Chemical Composition of *Corallina mediterranea* Areschoug and *Corallina granifera* Ell. et Soland

Salvatore De Rosa[a*], Zornitsa Kamenarska[b], Kamen Stefanov[b], Stefka Dimitrova-Konaklieva[b], Chavdar Najdenski[c], Iva Tzvetkova[c], Valeria Ninova[b], and Simeon Popov[b]

[a] Istituto di Chimica Biomolecolare, CNR, Via Campi Flegrei, 34, 80078 Pozzuoli, Naples, Italy. Fax: +39 08 18 04 17 70. E-mail: sderosa@icmib.na.cnr.i

[b] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

[c] Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

* Author for correspondence and reprint requests

Z. Naturforsch. **58 c**, 325–332 (2003); received December 6, 2002/January 8, 2003

The composition of sterols, volatiles and some polar compounds from three *Corallina* samples (*C. granifera* and *C. mediterranea* from the Black Sea and *C. mediterranea* from the Mediterranean Sea) was established. The sterol composition of the Black Sea samples was similar but it differs from that of the Mediterranean sample. The composition of the volatiles was very complex. The main groups of constituent were hydrocarbons, alcohols, carbonyl compounds, acids and their esters, terpenes. The composition of the polar components, soluble in *n*-butanol, was also established. There were some differences in the chemical composition of the two Black Sea species, which may be due to the biodiversity between them, while the differences in the composition of the two *C. mediterranea* samples could be due to the differences in the environment (salinity, temperature, pollution, etc.).

Key words: Corallina granifera, *Corallina mediterranea*, Sterols

Introduction

Corallina granifera Ell. et Soland., and *C. mediterranea* Areschoug are widely spread red algae (Rhodophyta) and belonging to family Corallinaceae, order Cryptonemiales, class Rhodophyceae, whose thalluses are impregnated with chalk.

Until now, the sterol composition was investigated in *C. officinalis* L., *C. granifera*, *C. mediterranea* and *C. elongata* (Palermo *et al.*, 1990; Sallam *et al.*, 1982), and generally the main sterol was cholesterol, while sterols with 28 and 29 carbon atoms were detected in low concentrations. Phosphatidylcholine was the main phospholipid in *C. granifera* (Dembitskii and Rozentzvet, 1990). The main fatty acids in the *Corallina* sp. were $C_{20:4}$(n-6), $C_{20:5}$(n-6), $C_{16:0}$, $C_{18:1}$(n-7) and $C_{18:1}$(n-9) acids (Dembitskii and Rozentzvet, 1990; Endo *et al.*, 1986; Mabrouk *et al.*, 1999). The long chain aldehydes (*E*)-2-tridecyl-2-heptadecanal and (2*E*,4*E*)-2-tridecyl-2,4-heptadecadienal were isolated from *C. mediterranea* (De Rosa *et al.*, 1995). Only from *C. rubens* were isolated terpenoids (Guven *et al.*, 1975). The composition of the amino acids (free and hydrolysed) was investigated in *C. squamata* (Haas, 1950) and *C. officinalis* (Madgwick *et al.*, 1970), collected at different locations. The main amino acids were asparagine and glutamine, followed by alanine, arginine, glycine, histidine and serine. The amino acid composition of marine algae was used for taxonomic purposes by Munda and Gubensek (1976). Polysaccharides were studied in different *Corallina* sp. (Cases *et al.*, 1994; Usov *et al.*, 1997). Some fractions of polysaccharide and polypeptide isolated from *C. rubens* showed antithrombin and fibrinolytic activities (Guven *et al.*, 1974). It was also found that lipophilic components from *C. pilulifera*, like hydrocarbons, triacyl-glycerides and free fatty acids ($C_{20:4}$ and $C_{20:5}$) induce larval metamorphosis of the sea urchins *Pseudocentrotus depressus* and *Anthocidaris crassispina* (Kitamura *et al.*, 1993; Kitamura *et al.*, 1992). Peptides from *C. officinalis* were found to possess antibiotic activity (Haas, 1950).

The taxonomic relations in the family Corallinaceae are still not established (Bailey *et al.*, 1998). We performed the analysis of three groups of compounds: sterols, volatiles and polar fraction, isolated from three *Corallina* samples (*C. granifera* and *C. mediterranea* from the Black Sea and *C. me-*

diterranea from the Mediterranean Sea). The two Black Sea samples inhabit the same environment and the differences in their chemical composition can be explained with the biodiversity. The differences between the two samples of *C. mediterranea* can be connected with the different environmental conditions between the Black Sea and the Mediterranean Sea (temperature, salinity, pollution, etc.).

