REVIEW

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Metabolites Produced by Nitrogen-Fixing *Nostoc* Species

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ABSTRACT. This paper provides a comprehensive overview of metabolites, including lipids and lipid-like compounds, boron-containing macrocycles, arsenolipids, oligopeptides and amino acid derivatives, produced by cyanobacteria of the genus *Nostoc*.

Abbreviations

AA	amino acid(s)
EPS	extracellular polysaccharide
MGDG	monogalactosyl diacylglycerol
PG	phosphatidylglycerol
SQDG	sulfoquinovosyl diacylglycerol
DGDG	digalactosyl diacylglycerol
FA	fatty acid(s)
PCC	*Pasteur Culture Collection*
ROS	reactive oxygen species

CONTENTS

1 INTRODUCTION

Members of the order *Nostocales* are broadly characterized by unbranched filaments and the production of up to three kinds of differentiated cells. All cyanobacteria are 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 reductant. *Nostoc* species are nitrogen-fixing cyanobacteria (Figs 1–4) belonging to the family *Nostocaceae* in the order *Nostocales* (Castenholz and Waterbury 1989). Heterocysts differentiate in response to the lack of combined nitrogen in the environment and are the sites of nitrogen fixation. *Nostoc* species also produce relatively short, motile filaments called hormogonia and this characteristic, in part, distinguishes them from members of the closely related genus *Anabaena*. In addition, *Nostoc* species differentiate spore-like structures termed akinetes in response to nutrient limitation other than nitrogen (cf. Sarma *et al*. 2004).

Nostoc species are widely distributed in illuminated portions of the biosphere, including fresh waters and tropical, temperate, and polar terrestrial systems; they are rarely found in marine habitats (Potts 2000; Shah *et al*. 2003). Growth in both aquatic and terrestrial habitats is often as a colony of filaments within a gelatinous matrix (Dodds *et al.* 1995). The size of the colonies ranges from microscopic to macroscopic dimensions (Liu and Chen 2003). Many *Nostoc* species occur in symbiotic associations with fungi to form lichens and with representatives of each of the major phylogenetic groups of plants (Meeks 1998; Rai *et al.* 2000). Nitrogen-fixing *Nostoc* species, in both free-living and symbiotic growth states, are major contributors to the sequestration of CO_2 in organic compounds, especially in nutrient poor and extreme environments.

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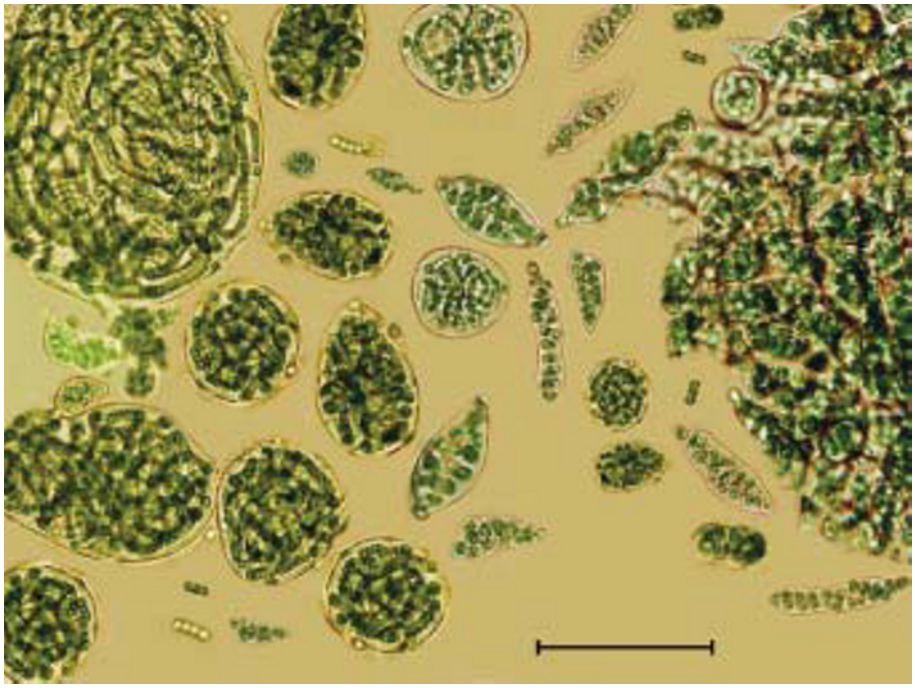


Fig. 1. Germination of *Nostoc* sp. The heterocysts containing threads occur in this species all tangled up in common gelatinous coat. A bigger one and several small (*Nostoc* baby) and medium sizes are seen; *bar* = 10 m.

