Volatile Substances of the Green Alga *Scenedesmus incrassatulus*

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Volatile substances of the green microalga *Scenedesmus incrassatulus*, cultivated in fresh and salt water, were studied. Cultivation in fresh water diversifies volatile secondary metabolites. Hydrocarbons and derivatives of the acetate pathway predominate when algae are grown in salt water; isoprenoids and aromatics are more abundant after fresh water cultivation.

Key words: Microalgae, *Scenedesmus*, Volatiles

Introduction

Volatile substances, which are excreted from algae in the water and then in the atmosphere, influence the environment to some extent. There are sufficient data about volatile substances from marine macrophytes. In red and brown algae, halogenated derivatives of acetate and mevalonate biosynthetic pathways and dimethyl sulfide were found (Jongaramruong and Blackman, 2000; Milkova *et al.*, 1997; Careri *et al.*, 2001; Yamamoto *et al.*, 2001). In green marine macrophytes acetate derivatives predominated, while the amount of isoprenoids was found to be relatively low (Sakagami *et al.*, 1991). Freshwater green macrophytes showed a more diverse volatile composition (Kamenarska *et al.*, 2000). The main volatiles in blue-green algae are hydrocarbons (Dembitsky *et al.*, 1999; Tellez *et al.*, 2001). The volatiles of only a few green freshwater microalgae have been investigated (Zolotovitch *et al.*, 1973; Rzama *et al.*, 1995).

The purpose of the present study is to investigate the quantity and composition of volatile substances from the industrially important green microalga *Scenedesmus incrassatulus* (Furnadzieva *et al.*, 1987). Volatiles of this alga should be compared when grown as high density cultures in fresh and Black Sea water.

Materials and Methods

Algal material

Green unicellular algae Scenedesmus incrassatulus Bohlin (R-83, Algal Culture Collection of the Plovdiv University). The algae were grown in the laboratory as non-sterile monoalgal culture at 33 °C, 9 klx uninterrupted light intensity and bubbled with $100\,l \cdot h^{-1}$ air enriched with 0.5 vol.% CO_2. Mineral nutrition medium and the same medium with $17\,g \cdot l^{-1}$ NaCl, analogously to Black Sea salinity were used. Proportions of both media were previously described (Petkov, 1995). Algae were harvested during the late exponential phase by centrifugation at $3000 \times g$.

Isolation of volatiles

The fresh biomass (5 g) was subjected to hydrodistillation in a Likens-Nickerson apparatus for 4 h, and the volatiles were collected in diethyl ether/*n*-pentane 1:1 (v/v) (50 ml).

GC-MS analysis

GC-MS analysis of the volatiles was performed on a Hewlett-Packard gas chromatograph 6890 equipped with a Hewlett-Packard MS 5973 detector. A HP5-MS capillary column was used (30 m × 0.25 mm, 0.25 mm film thickness). The temperature was programmed from 40 °C to 280 °C at a rate of $6\,°C \cdot min^{-1}$. Helium was used as a carrier

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Table I. Algal growth and yield of volatiles.

Parameter	Dimension	Fresh water	NaCl
Algal density	$g.l^{-1}$	8.43	6.33
Algal dry biomass	g	1.26	0.95
Volatile substances	mg	3.2	2.1
Percentage of volatiles	%	0.25	0.22

gas at 0.9 $ml \cdot min^{-1}$. The ion source was set at 250 °C and the ionization voltage was 70 eV.

Identification of compounds

Identification was accomplished using computer searches on a NIST98 MS data library (National Institute of Standards and Technology, Gaithersburg, MD, USA). 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. If available, reference compounds were co-chromatographed to confirm GC retention times.

Results and Discussion

The typical freshwater alga *S. incrassatulus* has easily been adapted to a medium containing 17 $g \cdot l^{-1}$ NaCl similar to coastal water of the Black Sea. At higher salinity, the growth rate of algal biomass decreased somewhat (Table I). The percentage of volatiles remained almost the same as in the fresh water cultivated algae.

In spite of the similarities in both samples, we found a significant difference in the ratio of the main classes of the determined substances (Table II). Diversity of the individual substances is greater in the fresh water cultivated algae.

Hydrocarbons (alkanes and alkenes) are abundantly present in the volatiles. All hydrocarbons have formerly been found in the cells of *Scenedesmus* by extraction with organic solvents (Furnadzieva *et al.*, 1987). The proportion of hydrocarbons found in volatiles and those found in solvent extracts are rather different. Hydrocarbons C_{15}, C_{17}, ΔC_{17}, C_{27}, ΔC_{27} predominate in lipophilic extracts, while in volatiles hydrocarbons $C_{11}-C_{30}$ are evenly distributed. All membranes contain hydrocarbons as we have previously established. Solvent extraction leads to isolation of the total hydrocarbons from all of the membranes to-

Table II. Chemical composition of volatiles of *Scenedesmus incrassatulus* (% of total ion current[a]).

