REVIEW

Folia Microbiol. **51** (3), 159–182 (2006) **http://www.biomed.cas.cz/mbu/folia/**

Metabolites Produced by Cyanobacteria Belonging to Several Species of the Family *Nostocaceae*

T. ŘEZANKA[a], V.M. DEMBITSKY[b]

[a]*Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20 Prague, Czechia*
e-mail rezanka@biomed.cas.cz

[b]*Department of Organic Chemistry, Hebrew University of Jerusalem, Jerusalem 91391, Israel*

Received 18 November 2004
Revised version 25 January 2006

ABSTRACT. This paper provides a comprehensive overview of metabolites, including lipids and lipid-like compounds, nitrogen metabolites, oligopeptides and amino acid derivatives, produced by cyanobacteria of the genera *Anabaenopsis*, *Aphanizomenon*, *Aulosira*, *Cylindrospermopsis*, *Cylindrospermum*, *Nodularia*, and *Richelia* of the family *Nostocaceae*.

Abbreviations

EC_{50} (effective concentration 50 %) – the plasma concentration required for obtaining 50 % of the maximum effect *in vivo*
IC_{50} (inhibitory concentration 50 %) – the concentration of a drug required for 50 % inhibition of replication *in vitro* (can be corrected for protein binding *etc*.)
LD_{50} (lethal dose 50 %) – the number of pathogens required to cause lethal disease in half of the exposed hosts

ELISA	enzyme-linked immunosorbent assay	PSP	paralytic shellfish poisoning toxins
FA	fatty acid(s)	WHO	*World Health Organization*
FABMS	fast atom bombardment mass spectrometry		

CONTENTS

1 INTRODUCTION

Cyanobacteria have been identified as one of the most promising groups of organisms from which were isolated novel, biologically active natural products (Shimizu 1996, 2003; Burja *et al.* 2001; Ascencio *et al.* 2004; Berlinck and Kossuga 2005; Dembitsky and Řezanka 2005; Dietrich and Hoeger 2005; Singh *et al.* 2005). The medicinal and nutrient qualities of cyanobacteria were first appreciated as early as 1500 BC, when *Nostoc* species were used to treat gout, fistula, and several forms of cancer (Bladon 2002; Pietra 1990; Liu and Chen 2003). Prior to the 1990s, limited investigations were undertaken on the isolation of biologically active natural products from cyanobacteria. Currently, published data indicate that the family *Nostocaceae* contained over 2500 strains of freshwater and marine cyanobacteria (Komárek and Hauer 2004). The very high incidence of novel, biologically active compounds isolated from *Nostocaceae* indicates that cyanobacteria are a rich source of potentially useful natural products (Liu and Chen 2003; Prasanna *et al.* 2004).

The family *Nostocaceae* (order *Nostocales*) consists of nitrogen-fixing cyanobacteria, which were characterized as eubacteria that grow as autotrophs with CO_2 as the carbon source, utilizing an oxygen-producing photosynthetic mechanism for the generation of ATP and reductants (Hrouzek *et al*. 2004). Members of the order *Nostocales* are broadly characterized by unbranched filaments and the production of up to three kinds of differentiated cells. Heterocysts differentiate in response to the lack of combined nitrogen in the environment and are the sites of nitrogen fixation (*cf*. Sarma *et al*. 2004). Heterocysts occur singularly in a semi-

regular spacing pattern in the filaments at a frequency of 3–10 % of the total cells (Castenholz and Waterbury 1989).

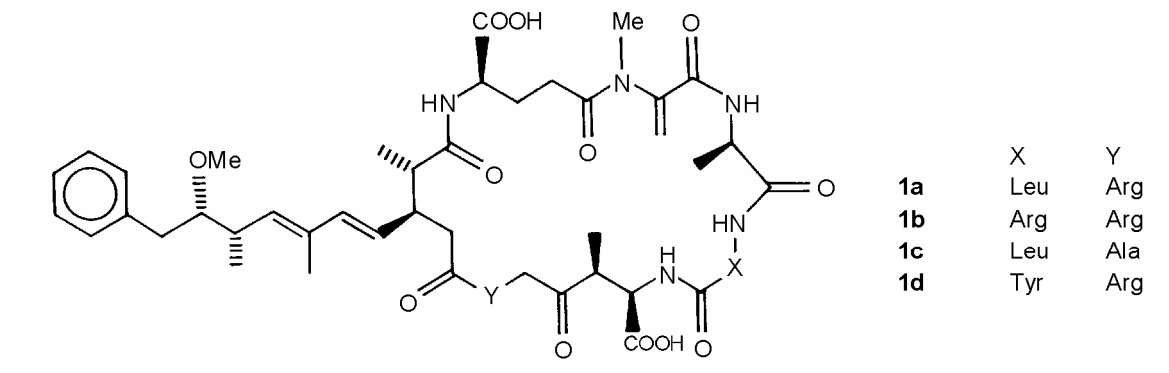
We discuss here the occurrence of secondary metabolites and biosynthetic mechanisms of certain metabolites, such as lipids, paralytic shellfish poisoning toxins, and polyether toxins in *Nostocaceae*.

2 GENUS *Anabaenopsis* (WOLOSZ.) V. MILL. 1923

Few papers focused on the isolation of some secondary metabolites in *Anabaenopsis* species have been reported. Thus, the biochemical composition and FA content of filamentous, heterocystous, nitrogen-fixing cyanobacterium *Anabaenopsis* sp. have been determined. Ten FA have been found, predominantly palmitic (45.5 %), oleic (8.2 %), linoleic (16.2 %), α-linolenic (16.8 %), γ-linolenic (3.6 %), and stearidonic (4.6 %) acids (Vargas *et al.* 1998). In addition, *Anabaenopsis* sp. cells contain 52.2 % (of total mass) proteins, 16.3 % of saccharides, and 11.4 % of lipids.

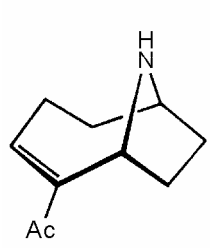
Anionic polysaccharides, with unknown structures, have been detected in the soil filamentous cyanobacterium *Anabaenopsis circularis* (Warrington *et al.* 1991).

The presence of microcystin-type toxins (**1a–d**) in extracts of a natural bloom of cyanobacteria composed predominantly of *Anabaenopsis milleri* has been reported (Lanaras and Cook 1994). The toxins have been extracted, purified, and compared to microcystin-LR (**1a**). The LD_{50} of *A. milleri* bloom material was 600–1500 mg lyophilized cells per kg body mass. Symptoms and pathological signs of poisoning in mice were characteristic of cyanobacterial hepatotoxins, with enlarged darkened livers with masses of 8–10 % of the total body mass. Comparison of the chromatographic behavior of the purified toxin with microcystin-LR by reversed phase HPLC indicated that an *A. milleri* bloom toxin was a microcystin type toxin and it is highly likely that the purified toxin is microcystin-LR. Capillary zone electrophoresis and micellar electrokinetic chromatography were applied to the simultaneous separation of cyanobacterial toxins, *i.e.* anatoxin-*a*, microcystin-LR, cylindrospermopsin (Vasas *et al.* 2004). Microcystins were also determined in 36 cyanobacterial (blue-green algal) bloom samples dominated by *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. Ten microcystins (MC), three major and seven minor variants, were detected in natural cyanobacterial samples by high-performance liquid chromatography. The structures of the microcystins were assigned on the basis of liquid chromatography–electrospray ionization mass spectrometry. The main microcystins detected in the blooms from freshwaters species were MC-LR, MC-RR and MC-YR. Microcystin content of the blooms varied from a few μg/g to 1687 μg/g freeze-dried material. Also some monodemethyl and didemethyl variants of MC-LR, MC-RR, MC-YR were found in a few analyzed samples (Jurczak *et al.* 2004).

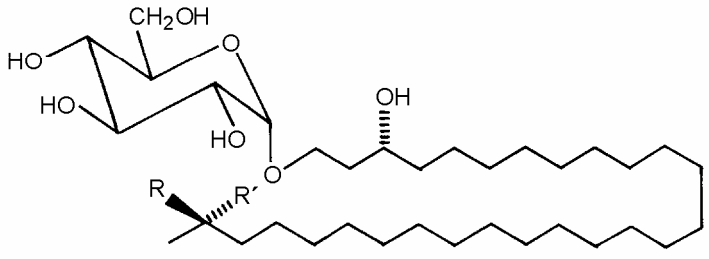


	X	Y
1a	Leu	Arg
1b	Arg	Arg
1c	Leu	Ala
1d	Tyr	Arg

1a–d



2



	R	R'
3a	H	OH
3b	=O	

3a,b

The phytoplankton communities and the production of cyanobacterial toxins were investigated in two alkaline Kenyan crater lakes, Lake Sonachi and Lake Simbi. Lake Sonachi was mainly dominated by the cyanobacterium *Arthrospira fusiformis*, Lake Simbi by *A. fusiformis* and *Anabaenopsis abijatae*. Using HPLC techniques, one structural variant of the hepatotoxin microcystin (microcystin-RR; **1b**) was found in Lake Sonachi and four variants (microcystin-LR, **1a**, -RR, **1b**, -LA, **1c** and -YR, **1d**) were identified in Lake Simbi. The neurotoxin anatoxin-A (**2**) was found in both lakes (Ballot *et al.* 2005).

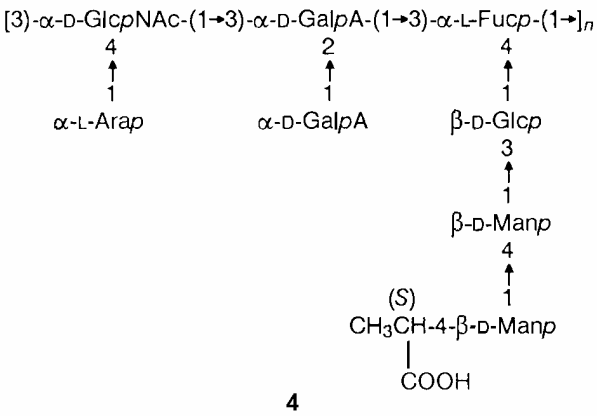
Cyanobacterial toxins in Lakes Bogoria, Nakuru and Elmenteita (Kenya) were analyzed both in the phytoplankton from these lakes and in isolated monocyanobacterial strains of *Arthrospira fusiformis*, *Anabaenopsis abijatae*, *Spirulina subsalsa* and *Phormidium terebriformis*. Using HPLC, the cyanobacterial hepatotoxins microcystin-LR, -RR, -YR, and -LA and the neurotoxin anatoxin-A were detected in phytoplankton samples. Total microcystin concentration amounted to 155 μg microcystin-LR (Lake Bogoria) and 4593 μg microcystin-LR (Lake Nakuru) (Ballot *et al.* 2004).

The genus *Cyanospira* was proved recently to be generically identical with the genus *Anabaenopsis* according to 16S rRNA and life form (Iteman *et al.* 2002; Rajaniemi *et al.* 2005).

The genus *Anabaenopsis* has been isolated from the alkaline soda lake Magadi in Kenya (Florenzano *et al.* 1985). Two species have been described, *viz. A. rippkae* and *A. capsulata*. The latter takes its name from the gelatinous capsule that surrounds the microbial cells that are arranged in colonies of helical thricomes.

Heterocyst glycolipids of the cyanobacterium *A. rippkae* have been isolated and their structures established to be 1-(*O*-α-D-glucopyranosyl)-3*R*,27*R*-octacosanediol (**3a**) and 1-(*O*-α-D-glucopyranosyl)-27-oxo-3*R*-octacosanol (**3b**) by spectroscopic and chemical means (Soriente *et al.* 1993).

The cyanobacterial strain *A. capsulata* was screened for its ability to remove Cu^{2+} from aqueous solutions; a quick and most effective heavy metal adsorption was observed (De Philippis *et al.* 2001). Unusual exopolysaccharide has been isolated from cultures of *A. capsulata* (Florenzano *et al.* 1985), and it was composed of arabinose, fucose, glucose, mannose, and galacturonic acid (GalA) (Flaibani *et al.* 1989). Two other papers reported by Marra *et al.* (1990) and Cesaro *et al.* (1990) have appeared dealing with the primary structure and they differ in the quantitative estimate of GalA. All five monosaccharides were reported to be present in equimolar ratio (Marra *et al.* 1990), whilst Flaibani *et al.* (1989) and Cesaro *et al.* (1990) reported that GalA was present in a 2 : 1 ratio with respect to the other neutral sugars. The batch culture of *A. capsulata* produced large amounts of a soluble exopolysaccharide and over a period 30 d under continuous illumination was studied. A mean exopolysaccharide productivity of about 6 g m^{-2} d^{-1} was attained. Purified exopolysaccharide exhibited a saccharidic composition consisting of four neutral sugars (glucose, mannose, fucose, and arabinose) and GalA in a molar ratio of 1 : 1 : 1 : 1 : 2, respectively. The exopolysaccharide was also characterized by the presence of pyruvic residues and by a protein content of about 2 % but *O*-acetyl groups and sulfate residues were not detected (Vincenzini *et al.* 1990). The presence of two monosaccharides, *N*-acetylglucosamine and the rare acidic sugar 4-*O*-(1-carboxyethyl)mannose was further described by Garozzo *et al.* (1995). The exocellular polysaccharide produced by a different strain of *A. capsulata* was studied by partial acid hydrolysis and *N*-deacetylation–nitrous acid deamination (Garozzo *et al.* (1998). The oligosaccharides have been isolated and characterized by a combination of 1D- and 2D-NMR, MS, and linkage analyses. The polysaccharide had an octasaccharide repeating unit (**4**).



4

3 GENUS *Aphanizomenon* MORR. ex BORN. & FLAH. 1886

Aphanizomenon flos-aquae (Fig. 1) is a colony-forming, filamentous, heterocystous cyanobacterium belonging to the family *Nostocaceae*, which is found in freshwater environments (Castenholz 1989). *A. flos-aquae* has a rather short history of consumption by humans. The exploitation of *A. flos-aquae* did not start until the early 1980s when natural blooms of this organism were first harvested on a large scale from

Klamath Lake in Oregon (USA) (Carmichael *et al.* 2000). Currently, two main harvesting strategies have been adopted on Klamath Lake. One involves an off-lake, large series of screens made of nylon mesh to remove the biomass from the water coming out of the lake and flowing into an aqueduct system. The other employs on-lake barges either equipped with rotating screens or fixed screens coupled with water pumps. The rotating screens can be lowered just under the surface of the lake to collect the biomass, whereas the fixed screens filter the water pumped from below the surface of the lake. It was reported that ≈2 Gg (*i.e.* 2000 t) of biomass of *A. flos-aquae* were harvested from Klamath Lake in 1998 (Carmichael *et al.* 2000).