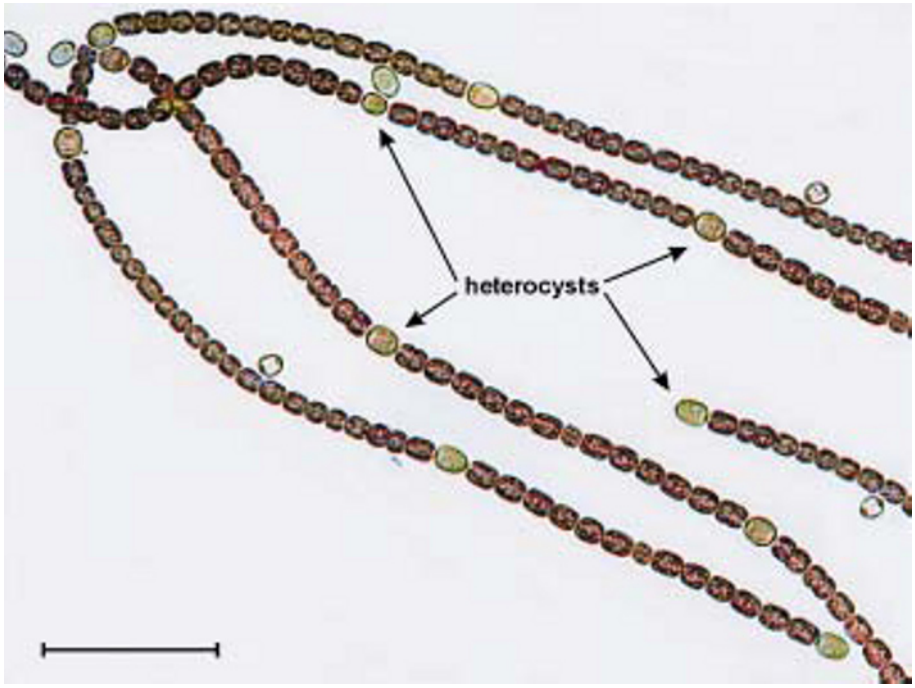


Fig. 2. *Nostoc caquena*; it is able to protect the machinery of nitrogen fixation from inactivation by producing specialized cells, called heterocysts (*arrows*), that rigorously exclude oxygen from within them. The *brown-green* cells are photosynthetic vegetative cells, *pale-green* ones are the heterocysts specialized for nitrogen fixation; *bar* = 20 m.

Nostoc species are terrestrial and benthic cosmopolitan microorganisms, which form extended mucilaginous layers on soil and in the aquatic environment on stones and mud. Many secondary metabolites with new structures have been isolated from these organisms. In nature, these components may be directed

against phototrophic competitors (cyanobacteria and algae) and grazers. The pharmacological value of these substances is that they have been shown to exhibit antiviral and antitumor properties, and *Nostoc*, as cyanobacteria in general, have gained much interest as a natural source of such compounds.