Materials and Methods

Collection of the samples

Sample of *C. granifera* was collected in May 2000, near the village at the Black Sea.

Samples of *C. mediterranea* were collected in May 2001, near the village of Varvara at Black Sea (sample B), and in the gulf of Naples, Italy (sample M).

Voucher-specimens of *C. granifera* and sample B of *C. mediterranea* were deposited in the herbarium of the Faculty of Pharmacy, Medical University, Sofia, and determined by Dr. Stefka Dimitrova-Konaklieva. Voucher-specimen of sample M *C. mediterranea* is maintained in the collection of marine organisms of ICB-CNR, Pozzuoli, Naples, and determined by Dr. Guido Villani.

Preparation of the extracts

Each sample of the investigated algae [365 g (dry weight) of *C. granifera*, 187 g (dry weight) of *C. mediterranea* sample B, and 450 g (dry weight) of *C. mediterranea* sample M] were consecutively extracted with 700 ml methanol, 700 ml methanol/chloroform (1:1 v/v) and 700 ml chloroform. The extracts were combined and concentrated. About 300 ml water was added. The chloroform layer was separated and dried under reduced pressure at 40 °C yielding 1.6 g, 0.68 g, and 1.3 g dry wt from *C. granifera*, sample B, and sample M of *C. mediterranea*, respectively. The aqueous residue was extracted twice with 300 ml *n*-butanol, that after elimination of solvent the *n*-butanol extract yielded 3.3 g, 0.86 g, and 0.2 from *C. granifera*, sample B, and sample M of *C. mediterranea*, respectively.

Isolation and analysis of sterols

About 200 mg of the chloroform extract of each alga was applied to a silica gel (20 g) column. The column was eluted with 100 ml petrol ether, followed by 100 ml petrol ether/acetone (10:1 v/v), 200 ml chloroform, and 100 ml chloroform/methanol (1:1 v/v). Fractions containing sterols were identified by thin layer chromatography (TLC) on silica gel G with petrol ether/acetone (8:1 v/v) and combined. GC and GC/MS investigated the total sterol mixture.

Quantitative analysis was performed on a Pye Unicam 304 gas chromatograph equipped with FID and a capillary column SPB-1 (30 m × 0.32 mm, 0.25 μm film thickness) at 230 °C and programmed to 300 °C at 4 °C min^{-1} and 10-min hold. Injector and detector were at 300 °C.

For GC/MS was used a Hewlett Packard 6890 + MS 5973 instrument equipped with a capillary column SPB-50 (30 m × 0.32 mm, 0.25 μm film thickness). The MS source was at 250 °C and the ionisation voltage at 70 eV. The GC oven temperature was at 270 °C and programmed to 290 °C at 4 °C.min^{-1} and 20-min hold.

Isolation and analysis of volatile compounds

Part of the chloroform extracts (230 mg from *C. granifera*, 180 mg from sample B, and 250 mg from sample M of C. *mediterranea*, respectively) was subjected to a four-hour distillation-extraction in a Lickens-Nickerson apparatus. The volatile compounds were extracted from the distillate with diethyl ether (yield: *C. granifera* – 8.0 mg, 3.5% from the total chloroform extract, sample B *C. mediterranea* – 14.25 mg, 7.9% from the total chloroform extract, sample M *C. mediterranea* – 15.0 mg, 6% from the total chloroform extract), and investigated by GC/MS equipped with a capillary column SPB 50-MS (30 m × 0.25 mm, 0.25 μm film thickness), at 40 °C and programmed to 280 °C at 6 °C min^{-1}.

Isolation and analysis of polar compounds

5 mg of each *n*-butanol extracts were subjected to a silylation with 50 μl pyridine and 75 μl of bis-(trimethylsilyl)trifluoroacetamide (BSTFA). The mixtures were heated at 80 °C for 30 min and analysed by GC/MS equipped with a capillary column HP-5 (25 m × 0.2 mm, 0.5 μm film thickness) at 100° C and programmed to 315° C at 5° C min^{-1} and 10-min hold.