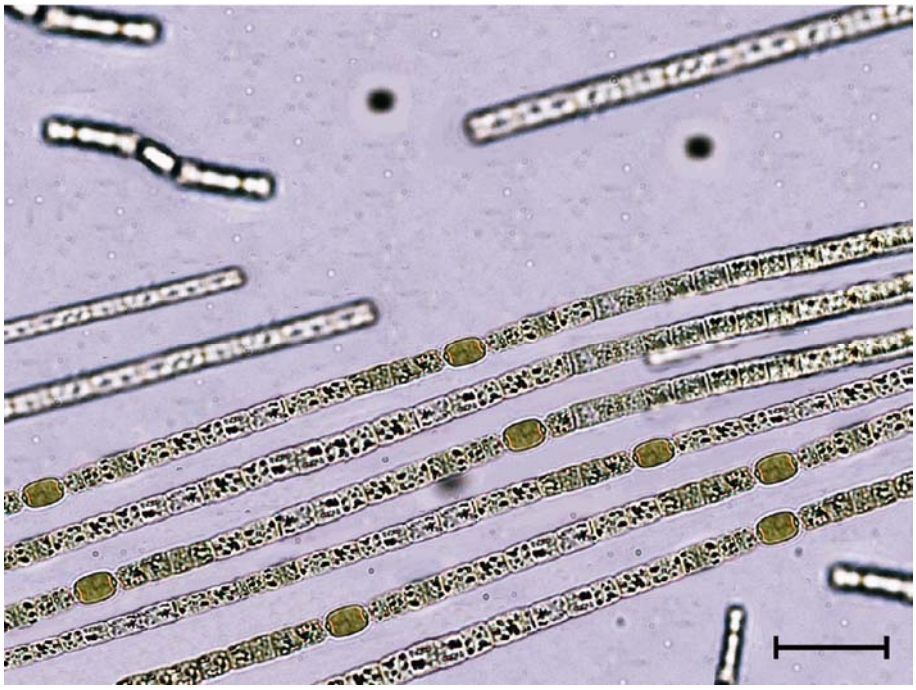


Fig. 1. *Aphanizomenon flos-aquae*; *bar* = 20 μm.

Analysis of *A. flos-aquae* from Klamath Lake showed that this cyanobacterium contains (% of total biomass): protein 62, saccharides 23, lipids 3, pigments (chlorophylls, xanthophylls, phycocyanins, and phycoerythrins); essential amino acids (mg/g): Gln 78, Leu 52, Ala 47, Asn 47, Arg 38, Lys 35, Tyr 33, Val 32, Gly 29, Ile 29, Pro 29, Ser 29, Phe 25, Thr 17, His 9, Asp 7, Met 7, Trp 7, Glu 4, Cys 2.

Vitamins (pyridoxine, nicotinamide, biotin, thiamine, tocopherol, riboflavin, folic, ascorbic, and pantothenic acids) were also found.

Using gradient HPLC and evaporative light-scattering detection, a variety of lipid classes of marine *Aphanizomenon* sp. isolated from spring bloom in the Northern Bothnian Sea (October 1996) have been separated and detected (Nordback *et al.* 1998). An extract of the cyanobacterium contains hydrocarbons, sterol esters, wax esters, sterols, triacylglycerols, chlorophyll *a*, monogalactosyldiacylglycerols, digalactosyldiacylglycerols, sulfoquinovosyldiacylglycerols, phosphatidylglycerols, and phosphatidylcholine. In an extract of *Aphanizomenon* sp. the dominant classes were found to be chlorophyll *a*, sulfoquinovosyldiacylglycerols, monogalactosyldiacylglycerols and nonpolar lipids (Nordback *et al.* 1998).

Palmitic acid was found as a major lipid compound (43.0 % of lipid mass), further linolenic (21.4), linoleic (12.4), stearic (2.9), and monounsaturated acids, *i.e.* palmitoleic (9.7), and oleic (5.0) were also detected.

The occurrence of dioic, hydroxy, branched, and unsaturated FA in cyanobacteria of the genus *Aphanizomenon* growing in different freshwater lakes has been studied. Dicarboxylic (4.52–7.14 %) and other FA were identified by GC–MS (Dembitsky *et al.* 2001) (Table I). An unusual finding was the identification of dioic acids, such as 2(*E*)-butene-1,4-dioic, 2-hydroxybutane-1,4-dioic, and 2-methylbutane-1,4-dioic acid. None of these acids had been found previously in *Aphanizomenon* strains. The results have shown for the first time the presence of fifteen dioic acids in cyanobacteria. Some dicarboxylic acids have the potential as antiproliferative and as general antitumor agents against primary invasive malignant melanoma (Tatman and Mo 2002). Aliphatic dicarboxylic acids surprisingly afforded potent cytotoxicity, antineoplastic activity

(Hall *et al.* 1999), and served as lipidic markers for identification some human and animal diseases (Singh 1997; Visentin *et al.* 1995). These acids are of major interest for medical specialists and biochemists (Edlund and Albertsson 2003).

Table I. Content (%, *M*/*M*) and structure of dimethyl esters of dioic acids from *Aphanizomenon* strains[a]

Dioic acid	*1*	*2*	*3*	*4*
Ethane-1,2-	0.06	0.21	0.32	0.18
Propane-1,3-	0.21	0.13	0.41	0.12
Butane-1,4-	0.87	1.49	1.18	2.01
(*E*)-2-Butene-1,4-	0.42	0.09	0.03	0.11
2-Hydroxybutane-1,4-	0.33	1.31	0.38	0.21
2-Methylbutane-1,4-	0.48	0.56	0.13	0.19
Pentane-1,5-	0.12	0.09	0.14	0.35
Hexane-1,6-	0.17	0.12	0.21	0.11
3-Methyl-hexane-1,6-	0.07	0	0.72	0.16
Heptane-1,7-	0.24	0.61	0.38	0.12
3-Methylheptane-1,7-	0.09	0.45	0.11	0.14
Octane-1,8-	0.11	0.26	0.17	0.31
Nonane-1,9-	1.21	1.68	0.92	0.64
Decane-1,10-	0.14	0.06	0.21	0.05
Undecane-1,11-	0	0.08	0.13	0.12
Total	4.52	7.14	5.44	4.82

[a] *1* – *Aphanizomenon flos-aquae* (Klamath Lake, USA)
2 – *A. flos-aquae* (Upper Klamath Lake, USA)
3 – *A. ovalisporum* (Tiberias Lake, Israel)
4 – *A. flos-aquae* (Queen Elizabeth Reservoir, UK)

Most of the FA were saturated $C_{4:0}$–$C_{18:0}$ (>65 %), but unsaturated FA were also found (26–39 %; Table II). Branched saturated FA were identified (7.10–17.2 %). The predominant unsaturation (38.9 % of the total monounsaturated FA) was at oleic acid (26.7 %). Other monounsaturated FA (palmitoleic and 11-hexadecenoic acid) were observed. Hydroxy FA were found as minor components (0.27–0.59 %) (Dembitsky *et al.* 2001).

The cellular FA content of 22 cyanobacterial strains belonging to the genera *Anabaena*, *Aphanizomenon*, *Calothrix*, *Cylindrospermum*, *Nostoc*, *Microcystis*, and *Planktothrix* has been studied. The strains of *Anabaena*, *Aphanizomenon*, and *Cylindrospermum* grouped tightly and were characterized by the presence of palmitoleic and *anteiso*-palmitic acids. Cluster analysis of *Anabaena*, *Aphanizomenon*, and *Cylindrospermum* showed that all hepatotoxic *Anabaena* strains grouped together, whereas the nontoxic and neurotoxic *Anabaena* strains grouped with the nontoxic *Aphanizomenon* strains (Gugger *et al.* 2002).

A. flos-aquae was cultivated in the laboratory under different growth conditions to search for long-chain alkan-1,15-diols and alkan-15-one-1-ols (Morris and Brassell 1988). These compounds which are ubiquitous in recent sediments were not present either free or bound in any of the analyzed cultures, indicating that results of chemotaxonomical studies based on field samples should be interpreted with great caution. A specific lipid, tentatively identified as 15-hydroxyhexacosanoic acid, was encountered in the cultured alga and has a potential importance as a biomarker (De Leeuw *et al.* 1992).

Some amides and further nitrogen-containing compounds have been isolated from *A. flos-aquae* (Dembitsky *et al.* 2000). Amides of FA are lipid bioregulators formed from long-chain saturated and unsaturated FA *via* amidation by the corresponding amines. Ethanolamides of FA are the most well studied species of this group; an alternative pathway for their biosynthesis includes hydrolysis of *N*-acylated phosphatidylethanolamines by phospholipase D. Ethanolamides of FA bind to the cannabinoid receptors of the central nervous system or peripheral tissues and can be considered as endogenous ligands of these receptors. Their pharmacological properties are similar to those of cannabimimetics. Simple amides of FA are also endogenous bioregulators acting as sleep-inducing (oleamide) or angiogenic (erucamide) factors. A new group of bioregulators comprises the amides of FA and biologically active amines (vanillinamine, dopamine, and serotonin) (Bezuglov *et al.* 1998).

A series of pterins (**5a**–**h**) has been isolated from *A. flos-aquae*. The glycoside-1 from the toxic strain NH-1 gave rise to xylose and glucose on hydrolysis, whereas glycoside-2 from *Cambridge Collection*

and nontoxic NH-1 strains gave mannose and glucose. The authors concluded that these pterins might be a useful marker for certain species of cyanobacteria (Ikawa *et al.* 1995).

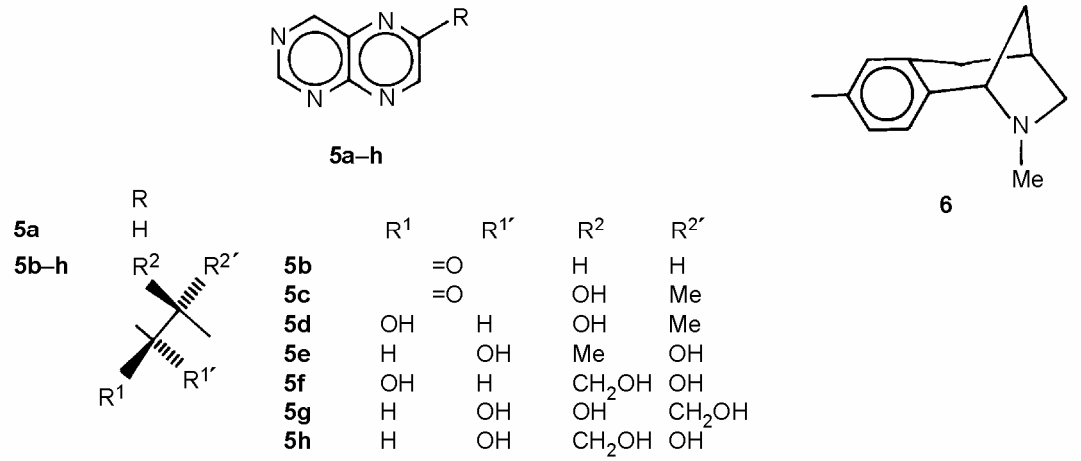
Table II. Hydroxy, *n*-saturated, branched saturated and unsaturated acids from *Aphanizomenon* strains[a] (%, *M*/*M*)

Fatty acid		*1*	*2*	*3*	*4*
Total hydroxy acids		**0.50**	**0.59**	**0.27**	**0.37**
2-Hydroxypropanoic	2-OH-3:0	0.12	0.15	0	0.04
3-Hydroxybutanoic	3-OH-4:0	0.14	0.11	0.06	0
2-Hydroxy-4-methylpentanoic	2-OH-4-Me-5:0	0.11	0.12	0.21	0.24
2-Hydroxy-3-methylpentanoic	2-OH-3-Me-5:0	0.13	0.21	0	0.09
***n*-Saturated**		**51.5**	**54.6**	**45.8**	**49.7**
Butanoic	4:0	0.19	0.15	0.11	0.05
Pentanoic	5:0	0.23	0.31	0.09	0.11
Hexanoic	6:0	0	0.18	0.24	0.16
Heptanoic	7:0	0.19	0.13	0.10	0.12
Octanoic	8:0	0.08	0.09	0.12	0.13
Nonanoic	9:0	0.18	0.23	0.22	0.19
Decanoic	10:0	0.19	0.11	0.21	0.29
Dodecanoic	12:0	0.44	0.26	0.31	0.18
Tetradecanoic	14:0	13.7	12.7	2.01	3.22
Pentadecanoic	15:0	3.15	1.51	0.95	0.84
Hexadecanoic	16:0	32.0	37.0	40.1	43.1
Heptadecanoic	17:0	0.68	0.18	0.22	0.31
Octadecanoic	18:0	0.51	1.74	1.14	0.98
Branched saturated		**17.21**	**10.32**	**9.53**	**7.10**
3-Methylbutanoic	3-Me-4:0	0.21	0.25	0	0
2-Methylbutanoic	2-Me-4:0	0	0.08	0	0.03
4-Methylhexanoic	4-Me-6:0	0.06	0	0.09	0.12
8-Methyldecanoic	8-Me-10:0	0.18	0.13	0.09	0.12
12-Methyltetradecanoic	12-Me-14:0	0.80	0.28	0.62	0.51
14-Methylpentadecanoic	14-Me-15:0	9.67	4.37	3.33	2.78
2-Methylhexadecanoic	2-Me-16:0	0.39	0.42	0.72	0.52
14-Methylhexadecanoic	14-Me-16:0	0.14	0.32	0.51	0.21
15-Methylhexadecanoic	15-Me-16:0	0.25	0.31	0.64	0.41
10-Methylheptadecanoic	10-Me-17:0	5.24	3.82	3.12	2.02
2-Hexylcyclopropaneoctanoic	cyc-17:0	0.27	0.34	0.41	0.38
Unsaturated		**26.2**	**27.4**	**38.9**	**37.9**
3-Methyl-(*Z*)-3-pentenoic	3-Me-3-5:1	0.09	0.03	0.11	0
(*Z*)-9-Hexadecenoic	9-16:1	2.39	1.02	2.34	1.85
11-Hexadecenoic	11-16:1	1.24	3.19	2.14	2.55
(*Z*)-9-Octadecenoic	9-18:1	20.9	19.9	26.7	26.1
9,12-Octadecadienoic	9,12-18:2	0.28	0.41	4.14	5.21
10,13-Octadecadienoic	10,13-18:2	0.19	1.68	2.05	1.19
12,15-Octadecadienoic	12,15-18:2	0.16	0.34	0.61	0.43
9,12,15-Octadecatrienoic	9,12,15-18:3	0.94	0.75	0.81	0.74

[a]*See* footnote to Table I.

An alkaloid with a benzazepine skeleton, aphanorphine (**6**) was isolated from *A. flos-aquae* together with its previously known constituents (Gulavita *et al.* 1988). The anatoxins are a group of neurotoxic alkaloids produced by a number of cyanobacterial genera including *Aphanizomenon*, *Anabaena*, and *Plankothrix.* All planktic *Oscillatoria* species were transferred (Nadeau *et al.* 2001) into the genus *Plankothrix* according to molecular (Suda *et al.* 2002) and phenotype (Anagnostidis and Komárek 1988) criteria already several years ago. The toxicity of these compounds (LD_{50}) begins at 20 μg/kg (i.p. mouse). Anatoxin-A (**2**) as a low-molar-mass alkaloid was isolated from *A. flos-aquae* (Sivonen and Jones 1996). The biosynthesis of anatoxin-A produced by this cyanobacterium involves the loss of glycine from arginine. Significantly, this retroaldol-type cleavage is analogous to the Claisen condensation of acetate to arginine in the saxitoxin bio-

synthesis. According to feeding experiments carried out with *A. flos-aquae*, the molecule is built from arginine and acetate in an extraordinary manner (Shimizu 1996).



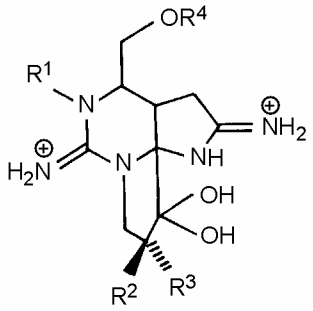
The cytotoxic toxin cylindrospermopsin (**16b**; *see* p. 171) has been isolated from the cyanobacteria *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, and *Umezakia natans* (Kaebernick and Neilan 2001). Toxicity of **18** in mammals has been reported by Seawright *et al.* (2000). In white Swiss mice, the median acute lethal dose *via* i.p. injection was 0.2 mg/kg over a period of 5 d, while the 5-d oral median lethal dose was 6 mg/kg (Terao *et al.* 1994; Seawright *et al.* 1999). The acute toxicity mechanism has been ascribed to inhibition of protein synthesis (Terao *et al.* 1994), which further suggested that activation through hepatic microsomal mixed oxygenation also contributes to the essentially periacinar hepatotoxicity.