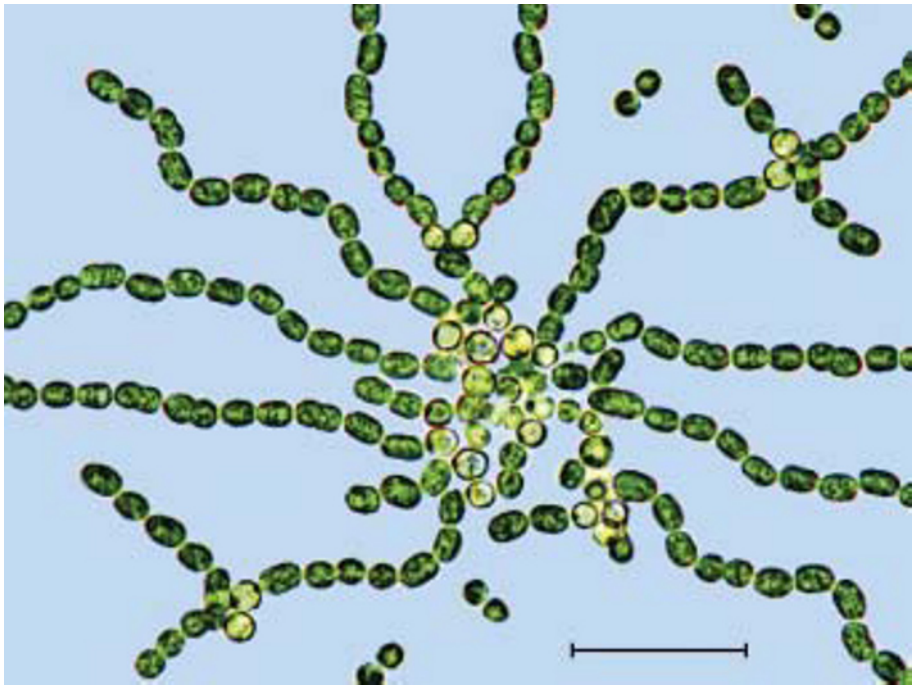


Fig. 3. *Nostoc* sp. PCC 6719 (ATCC 29105). The *green* cells are photosynthetic vegetative cells, *yellow-green* ones are the heterocysts; *bar* = 40 m.

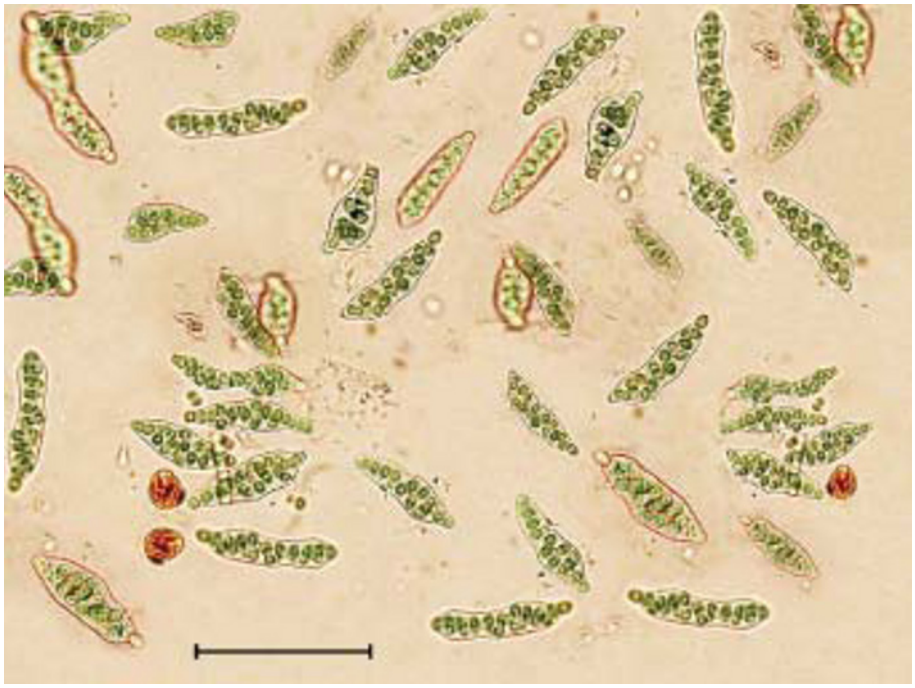


Fig. 4. Germination of *Nostoc* sp. The heterocysts containing threads occur all tangled up in common gelatinous coat; several small and medium sizes of *Nostoc* baby; *bar* = 10 m.

