Volatile Components of the Freshwater Algae *Spirogyra* and *Mougeotia*

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Several species of freshwater green algae belonging to the order Zygnematales (*Spirogyra crassa* (Ktz.) Czurda, *S. longata* (Vauch.) Ktz., and *Mougeotia viridis* (Ktz.) Wittr.) were found to have a specific composition of the volatile fraction, which confirms an earlier proposal for the existence of two groups in the genus *Spirogyra*. Antibacterial activity was found in volatiles from *S. longata*.

Introduction

While the chemical composition and biological activity of marine algae have been studied in depth, freshwater algae have been investigated less intensively, especially those belonging to Zygnemaceae (order Zygnematales). The most numerous representatives of this family in the Bulgarian flora are genera *Spirogyra, Mougeotia* and *Zygnema*, which inhabit rivers and ponds. In the phylogenetic scheme of Chlorophyta, these algae have a special position between Bryopsidales and Charophyta. Recently we investigated the sterol and polysaccharide composition of some *Spirogyra* and *Mougeotia* species, growing in Bulgaria (Mitova *et al.*, 1999, Stefanov *et al.*, 1996) and found that both groups of compounds show characteristic patterns in the species of *Spirogyra* and *Mougeotia* while *S. crassa* differs from the other investigated *Spirogyra* species, especially in its sterol composition. The differences obtained may be used in chemical taxonomy of these freshwater green algae. Little is known about other metabolites of these algae. In the lipophylic fraction of *S. crassa* different lipid classes and their fatty acid composition (Stefanov *et al.,* 1996) were analyzed. Phospholipids (Nakanishi, 1995) and glycolipids (Brush and Percival, 1972) were analyzed in *Spirogyra sp.* and *Mougeotia sp.,* respectively. Sugars, amino acids and some aliphatic amines were also identified in *Spirogyra sp*. (Cannel *et al.*,1988; Kull and Hentschel, 1966). Tannins (Nishizawa *et al.*, 1985; Nakabayashi and Hada, 1954) and fatty acids (Pettko and Szotyori, 1967) were also found in *Spyrogyra sp.* Evidently, research on chemical composition of *Spirogyra* and *Mougeotia* species is very limited, especially on their secondary metabolites, which often possess biological activity.

The volatile constituents of Zygnemaceae algae are of interest, because such compounds often possess a valuable biological activity and have been investigated only in a few algal species (Mahran *et al.*, 1993; Gally *et al.*, 1993; Sugisawa *et al.*, 1990). These compounds are also of importance, because they are being continually emitted into the atmosphere by algae. Volatiles from Zygnemaceae were not investigated till now.

The purpose of this research is to investigate the chemical composition of the volatiles from two *Spirogyra* species (*S. longata* and *S. crassa*), which, according to our recent investigations posses a sterol and polysaccharide composition (Mitova *et al.*, 1999), and volatiles different from *Mougeotia viridis.* The comparison between the three samples of volatiles allows some taxonomic conclusions and confirms our previous suggestions that *S. crassa*, other *Spirogyra* species investigated and *Mougeotia viridis* belong to three different groups. It is also of interest to investigate the antibacterial and antifungal activity of the volatiles from different species in order to obtain information about the functions of these compounds in the investigated algae.

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Materials and Methods

Algal material

Sample of *M. viridis* was collected from a lake at the south part of Vitosha mountain in May (Voucher specimen N° 21051996).

Sample of *S. crassa* was collected in May (Voucher specimen N° 10051997) at the same region.

Sample of *S. longata* was collected in June in a pond at the northern part of Vitosha mountain (Voucher specimen N° 03061995).

Voucher specimens were determined by Dr. St. Dimitrova-Konaklieva and deposited in the herbarium of the Faculty of Pharmacy, Medical University, Sofia.

All algal samples were investigated microscopically. Bacterial or other cells, different from these of the investigated species, were not found.

Isolation and identification of the volatile compounds

About 50 g of the fresh algae were homogenized with ethanol and refluxed for 10 minutes in order to inactivate the enzymes. An equal volume of chloroform was added, the mixture was filtered and an equal amount of water was added. The lower layer, containing the total lipophylic substances [683 mg (*S. longata*), 192 mg (*S. crassa*), 201 mg (*M. viridis*)], was subjected to a hydrodistillation for four h, and the volatiles were extracted from the distillate with diethyl ether (yield: 26.9 mg (3.9% from the lipophylic extract) in *S. longata*; 12.41 mg (6.5%) in *S. crassa;* 32 mg (16%) in *M. viridis*). They were investigated by analytical GC/MS by a Hewlett Packard gas chromatograph 6890 equipped with an Hewlett Packard MS 5973 detector. A HP5-MS capillary column was used (30 m x 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 at 0.9 $ml.min^{-1}$. The ion source was set at 250 °C and the ionization voltage was 70 e V.