Cylindrospermopsin (**16b**), a cyanobacterial guanidine alkaloid hepatotoxin and protein-synthesis-inhibitor, was assayed for its effects on the germination of pollen from tobacco. Pollen germination, measured by Alcian Blue binding, was inhibited by **16b** between 5 and 1000 μg/mL. As a protein-synthesis-inhibitor, **16b** did not inhibit pollen germination to the same extent as cycloheximide on a gravimetric basis, but significantly reduced the amount of *U*-^{14}C-L-leucine labeling in pollen tubes. The inhibition of tobacco pollen germination may be amenable for development as a bioassay for **16b**, although this would require a preconcentration step for the monitoring of environmental samples (Metcalf *et al.* 2004).

The presence of PSP toxins in cultures of *A. flos-aquae*, isolated from the Crestuma-Lever reservoir, was found by reversed-phase HPLC employing two isocratic elution systems for the separation (Ferreira *et al.* 2001). With the first isocratic elution protocol, the presence of apolar toxins, such as saxitoxin (**7g**), decarbamoylsaxitoxin (**7a**) and neosaxitoxin (**7h**), was not detected. On the other hand, GTX1 (gonyautoxin; **7j**), deGTX2 (**7c**), and GTX4 (**7l**) were present in the *A. flos-aquae* cells collected either directly from the bloom or in the other toxic isolates previously cultivated in the laboratory. Detection of toxins in an algal bloom in Lake Crato (Portugal) drinking water reservoir was followed by isolation of a strain of *Aphanizomenon gracile*. The strain, coded as LMECYA40, was cultured and identified by combining a morphological study with 16S rRNA gene sequencing. The toxin profile of this isolate, as revealed by HPLC-FLD analysis, was similar to that of other *Aphanizomenon* strains, consisting of two analogues: neosaxitoxin (0.27 fmol per cell) and saxitoxin (0.05 fmol per cell) (Pereira *et al.* 2004). The report of **7g** in cyanobacterial blooms in Finland was published (Rapala *et al.* 2005). Bloom samples were collected from Finnish freshwater and showed the presence of paralytic shellfish toxin (**7g**) in a high concentration, as much as 1 mg/L.

Montargil reservoir, located in a dry flat area in the center of Portugal, was filled in 1958 to meet agricultural, electric, and industrial requirements. In May 1996, an intensive bloom of phytoplankton was detected. The algal community was strongly dominated by cyanobacteria with predominance of *A. flos-aquae* from May to June and *Microcystis aeruginosa* from July to August. Extracts of samples collected during the bloom period showed high toxicity by mouse bioassay. During the *M. aeruginosa* predominance period, the toxicity was ascribed to the presence of hepatotoxins, but clear symptoms of paralytic shellfish poison were observed when *A. flos-aquae* was the dominant species (Pereira *et al.* 2000). In order to confirm the production of toxins, the culture of the strain *A. flos-aquae* was isolated and established. Identification of the **7g** analogs was achieved using HPLC with postcolumn fluorescence derivatization and LC–MS technique. The toxins found in the culture extract were C4 (**7r**; 64.5 molar %), neosaxitoxin (**7h**; 23.0), decarbamoylsaxitoxin (**7a**; 6.1), saxitoxin (**7g**; 5.4), and GTX8 (**7q**; (1.1). The toxin profile is rather different from the pre-

viously reported PSP producing *A. flos-aquae* and demonstrates its diversity in terms of toxin production (Pereira *et al.* 2000).



7a–r

	R^1	R^2	R^3	R^4
7a	H	H	H	H
7b	OH	H	H	H
7c	H	H	OSO_3^-	H
7d	OH	H	OSO_3^-	H
7e	H	OSO_3^-	H	H
7f	OH	OSO_3^-	H	H
7g	H	H	H	$CONH_2$
7h	OH	H	H	$CONH_2$
7i	H	H	OSO_3^-	$CONH_2$
7j	OH	H	OSO_3^-	$CONH_2$
7k	H	OSO_3^-	H	$CONH_2$
7l	OH	OSO_3^-	H	$CONH_2$
7m	H	H	H	$CONHSO_3^-$
7n	OH	H	H	$CONHSO_3^-$
7o	H	H	OSO_3^-	$CONHSO_3^-$
7p	OH	H	OSO_3^-	$CONHSO_3^-$
7q	H	OSO_3^-	H	$CONHSO_3^-$
7r	OH	OSO_3^-	H	$CONHSO_3^-$

A single run HPLC method has been developed and shown to be applicable to the quantitative analysis of PSP produced by Australian cyanobacteria (*Anabaena circinalis*) and other cyanobacteria. The daily precision of this method was adequate for it to be considered as a routine analytical tool for direct PSP analysis of cyanobacterial extracts and water bodies containing C3, C4, GTX2, GTX3, neoSTX in the 10–70 ppb concentration range (Papageorgiou *et al.* 2005) (Table III).

The distribution pattern and seasonal dynamics of microcystin-LR, -YR and -RR in various organs of four edible freshwater mussels (*Anodonta woodiana*, *Hyriopsis cumingii*, *Cristaria plicata*, and *Lamprotula leai*) were studied monthly during Oct 2003–Sep 2004 in Lake Taihu (China) with toxic cyanobacterial blooms in the summer. Qualitative and quantitative determinations of microcystins in the organs were done by LC–MS and HPLC. The major toxins were present in the hepatopancreas, followed by visceral mass with substantial amount in gonads, whereas gill and foot were the least. Among the foot samples analyzed, 54 % were above the provisional, WHO tolerable daily intake level, and the mean daily intakes from the four bivalves were 8.0–23.5 times the tolerable daily intake value when the bivalves are eaten as a whole, suggesting the high risk of consuming bivalves in Lake Taihu (Chen and Xie 2005).

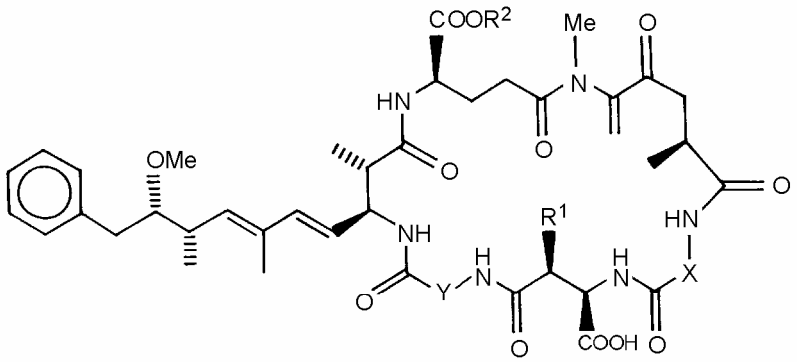
In a survey in Greece 1987–2000 hepatotoxic cyanobacterial blooms were observed in 9 of 33 freshwater reservoirs. Microcystins (**1a**–**d**) were detected by HPLC in seven of these lakes, and the total microcystin concentration per scum dry mass ranged from 50.3 to 1638 μg/g. Cyanobacterial genera, such as *Microcystis*, *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, and *Cylindrospermopsis* were present in 31 fresh water (Cook *et al.* 2004). From published data it would appear that Mediterranean countries are more likely to have a low level of cyanobacterial toxins, such as microcystin. A case study in Lake Kastoria was used to highlight the seasonal patterns of cyanobacterial and microcystin-LR occurrence and to assess cyanotoxin risk (Cook *et al.* 2004). Cyanobacterial biovolume was high (>11 μL/L) throughout the year and was in excess

of Guidance Level 2 (10 μL/L) proposed by WHO for recreational waters and Alert Level 2 for drinking water. Furthermore, surface water samples from April to November exceeded Guidance Level 3, with the potential for acute cyanobacterial poisoning. Intracellular microcystin-LR concentration (maximum 3186 μg/L) exceeded the WHO guideline for drinking water (1 μg/L) from September to November with a high risk of adverse health effects. Preliminary evidence indicates that in three lakes microcystins are accumulated in some aquatic organisms. Generally, a high-risk level can be deduced from the data for the Mediterranean region (Cook *et al.* 2004).

Table III. Toxicity of paralytic shellfish poisoning toxins **7a**–**r**

Toxin	No.	Net charge	Relative toxicity
Decarbamoylsaxitoxin deSTX	**7a**	+2	0.513
Decarbamoylneosaxitoxin deneoSTX	**7b**	+2	–
Decarbamoylgonyautoxin deGTX2	**7c**	+1	0.651
Decarbamoylgonyautoxin deGTX1	**7d**	+1	–
Decarbamoylgonyautoxin deGTX3	**7e**	+1	0.754
Decarbamoylgonyautoxin deGTX4	**7f**	+1	–
Saxitoxin STX	**7g**	+2	1.000
Neosaxitoxin neoSTX	**7h**	+2	0.924
Gonyautoxin GTX2	**7i**	+1	0.359
Gonyautoxin GTX1	**7j**	+1	0.994
Gonyautoxin GTX3	**7k**	+1	0.638
Gonyautoxin GTX4	**7l**	+1	0.726
Gonyautoxin GTX5	**7m**	+1	0.064
Gonyautoxin GTX6	**7n**	+1	–
Epigonyautoxin GTX8	**7o**	0	0.006
C3	**7p**	0	0.013
Gonyautoxin GTX8	**7q**	0	0.096
C4	**7r**	0	0.058

Many different microcystins LR (**8a**–**d**) were isolated and identified in the *A. flos-aquae*. The toxicity was determined i.p. in mouse; the LD_{50} values were 40–1000 μg/kg (Krishnamyrthy *et al.* 1989; Harada *et al.* 1990, 1991; Rinehart *et al.* 1988, 1994; Sivonen *et al.* 1992; Bateman *et al.* 1995).



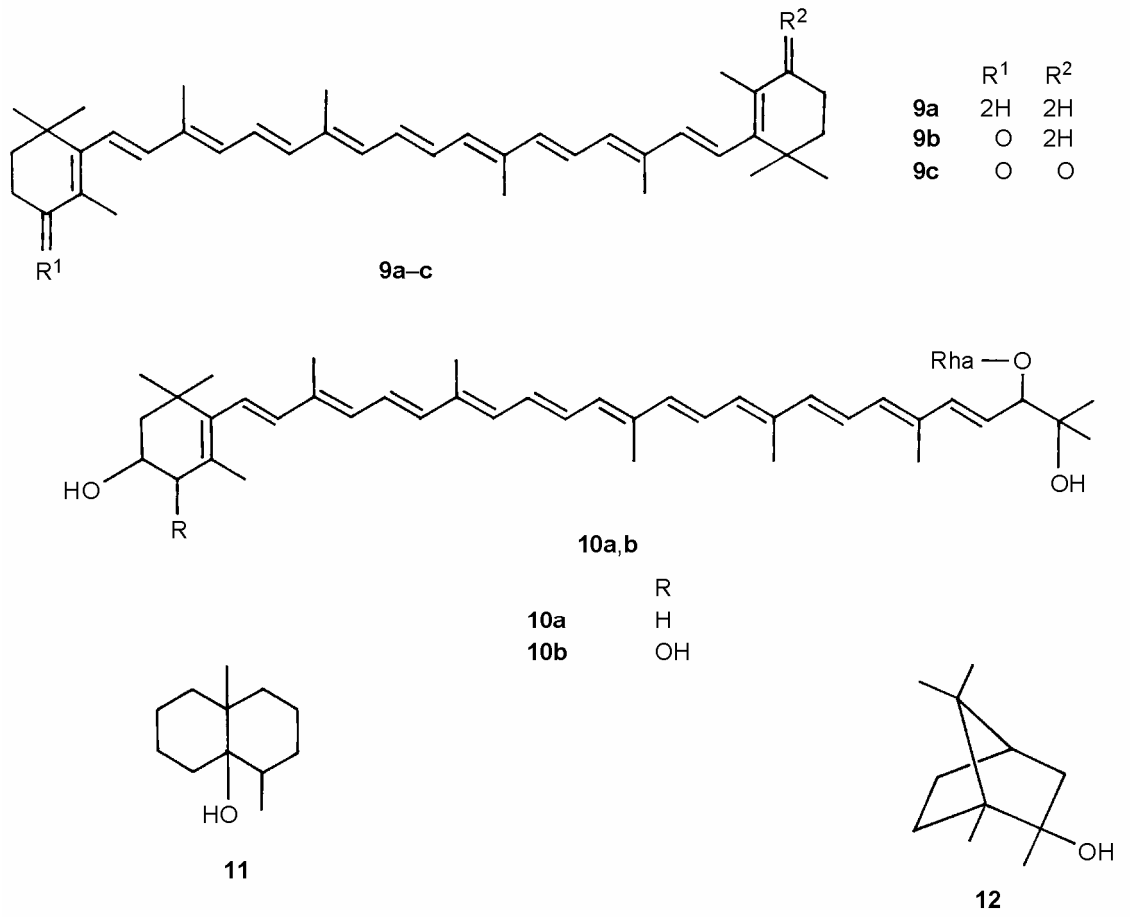
8a–f

	X	Y	R^1	R^2
8a	Leu	Arg		
8b	Leu	Ala		Me
8c	Leu	Arg	Me	
8d	Hty	Arg		
8e	Arg	Arg		
8f	Tyr	Arg		

The concentration of a cyanobacterial toxin, nodularin (**13b**), was measured in the Baltic Sea in 1998–1999. Statistical associations of the concentration of **13b** with environmental factors were tested by multiple regression analysis. To reveal the toxin-producing organism, colonies of *Aphanizomenon* and *Nodularia* were picked and analyzed for peptide toxins (Repka *et al.* 2004). All the measured samples contained

13b, but other toxins were not detected by the HPLC analysis. In both years, the highest **13b** concentration was found in the surface water layer. The concentration of **13b** was positively correlated with silicate concentration in water. High concentration of silica in surface water may indicate recent upwelling, which in turn renders surface water rich in nutrients. This upwelling is likely to intensify cyanobacterial growth and toxin production which may explain this rather unexpected result. The picked *Aphanizomenon* colonies did not contain **13b** or the concentration was below the detection limit. Thus, it was concluded that most of **13b** was bound to *Nodularia* cells. The abundances of zooplankton (copepods, rotifers, and cladocerans) were unrelated to *Nodularia*, but were positively associated with *Aphanizomenon*.

For the first time carotenoid composition of the *A. flos-aquae* has been studied by Tischer (1938, 1939) more than 65 years ago, who reported the presence of β-carotene (**9a**) and four pigments, designated as flavacin, aphanin, aphanicin, and aphanizophyll. Later, Hertzberg and Liaaen-Jensen (1966, 1967) have re-examined the carotenoid composition of the *A. flos-aquae*. The epiphasic fraction comprised β-carotene, flavacin, aphanin, and aphanicin. The last two were shown to be identical with echinenone (**9b**) and cantha-xanthin (**9c**), respectively. The hypophasic fraction contained in addition to aphanizophyll (**10b**) (later was also established as oscillaxanthin) small amounts of myxoxanthophyll (**10a**). Fiksdahl *et al.* (1983) shown that the Kezar Lake strain of *A. flos-aquae* contained seven different carotenoids.