2 HYDROCARBONS

Hydrocarbons were found in different cyanobacterial species and partly reviewed in some papers (Brocks *et al.* 2003, 1999; Dembitsky and Srebnik 2002; Kenig 2000; Summons *et al.* 1999; Murata and Nishida 1987). Most cyanobacterial species contain small amounts of medium-chain hydrocarbons $C_{13–21}$, often with either *n*-heptadecane (*n*-$C_{17:0}$) or $C_{17:1}$ predominating (Gelpi *et al.* 1970; Nevenzel 1989). Nevertheless, some cyanobacterial species produce very long-chain and highly branched hydrocarbons $C_{19–29}$ (Koster *et al.* 1999; Schouten *et al.* 2001; Kenig 2000; 1995; Shiea *et al.* 1990). Han *et al.* (1969) demonstrated that *N. muscorum* culture enzymically decarboxylated stearate to *n*-heptadecane. Han *et al.* (1968) have found that *N. muscorum* contains besides *n*-heptadecane (83 %) also 7- and 8-methylheptadecanes (each about 16 %). Marine *N. endophylum* contains 80 % *n*-$C_{17:0}$ and 20 % branched-methyl-$C_{17:0}$ (position of methyl group not determined) hydrocarbons (Murray *et al.* 1977). Paoletti *et al.* (1975) reported that *N. commune* contains 24.2 % 2-methylheptadecane, and 50.4 % *n*-$C_{17:0}$, 1.5 % $C_{15:0}$, 1.6 % $C_{16:0}$, and 3.2 % $C_{17:1}$.

Cells of *N. muscorum* have been subjected to thermal alteration over varying periods of time and the isoprenoid hydrocarbons produced in these experiments have been examined. The major hydrocarbons were phytane and 5 isomeric phytenes, montmorillonite increasing the amount of phytane. No phytadienes, pristane or pristenes were detected in the products (Philp *et al.* 1978).

Investigation of hydrocarbon production by 12 species of cyanobacteria and algae, morphologically similar to fossil forms, including *Nostoc* sp., along with confirmatory data on *Anacystis nidulans* and *Botryococcus braunii* have been reported (Gelpi *et al.* 1970). The normal hydrocarbon range was $C_{15–19}$ in most species, *n*-C_{17} being predominant in all cultures. However, a few of the species showed a bimodal distribution of aliphatic hydrocarbons with maxima at C_{17} and C_{27}, with significant amounts of C_{23}, C_{27}, and C_{29} straight-chain alkanes and alkenes. Similar bimodal distributions of saturated hydrocarbons have been observed in both Tertiary and Precambrian sediments, supporting the interpretation of biological origin. Squalene was the only isoprenoid reported.

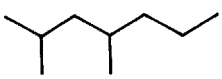
The odor-producing filamentous cyanobacterium in water-works in central Taiwan (Hu and Chiang 1996) was identified as *Nostoc* sp. Geosmin, 1-chlorooctane, 1-chlorodecane, 1-chlorododecane, and methylisoborneol were produced as the population reached its declining stage. Besides geosmin and methylisoborneol, there were other unidentified organic compounds produced by *Nostoc* sp.

The photosynthesizing and nitrogen-fixing cyanobacterial genus *Nostoc* participates in a wide range of symbiotic associations with hosts from different organism groups, including lichen species. About 10 % of lichens have *Nostoc* species as the main or the only one photosynthetic partner and their role is probably to exploit the nitrogen-fixing ability (Tschermak-Woess 1988). The complex hydrocarbons and volatile compounds produced by cultured cyanobacterial photobiont *Nostoc* sp. isolated from lichen *Collema* sp. has been reported (Dembitsky *et al.* 1999). More than 130 volatile compounds including short-chain hydrocarbons ($C_{7–10}$), medium- and long-chain hydrocarbons ($C_{15–30}$), isoprenoid hydrocarbons (**1**–**4d**), and also cyclopentane (**5a**–**h**), cyclohexane (**6a**–**h**, **7a**–**q**), and cyclooctane (**8**) homologues and their isomers were identified by GC–MS (Fig. 5).

3 LIPIDS

The lipids not only form the bilayer structure of biomembranes, but also associate with some membrane proteins to assist the proper activities of the membranes in photosynthesis (Gosbos and Murata 1998). Triacylglycerols are the most common storage lipids and may constitute up to 80 % of the total lipid fraction in cyanobacteria (Murata and Nishida 1987). Besides triacylglycerols, the other major lipids detected in cyanobacteria including *Nostoc* species (Fig. 6), were SQDG, MGDG, DGDG and PG. These 4 major polar lipids can be identified on the basis of their R_F values in TLC or ^{1}H- and ^{13}C-NMR (Döhler and Datz 1980; Sato and Murata 1981; Piorreck and Pohl 1984; Harwood *et al.* 1988). All cyanobacteria as well as *Nostoc* species, do not contain phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and diphosphatidylglycerol. Among the neutral lipids some minor lipids, except for triacylglycerols, were found in *N. commune*, *e.g.* sterol esters, waxes and free sterols (Bychek and Bychek 1997).