Identification of compounds by GC/MS analyses

The identification was accomplished using computer searches on a NIST98 MS Data library. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the basis of its mass-spectral fragmentation. When possible reference compounds were co-chromatographed to confirm GC retention times especially when isomeric compounds have similar spectra.

Results and Discussion

Sterol composition

Sterols are important for the functioning of the cell membranes. Their composition depends on the environment and on the specificity of the organism and in some cases is used for chemotaxonomic investigations. The sterols were investigated by GC/MS (qualitative analysis) and by GC (qualitative and quantitative analyses). The data obtained is reported in Table I. It is evident that the sterol composition of the three samples investigated is characteristic for the red algae. Like in other *Corallina* species previously studied (Palermo *et al.*, 1990; Sallam *et al.*, 1982), cholesterol was the main sterol in the investigated samples but it was in relatively lower concentrations in the two Black Sea species. The C-24 alkylated sterols and those with C-22 double bound, were in low concentrations especially in the Mediterranean sample. Two steroidal ketones together with traces of 5α-cholest-7-en-3β-ol were identified only in the Mediterranean sample. We can assume that the two steroidal ketones identified are due to oxidation processes within the algal organism, because the same work-up was used for all samples. Steroidal ketones have never been identified in Black Sea algae but these two steroidal ketones were earlier found in the Adriatic brown alga *Padina pavonia* (Kamenarska *et al.*, 2002).

From the results obtained it can be concluded that in the genus *Corallina* the sterol composition does not depend on the biodiversity (the two Black Sea species possessed almost identical sterol pattern). The differences in the sterol composition of the two *C. mediterranea* samples, is an indication that the sterol composition in the species investigated depends on environmental factors. The elevated concentration of cholesterol in the Mediterranean sample will cause some decrease in the permeability of the cell membranes, which might be due to the higher salinity of this sea. The higher temperature of the Mediterranean Sea water might stimulate the oxidation processes in the organisms and thus the production of the two steroidal ketones can be explained.

Volatile compounds

The volatile compounds, which are part of the chloroform extract, were analysed by GC/MS, and the data are reported in Table II. The composition of the volatile compounds from the three algae

Table I. Sterol composition (% of the total sterol fraction).*

Sterols	*C. granifera*	*C. mediterranea* (sample B)	*C. mediterranea* (sample M)
27-nor-24-Methyl-cholesta-5,22-dien-3β-ol or (22*Z*)-Cholesta-5,22-dien-3β-ol	–	–	<0.1
(22*E*)-Cholesta-5,22-dien-3β-ol	0.9	1.9	<0.1
Cholesterol	91.8	87.8	95.7
24-Methyl-cholesta-5,22-dien-3β-ol	1.1	4.1	0.3
5α-Cholest-7-en-3β-ol	–	–	0.5
24-Methyl-cholest-5-en-3β-ol and 24-Methyl-cholesta-5,24(28)-dien-3β-ol	3.2	3.8	1.6
Cholest-4-en-3-one	–	–	0.5
Cholesta-4,6-dien-3-one	–	–	0.8
24-Ethyl-cholesta-5,22-dien-3β-ol	0.7	–	–
24-Ethyl-cholesta-5-en-3β-ol	2.3	2.4	1.0

* Values obtained from three parallel measurements. The standard deviations (related to peak proportion on the chromatograms) are as follows: ± 0.3 for cholesterol and ± 0.1 for the others.