Antibacterial tests

For the investigation of the antibacterial activity we used a modification of bioautography, developed in our laboratory (Kujumgiev *et al.*, 1993). *Staphylococcus aureus* 209 and *Escherichia coli* WF+ were used as test organisms. For every test were used 0.5 mg from the total volatile compounds. The antibacterial activity was measured by the diameter of the inhibitory zones in the soft agar layer after a 72-h throughout incubation at 37 °C. An inhibitory zone with a diameter of less than 5 mm indicated lack of activity.

Antifungal tests

For these investigations we used the agar cup method (Spooner and Sykes, 1972). Parts of the investigated extracts (0.5 mg) were placed in a pit with a diameter 10 mm, 10^7 yeast cells were used per Petri dish and after an incubation at 37 °C for 72 h the diameters of the inhibition zones were determined.

Results and Discussion

The volatiles were obtained as usually – part of the lipophylic extract was subjected to a hydrodistillation and the volatiles were extracted with diethyl ether from the distillate. They were analyzed by GC/MS and the identification of the compounds was accomplished by using computer searches on commercial libraries. The results obtained are summarized in Table I. When GC/MS is used, the size of the peaks are proportional to the corresponding ion currents, which depend on the characteristics of the compound (intensity of the mass spectral fragmentation). For this reason the results obtained are semiquantitative. When we use GC/MS to compare the chemical composition of different organisms the deviations are identical and comparisons can be made.

The antibacterial and antifungal activities were investigated only for volatiles from *Spirogyra crassa* and *Spirogyra longata*.

Both investigated samples show no activity against *Echerichia coli* and *Candida albicans*. Only volatiles from *Spirogyra longata* show a moderate activity against *Staphylococcus aureus* (inhibition zone 12 mm for 0.5 mg of volatiles), while volatiles from *S. crassa* possess no activity.

The composition of the volatiles from the three investigated algae appeared to be very complex. Different groups of compounds were found (Table I).

Table I. Composition of the volatile substances (in %* of the total volatiles).

Compounds	*Spirogyra longata*	*Spirogyra crassa*	*Mougeotia viridis*
Aldehydes:	0.19	0.58	–
Benzaldehyde	<0.05	0.58	–
Hexanal	<0.05	–	–
2,4-Heptadienal	<0.05	–	–
2,6-Nonadienal	<0.05	–	–
2,4-Decadienal	0.19	–	–
5,5-Dimethyl-3-oxo-1-Cyclohexene-1-Carbaldehyde	<0.05	–	–
Ketones:	0.12	0.3	–
3,5-Octadiene-2-one	0,12	–	–
Acetophenone	<0.05	0.3	–
Terpenes:	2.19	–	1
Pristane	2.1	–	–
Isobornylpropionate	<0.05	–	–
Beta-ionone	<0.05	–	–
Dihydroactinidiolide	0.09	–	0.2
Hexahydrofarnesylacetone	–	–	0.8
Alcohols:	–	1.98	–
7-Dodecenol	–	1.3	–
Benzyl alcohol	–	0.68	–
Halogenated compounds:	–	3.33	14.29
1,1,2-Trichloroethane	–	3.33	–
M+ 122/24	–	–	14.29
Esters:	1.15	2.9	1.6
Isopropylmyristate	1.09	2.9	–
Methyl esters of:			
Octanedioic acid	0.06	–	–
Pentadecanoic acid	–	–	0.2
Hexadecanoic acid	–	–	0.8
Heptadecanoic acid	–	–	0.2
Octadecanoic acid	–	–	0.1
9,12-Octadecadienoic acid	–	–	0.2
11-Octadecenoic acid	–	–	0.1
5,8,11,14-Eicosatetraenoic acid	–	–	<0.05
Eicosa-5,8,11,14,17-pentaenoic acid	–	–	<0.05
Aromatic Compounds:	1.48	1.3	–
Benzoic acid	–	0.19	–
Benzylbenzoate	0.61	–	–
Tert.-butyl ester of benzoic acid	–	0.77	–
Phenol	–	0.34	–
1-Pentylheptyl benzene	0.22	–	–
1-Propylnonyl benzene	0.23	–	–
Phenanthrene	0.4	–	–
1-Allylnaphthalene	<0.05	–	–
Hydrocarbons:	12.56	3.81	1.1
3-Methylnonane	<0.05	–	–
Undecane	0.12	–	–
Dodecane	0.13	–	–
Tridecane	0.1	–	–
Tetradecane	0.09	–	–
Pentadecane	0.1	–	–
Hexadecane	0.5	–	0.2
Heptadecane	1.23	–	0.1
Octadecane	2.46	–	0.1
Nonadecane	3.16	–	0.2
Eicosane	2.7	–	0.1
Heneicosane	1.9	–	–