Mercer and Davies (1975, 1979) discovered the do-, tri-, and tetracosane series of unusual chlorosulfolipids **9a**–**f**, **10a**–**c**, **11** in *Nostoc* sp., as well as in two members of the *Xanthophyceae*, (*Botrydium granulatum* and *Monodus subterraneus*), and 2 members of the *Chlorophyceae* (*Elakatothrix viridis* and *Zygnema* sp).



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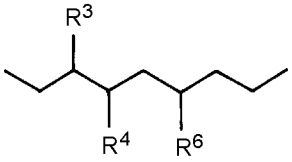
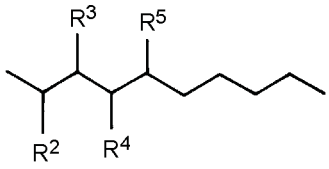


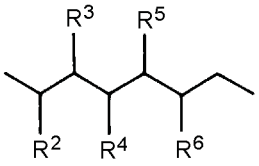
3a,**b**

	R^3	R^4	R^6
3a	Me		Me
3b		Me	Me



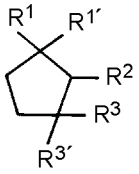
4a–d

	R^2	R^3	R^4	R^5
4a	Me			
4b		Me		
4c			Me	
4d				Me



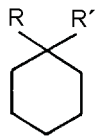
2a–f

	R^2	R^3	R^4	R^5	R^6
2a	Me				
2b		Me			
2c			Me		
2d	Me			Me	
2e	Me				Et
2f			Et		



5a–h

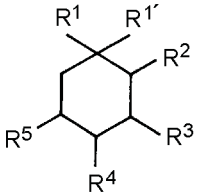
	R^1	$R^{1'}$	R^2	R^3	$R^{3'}$
5a	Me		Me		
5b	Et				
5c	Pe				
5d	OH		Me		
5e	Pr		Me		
5f	Bu		Et		
5g	Bu	Me			
5h	Me	Me		Me	Me



6a–h

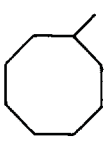
	R	R′
6a	Me	
6b	Et	
6c	Pr	
6d	Bu	
6e	Cro	
6f	2,3-Me_2Pr	
6g	Et	Me
6h	Pr	Me

Cro = crotyl (but-2-en-1-yl)



7a–q

	R^1	$R^{1'}$	R^2	R^3	R^4	R^5	
7a	Me		Me				*trans*
7b	Et		Me				
7c	Pr		Me				
7d	Pr		Et				*cis*
7e	Pr		Et				*trans*
7f	Me			Me			*trans*
7g	Pr			Me			
7h	Bu			Me			
7i	Et				Me		
7j	Et	Me		Me			
7k	Et	Me			Me		
7l	Me		Me	Me			*cis, trans*
7m	Me		Me	Me			*trans, trans*
7n	Me		Me		Me		*trans, cis*
7o	Et		Me		Me		
7p	Me			Me		Me	*cis, cis*
7q	Me			Me		Me	*cis, trans*