	R^1	R^2
9a	2H	2H
9b	O	2H
9c	O	O

	R
10a	H
10b	OH

11 **12**

The profiles of geosmin (**11**), of which the dissolved and the particle-bound fractions were distinguished, were analyzed monthly in a seasonal study in a stratified mesotrophic prealpine lake Zürichsee (Durrer *et al.* 1999). Remarkable seasonal and spatial differences in the total amounts and ratios between the two fractions were observed. Equally low concentrations of dissolved and particle-bound **11** were found in the winter season during turnover conditions (maximum total concentration of **11** being 3.1 ng/L). In the clear-water period the dissolved fraction of **11** increased dramatically (≈93 %) whereas the particle-bound **11** showed only minor changes. In the autumn and first part of the winter surface films contributed essentially to the particle-bound fraction of **11** exhibiting maximum concentrations of 21 ng/L. Possible producers of **11** in Zürichsee are *Aphanizomenon* and *Planktothrix*. Feeding experiments with *Daphnia* and *Simocephalus*

simulating the phenomenon of the clear-water period apparently demonstrated that grazing could be regarded as a major mechanism for the liberation of **11** from particles.

Levels of **11** and 2-methylisoborneol (**12**) and composition of phytoplankton communities were determined for water samples collected from 35 fishponds at Auburn (Alabama), and 34 ponds in west-central Mississippi (Vanderploeg *et al.* 1992). In Auburn ponds, the level of 37 ranged from 0.05 to 8.9 μg/L but **12** was never detected. The highest concentration of **11** was measured in ponds with plankton blooms where *Anabaena* species dominated. In Mississippi ponds, the level of **12** ranged from 0.05 to 76 μg/L. The highest concentration of **12** was associated with blooms of *Plankothrix chalybea*, a cyanobacterium that produces **12** in unialgal culture. Geosmin at 0.05–6.25 μg/L could be associated with blooms of *Anabaena spiroides* and always concurred with the concentration of **12** (0.45–3.25 μg/L). Neither **11** nor **12** at either location could be associated with phytoplankton blooms of *A. flos-aquae*, *Plankothrix agardhii*, *Raphidiopsis brookii*, or *Microcystis aeruginosa* (Vanderploeg *et al.* 1992).

Flavor profile analysis results were compared with water quality data for two storage reservoirs of the East Bay in Oakland (California) to determine what conditions exist at the time of an earthy or musty taste and odor episode. Strong musty and earthy aromas coincided most frequently with fall *Anabaena* sp. blooms and summer *Synechococcus* (*Anacystis*) sp. blooms. Occasional *Aphanizomenon* sp. blooms were concurrent with *Anabaena* sp. blooms, so odor problems associated with *Aphanizomenon* sp. could not be distinguished (Seligman *et al.* 1992).

Recently the identification of three new high-molar-mass polysaccharides isolated from food-grade microalgae was described; these are potent activators of human monocytes and/or macrophages: "Immulina" from *Arthrospira platensis* (Rippka *et al.* 1979), "Immunon" from *A. flos-aquae*, and "Immurella" from *Chlorella pyrenoidosa* (Pugh *et al.* 2001). These polysaccharides are structurally complex and have an estimated molar mass of >10 MDa. All three polysaccharides are highly water-soluble and comprise 0.5–2.0 % of microalgal dry mass. Immunostimulatory activity was measured using a transcription factor-based bioassay for nuclear factor κB activation in THP-1 human monocytes and/or macrophages. Using this system the EC_{50} values of these polysaccharides are 20–110 ng/mL. THP-1 activation was confirmed by measuring immune cytokine mRNA induction using reverse transcriptase-polymerase chain reaction. Each polysaccharide substantially increased the mRNA level of interleukin-1β and tumor necrosis α-factor. These polysaccharides are 100–1000 times more active for *in vitro* monocyte activation than polysaccharide preparations that are currently used clinically for cancer immunotherapy (Pugh *et al.* 2001).

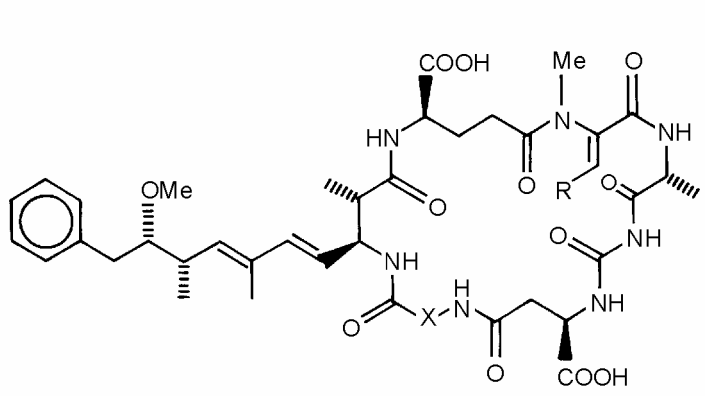
The amino acid sequence of ferredoxin I from *A. flos-aquae* was determined and its sequence has been analyzed (Lee *et al.* 1983). Ferredoxin is composed of 97 amino acid residues with a molar mass of 10 384 Da, excluding two iron and two sulfur atoms in the 2Fe–2S cluster. The numbers of amino acid differences among blue-green algal ferredoxins indicated that *A. flos-aquae* ferredoxin has considerable similarity to other filamentous algal ferredoxins.

An isolate of *A. flos-aquae* was made from a water bloom sample taken at a small pond near Durham (New Hampshire) in 1980. The batch cultured strain was toxic to mice and had an i.p. LD_{50} of ≈5.0 mg/kg (Mahmood and Carmichael 1986). TLC and HPLC indicated that *A. flos-aquae* produced PSP, mainly neosaxitoxin and saxitoxin. Three labile toxins were also detected which were not similar to any of the known PSP.

More than 100 samples of blue-green algae products, consisting of *Aphanizomenon*, *Arthrospira* (Wehr and Sheath 2003), and unidentified blue-green algae, in the form of pills, capsules, and powders were collected from retail outlets from across Canada (Lawrence *et al.* 2001). The samples were extracted with 75 % methanol and centrifuged to remove solids. The results obtained by ELISA and LC-MS–MS agreed very well over a concentration range of ≈0.5–35 μg/g toxins. The colorimetric phosphatase results generally agreed with the previous methods. While the two-biochemical assays measured the total microcystin content compared with a standard of microcystin-LR (**1a**), the LC-MS–MS method measured specific microcystins (-LR, -RR, -LA, -YR; **1a**–**d**) using external standards of these for identification and quantification. Microcystins **1a** and **1c** were found in all samples by LC-MS–MS. Otherwise, the LC-MS–MS results were significantly lower than the results of the biochemical assays and other unknown microcystins that have would been present.

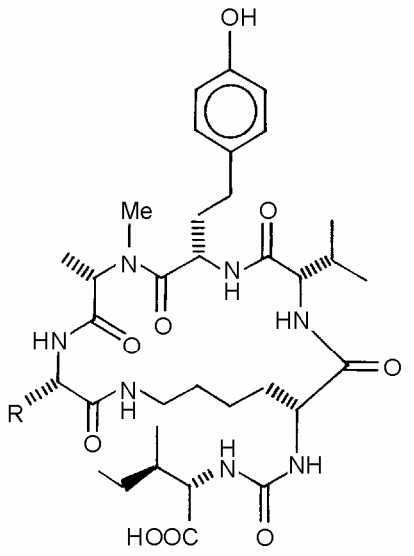
A hepatotoxic cyanobacterial water bloom was collected from a dam in Finland. The water bloom contained two cyanobacterial species, *A. flos-aquae* and *Microcystis aeruginosa* (Namikoshi *et al.* 1992). Two hepatotoxins, 1 and 2, were isolated from extracts of lyophilized cells. The structures of 1 and 2 were assigned based upon their amino acid analyses on an HPLC system, a chiral GC capillary column, FABMS, high resolution FABMS, and tandem FABMS. Toxin 1 was identical with a previously reported compound, [D-Asp3]microcystin-RR (**8e**). Toxin 2 was new and was assigned as [D-Asp3]microcystin-YR (**8f**).

Cyanobacterial bloom samples from the Gulf of Finland (northern Baltic Sea) were collected in July 2003 and analyzed for microcystins and nodularins, *i.e.* cyanobacterial peptide hepatotoxins, by ELISA, HPLC–UV, and LC–MS (Karlsson *et al.* 2004). The blooms consisted mainly of the genera *Nodularia*, *Anabaena*, and *Aphanizomenon*. The main hepatotoxin in the samples was nodularin (**13b**), all the samples also contained demethylnodularin (**13a**). The presence of microcystin-LR (**1a**) was confirmed in three locations out of nine by multiple reactants monitoring on the triple quadrupole mass spectrometer. This is the first reported finding of microcystins in the Baltic Sea from the open sea area.



13a–c

	X	R
13a	Arg	H
13b	Arg	Me
13c	Har	Me



14a,b

	R
14a	iPr
14b	Phe

Anabaenopeptins I (**14a**) and J (**14b**), two new ureido bond-containing cyclic peptides, were isolated from the cultivated *A. flos-aquae* as potent carboxypeptidase-A inhibitors with IC_{50} of 5.2 and 7.6 ng/mL, respectively (Murakami *et al.* 2000).

4 GENUS *Aulosira* KIRCHN. ex BORN. & FLAH. 1886

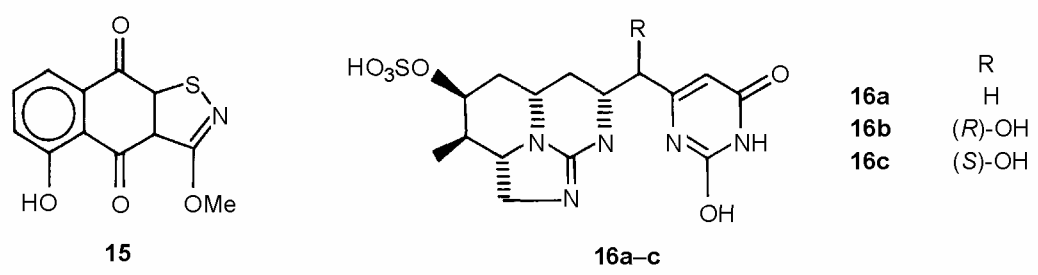
A few papers on metabolites from the genus *Aulosira* have been published. *A. fertilissima* isolated from a soil sample near Moon Beach (Okinawa) in 1986 and its culture has been studied by the Corbett assay (Corbett *et al.* 1992). Using a bioassay-guided fractionation procedure, a novel yellow pigment, aulosirazole (**15**), was isolated which accounted for the solid-tumor-selective activity. The lipophilic extract of *A. fertilissima* was fractionated by size-exclusion chromatography and its activity was monitored by human nasopharyngeal carcinoma cytotoxicity. Aulosirazole, the major cytotoxin in this cyanobacterium, shows solid-tumor-selective activity in the Corbett assay (Stratmann *et al.* 1994) with IC_{50} values against human nasopharyngeal carcinoma and human colorectal adenocarcinoma cell line at 350 and 45 ng/mL, respectively.

5 GENUS *Cylindrospermopsis* SEENAYYA & SUBBA RAJU 1972

This genus is an ancient group of the cyanobacteria which tolerates a wide range of environmental conditions and has even been found growing in hot springs, Antarctic lakes under permanent ice cover, and in extremely salty pools (Chonudomkul *et al.* 2004). Some species form dormant cells that can withstand dry or harsh conditions for extended periods of time. A number of cyanobacterial species release toxins that can cause death in mammals, birds, and fish and illness in humans (Newcombe and Nicholson 2004).

The species of *Cylindrospermopsis* grow abundantly as blooms in subtropical freshwater lakes and rivers with high levels of phosphorus and other nutrients. In recent years, this species has begun replacing

other bloom-forming algae in lakes, pounds, reservoirs, and rivers around the world and appears to be moving into more temperate climates. States where *Cylindrospermopsis* has been found, but not in bloom condition, include Michigan, Illinois, and Ohio (Duy *et al.* 2000; Svrcek and Smith 2004; Wood and Stirling 2003).



Cylindrospermopsin (**16b**) is a potent cyanobacterial hepatotoxin produced by *C. raciborskii* (Fig. 2) (Ohtani *et al.* 1992) and other cyanobacteria such as *Umezakia natans* (Harada *et al.* 1994), *Aphanizomenon ovalisporum* (Banker *et al.* 1997) and *Raphidiopsis curvata* (Li *et al.* 2001). The absolute configuration of (–)-7-epicylindrospermopsin (**16c**), a toxic metabolite of the freshwater cyanobacterium *Aphanizomenon ovalisporum*, was determined by total synthesis (White and Hansen 2005).

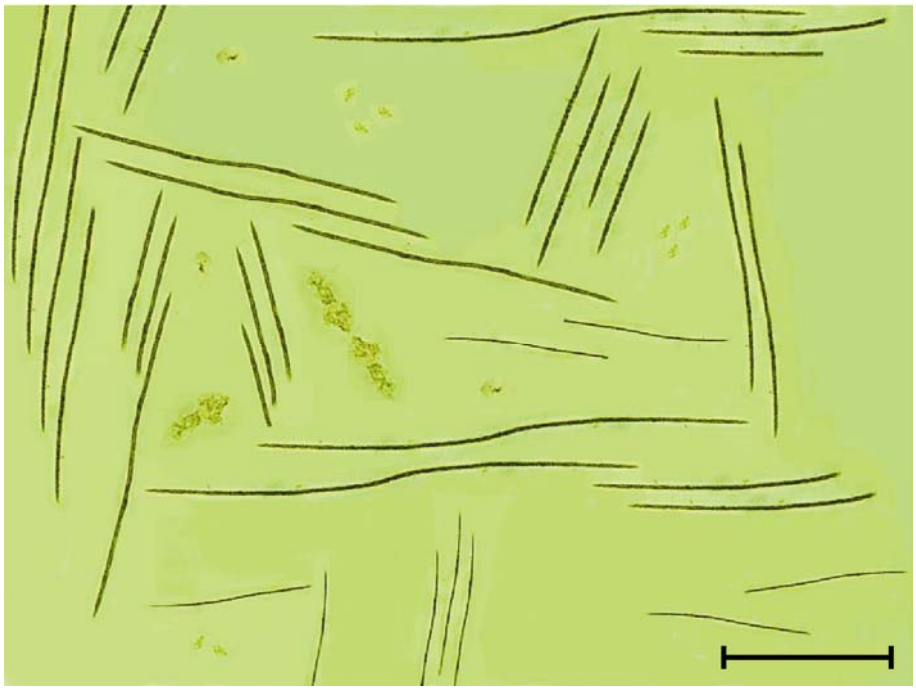


Fig. 2. *Cylindrospermopsis raciborskii*; toxic strain producing hepatotoxin cylindrospermopsin; *bar* = 40 μm.

In tropical and subtropical waters of Australia, toxin **18** with a completely different mechanism of toxicity has caused health problems in drinking water (Hawkins *et al.* 1985, 1997; Harada *et al.* 1994; Banker *et al.* 1997). In a pure form, **18** mainly affects the liver, although crude extracts of *C. raciborskii* injected or given orally to mice also induce pathological symptoms in the kidneys, spleen, thymus and heart. Pure **16b** has an LD_{50} in mice (i.p.) of 2.1 mg/kg at 1 d and 0.2 mg/kg at 5–6 d (Ohtani *et al.* 1992). Recently, new structural variants of **16b** have been isolated from an Australian strain of *C. raciborskii*, with one being identified as deoxycylindrospermopsin (**16a**) (Norris *et al.* 1999).

