characterization of branched chain fatty acids from subcutaneous triacylglycerols of barley-fed lambs. Lipids 9: 669–673.

Fay, L. &U. Richli, 1991. Location of double bonds in polyunsaturated fatty acids by GC-MS after 4,4-dimethyloxazoline derivatization. J. Chromatogr. 541: 89–98.

Gally, A., N. Yannovits &C. Poulos, 1993. The aroma volatiles from *Cystoseira stricta* var. amentaceae. J. Essenial Oil Res. 5: 27–32.

Hendriks, H., J. Geerts &Th. Malingre, 1981. The occurrence of valeranone and cryptofauronol in the essential oil of Valeriana officinalis L. S. l. collected in the northern part of The Netherlands. Pharm. Weekblad, Sci. Edition, 116: 1316–1320.

Imre, S. &M. Acikkol, 1981. Free Fatty Acids Composition of Some Marine Invertebrates Collected from Turkish Waters. Istanbul Univ. Eczacilik Fak. Mecm. 17: 202–207 (Eng).

Jiang, Y., T. J. Ridsdill-Smith &E. L. Ghisalberti, 1997. The effect of volatile metabolites and lipid peroxidation on the aggregation of redlegged earth mites *Halotigeus destructor* (Acarina: penpanthalidae) on damaged cotyledons of subterranean clover. J. Chem. Ecol. 23: 163–174.

Kamenarska, Z. G., S. Dimitrova-Konaklieva, C. H. Nicolova, A. Kujumgiev, K. Stefanov &S. Popov, 2000. Volatile components of the freshwater algae *Spirogyra* and *Mougetia*. Z. Naturforsch. 55. 495–499.

Kanias, G. D., H. Skaltsa, E. Tsitsa, A. Loukis &J. Bitis, 1992. Study of the correlation between trace elements, sterols and fatty acids in brown algae from the Saronikos Gulf of Greece. Fresenius J. Anal. Chem. 344: 334–339.

Kubo, I., M. Ochi, K. Shibata, F. J. Hanke, T. Nakatsu, K. S. Tan, M. Taniguchi, T. Kamikawa, Y. Yamagiwa, M. Arikuza &W. F. Wood, 1990. Effect of a marine algal constituent on the growth of lettuce and rice seedings. J. Nat. Prod. 53: 50–56.

Litchfield, C., J. Tyszkiewicz &V. Dato, 1980. 5,9,23-Triacontatrienoic Acid, Principal Fatty Acid of the Marine Sponge *Chondrilla nucula*. Lipids 15: 200–202.

Mahran, G. H., F. M. Soliman &E. A. El-Kashouy, 1993. Study of volatile constituents of *Dictyota dichotoma* var. implexa. Indian Perfum. 37: 240–244.

Seyama, Y., H. Otsuka, A. Kawaguchi &T. Yamakawa, 1981. Fatty Acid Synthetase from the Harderian Gland of Guinea Pig: Biosynthesis of Methyl-Branched Fatty Acids. J. Biochem. 90: 789–797.

Seyama, Y., K. Ohashi, T. Imamura, T. Kasama &H. Otsuka, 1983. Branched Chain Fatty Acids in Phospholipids of Guinea Pig Harderian Gland. J. Biochem. 94: 1231–1239.

Sica, D., F. De Simone, E. Ramundo &F. Zollo, 1978. Sterols from Some Sponges. Biochem. Syst. Ecol. 6: 77–79.

Thiel, V., A. Jenisch, G. Worheide, A. Lowenberg, J. Reitner &W. Michaelis, 1999. Mid-chain branched alkanoic acids from 'living fossil' demosponges: a link to ancient sedimentary lipids? Organic Geochemistry 30: 1–14.

Walkup, R. D., G. C. Jamieson, M. R. Ratcliff &C. Djerasi, 1981. Phospholipid Studies of Marine Organisms: Phospholipids, Phospholipid-Bound Fatty Acids and Free Sterols of the Sponge *Aplysina fistularis* (Pallas) forma fulva (Pallas) (=*Verongia thiona*). Isolation and Structure Elucidation of Unprecedented Branched Fatty Acids. Lipids 16: 631–646.

Wang, S., E. L. Ghisalberti &J. Ridsdill-Smith, 1999. Volatiles from *Trifolium* as feeding detergents of redlegged earth mites. Phytochemistry 52: 601–605.

Yasumoto, M., K. Mada, T. Ooi &T. Kusumi, 2000. New terpenoid components from the volatile oils of the soft corals *Clavularia viridis* and *Sarcophyton acutangulum*. J. Nat. Prod. 63: 1534–1536.