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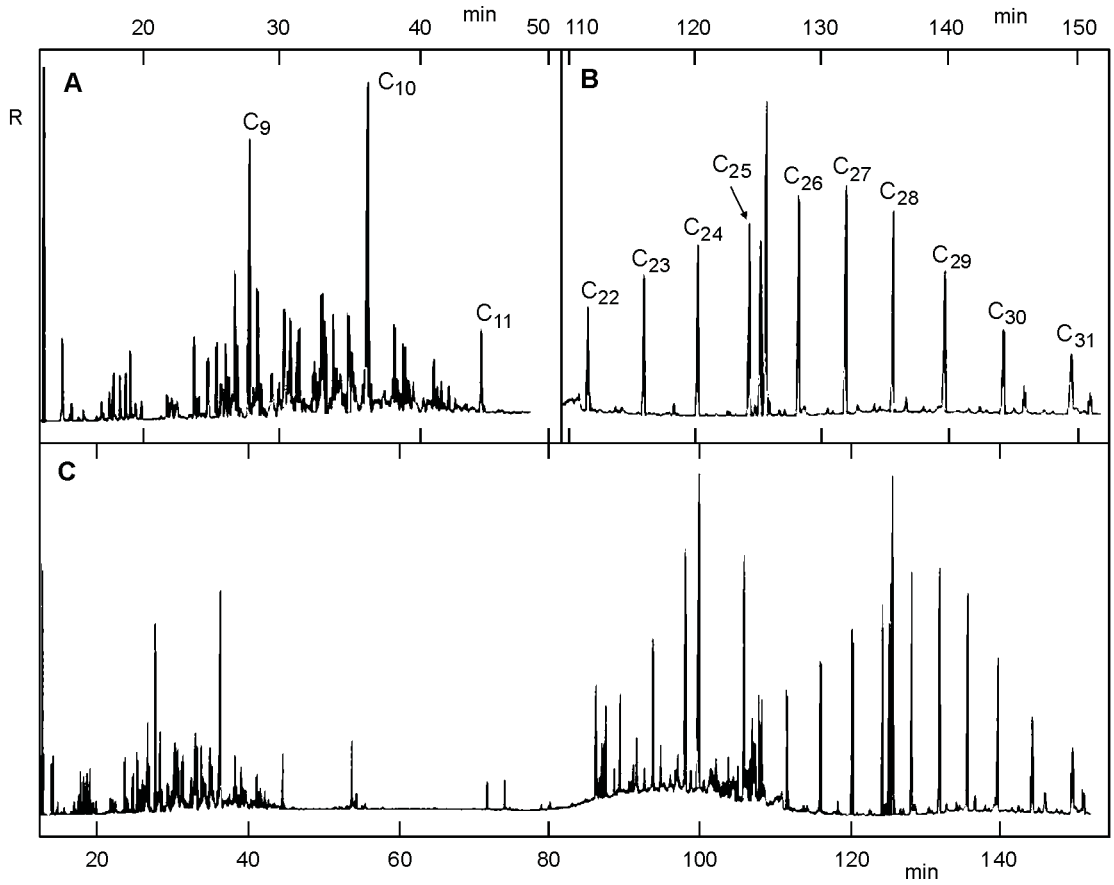


Fig. 5. GC separation (min) of light hydrocarbons, including cyclopentane and cyclohexane isomers (**A**), very long-chain hydrocarbons (**B**), and total separation of hydrocarbons, fatty acids and other metabolites (**C**) produced by *Nostoc* sp. (Dembitsky *et al.* 1999); R – detector response.

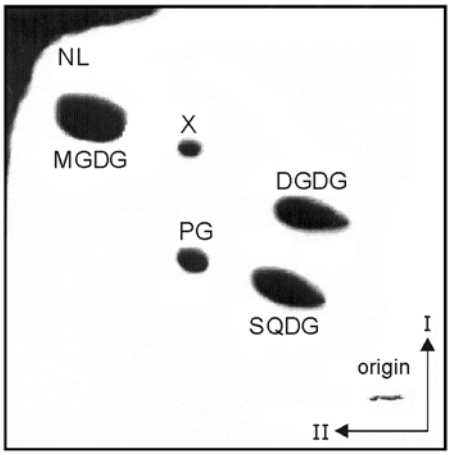
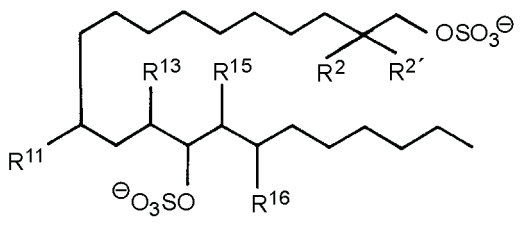


Fig. 6. The 2D-TLC of polar lipids of cultured *Nostoc* sp. Lipids were separated on precoated silica gel plates (*Merck* 5715) with solution mixture: 1st dimension, chloroform–methanol–water (65 : 25 : 4, *V/V/V*); 2nd dimension, chloroform–methanol–conc. ammonia–isopropylamine (65 : 35 : 5 : 0.5, *V/V/V/V*) (Döhler and Datz 1980).