Table II. Composition of the volatile compounds (% of the total volatiles).*

Compounds	*C. granifera*	*C. mediterranea* (sample B)	*C. mediterranea* (sample M)
Hydrocarbons	**3.2**	**5.1**	**18.4**
2-Tetradecene	–	0.1	–
Tetradecane	–	0.3	–
1-Pentadecene	–	–	0.7
Pentadecane	0.2	0.3	–
1-Hexadecene	–	0.5	–
Hexadecane	0.1	0.5	–
Heptadecane	2.9	2.3	4.9
Octadecane	–	0.4	–
Nonadecane	0.5	0.2	0.7
Eicosane	–	–	0.9
Heneicosane	–	0.4	2.1
Docosane	–	0.2	1.5
Tricosane	–	0.1	0.6
Tetracosane	–	0.5	2.8
Pentacosane	–	0.2	2.4
Hexacosane	–	–	1.8
Alcohols	**0.2**	**1.1**	**0.4**
2,3-Butanediol	–	0.1	0.4
1,3-Butanediol	0.2	0.6	–
2-Pentanol	<0.1	–	–
2-Propyl-1-pentanol	–	0.3	–
1-Octenol	–	0.1	–
Phenols	**–**	**<0.1**	**<0.1**
Phenol	–	<0.1	–
p-tert.-Butylphenol	–	–	<0.1
Aldehydes	**<0.1**	**<0.1**	**<0.1**
2,4-Heptadienal	<0.1	–	
Nonanal	–	–	<0.1
Decanal	–	–	<0.1
Pentadecanal	–	–	<0.1
3,4-Dihydroxybenzaldehyde	–	<0.1	–
Ketones	**<0.1**	**4.1**	**–**
3-Methyl-2-pentanone	–	<0.1	–
4-Hydroxy-4-methyl-2-pentanone	–	3.7	–
4-Methoxy-4-methyl-2-pentanone	–	<0.1	–
6-Methyl-3,5-hepdadien-2-one	–	0.4	–
2,6-bis-(1,1-Dimethyl)-2,5-cyclohexadien-1,4-dione	<0.1	–	–
Acids	**4.2**	**2.0**	**2.4**
Propionic acid	–	0.8	–
Myristic acid	–	0.9	–
Palmitic acid	0.7	–	2.4
Linoleic acid	3.5	–	–
Pyruvic acid	<0.1	–	–
Benzoic acid	–	0.3	–
Esters	**76.4**	**0.4**	**9.9**
5-Oxovaleric acid, methyl ester	<0.1	–	–
Benzeneacetic acid, methyl ester	0.1	–	–
Caprinic acid, methyl ester	<0.1	–	–
Lauric acid, methyl ester	0.2	–	–
Myristic acid, methyl ester	7.5	–	–
Pentadecanoic acid, methyl ester	0.4	–	–
(9)16:1 acid, methyl ester	<0.1	–	–
(9,12)16:2 acid, methyl ester	19.6	–	–
Palmitic acid, methyl ester	18.8	–	–
Oleic acid (isomer), methyl ester	4.1	–	–

Table II (content).

Compounds	*C. granifera*	*C. mediterranea* (sample B)	*C. mediterranea* (sample M)
Oleic acid, methyl ester	1.6	–	–
Linoleic acid, methyl ester	2.8	–	–
Linolenic acid, methyl ester	0.9	–	–
Stearic acid, methyl ester	0.4	–	–
Arachidonic acid, methyl ester	2.2	–	–
Eicosapentaenoic acid, methyl ester	17.8	–	9.9
Acetic acid, methylpropyl ester	–	0.1	–
2-Methyl-2-propenic acid, butyl ester	–	<0.1	–
Isopropylmyristate	–	0.3	–
Terpenes	**1.1**	**1.7**	**5.8**
Eucaliptol	–	0.3	–
Dihydroactinidiolide	0.2	0.2	<0.1
Hexahydrofarnesyl acetone	0.4	0.3	–
Phytol	0.5	0.9	5.8
N-containing compounds	**–**	**–**	**<0.1**
Benzothiazole	–	–	<0.1
Others	**–**	**0.4**	
3,5,5-Trimethyl-2(5H)-furanone	–	0.4	

* The ion current generated depends on the characteristics of the compound and is not a true quantification. This method is suitable for comparing the chemical composition of different organisms, because the deviations caused by the differences in the intensity of the mass spectral fragmentation will be identical.

was very complex. The main groups of constituent were hydrocarbons, alcohols, carbonyl compounds, acids, esters and terpenes.

The hydrocarbons were in higher concentration in the Mediterranean sample. As it was expected straight chain compounds with an odd number of the carbon atoms predominated. Compared to the Mediterranean sample, the two Black Sea samples contained lower concentrations of hydrocarbons. *C. granifera* was characterized by the smallest diversity of hydrocarbons. This species also contained the lowest concentration of hydrocarbons with an even number of carbon atoms. Hydrocarbons from *C. granifera* contained up to 19 carbon atoms, while the two *C. mediterranea* samples had hydrocarbons with up to 26 carbon atoms. From these data, it is clear that apparently the concentration of the hydrocarbons in the two *Corallina* sp. depends more on the ecological factors while the variety of the hydrocarbons depends more on their biodiversity. Unsaturated hydrocarbons were found only in both samples of *C. mediterranea*.