Cylindrospermopsis, when found in large quantities, can produce several substances that show toxicity in laboratory studies, including cylindrospermopsin (**16b**), saxitoxin, and anatoxin-A. These and other toxins can also be produced by several other species of cyanobacteria. Humans and animals are primarily exposed to toxic effects by drinking or swimming in untreated water (Carmichael 2001; Moore *et al.* 1998).

6 GENUS *Cylindrospermum* KÜTZING ex BORNET et FLAH. 1886

Cylindrospermum cells (Fig. 3) are large, ornamental, ellipsoidal or spherical akinetes that usually develop from the lowermost vegetative cell adjacent to the terminal heterocysts, but are not always present in culture. There may be a single akinete or as many as seven. Many species can be differentiated based on akinete shape.

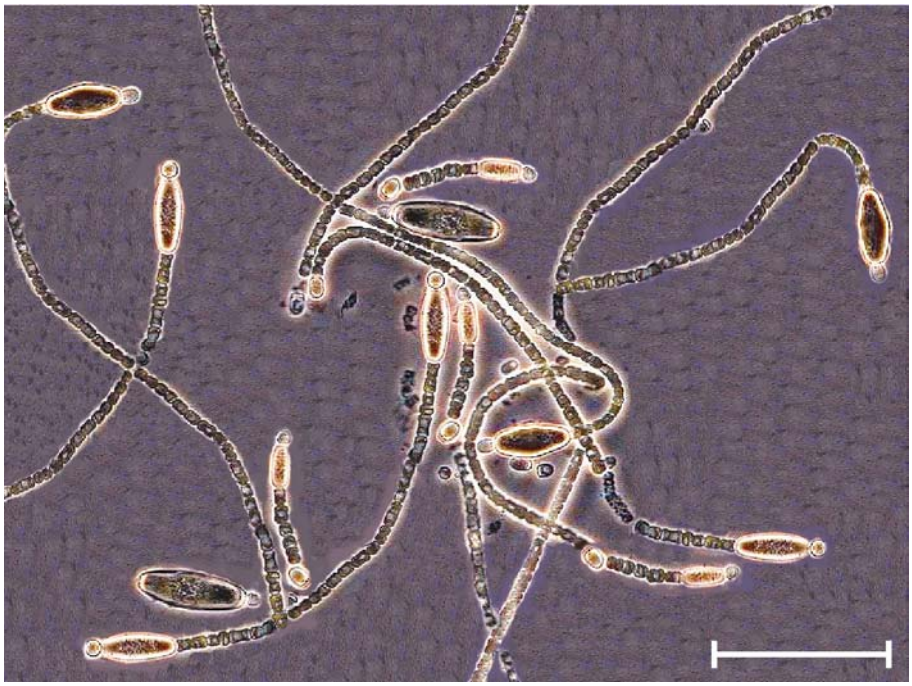


Fig. 3. *Cylindrospermum* sp.; producer of bridged aromatic compounds and toxic macrolides; *bar* = 20 μm.

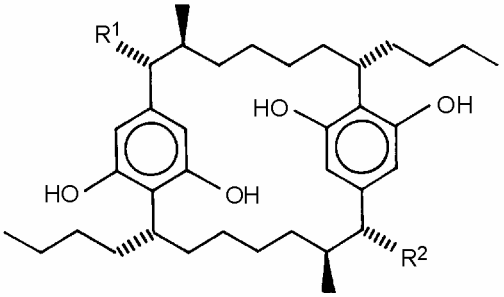
Nontoxic cyanobacterium *C. stagnale* contains palmitic (26.2 %), palmitoleic (14.2), octadecadienoic (15.2), and octadecatrienoic (13.4) acids and also minority FA (Gugger *et al.* 2002).

Five new [7,7]paracyclophanes, cylindrocyclophanes A–F (**17a**–**f**) have been isolated from three strains of the terrestrial blue-green alga *C. licheniforme* and their total structure was elucidated (Moore *et al.* 1992). These 22-member [7,7]paracyclophanes display *in vitro* cytotoxicity against tumor cell lines (IC_{50} 2–10 μg/mL) (Moore *et al.* 1990, 1992). The biosynthesis of nostocyclophane D (**18**) and cylindrocyclophane D (**17f**) has been investigated by the administration of labeled (^{2}H, ^{13}C, ^{18}O) sodium acetate and labeled methionine (^{2}H, ^{13}C) to cultures of the cyanobacteria *Nostoc linckia* and *C. licheniforme*, respectively. The isotopically labeled compounds produced indicate that the structural skeletons of these [7,7]paracyclophanes are oligoketides that have been constructed by dimerization of acetate-derived nonaketides. Acetate has also been identified as the source of the branched methyl groups in **17f** (Bobzin and Moore 1993).

Scytophycin B (**19a**), 6-hydroxyscytophycin B (**19b**), scytophycin E (**20**), and tolytoxin (**19c**) account for the cytotoxicity and fungicidal activity of the terrestrial blue-green alga *C. muscicola* (Jung *et al.* 1991). Toxin **19c**, a macrocyclic lactone, is a potent antifungal antibiotic, exhibiting LD_{50} (i.p.) of 1.5 mg/kg. It also inhibits the growth of a variety of mammalian cells at similar doses, without specific inhibition of macromolecular synthesis. The effects in mammalian cells are primarily cytostatic, with cell death being time- and dose-dependent. No antibacterial, antiviral, or hemolytic activities have been observed (Patterson and Carmeli 1992).

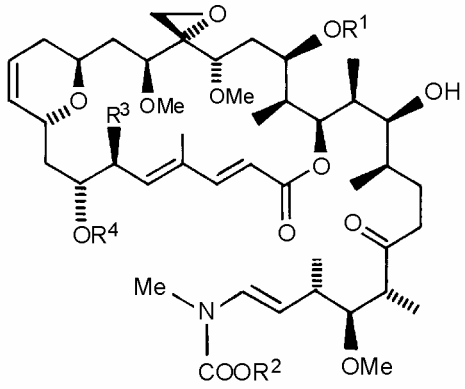
An effective glucosidase inhibitor was isolated from the cyanobacterial genus *Cylindrospermum.* Its chemical structure was determined to be bis(2,5-hydroxymethyl)pyrrolidine-3,4-diol (**21**) (Juttner and Wessel 2003). All five species of *Cylindrospermum* investigated synthesized this compound but accumulated it to a different extent intracellularly. Particularly active producers were the axenic *C. licheniforme* and a monoxenic unknown species of *Cylindrospermum*. The major part of **21** was found to be extracellular for all investigated species. The isolated compound inhibited digestive α- and β-glucosidases isolated from crustacean zooplankton (IC_{50} 19 and 49 nmol/L, respectively). Digestive enzymes of macrozoobenthos (chirono-

mids, trichoptera, and ephemeroptera) were less sensitive to **21**. The insect digestive β-glucosidase was more effectively inhibited than the α-glucosidase. Beside others, the ecological function of the glucosidase inhibitor may be the reduction of the digestibility of the cyanobacterium for grazers (Juttner and Wessel 2003). The alkaloid anatoxin-A has also been isolated from *Cylindrospermum* sp. in Finland by Sivonen *et al.* (1989).



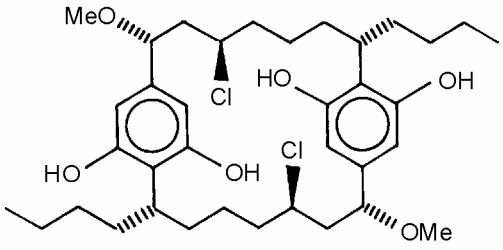
17a–f

	R^1	R^2
17a	H	H
17b	OH	H
17c	OH	OH
17d	OAc	H
17e	OH	OAc
17f	OAc	OAc

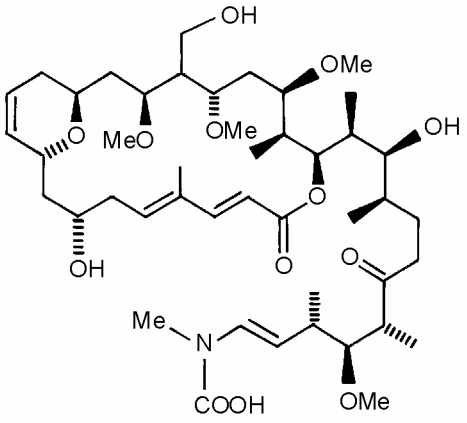


19a–c

	R^1	R^2	R^3	R^4
19a	Me	H	H	H
19b	Me	H	OH	H
19c	H	Me	OH	Me



18



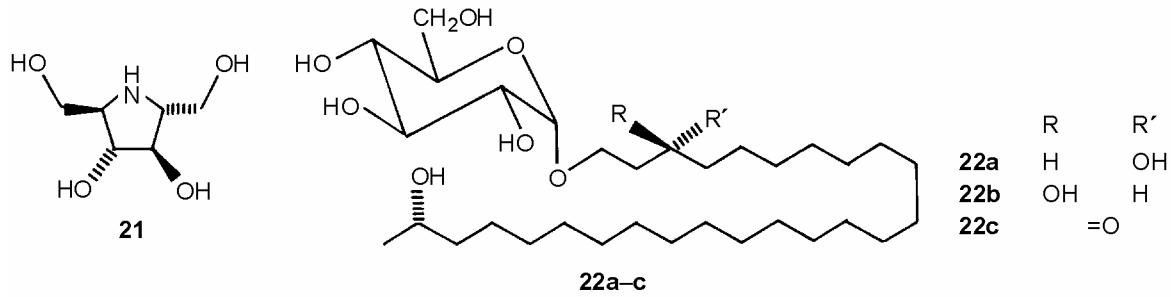
20

7 GENUS *Nodularia* MERT. ex BORN. & FLAH. 1886

Members of this genus are cyanobacteria and have been recorded as the cause of cyanobacterial toxicosis due to pronounced growth "water bloom", particularly when the water is fertile and there is accelerated eutrophication and release of either hepatotoxins or neurotoxins (Fig. 4). Some cyanobacteria may become buoyant in certain conditions and form dense floating mats on the water surface. *N. spumigena* and other members of this genus are known worldwide as planktonic, gas-vesiculate organisms which are often dominant in inland saline lakes and in brackish marine waters.

The occurrence of dioic, hydroxy, branched, and unsaturated FA in cyanobacteria of the genus *Nodularia* as well as in *Apanizomenon* species has been studied (Řezanka *et al.* 2003). Most of the FA were saturated (C_4–C_{18}; >49 %, *M*/*M*), but unsaturated FA were also identified (33–37 %, *M*/*M*; Table IV). Saturated branched FA were identified as a minority. The predominant unsaturated member (33–37 % of the total monounsaturated FA) was oleic acid, the other being linoleic acid; hydroxy FA were found as minor components (Řezanka *et al.* 2003).

The "heterocyst glycolipids" have been reported to take part in the protection of the specialized cyanobacterial cells capable of N_2 fixation against the penetration of O_2. Such glycolipids have been isolated in a pure form from *Nodularia harveyana*, and their structures have been established by spectroscopic and chemical means to be 1-(*O*-α-D-glucopyranosyl)-3*R*,25*R*-hexacosanediol (**22a**), 1-(*O*-α-D-glucopyranosyl)-3*S*,25*R*-hexacosanediol (**22b**) and 1-(*O*-α-D-glucopyranosyl)-3-oxo-25*R*-hexacosanol (**22c**) (Soriente *et al.* 1992).



A novel glycosidic compound, suomilide (**23**), was isolated as "heterocyst glycolipid" from the non-toxic *N. spumigena* (Fujii *et al.* 1997*a*).

Fig. 4. *Nodularia chucula*; producer of toxic peptides; *bar* = 20 μm.

N. spumigena strain KAC 66 contained chlorophyll *a* and a few carotenoids, such as echinenone, β-carotene, and canthaxanthin (Henriksen 2005). A 4-oxomyxoxanthophyll-like pigment was found in two strains of the toxic *N. spumigena* isolated from the Baltic Sea. The pigment was also found in samples taken during intense blooms of *N. spumigena* and was found to be correlated with the concentration of the algal toxin nodularin ($r = 0.97$). *N. spumigena* could be detected by the 4-oxomyxoxanthophyll-like pigment at very low abundances by HPLC, *i.e.* <0.4 μg chlorophyll *a* per L (Schluter *et al.* 2004).

Three filamentous and heterocystous cyanobacterial strains, *N. baltica*, *N. harveyana*, and *N. spumigena*, have been tested for the presence and induction of UV-absorbing/screening mycosporine-like amino acids (MAAs) by simulated solar radiation in combination with 395 nm photosynthetically active radiation cut-off filters. Absorption spectroscopic analysis of methanolic extracts revealed a typical MAA peak at 334 nm in all three cyanobacteria. Specific contents of MAAs had a pronounced induction in samples covered with 295 nm cut-off filters after 3 d of irradiation. HPLC studies revealed the presence of two types

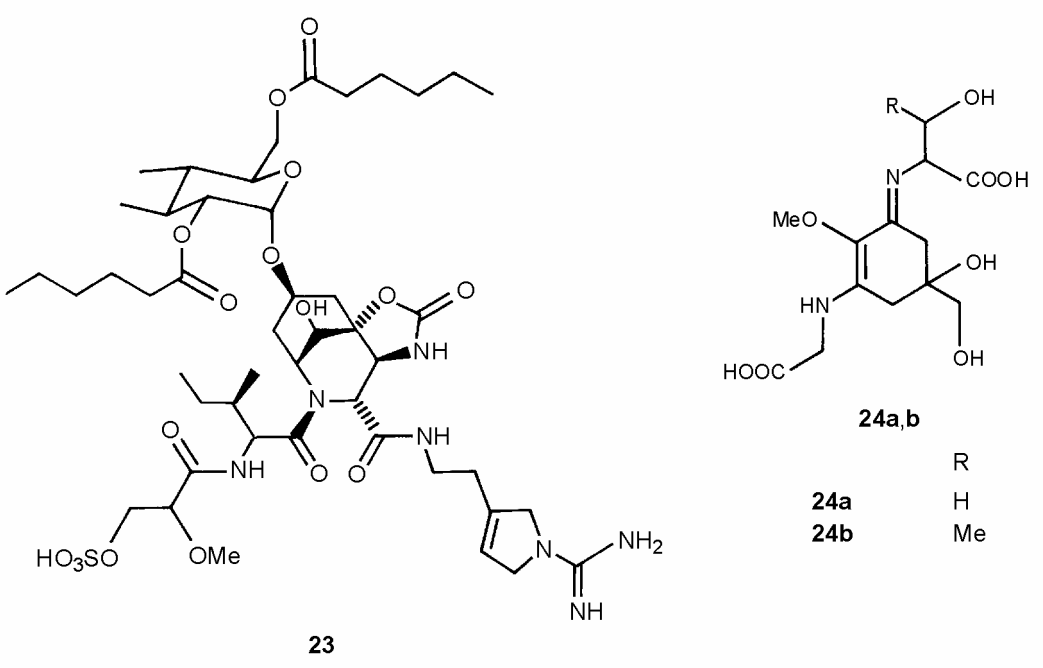
of MAAs in all three cyanobacteria, which were identified as shinorine (**24a**) and porphyra-334 (**24b**), both absorbing maximally at 334 nm. The occurrence of porphyra-334 is rare in cyanobacteria. The results indi

Table IV. Hydroxy, *n*-saturated, branched and unsaturated acids from *Nodularia* strains[a] (%, *M*/*M*)