3.1 Fatty acids

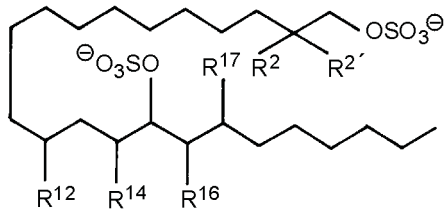
Representatives of the genus *Nostoc*, almost 50 different strains collected in different regions of the world have been investigated regarding their content of various FA (Table I). Most of the FA were *n*-saturated (16.9–51.8 %), monounsaturated (7.7–51.5 %) but also di-, tri- and oligounsaturated (16.3–56.8 %) FA were identified (Table I). The species of cyanobacteria appear to synthesize short (C_3–C_{10}) carboxylic acids, whose content is usually lower than that of long-chain FA (C_{14}–C_{18}). The major FA was 16:0, ranging from 14.9 to 50.8 % of total FA. The predominant monounsaturated (39 % of the total mono-unsaturated FA) was oleic acid (*Z*-9-18:1). Other monounsaturated FA were also observed, *e.g.*, *Z*-9-hexadecenoic and 11-hexadecenoic acids. Oligoenoic FA present in various species of *Nostoc* were represented by a large spectrum of

acids, mainly linoleic and - and -linolenic acids. The principal FA in the investigated *Nostoc* species did not essentially differ from those of most previously studied cyanobacteria (Schneider *et al.* 1970; Murata and Nishida 1987).



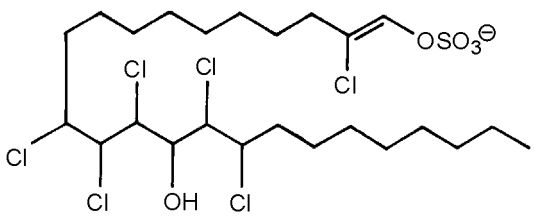
9a–f

	R^2	$R^{2'}$	R^{11}	R^{13}	R^{15}	R^{16}
9a						Cl
9b					Cl	Cl
9c	Cl	Cl	Cl	Cl	Cl	Cl
9d	Cl	Cl	Cl	Cl	Cl	
9e	Cl	Cl	Cl	Cl		Cl
9f	Cl	Cl				



10a–c

	R^2	$R^{2'}$	R^{12}	R^{14}	R^{16}	R^{17}
10a					Cl	
10b						Cl
10c	Cl	Cl	Cl	Cl	Cl	Cl



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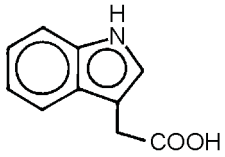

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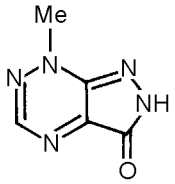

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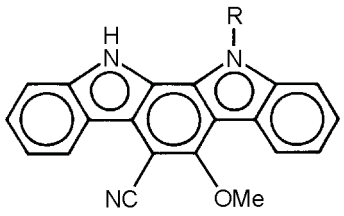
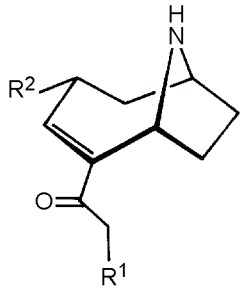


14a,b

	R
14a	H
14b	Me



15a–c

	R^1	R^2
15a	H	H
15b	Me	H
15c	Me	OH

Hydroxy acids (0.4–0.7 %) were found as minor components. These acids were previously isolated by GC–MS from freshwater cyanobacteria belonging to the genus *Aphanizomenon* (Dembitsky *et al.* 2001) and symbiotic *Nostoc* sp. (Dembitsky *et al.* 1999). Branched saturated FA that were identified in the *Nostoc* ranged from 1.1 to 8.6 %.

Special attention was given to dioic acids. The analysis of published data on *Nostoc* has shown that only Řezanka *et al.* (2003) have found some dioic acid in *N. linkia*. The detection of these is rather problematic with the usual columns. However, the use of coupled two columns with different polarity solves the problem perfectly. Aliphatic dicarboxylic acids surprisingly afforded potent cytotoxicity and antineoplastic activity (Hall *et al.* 1999), and could serve as lipidoic markers for identification of some human and animal diseases (Ma *et al.* 1999; Singh 1997). These acids are of major interest for medical specialists and biochemists.