Alcohols and phenols predominated in the *C. mediterranea* sample B. Phenols were identified only in the volatile compounds of the two *C. mediterrane* samples. It is known that they possess an antibacterial and antifungal activity. The main alcohols are the two isomers of butandiol. It is known that 2,3-butandiol is an insect pheromone, while 1-octenol is an insect attractant, but the role of these compounds in the marine organisms is unclear.

Traces of aldehydes were identified in the three species, while ketones appeared to be characteristic only for *C. mediterranea* sample B. The aldehydes and ketones are known to take an active part in higher plant-insect relationships, serving as attractants or repellents (Wang *et al.*, 1999). Their functions in the marine organisms are unknown but it could be mentioned that the terrestrial insects as well as the marine Crustaceans (main predators on algae) belong to the same type Arthropoda.

The main group of volatile compounds identified in *C. granifera* were fatty acid methyl esters (FAME). They were totally absent in the *C. mediterranea* sample B and only one methyl ester was found in the sample M. The methyl ester of 5-oxovaleric acid is of interest, because it is an ester of oxoacid rarely found in algae. Methylpropyl ester of the acetic acid was found in the *C. mediterranea* sample B, and methyl ester of the phenylacetic

acid was found in *C. granifera.* These two compounds could have defensive functions. Different esters, especially acetates are important for the plant – insect interactions, but their functions in marine organisms are not investigated.

Terpenoids are often found in low concentrations in marine algae. Eucalyptol was identified in the volatile compounds of *C. mediterranea* sample B. Dihydroactinidiolide, hexahydrofarnesylacetone and phytol were found in similar amounts in the two Black Sea algae, while phytol was found in high concentration in the Mediterranean sample.

Polar compounds

The main components, identified in the *n*-butanol extracts of the two Black Sea algae were free fatty acids (FFA), while their concentration in the Mediterranean sample was lower. This is an indication that the environmental conditions and not biodiversity determine the concentrations of the FFA in the species from genus *Corallina.* Similarly to other marine organisms palmitic acid is the main fatty acid. Hydroxy acids were identified in the three algae investigated. 2-Hydroxy- and 2,3-dihydroxy-propionic acids are present in most of early investigated algae (De Rosa *et al.*, 2001; Kamenarska *et al.*, 2002). Several dicarboxylic acids were identified only in the *C. mediterranea* sample B.

Free amino acids at different concentration were identified in the three *Corallina* samples. The main free amino acids were 5-oxoproline, valine, leucine, and isoleucine. 5-Oxoproline was detected in all samples and it is much higher in *C. granifera*, and the only free amino acid, identified in the *C. mediterranea* sample B.

The biggest amount of N-containing compounds was identified in the *C. mediterranea* sample M.

A bigger diversity of free mono and disaccharides was observed in the Black Sea species. Sucrose is the main carbohydrate detected in all samples.

Biological activity

The Gram (+) bacteria *Staphylococcus aureus*, the Gram (–) bacteria *Echerichia coli* and the fungi *Candida albicans* were used for the investigation of the antibacterial and antifungal activity. The chloroform, *n*-butanol and volatile compounds were tested by bioautography test (Kujumgiev *et al.* 1993). Significant activities were observed only against the Gram (+) *Staphylococcus aureus*, which is characteristic for most of the marine organisms. The chloroform extracts (18 ± 1.7 mm, and 15.7 ± 0.6 mm, for *C. granifera* and *C. mediterranea* sample B, respectively) and volatiles (21.3 ± 2.3 mm, and 15.3 ± 0.6 mm, for *C. granifera* and *C. mediterranea* sample B, respectively) from the two Black Sea *Corallina* species were active, while no activity was observed in the corresponding extracts from *C. mediterranea* sample M. The *n*-butanol extract from *C. mediterranea* sample B showed no activity, contrary to the Mediterranean sample that showed a significant activity (13.7 ± 0.6 mm). Also the *n*-butanol extract from *C. granifera* had activity (30 ± 0 mm). The observed differences are in accordance with the significant differences in the chemical composition of the investigated samples and might be explained with the different enemies of *C. mediterranea* in both seas. The *n*-butanol extract from *C. mediterranea* sample M contains much more N-containing compounds and oxidised fatty acids than the Black Sea sample and this could explain the differences in the antibacterial activities of both samples of *C. mediterranea.* The bigger content of eucalyptol in *C. mediterranea* sample B could explain the antibacterial activity of the volatile compounds from this sample.