Table I. (continued).

Compounds	*Spirogyra longata*	*Spirogyra crassa*	*Mougeotia viridis*
Pentacosane	0.07	–	–
N-propylcyclohexane	<0.05		–
Docosane	–	–	0.2
Tricosane	–	–	0.2
Pentacosane	–	–	0.1
Heptacosane	–	–	<0.05
Hexacosane	–	–	<0.05
1-Hexadecyne	–	1.11	–

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

Terpenoids: Contrary to the essential oils of the higher plants, containing mainly terpenoids, in the volatiles of algae terpenoids are in low concentrations. The same is true for the volatiles from terrestrial plants, which do not contain essential oils. In the investigated volatiles we found five terpenoids. It must be mentioned that dihydroactinidiolide and hexahydrofarnesylacetone are often found in marine green algae (Sakagami *et al.*, 1991), while ionone derivatives have been determined in some unicellular freshwater algae (Rzama *et al.*, 1995) and marine green algae (Sakagami *et al.*, 1991). We found terpenoids mainly in the volatiles from *S. longata*, while no terpenoids were identified in *S. crassa*. Only the common marine terpenoids dihydroactinidiolide and hexahydrofarnesylacetone were identified in *Mougeotia viridis*.

Carbonyl compounds: Six aldehydes were identified. Most of them were concentrated mainly in *S. longata*. Significant amount of benzaldehyde was found in *S. crassa*. Another group of carbonyl compounds, ketones, are mainly in *S. crassa*. *M. viridis* did not contain carbonyl compounds.

Alcohols: Two alcohols were identified only in *S. crassa*. It must be mentioned that this algae contains the highest concentrations of benzyl alcohol and the biogenetically related benzaldehyde.

Aromatic compounds: They are concentrated mainly in *S. longata* and totally absent in *M. viridis*. Part of them are alkylated benzenes. Another interesting compound is the *tert.*-butyl ester of benzoic acid. Recently *tert.*-butyl derivatives of different compounds were found in some marine algae and invertebrates (Popov *et al.*, unpubl. results). Benzoic acid and phenol may have defensive functions.

Halogenated compounds: Unexpectedly we found two chlorinated compounds in significant amounts. 1,1,2-trichloroethane in *S. crassa* can be emitted into the atmosphere.

Esters: Besides the aromatic esters we found isopropylmyristate only in the *Spirogyra* species. In *Mougeotia viridis* significant concentrations of methyl esters of characteristic for algae fatty acids were found. The presence of fatty acid methyl esters in marine organisms is still disputed. In some cases it is proved that they are artifacts, formed through the extraction procedure by methanolysis of the lipids. In our case the extraction was made with ethanol confirming the fact that the methyl esters are natural products. Their appearance only in *Mougeotia viridis* can have a taxonomic value.

Hydrocarbons: The hydrocarbon composition of the investigated algae corresponds to their proposed separation into three groups (Mitova *et al.*, 1999). The concentrations of hydrocarbons are higher in *S. longata*. As expected they consist mainly of compounds with an odd number of carbon atoms. They appeared to be saturated and the main components possess 17–21 carbon atoms. The situation is similar in *M. viridis* hydrocarbons, but they appeared in much lower concentrations. Surprisingly, the concentration of saturated hydrocarbons in *S. crassa* was very low and no individual compound from this group was identified. Instead two acetylenic compounds were found in significant concentrations. This confirms the proposed specific taxonomic position of *S. crassa*. When the chemical composition is used for taxonomic conclusions we always must keep in mind that the composition may be influenced by external factors (mineral nutrition, light, etc.). The collection sites of the algae possess similar ecological

conditions, but even some small differences may cause changes in the chemical composition. So, there always are some doubts in the chemotaxonomic conclusions.

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