Fatty acid		*1*	*2*	*3*
Hydroxy acids		**0.71**	**0**	**0**
3-Hydroxybutanoic	3-OH-4:0	0.32	0	0
2-Hydroxy-4-methylpentanoic	2-OH-4-Me-5:0	0.39	0	0
***n*-Saturated**		**49.1**	**44.2**	**31.9**
Butanoic	4:0	0.13	0	0
Octanoic	8:0	0.23	0	0
Decanoic	10:0	0.37	0	0
Dodecanoic	12:0	0.72	0	0
Tridecanoic	13:0	0.36	0	0
Tetradecanoic	14:0	0.89	0.3	15.1
Pentadecanoic	15:0	1.13	0	0
Hexadecanoic	16:0	39.7	42.8	14.2
Heptadecanoic	17:0	0.69	0	0
Octadecanoic	18:0	4.86	1.1	2.6
Branched saturated		**12.8**	**0**	**0**
4-Methylhexanoic	4-Me-6:0	0.25	0	0
8-Methyldecanoic	8-Me-10:0	0.68	0	0
12-Methyltridecanoic	12-Me-13:0	0.32	0	0
12-Methyltetradecanoic	12-Me-14:0	0.89	0	0
14-Methylpentadecanoic	14-Me-15:0	1.36	0	0
14-Methylhexadecanoic	14-Me-16:0	1.34	0	0
15-Methylhexadecanoic	15-Me-16:0	1.21	0	0
10-Methylheptadecanoic	10-Me-17:0	1.11	0	0
2-Hexylcyclopropaneoctanoic	cyc-17:0	2.17	0	0
13-Methyltetradecanoic	13-Me-13:0	1.18	0	0
16-Methylheptadecanoic	16-Me-17:0	0.93	0	0
15-Methylheptadecanoic	15-Me-17:0	0.78	0	0
Unsaturated		**33.0**	**36.9**	**50.2**
(*Z*)-7-Hexadecenoic	7-16:1	0	9.5	6.9
(*Z*)-9-Hexadecenoic	9-16:1	2.58	0	0
(*Z*)-7-Octadecenoic	7-18:1	0	0	2.3
11-Hexadecenoic	11-16:1	0.21	0	0
(*Z*)-9-Octadecenoic	9-18:1	12.49	4.2	3.2
9,12-Hexadecadienoic	9,12-16:2	0.59	0.4	0
9,12-Octadecadienoic	9,12-18:2	13.46	22.8	4.2
10,13-Octadecadienoic	10,13-18:2	1.29	0	0
9,12,15-Octadecatrienoic	9,12,15-18:3	2.38	0	5.4
6,9,12,15-Octadecatetraenoic	6,9,12,15-18:4	0	0	4.3
11,14,17-Icosatrienoic	11,14,17-20:3	0	0	5.7
5,8,11,14,17-Icosapentaenoic	5,8,11,14,17-20:5	0	0	6.2
4,7,10,13,16,19-Docosahexaenoic	4,7,10,13,16,19-22:6	0	0	12.0
Dioic		**4.40**	**0**	**0**
Butane-1,4-dioic		0.77	0	0
2-Hydroxybutane-1,4-dioic		0.65	0	0
Hexane-1,6-dioic		0.48	0	0
Heptane-1,7-dioic		0.41	0	0
Octane-1,8-dioic		0.67	0	0
Nonane-1,9-dioic		1.42	0	0

[a] *1* – *Nodularia sphaerocarpa* (Řezanka *et al.* 2003)
2 – *Nodularia* sp. (Vargas *et al.* 1998)
3 – *N. spumigena* + unidentified phytoplankton (Engstrom-Ost *et al.* 2002)

cate that UV-A is more effective than UV-B in eliciting MAAs induction in the studied cyanobacteria (Sinha *et al.* 2003).



23

The effect of salinity on growth, toxin production, and morphology of *N. spumigena* isolated from the Gulf of Gdansk (southern Baltic Sea) was determined by Mazur-Marzec *et al.* (2005).

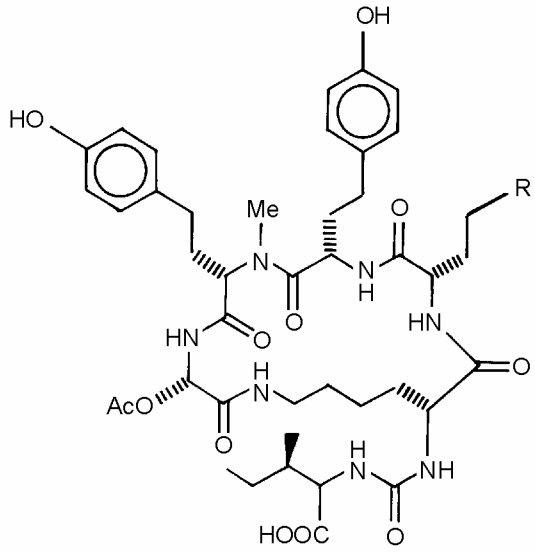
The cyanotoxin **13b** was found in waters where *N. spumigena* was present; the most prominent areas are the Baltic Sea and brackish water estuaries and coastal lakes of Australia and New Zealand. However, the best known *N. spumigena* bloom location, Lake Alexandrina (Australia), has salinity which is only slightly elevated above normal river water and at levels still suitable for drinking water. The presence of **13b** in environmental samples is usually rather insignificant. In the Baltic Sea, collection of samples over several years showed **13b** to be present as the major compound. The same is true for the almost 90 hepatotoxic *Nodularia* strains isolated from the same source (Lehtimaki *et al.* 1997). Analyses of several strains isolated from blooms across Australia have revealed similar results, but nodularin-producing being found rarely, and then only at a low relative abundance (Jones *et al.* 1994; Hobson and Fallowfield 2003).

Nodularia sp., an axenic, non-gas-vacuolated strain from a freshwater source produces several metabolites with cyanobacterial hepatotoxin characteristics. The most abundant is a cyclic pentapeptide, (L-Har$_2$)nodularin (**13c**; *see* p. 170) had similar toxicity by *in vivo* bioassay as **13b**, which was present in lower amounts in the cultures (Beattie *et al.* 2000).

A peptide toxin was isolated from *N. spumigena* by HPLC. The i.p. LD_{50} of the toxin was 50 μg/kg for mice with death within 1–3 h. The major effects of the toxin were seen in the liver in the form of extensive hemorrhages. Amino acid analysis showed the presence of equimolar amounts of glutamic acid, β-methylaspartic acid, and arginine. The toxicological and some of the chemical properties of the isolated toxin were similar to those reported for hepatotoxins isolated from the cyanobacterium *Microcystis aeruginosa* (Eriksson *et a*l. 1988). The structure of a hepatotoxic peptide from *N. spumigena* was determined: The toxin is a cyclic pentapeptide (molar mass 824.5 g/mol) with the following structure: cyclo-(β-methylisoAsp-Arg-Adda-isoGlu-*N*-methyldehydrobutyric acid) (Adda: 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid) (Sandstrom *et al.* 1990).

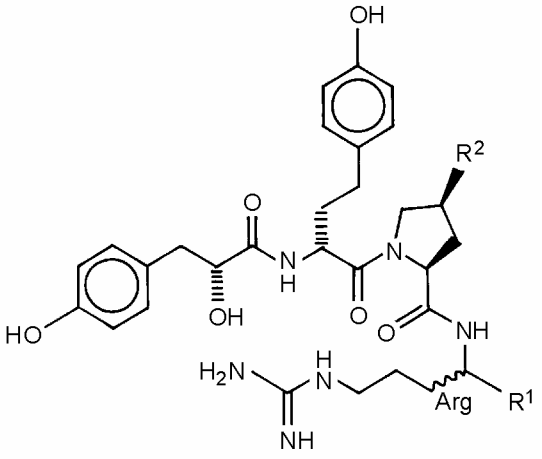
Two types of novel cyclic peptides, nodulapeptins A (**25b**) and B (**25a**) and linear peptides spumigins A–D (**26a–d**), were isolated together with **13b** from toxic *N. spumigena* (Fujii *et al.* 1997*b*).

Linear peptides, as Adda-D-Glu(γ)-Mdhb-D-MeAsp(β)-L-Arg-OH, Adda-D-Glu(γ)-Mdha-D-Ala-L-Leu-D-MeAsp(β)-L-Arg-OH, L-Leu-D-MeAsp(β)-L-Arg-Adda-D-Glu(γ)-Mdha-D-Ala-OH, and L-Phe-D-MeAsp(β)-L-Arg-Adda-D-Glu(γ)-Mdha-D-Ala-OH were isolated from cultured *N. spumigena* and were presented in the cultivated cells after 1–8 weeks. Some of these peptides are thought to be biogenetic precursors of nodularin (**13b**) and microcystins (**1a–d**). Feeding experiments using ^{13}C-labeled precursors established that the 2-, 6-, 8-methyl, and 9-methoxy carbons of the unusual Adda unit were clearly derived from L-methionine (Choi *et al.* 1993).



25a,b

	R
25a	-S(Me)O
25b	-S(Me)O_2



26a–d

	R^1	R^2	Arg
26a	CH_2OH	Me	DL-
26b	COOH	Me	L-
26c	COOH	Me	D-
26d	COOH	H	DL-

8 GENUS *Richelia* JOHS.-SCHMIDT 1901

This prokaryotic nostocalean genus is characterized by short trichomes with terminal heterocysts and by the absence of akinetes. The only described species, *R. intracellularis*, occurs in warm oceans symbiotically in the frustules of diatoms *Rhizosolenia* or *Hemiaulus*, or epiphytically on *Chaetoceros*. The heterocystous endosymbiont *Richelia* has traditionally been considered the dominant marine N_2 fixers and are important in regulating biological productivity of secondary metabolites in marine environments (Montoya *et al.* 2004). Natural compounds have not yet been isolated from endosymbiont *Richelia*, but many biological active metabolites have been identified from the host-diatoms of the genus *Rhizosolenia*, *e.g.* sterols $C_{28}^{\Delta 5,22}$, $C_{28}^{\Delta 5,24(28)}$, $C_{27}^{\Delta 5}$, $C_{29}^{\Delta 5}$, $C_{27}^{\Delta 5,22}$ (Volkman *et al.* 1998), C_{25} and C_{30} highly branched isoprenoid alkenes (Belt *et al.* 2000, 2001; Sinninghe Damste *et al.* 1999*a*,*b*; Volkman *et al.* 1994), oligounsaturated terpenoids (Rowland *et al.* 2001), monocyclic sester- and triterpenoids (Belt *et al.* 2003), and unusual monocyclic alkenes (Masse *et al.* 2004*a*,*b*). It is possible that the endosymbiont *Richelia* supported to synthesis of these compounds.

Cyanobacteria are one of the largest producers of biomass in the marine and also in fresh-water environment. They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against other settling organisms. These active metabolites, also known as biogenic compounds, produced by several species of marine and freshwater cyanobacteria (blue-green algae), have antibacterial, antialgal, and antifungal properties and have likely uses, *e.g.* in therapeutics. The isolated substances with potent pharmacological activity belong to groups of FA, lipopeptides, amides, alkaloids, terpenoids, lactones, nitrogen heterocycles and toxins. This review mainly discusses the successes of such research, and the future applications in the context of understanding the system biology of cyanobacteria.

The authors express their appreciation to Mr. P. Řezanka (student of the *Institute of Chemical Technology* and *Faculty of Science, Charles University*, Prague) for technical assistance. The research was supported by *Institutional Research Concept* AV 0Z 502 0903 of the *Institute of Microbiology, Acad. Sci. Czech Rep*.

REFERENCES

ANAGNOSTIDIS K., KOMÁREK J.: Modern approach to the classification system of cyanophytes. 3 – *Oscillatoriales*. *Arch.Hydrobiol. Suppl.* **80** (*Algol.Stud.* 50–53), 327–472 (1988).

ASCENCIO F., GAMA N.L., DE PHILIPPIS R., HO B.: Effectiveness of *Cyanothece* spp. and *Cyanospira capsulata* exocellular polysaccharides as antiadhesive agents for blocking attachment of *Helicobacter pylori* to human gastric cells. *Folia Microbiol.* **49**, 64–70 (2004).

BALLOT A., KRIENITZ L., KOTUT K., WIEGAND C., METCALF J.S., CODD G.A., PFLUGMACHER S.: Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya – Lakes Bogoria, Nakuru and Elmenteita. *J.Plankton Res*. **26**, 925–935 (2004).

BALLOT A., KRIENITZ L., KOTUT K., WIEGAND C., PFLUGMACHER S.: Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful Algae* **4**, 139–150 (2005).

BANKER R., CARMELI S., HADAS O., TELTSCH B., PORAT R., SUKENIK A.: Identification of cylindrospermopsin in the cyanobacterium *Aphanizomenon ovalisporum* (*Cyanophyceae*) isolated from Lake Kinneret, Israel. *J.Phycol.* **33**, 613–616 (1997).

BATEMAN K.P., THIBAULT P., DOUGLAS D.J., WHITE R.L.: Mass spectral analyses of microcystins from toxic cyanobacteria using online chromatographic and electrophoretic separations. *J.Chromatogr. A* **712**, 253–268 (1995).

BEATTIE K.A., KAYA K., CODD G.A.: The cyanobacterium *Nodularia* PCC 7804, of freshwater origin, produces [L-Har_2]nodularin. *Phytochemistry* **54**, 57–61 (2000).

BELT S.T., ALLARD W.G., MASSE G., ROBERT J.M., ROWLAND S.J.: Highly branched isoprenoids (HBIs): identification of the most common and abundant sedimentary isomers. *Geochim.Cosmochim.Acta* **64**, 3839–3851 (2000).

BELT S.T., ALLARD W.G., MASSE G., ROBERT J.M., ROWLAND S.J.: Structural characterization of C_{30} highly branched isoprenoid alkenes (rhizenes) in the marine diatom *Rhizosolenia setigera*. *Tetrahedron Lett.* **42**, 5583–5585 (2001).

BELT S.T., MASSE G., ALLARD W.G., ROBERT J.M., ROWLAND S.J.: Novel monocyclic sester- and triterpenoids from the marine diatom, *Rhizosolenia setigera*. *Tetrahedron Lett.* **44**, 9103–9106 (2003).

BERLINCK R.G.S., KOSSUGA M.H.: Natural guanidine derivatives. *Natural Prod.Rep.* **22**, 516–550 (2005).

BEZUGLOV V.V., BOBROV M.Y., ARCHAKOV A.V.: Bioactive amides of fatty acids. *Biochemistry (Moscow)* **63**, 27–37 (1998).

BLADON C.M.: *Pharmaceutical Chemistry*. Wiley, UK 2002.

BOBZIN S.C., MOORE R.E.: Biosynthetic origin of [7,7]paracyclophanes from cyanobacteria. *Tetrahedron* **49**, 7615–7626 (1993).

BURJA A.M., BANAIGS B., ABOU-MANSOUR E., BURGESS J.G., WRIGHT P.C.: Marine cyanobacteria – a prolific source of natural products. *Tetrahedron* **57**, 9347–9377 (2001).

CARMICHAEL W.W.: Health effects of toxin-producing cyanobacteria: "The cyanoHABs". *Human Ecol.Risc.Assessment* **7**, 1393–1407 (2001).

CARMICHAEL W.W., DRAPEAU C., ANDERSON D.M.: Harvesting of *Aphanizomenon flos-aquae* RALFS ex BORN. & FLAH. var. *flos-aquae* (cyanobacteria) from Klamath Lake for human dietary use. *J.Appl.Phycol.* **12**, 585–595 (2000).