The cytotoxicity of the extracts was also investigated by using brine shrimp (*Artemia salina*) test (Solis *et al.*, 1993). The *n*-butanol extract of *C. granifera* (LC_{50} 18.1 ± 4.6 μg/ml) showed a stronger cytotoxic activity than the *n*-butanol extract of *C. mediterranea* (LC_{50} 245.6 ± 16 and > 1000 μg/ml, for sample B and M, respectively). The chloroform extract of *C. mediterranea* sample M (LC_{50} 18.4 ± 4.4 μg/ml) possesses significantly higher activity than the corresponding extract of sample B (LC_{50} 59.6 ± 6.2 μg/ml).

Acknowledgements

This research was supported by CNR-Rome and by the Bulgarian National Foundation for Scientific Research (Contract X-1101). The technical assistance of Mr. C. Iodice is gratefully acknowledged.

Table III. Composition of the *n*-butanol extracts (% of the total silylated compounds in the extracts).*

Compounds	*C. granifera*	*C. mediterranea* (sample B)	*C. mediterranea* (sample M)
Alcohols	**4.4**	**4.4**	**3.0**
Octanol	–	–	1.5
1,3-Butanediol	–	–	<0.1
1,4-Butanediol	–	–	<0.1
Glycerol	4.0	4.4	1.5
Aldehydes	**–**	**–**	**<0.1**
2,3,4-Trihydroxybutanal	–	–	<0.1
Monocarboxylic acids	34.7	28.3	0.4
Lauric acid	–	<0.1	–
Myristic acid	3.4	2.2	–
Pentadecanoic acid	–	0.3	–
Palmitoleic acid	2.1	1.6	–
Palmitic acid	21.4	16.9	0.4
Margarinic acid	–	0.5	–
Stearic acid	0.6	2.2	–
Elaidic acid	1.3	1.7	–
Oleic acid	3.5	1.6	–
Linoleic acid	1.4	–	–
Arachidonic acid	1.0	1.3	–
Hydroxy acids	0.9	0.9	3.3
2-Hydroxypropionic acid	0.7	0.5	0.4
Hydroxy acetic acid	–	0.4	0.1
3-Hydroxypropionic acid	–	–	<0.1
2-Hydroxybutyric acid	–	<0.1	–
2,3-Dihydroxypropionic acid	0.2	<0.1	1.0
2-Hydroxy ethanesulfonic acid	–	–	1.8
Dicarboxylic acids	**–**	**1.8**	**–**
Succinic acid	–	0.5	–
Glutaric acid	–	1.3	–
Adipinic acid	–	<0.1	–
Heptanedioic acid	–	<0.1	–
Octanedioic acid	–	<0.1	–
Azelaidic acid	–	<0.1	–
Amino acids	**3.6**	**0.9**	**1.4**
Alanine	0.2	–	–
Valine	0.4	–	0.2
Leucine	<0.1	–	0.2
Isoleucine	0.3	–	0.4
Proline	–	–	0.2
Threonine	0.2	–	–
5-Oxoproline	2.5	0.9	0.4
Phenylalanine	–	–	<0.1
Aromatic acids	**–**	**<0.1**	**–**
4-Hydroxybenzoic acid	–	<0.1	–
Esters	**–**	**2.0**	
2,3-Dihydroxypalmitic acid, propyl ester	–	2.0	
N-containing compounds	**2.6**	**1.6**	**5.7**
2-Pyridinecarboxylic acid	–	0.5	4.0
2-Piperidinecarboxylic acid	–	–	0.4
Niacinamide	–	–	0.8
Urea	–	–	0.5
Uridine	2.6	1.1	–
Carbohydrates	**13.6**	**7.7**	**8.6**
2-O-Methylascorbic acid	–	–	0.2
5-Deoxymyoinositol	–	–	0.2
Glucose	3.5	2.3	0.6
Sucrose	8.0	3.7	7.6
Fructose	<0.1	–	–
Mannitol	2.1	1.7	–
Ribonic acid	–	<0.1	–

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

Bailey J., Chapman C., and Russell L. (1998), A phylogenetic study of Corallinales (Rhodophyta) based on nuclear small – subunit rRNA gene sequences. J. Phycol. **34**, 692–705.

Cases M., Stortz C. A., and Cerezo A. S. (1994), Structure of the "corallinans"-sulfated xylogalactans from *Corallina officinalis*. Int. J. Biol. Macromol. **16**, 93–97.