Substances	Fresh water (%)	NaCl (%)
Hydrocarbons	***19.1***	***50.3***
Undecane	0.9	–
Dodecane	2.1	1.6
Tridecane	2.4	3.9
Tetradecane	0.7	1.8
Pentadecane	0.4	1.2
Hexadecane	0.6	2.3
Heptadecene	0.7	1.5
Heptadecane	1.0	2.0
Octadecane	0.4	1.3
Nonadecene	1.0	–
Eicosane	0.4	–
Heneicosane	0.4	0.6
Docosane	0.4	0.9
Tricosane	0.3	2.4
Pentacosane	0.5	2.6
Hexacosane	–	0.7
Heptacosene	6.9	1.8
Heptacosane	–	2.6
Hentriacontane	–	4.7
Hentriacontane-isomer	–	4.8
Tritriacontene	–	13.6
Derivatives of acetylcoenzyme A with different degree of oxygenation	***8.3***	***8.9***
Butanediol	0.5	1.0
Butanediol-isomer	0.9	1.7
Heptanol	0.3	–
Octanol	0.2	–
Nonanol	1.0	2.3
Pentadecanone	1.6	–
Ethylpalmitate	0.8	–
Oleic acid	2.4	–
Stearic acid	0.8	0.5
Ethyl oleate	1.4	–
Octadecyl 2-ethylhexanoate	–	1.8
Isoprenoids	***57.8***	***22.4***
Isoprenyl acetate	–	0.8
Carene	0.6	–
Cedrol	0.5	–
2,6,10,14-Tetra-methylpentadecane	–	0.9
Manool	4.9	–
Phytol	26.5	11.7
Ferruginol	18.1	0.6
Ferruginol-isomer	2.6	–
Stigmastane	–	1.1
Squalene	2.6	3.5
Triterpenic hydrocarbon	0.5	1.1
28-Nor-17-β-(H)-hopane	0.7	1.4
Cholesterol	0.8	1.3
Aromatics	***6.0***	***1.9***
p-Xylene	0.8	–
Phenylethanol	0.4	–
1,4-Dimethoxybenzene	0.4	–
Naphthalene	1.7	0.7
4-Chloro-3-methylphenol	0.6	0.3
Benzyl benzoate	0.4	0.7
Benzyl cinnamate	1.2	0.2
1-(2,6-Dihydroxy-4-methoxyphenyl)-3-phenyl-(*E*)-2-propen-1-one	0.5	–

[a] The total ion current generated depends on the characteristics of the compound concerned and is not a true quantitation.

gether (Petkov, 1990). The observed differences in hydrocarbon composition might be explained by the assumption that steam distillation yields substances from the plasma membrane only but not from the intracellular membranes. This suggestion could be supported by the absence of methyl esters of long chain fatty acids in the volatiles. Studying the lipids of *S. incrassatulus*, we have repeatedly found these substances (Petkov and Furnadzieva, 1993). In the present study we did not find them among other fatty acids and their esters which have similar chromatographic behavior. Probably they are localized in inner membranes and do not leave the cell if it is not disintegrated.

Until now, among the lipids of species of the genus *Scenedesmus* we have found neither 2-ethylhexanoic acid nor its esters. We found an ester of this acid, namely octadecyl 2-ethylhexanoate, which could be an artefact (Table II). Usually studies of this kind give a positive detection for phthalic acid esters, detergents, and other non-algal material, which we tried to minimize using all-glass equipment and re-distilled solvents.

Hydrocarbons are about 50% of the volatiles in the NaCl sample, while their concentration is rather lower in the fresh water sample. The quantity of all lipophilic substances, which are derivatives of the acetate pathway, is increased two-fold in the NaCl sample. Hydrocarbons longer than C_{27} were found only in the NaCl sample.

Salt water markedly suppressed biosynthetic pathways of secondary metabolism other than the acetate one. This becomes obvious looking at the quantity and abundance of isoprenoids. The pathway of mevalonic acid was present more expressively in the algae grown in fresh water.

Phytol was the most abundant isoprenoid and even the most abundant component of the volatiles – more than a quarter of the mixture weight (Table II). In our previous studies, using solvent extraction, we have found that about 7% of the total phytol of *Scenedesmus* is free, not bound to the porphyrin nucleus of chlorophyll (Petkov, 1990). Here the quantity of phytol in volatiles is in good accordance with the previous studies, and the chlorophyll content was 3.5% of dry weight (DW) in the fresh water sample and 2.0% DW in the NaCl sample. There are data about significant amounts of phytol in volatiles of other green algae (Rzama *et al.*, 1995; Sakagami *et al.*, 1991). These facts raise the question whether phytol possesses other functions besides being part of chlorophyll.

The above mentioned trend of differences was also apparent with the aromatic compounds (Table II). Their amount was three times higher and they had twice more representatives in the freshwater sample.

The other substances to 100% were identified as 8.8% dienoic, trienoic and branched fatty hydrocarbons in the fresh water sample, and 16.5% in the NaCl sample. Two diterpenic hydrocarbons 0.7 and 0.8%, respectively, were found in the fresh water sample.

The presence of isoprenyl acetate, carene, cedrol and most of the aromatics may explain the agreeable odour of *Scenedesmus* fresh biomass although they represent only 0.02% of algal dry weight.

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