CASTENHOLZ R.W., WATERBURY J.B.: Oxygenic photosynthetic bacteria. Group I. Cyanobacteria, pp. 1710–1789 in *Bergey's Manual of Systematic Bacteriology, Vol. 3* (J.T. Staley, M.P. Bryant, N. Pfenning, J.G. Holt, Eds). Williams & Wilkins, Baltimore 1989.

CESARO A., LIUT G., BERTOCCHI C., NAVARINI L., URBANI L.: Polysaccharides from cyanobacteria. 5. Physicochemical properties of the exocellular polysaccharide from *Cyanospira capsulata*. *Internat.J.Biol.Macromol.* **12**, 79–84 (1990).

CHEN J., XIE P.: Seasonal dynamics of the hepatotoxic microcystins in various organs of four freshwater bivalves from the large eutrophic lake Taihu of subtropical China and the risk to human consumption. *Environ.Toxicol.* **20**, 572–584 (2005).

CHOI B.W., NAMIKOSHI M., SUN F., RINEHART K.L., CARMICHAEL W.W., KAUP A.M., EVANS W.R., BEASLEY V.R.: Isolation of linear peptides related to the hepatotoxins nodularin and microcystins. *Tetrahedron Lett.* **34**, 7881–7884 (1993).

CHONUDOMKUL D., YONGMANITCHAI W., THEERAGOOL G., KAWACHI M., KASAI F., KAYA K., WATANABE M.M.: Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (*Nostocales*, cyanobacteria) strains from Thailand and Japan. *FEMS Microbiol.Ecol.* **48**, 345–355 (2004).

COOK C.M., VARDAKA E., LANARAS T.: Toxic cyanobacteria in Greek freshwaters, 1987–2000: occurrence, toxicity, and impacts in the Mediterranean region. *Acta Hydrochim.Hydrobiol.* **32**, 107–124 (2004).

CORBETT T.H., VALERIOTE F.A., POLIN L., PANCHAPOR C., PUGH S., WHITE K., LOWICHIK N., KNIGHT J., BISSERY M.C., WOZNIAK A., LORUSSO P., BIERNAT L., POLIN D., KNIGHT L., BIGGAR S., LOONEY D., DEMCHIK L., JONES J., JONES L., BLAIR S., PALMER K., ESSENMACHER S., LISOW L., MATTES K.C., CAVANAUGH P.F., RAKE J.B., BAKER L.: Discovery and development of anticancer agents, pp. 35–87 in *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development* (F.A. Valeriote, T.H. Corbett, L H. Baker, Eds). Kluwer Academic Publishers, Nonvell 1992.

DE LEEUW J.W., RIJPSTRA W.I.C., MUR L.R.: The absence of long-chain alkyl diols and alkyl keto-1-ols in cultures of the cyanobacterium *Aphanizomenon flos-aquae*. *Org.Geochem.* **18**, 575–578 (1992).

DE PHILIPPIS R., SILI C., PAPERI R., VINCENZINI M.. Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *J.Appl.Phycol.* **13**, 293–299 (2001).

DEMBITSKY V.M., ŘEZANKA T.: Metabolites produced by nitrogen-fixing *Nostoc* species. *Folia Microbiol*. **50**, 363–392 (2005).

DEMBITSKY V.M., SHKROB I., LEV O.: Occurrence of volatile nitrogen-containing compounds in nitrogen-fixing cyanobacterium *Aphanizomenon flos-aquae. J.Chem.Ecol*. **26**, 1359–1366 (2000).

DEMBITSKY V.M., SHKROB I., GO J.V.: Dicarboxylic and fatty acid compositions of cyanobacteria of the genus *Aphanizomenon*. *Biochemistry (Moscow)* **66**, 92–97 (2001).

DIETRICH D., HOEGER S.: Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach? *Toxicol.Appl.Pharmacol.* **203**, 273–289 (2005).

DURRER M., ZIMMERMANN U., JUTTNER F.: Dissolved and particle-bound geosmin in a mesotrophic lake, Zürichsee, spatial and seasonal distribution and the effect of grazers. *Water Res.* **33**, 3628–3636 (1999).

DUY T.N., LAM P.K.S., SHAW G.R., CONNELL D.W.: Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Rev.Environ.Contamin.Toxicol.* **163**, 113–186 (2000).

EDLUND U., ALBERTSSON A.C.: Polyesters based on diacid monomers. *Adv.Drug Delivery Rev*. **55**, 585–609 (2003).

ENGSTROM-OST J., KOSKI M., SCHMIDT K., VIITASALO M., JONASDOTTIR S.H., KOKKONEN M., REPKA S., SIVONEN K.: Effects of toxic cyanobacteria on plankton assemblage: community development during decay of *Nodularia spumigena*. *Marine Ecol.Progr. Ser*. **232**, 1–14 (2002).

ERIKSSON J.E., MERILUOTO J.A.O., KUJARI H.P., OSTERLUND K., FAGERLUND K., HALLBOM L.: Preliminary characterization of a toxin isolated from the cyanobacterium *Nodularia spumigena*. *Toxicon* **26**, 161–166 (1988).

FERREIRA F.M.B., SOLER J.M.F., FIDALGO M.L., FERNANDEZ-VILA P.: PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro River, northern Portugal). *Toxicon* **39**, 757–761 (2001).

FIKSDAHL A., FOSS P., LIAAEN-JENSEN S., SIEGELMAN H.W.: Carotenoids of blue-green algae. 11. Carotenoids of chromatically-adapted cyanobacteria. *Comp.Biochem.Physiol. B* **76**, 599–601 (1983).

FLAIBANI A., OLSEN Y., PAINTER T.J.: Polysaccharides in desert reclamation: composition of extracellular proteoglycan complexes produced by filamentous blue-green and unicellular green soil algae. *Carbohydr.Res.* **190**, 235–248 (1989).

FLORENZANO G., SILI C., PELOSI E., VINCENZINI M.: *Cyanospira rippkae* and *Cyanospira capsulata* (gen.nov. and spp.nov.) – new filamentous heterocystous cyanobacteria from Magadi Lake (Kenya). *Arch.Microbiol.* **140**, 301–306 (1985).

FUJII K., SIVONEN K., ADACHI K., NOGUCHI K., SHIMIZU Y., SANO H., HIRAYAMA K., SUZUKI M., HARADA K.I.: Comparative study of toxic and non-toxic cyanobacterial products: a novel glycoside, suomilide, from non-toxic *Nodularia spumigena* HKVV. *Tetrahedron Lett.* **38**, 5529–5532 (1997*a*).

FUJII K., SIVONEN K., ADACHI K., NOGUCHI K., SANO H., HIRAYAMA K., SUZUKI M., HARADA K.I.: Comparative study of toxic and non-toxic cyanobacterial products: novel peptides from toxic *Nodularia spumigena* AV1. *Tetrahedron Lett.* **38**, 5525–5528 (1997*b*).

GAROZZO D., IMPALLOMENI G., SPINA E., STURIALE L., CESARO A., CESCUTTI P.: Identification of *N*-acetylglucosamine and 4-*O*-1-carboxyethyl]mannose in the exopolysaccharide from *Cyanospira capsulata*. *Carbohydr.Res*. **270**, 97–106 (1995).

GAROZZO D., IMPALLOMENI G., SPINA E., STURIALE L.: The structure of the exocellular polysaccharide from the cyanobacterium *Cyanospira capsulata*. *Carbohydr.Res.* **307**, 113–124 (1998).

GUGGER M., LYRA C., SUOMINEN I., TSITKO I., HUMBERT J.F., SALKINOJA-SALONEN M.S., SIVONEN K.: Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). *Internat.J.Syst.Evol.Microbiol.* **52**, 1007–1015 (2002).

GULAVITA N., HORI A., SHIMIZU Y., LASZLO P., CLARDY J.: Aphanorphine, a novel tricyclic alkaloid from the blue-green alga *Aphanizomenon flos-aquae*. *Tetrahedron Lett.* **29**, 4381–4384 (1988).

HALL I.H., IZYDORE R.A., WARREN A.E., BARNES C.R.: Cytotoxicity and mode of action of aliphatic dicarboxylic acids in L1210 lymphocytic leukemia cells. *Anticancer Res.* **19**, 205–211 (1999).

HARADA K.I., MATSUURA K., SUZUKI M., WATANABE M.F., OISHI S., DAHLEM A.M., BEASLEY V.R., CARMICHAEL W.W.: Isolation and characterization of the minor components associated with microcystins-LR and -RR in the cyanobacterium (blue-green algae). *Toxicon* **28**, 55–64 (1990).

HARADA K.I., OGAWA K., KIMURA Y., MURATA H., SUZUKI M., THORN P.M., EVANS W.R., CARMICHAEL W.W.: Microcystins from *Anabaena flos-aquae* NRC 525-17. *Chem.Res.Toxicol.* **4**, 535–540 (1991).

HARADA K.I., OHTANI I., IWAMOTO K., SUZUKI M., WATANABE M.F., WATANABE M., TERAO K.: Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. *Toxicon* **32**, 73–84 (1994).

HAWKINS P.R., RUNNEGAR M.T.C., JACKSON A.R.B., FALCONER I.R.: Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (WOLOSZYNSKA) seenaya and subba raju isolated from a domestic water-supply reservoir. *Appl.Environ.Microbiol*. **50**, 1292–1295 (1985).

HAWKINS P.R., CHANDRASENA N.R., JONES G.J., HUMPAGE A.R., FALCONER I.R.: Isolation and toxicity of *Cylindrospermopsis raciborskii* from an Ornamental Lake. *Toxicon* **35**, 341–346 (1997).

HENRIKSEN P.: Estimating nodularin content of cyanobacterial blooms from abundance of *Nodularia spumigena* and its characteristic pigments – a case study from the Baltic entrance area. *Harmful Algae* **4**, 167–178 (2005).

HERTZBERG S., LIAAEN-JENSEN S.: The carotenoids of blue-green algae. II. The carotenoids of *Aphanizomenon flos-aquae*. *Phytochemistry* **5**, 565–570 (1966).

HERTZBERG S., LIAAEN-JENSEN S.: The carotenoids of blue-green algae. III. A comparative study of mutatochrome and flavacin. *Phytochemistry* **6**, 1119–1126 (1967).

HOBSON P., FALLOWFIELD H.J.: Effect of irradiance, temperature, and salinity on growth and toxin production by *Nodularia spumigena*. *Hydrobiologia* **493**, 7–15 (2003).

HROUZEK P., LUKEŠOVÁ A., ŠIMEK M.: Comparison of light and dark nitrogenase activity in selected soil cyanobacteria. *Folia Microbiol.* **49**, 435–440 (2004).

IKAWA M., SASNER J.J., HANEY J.F., FOXALL T.L.: Pterins of the cyanobacterium *Aphanizomenon flos-aquae*. *Phytochemistry* **38**, 1229–1232 (1995).

ITEMAN I., RIPPKA R., TANDEAU DE MARSAC N., HERDMAN M.: rDNA analyses of planktonic heterocystous cyanobacteria, including members of the genera *Anabaenopsis* and *Cyanospira*. *Microbiology* **148**, 481–496 (2002).

JONES G.J., BLACKBURN S.I., PARKER N.S.: A toxic bloom of *Nodularia spumigena* in Orielton Lagoon, Tasmania. *Austral.J.Mar. Freshwater Res.* **45**, 787–800 (1994).

JUNG J.H., MOORE R.E., PATTERSON G.M.L.: Scytophycins from a blue-green alga belonging to the *Nostocaceae*. *Phytochemistry* **30**, 3615–3616 (1991).

JURCZAK T., TARCZYNSKA M., KARLSSON K., MERILUOTO J.: Characterization and diversity of cyanobacterial hepatotoxins (microcystins) in blooms from polish freshwaters identified by liquid chromatography–electrospray ionisation mass spectrometry. *Chromatographia* **59**, 571–578 (2004).

JUTTNER F., WESSEL H.P.: Isolation of di(hydroxymethyl)dihydroxypyrrolidine from the cyanobacterial genus *Cylindrospermum* that effectively inhibits digestive glucosidases of aquatic insects and crustacean grazers. *J.Phycol.* **39**, 26–32 (2003).

KAEBERNICK M., NEILAN B.A.: Ecological and molecular investigations of cyanotoxin production. *FEMS Microbiol.Ecol.* **35**, 1–9 (2001).

KARLSSON K.M., KANKAANPAA H., HUTTUNEN M., MERILUOTO J.: First observation of microcystin-LR in pelagic cyanobacterial blooms in the northern Baltic Sea. *Harmful Algae*, available on-line 31 March 2004.

KOMÁREK J., HAUER T.: CyanoDB.cz – on-line database of cyanobacterial genera; **`http://www.cyanodb.cz`** (2004).

KRISHNAMYRTHY T., SZAFRANIEC L., HUNT D.F., SHABANOWITZ J., YATES J.R., HAUER C.R., CARMICHAEL W.W., SKULBERG O., CODD G.A., MISSLER S.: Structural characterization of toxic cyclic peptides from blue-green algae by tandem mass spectrometry. *Proc.Nat.Acad.Sci.USA* **86**, 770–774 (1989).

LANARAS T., COOK C.M.: Toxin extraction from an *Anabaenopsis milleri* – dominated bloom. *Sci.Total Environ.* **142**, 163–169 (1994).

LAWRENCE J.F., NIEDZWIADEK B., MENARD C., LAU B.P.Y., LEWIS D., KUPER-GOODMAN T., CARBONE S., HOLMES C.: Comparison of liquid chromatography/mass spectrometry, ELISA, and phosphatase assay for the determination of microcystins in blue-green algae products. *JAOAC Internat.* **84**, 1035–1044 (2001).

LEE I.S., HASE T., MATSUBARA H., HO K.K., KROGMANN D.W.: Amino acid sequence of ferredoxin I from *Aphanizomenon flos-aquae*. *Biochim.Biophys.Acta* **744**, 53–56 (1983).

LEHTIMAKI J., MOISANDER P., SIVONEN K., KONONEN K.: Growth, nitrogen fixation, and nodularin production by two Baltic Sea cyanobacteria. *Appl.Eenviron.Microbiol.* **63**, 1647–1656 (1997).

LI R., CARMICHAEL W.W., BRITTAIN S., EAGLESHAM G.K., SHAW G.R., LIU Y., WATANABE M.M.: First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria). *J.Phycol.* **37**, 1121–1126 (2001).

LIU X.J., CHEN F.: Cell differentiation and colony alteration of *Nostoc flagelliforme*, an edible terrestrial cyanobacterium, in different liquid suspension cultures. *Folia Microbiol.* **48**, 619–626 (2003).

MAHMOOD N.A., CARMICHAEL W.W.: Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. *Toxicon* **24**, 175–186 (1986).

MARRA M., PALMERI A., BALLIO A., SEGRE A., SLODKY M.E.: Structural characterization of the exocellular polysaccharide from *Cyanospira capsulata*. *Carbohydr.Res.* **197**, 338–344 (1990).

MASSE G., BELT S.T., ALLARD W.G., LEWIS C.A., WAKEHAM S.G., ROWLAND S.J.: Occurrence of novel monocyclic alkenes from diatoms in marine particulate matter and sediments. *Org.Geochem.* **35**, 813–822 (2004*a*).

MASSE G., BELT S.T., ROWLAND S.J.: Biosynthesis of unusual monocyclic alkenes by the diatom *Rhizosolenia setigera* (BRIGHTWELL). *Phytochemistry* **65**, 1101–1106 (2004*b*).