De Rosa S., De Guilio A., Iodice C., Alcaraz M. J., and Paya M. (1995), Long chain aldehydes from the red algae *Corallina mediterranea*. Phytochemistry, **40**, 995–996.

De Rosa S., Kamenarska Z., Bankova V., Stefanov K., Dimitrova-Konaklieva St., Najdenski H., Tzvetkova I., and Popov, S. (2001), Chemical composition and biological activity of the Black Sea algae *Polysiphonia denudata* (Dillw.) Kütz and *Polysiphonia denudata f.fragilis* (Sperk) Woronich. Z. Naturforsh. **56 c**, 1008–1014.

Dembitskii V. M., and Rosentsvet O. A. (1990), Lipids of marine macrophytic algae I. Fatty acid and phospholipid composition of Rhodophyceae. Khim. Prir. Soedin. **1**, 113–115.

Endo S., Kishi Y., and Mitsuashi, T. (1986), Fatty acid composition of four marine algae. Tokyo Gakugei Daigaku Kiyo, Dai 4-bumon **38**, 17–20. (in Japanese)

Guven K. C., Mutluay E., Aktin E., and Koyuncuoglu H. (1975), Chemical and pharmacological investigations on *Corallina rubens*. Planta Med. **27**, 5–13.

Guven K. C., Ulutin S. B., Mutluay E., and Ulutin O. N. (1974), Anticoagulant-anttithrombin and fibrinolytic actions of extract of marine *Corallina rubens*. Haemostasis **2**, 260–268.

Haas P. (1950), Certain peptides occurring in marine algae. Biochem. J. **46**, 503–505.

Kamenarska Z., Gasic M. J., Zlatovic M., Rasovic A., Sladic D., Kljajic Z., Stefanov K., Seizova K., Najdenski H., Kujumgiev A., Tsvetkova I., and Popov S. (2002), Chemical composition of the brown alga *Padina pavonia* (L.) Gaill. from the Adriatic Sea. Bot. Mar. **45** (in press).

Kitamura H., Kitahara S., and Hirayama K. (1992), Lipophilic inducers extracted from *Corallina pilulifera* for larval settlement and metamorphosis of two sea urchins *Pseudocentrotus depressus* and *Anthocidaris crassispina*. Nippon Suisan Gakkaishi **58**, 75–78.

Kitamura H., Kitahara S., and Koh H. B. (1993), The induction of larval settlement and metamorphosis of two sea urchins *Pseudocentrotus depressusans* and *Anthiociadaris crassispina* by free fatty acids extracted from the red alga *Corallina pilulifera*. Mar. Biol. **115**, 387–392.

Kujumdjiev A., Bankova V., Ignatova A., and Popov S. (1993), Antibacterial activity of propolis, some of its components and analogs. Pharmazie **48**, 785–786.

Mabrouk S. S., Hashem A. M., El-Sayed L., and El-Shayeb N. M. A. (1999), Screening of some Mediteranean red algae as sources of polyunsaturated fatty acids. Adv. Food Sci. **21**, 30–33.

Madgwick J. C., Ralph B. J., Shannon J. S., and Simes J. H. (1970), Non-protein amino acids in Australian seaweeds. Arch. Biochem. Biophys. **141**, 766–767.

Munda I. M., and Gubensek F. (1976), The amino acid composition of some common marine algae from Iceland. Bot. Mar.**19**, 85–92.

Palermo J. A., Gros E. G., and Seldes A. M. (1990), Steroids from aquatic organism. Sterols of three algae of the family Corallinaceae. An Asoc. Quim. Argent. **78**, 157–159.

Sallam L. A., Hamdy A. A., Naim N., El-Refai A. H., and Karawya M. S. (1982), Chemical composition of some Egyptian marine algae. 1. Sterol constituents. Egypt. J. Pharm. Sci., **23**, 179–186.

Solis P., Wright C. W., Anderson M. M., Gupta M. P., and Phillipson J. D. (1993), A microwell citotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. **59**, 250–252.

Usov A. I., Bilan M. I., and Shashkov A. S. (1997), Polysacharides of algae. Carbohydr. Res. **303**, 93–102.

Wang S., Ghisalberti E. L., and Ridsdill-Smith J. (1999), Volatiles from *Trifolium* as feeding deterents of redlegged earth mites. Phytochemistry **52**, 601–605.