MAZUR-MARZEC H., ZEGLINSKA L., PLINSKI M.: The effect of salinity on the growth, toxin production, and morphology of *Nodularia spumigena* isolated from the Gulf of Gdansk, southern Baltic Sea. *J.Appl.Phycol.* **17**, 171–179 (2005).

METCALF J.S., BARAKATE A., CODD G.A.: Inhibition of plant protein synthesis by the cyanobacterial hepatotoxin, cylindrospermopsin. *FEMS Microbiol.Lett.* **235**, 125–129 (2004).

MONTOYA J.P., HOLL C.M., ZEHR J.P., HANSEN A., VILLAREAL T.A., CAPONE D.G.: High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* **430**, 1027–1031 (2004).

MOORE B.S., CHEN J.L., PATTERSON G.M.L., MOORE R.E.: Structures of cylindrocyclophanes A–F. *Tetrahedron* **48**, 3001–3006 (1992).

MOORE B.S., CHEN J.L., PATTERSON G.M.L., MOORE R.E., BRINEN L.S., KATO Y., CLARDY J.: [7,7]Paracyclophanes from blue-green algae. *J.Amer.Chem.Soc.* **112**, 4061–4063 (1990).

MOORE M.R., SHAW G.R., SEAWRIGHT A.A., NEVILLE G.R.: A human health risk assessment of exposure to the cyanobacterial toxin, cylindrospermopsin in drinking water. *Toxicol.Lett.* **95**, 91 (1998).

MORRIS R.J., BRASSELL S.C.: Long-chain alkanediols – biological markers for cyanobacterial contributions to sediments. *Lipids* **23**, 256–258 (1988).

MURAKAMI M., SUZUKI S., ITOU Y., KODANI S., ISHIDA K.: New anabaenopeptins, potent carboxypeptidase-A inhibitors from the cyanobacterium *Aphanizomenon flos-aquae*. *J.Nat.Prod.* **63**, 1280–1282 (2000).

NADEAU T.L., MILBRANDT E.C., CASTENHOLZ R.W.: Evolutionary relationships of cultivated antarctic oscillatorians (cyanobacteria). *J.Phycol.* **37**, 650–654 (2001).

NAMIKOSHI M., SIVONEN K., EVANS W.R., SUN F., CARMICHAEL W.W., RINEHART K.L.: Isolation and structures of microcystins from a cyanobacterial water bloom (Finland). *Toxicon* **30**, 1473–1479 (1992).

NEWCOMBE G., NICHOLSON B.: Water treatment options for dissolved cyanotoxins. *J.Water Supp.Res.Technol.* **53**, 227–239 (2004).

NORDBACK J., LUNDBERG E., CHRISTIE W.W.: Separation of lipid classes from marine particulate material by HPLC on a polyvinyl alcohol-bonded stationary phase using dual-channel evaporative light-scattering detection. *Mar.Chem.* **60**, 165–175 (1998).

NORRIS R.L., EAGLESHAM G.K., PIERENS G., SHAW G.R., SMITH M.J., CHISWELL R.K., SEAWRIGHT A.A., MOORE M.R.: Deoxy-cylindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. *Environ.Toxicol.* **14**, 163–165 (1999).

OHTANI I., MOORE R.E., RUNNEGAR M.T.: Cylindrospermopsin, a potent hepatotoxin from the blue-green alga *Cylindrospermopsis raciborskii*. *J.Amer.Chem.Soc.* **114**, 7941–7942 (1992).

PAPAGEORGIOU J., NICHOLSON B.C., LINKE T.A., KAPRALOS C.: Analysis of cyanobacterial-derived saxitoxins using high-performance ion exchange chromatography with chemical oxidation/fluorescence detection. *Environ.Toxicol.* **20**, 549–559 (2005).

PATTERSON G.M., CARMELI S.: Biological effects of tolytoxin (6-hydroxy-7-*O*-methylscytophycin *b*), a potent bioactive metabolite from cyanobacteria. *Arch.Microbiol.* **157**, 406–410 (1992).

PEREIRA P., ONODERA H., ANDRINOLO D., FRANCA S., ARAJO F., LAGOS N., OSHIMA Y.: Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal. *Toxicon* **38**, 1689–1702 (2000).

PEREIRA P., LI R.H., CARMICHAEL W.W., DIAS E., FRANCA S.: Taxonomy and production of paralytic shellfish toxins by the freshwater cyanobacterium *Aphanizomenon gracile* LMECYA40. *Eur.J.Phycol.* **39**, 361–368 (2004).

PIETRA F.: *A Secret World: Natural Products of Marine Life*, 1st ed. Birkhäuser, Basel 1990.

PRASANNA R., PABBY A., SINGH P.K.: Effect of glucose and light–dark environment on pigmentation profiles in the cyanobacterium *Calothrix elenkenii. Folia Microbiol.* **49**, 26–30 (2004).

PUGH N., ROSS S.A., ELSOHLY H.N., ELSOHLY M.A., PASCO D.S.: Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. *Planta Medica* **67**, 737–742 (2001).

RAJANIEMI P., HROUZEK P., KASTOVSKA K., WILLAME R., RANTALA A., HOFFMANN L., KOMÁREK J., SIVONEN K.: Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (*Nostocales*, cyanobacteria). *Internat.J.Syst.Evol.Microbiol.* **55**, 11–26 (2005).

RAPALA J., ROBERTSON A., NEGRI A.P., BERG K.A., TUOMI P., LYRA C., ERKOMAA K., LAHTI K., HOPPU K., LEPISTO L.: First report of saxitoxin in Finnish lakes and possible associated effects on human health. *Environ.Toxicol.* **20**, 331–340 (2005).

REPKA S., MEYERHOFER M., VON BROCKEL K., SIVONEN K.: Associations of cyanobacterial toxin, nodularin, with environmental factors and zooplankton in the Baltic Sea. *Microb.Ecol.* **47**, 350–358 (2004).

ŘEZANKA T., DOR I., PRELL A., DEMBITSKY V.M.: Fatty acid composition of six freshwater wild cyanobacterial species. *Folia Microbiol*. **48**, 71–75 (2003).

RIPPKA R., DERUELLES J., WATERBURY J.B., HERDMAN M., STANIER R.Y.: Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J.Gen.Microbiol*. **111**, 1–61 (1979).

RINEHART K.L., HARADA K.I., NAMIKOSHI M., CHEN C., HARVIS C.A., MUNRO M.H.G., BLUNT J.W., MULLIGAN P.E., BEASLEY V.R., DAHLEM A.M., CARMICHAEL W.W.: Nodularin, microcystin, and the configuration of Adda. *J.Amer.Chem.Soc.* **110**, 8557–8558 (1988).

RINEHART K.L., NAMIKOSHI M., CHOI B.W.: Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *J.Appl.Phycol.* **6**, 159–176 (1994).

ROWLAND S.J., ALLARD W.G., BELT S.T., MASSE G., ROBERT J.-M., BLACKBURN S., FRAMPTON D., REVILL A.T., VOLKMAN J.K.: Factors influencing the distributions of polyunsaturated terpenoids in the diatom, *Rhizosolenia setigera*. *Phytochemistry* **58**, 717–728 (2001).

SANDSTROM A., GLEMAREC C., MERILUOTO J.A.O., ERIKSSON J.E., CHATTOPADHYAYA J.: Structure of a hepatotoxic pentapeptide from the cyanobacterium *Nodularia spumigena*. *Toxicon* **28**, 535–540 (1990).

SARMA T.A., GURPREET AHUJA, KHATTAR J.I.S.: Nutrient stress causes akinete differentiation in cyanobacterium *Anabaena torulosa* with concomitant increase in nitrogen reserve substances. *Folia Microbiol.* **49**, 557–562 (2004).

SCHLUTER L., GARDE K., KAAS H.: Detection of the toxic cyanobacteria by *Nodularia spumigena* means of a 4-keto-myxoxanthophyll-like pigment in the Baltic Sea. *Mar.Ecol.-Progr.Ser.* **275**, 69–78 (2004).

SEAWRIGHT A., NOLAN C., SHAW G., CHISWELL R., NORRIS R., MOORE M., SMITH M.: *Environ.Toxicol*. **14**, 135–142 (1999).

SEAWRIGHT A., NORRIS R., SHAW G., MOORE M., BURGESS V.: Toxicity of the cyanobacterial toxin, cylindrospermopsin in mammals. *Toxicology* **148**, 75–76 (2000).

SELIGMAN K., ENOS A.K., LAI H.H.: A comparison of 1988–1990 flavor profile analysis results with water conditions in 2 northern California reservoirs. *Water Sci.Technol.* **25**, 19–25 (1992).

SHIMIZU Y.: Microalgal metabolites: a new perspective. *Ann.Rev.Microbiol.* **50**, 431–465 (1996).

SHIMIZU Y.: Microalgal metabolites. *Curr.Opin.Microbiol.* **6**, 236–243 (2003).

SINGH I.: Biochemistry of peroxisomes in health and disease. *Mol.Cell.Biochem*. **167**, 1–29 (1997).

SINGH S., KATE B.N., BANERJEE U.C.: Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit.Rev.Biotechnol.* **25**, 73–95 (2005).

SINHA R.P., AMBASHT N.K., SINHA J.P., KLISCH M., HADER D.P.: UV-B-induced synthesis of mycosporine-like amino acids in three strains of *Nodularia* (cyanobacteria). *J.Photochem.Photobiol.Biol*. **71**, 51–58 (2003).

SINNINGHE DAMSTE J.S., RIJPSTRA W.I.C., SCHOUTEN S., PELETIER H., VAN DER MAAREL M.J.E.C., GIESKES W.W.C.: A C_{25} highly branched isoprenoid alkene and C_{25} and C_{27} *n*-polyenes in the marine diatom *Rhizosolenia setigera*. *Org.Geochem.* **30**, 95–100 (1999*a*).

SINNINGHE DAMSTE J.S., SCHOUTEN S., RIJPSTRA W.I.C., HOPMANS E.C., PELETIER H., GIESKES W.W.C., GEENEVASEN J.A.J.: Structural identification of the C_{25} highly branched isoprenoid pentaene in the marine diatom *Rhizosolenia setigera*. *Org.Geochem.* **30**, 1581–1583 (1999*b*).

SIVONEN K., JONES G.: Cyanobacterial toxins, pp. 41–111 in *Toxic Cyanobacteria in Water* (I. Chorus, J. Bartram, Eds). Word Health Organization Press, Geneva 1996.

SIVONEN K., HIMBERG K., LUUKKAINEN R., NIEMEL S.I., POON G.K., CODD G.A.: Preliminary characterization of neurotoxic blooms and strains from Finland. *Tax.Assess.* **4**, 339–352 (1989).

SIVONEN K., SKULBERG O.M., NAMIKOSHI M., EVANS W.R., CARMICHAEL W.W., RINEHART K.L.: Two methyl ester derivates of microcystins, cyclic heptapeptide hepatotoxins, isolated from *Anabaena flos-aquae* strain CYA 83/1. *Toxicon* **30**, 1465–1471 (1992).

SORIENTE A., SODANO G., CAMBACORTA A., TRINCONE A.: Structure of the "heterocyst glycolipids" of the marine cyanobacterium *Nodularia harveyana*. *Tetrahedron* **48**, 5375–5384 (1992).

SORIENTE A., GAMBACORTA A., TRINCONE A., SILI C., VINCENZINI M., SODANO G.: Heterocyst glycolipids of the cyanobacterium *Cyanospira rippkae*. *Phytochemistry* **33**, 393–396 (1993).

STRATMANN K., BELLI J., JENSEN C.M., MOORE R.E., PATTERSON G.M.L.: A aulosirazole, a novel solid tumor selective cytotoxin from the blue-green alga *Aulosira fertilissima*. *J.Org.Chem.* **59**, 6279–6281 (1994).

SUDA S., WATANABE M.M., OTSUKA S., MAHAKAHANT A., YONGMANITCHAI W., NOPARTNARAPORN N., LIU Y., DAY J.G.: Taxonomic revision of water-bloom-forming species of oscillatorioid cyanobacteria. *Internat.J.Syst.Evol.Microbiol*. **52**, 1577–1595 (2002).

SVRCEK C., SMITH D.W.: Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *J.Environ. Eng.Sci.* **3**, 155–185 (2004).

TATMAN D., MO H.B.: Volatile isoprenoid constituents of fruits, vegetables and herbs cumulatively suppress the proliferation of murine B16 melanoma and human HL-60 leukemia cells. *Canc.Res*. **175**, 129–139 (2002).

TERAO K., OHMORI S., IGARASHI K., OHATANI I., WATANABE M., HARADA K., ITO E., WATANABE M.: Electron-microscopic studies on experimental poisoning in mice induced by cylindrospermopsin isolated from blue-green alga *Umezakia natans*. *Toxicon* **32**, 833–843 (1994).

TISCHER J.: Carotenoids of the freshwater algae. IV. The polyene pigments of the blue-green algae *Aphanizomenon flos-aquae. Z.Physiol.Chem*. **251**, 109–128 (1938).

TISCHER J.: Carotenoids of freshwater algae. *Z.Physiol.Chem*. **252**, 257–269 (1939).

VANDERPLOEG M., TUCKER C.S., BOYD C.E.: Geosmin and 2-methylisoborneol production by cyanobacteria in fish ponds in the southeastern United States. *Water Sci.Technol.* **25**, 283–290 (1992).

VARGAS M.A., RODRIGUEZ H., MORENO J., OLIVARES H., DEL CAMPO J.A., RIVAS J., GUERRERO M.G.: Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *J.Phycol.* **34**, 812–817 (1998).

VASAS G., GASPAR A., PAGER C., SURANYI G., MATHE C., HAMVAS M.M., BORBELY G.: Analysis of cyanobacterial toxins (anatoxin-A, cylindrospermopsin, microcystin-LR) by capillary electrophoresis. *Electrophoresis* **25**, 108–115 (2004).

VINCENZINI M., DEPHILIPPIS R., SILI C., MATERASSI R.: Studies on exopolysaccharide release by diazotrophic batch cultures of *Cyanospira capsulata. Appl.Microbiol.Biotechnol.* **34**, 392–396 (1990).

VISENTIN M., SALMONA M., TACCONI M.T.: Reyes and reye-like syndromes, drug-related diseases (causative agents, etiology, pathogenesis, and therapeutic approaches). *Drug Metab.Rev.* **27**, 517–539 (1995).

VOLKMAN J.K., BARRETT S.M., DUNSTAN G.A.: C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org.Geochem.* **21**, 407–414 (1994).

VOLKMAN J.K., BARRETT S.M., BLACKBURN S.I., MANSOUR M.P., SIKES E.L., GELIN F.: Microalgal biomarkers: a review of recent research developments. *Org.Geochem.* **29**, 1163–1179 (1998).

WARRINGTON D., SHAINBERG I., LEVY G.J., BAR-OR Y.: Polysaccharide and salt effects on infiltration and soil erosion. A rainfall simulation study. *Soil Technol.* **4**, 79–91 (1991).

WEHR J.D., SHEATH R.G.: *Freshwater Algae of North America: Ecology and Classification*. Academic Press, San Diego (USA) 2003.

WHITE J.D., HANSEN J.D.: Total synthesis of (–)-7-epicylindrospermopsin, a toxic metabolite of the freshwater cyanobacterium *Aphanizomenon ovalisporum*, and assignment of its absolute configuration. *J.Org.Chem.* **70**, 1963–1977 (2005).

WOOD S.A., STIRLING D.J.: First identification of the cylindrospermopsin-producing cyanobacterium *Cylindrospermopsis raciborskii* in New Zealand. *New Zealand J.Mar.Freshwater Res.* **37**, 821–828 (2003).