Table I. *Nostoc* species investigated for their content of fatty acids(%, *W/W*)

Strain		Origin, place	Habitat[a]	Straigh saturated	Branched saturated	Mono-unsaturated	Oligo-unsaturated	Hydroxy	Dioic	Reference
N. commune	UTEX 584	Gulf of Eilat (Israel)	MA	33.6	8.6	28.7	21.1	0	0	Potts *et al.* 1987
	UTEX 584	*Texas University*, collection	FW	35.6	0	7.7	56.7	0	0	Olie and Potts 1986
	FACHB261	*Hong Kong University*, collection	TE	30.6	0	39.3	30.1	0	0	Liu *et al.* 2003
	CCAP1453/2	*ditto*	TE	29.6	0	40.3	30.1	0	0	*ditto*
		Göttingen University, collection	FW	41.2	0	16.6	42.2	0	0	Vargas *et al.* 1998
		Negev Desert (Israel)	TE	30.0	4.1	28.0	36.5	0	1.4	Temina *et al.* 2006
N. ellipsosporum	CCAP1453/2	*Hong Kong University*, collection	TE	28.9	0	38.9	32.2	0	0	Liu *et al.* 2003
	CCAP1453/11	*ditto*	TE	31.2	0	34.8	34.0	0	0	*ditto*
	CCAP1453/15	*ditto*	TE	29.6	0	38.4	32.0	0	0	*ditto*
	CCAP1453/16	*ditto*	TE	31.4	0	36.4	32.2	0	0	*ditto*
	CCAP1453/17	*ditto*	TE	31.2	0	36.5	32.3	0	0	*ditto*
	CCAP1453/18	*ditto*	TE	30.6	0	39.7	29.7	0	0	*ditto*
	CCAP1453/19	*ditto*	TE	27.7	0	42.4	29.9	0	0	*ditto*
N. flagelliforme		Natural colony, China	TE	24.2	0	33.1	42.7	0	0	Liu *et al.* 2004
	FACHB838	*Hong Kong University*, collection	TE	28.1	0	37.5	34.4	0	0	*ditto*
	CCAP1453/33	*ditto*	TE	26.9	0	39.4	33.7	0	0	*ditto*
N. linckia		Ein Boqeq Springs (Israel)	FW	40.6	6.1	24.5	24.4	0.7	3.7	Řezanka *et al.* 2003
N. muscorum	UTEX389	*Hong Kong University*, collection	TE	25.9	0	48.9	25.2	0	0	Liu *et al.* 2003
		Texas University, collection	FW	35.0	0	38.1	26.9	0	0	Parker *et al.* 1967
		Tennessee University, collection	FW	40.5	0	30.4	29.1	0	0	Holton *et al.* 1968
N. paludosum		Lagoon Albufera de Valencia (Spain)	MA	36.8	0	31.2	32.0	0	0	Vargas *et al.* 1998
		Dead Sea area (Israel)	TE	29.2	1.1	27.2	40.1	0	2.4	Temina *et al.* 2006
N. pruniforme		Lake Kinneret (Israel)	FW	27.8	2.8	17.3	48.9	0	3.2	*ditto*
N. punctiforme	UTEX1833	*Hong Kong University*, collection	TE	26.1	0	51.1	22.8	0	0	Liu *et al.* 2003
		University of Tokyo, collection	FW	29.9	0	28.6	41.5	0	0	Watanabe *et al.* 1968
N. verrucosum		Hula Lake (Israel)	FW	32.8	0.9	15.2	49.4	0	1.7	Temina *et al.* 2006
Nostoc sp.		*Collema* sp. (lichen)	PB	67.5	0	10.3	22.2	0	0	Dembitsky *et al.* 1999
		C. cristatum (lichen)	PB	26.1	1.0	17.5	53.6	0.4	1.4	Temina *et al.* 2006
		Peltigera horizontalis (lichen)	PB	40.2	0	29.6	30.2	0	0	Guschina *et al.* 2003
		P. hydrothyria (lichen)	PB	31.8	1.4	20.1	43.7	0.6	2.4	Temina *et al.* 2006
		Azolla caroliniana (fern)	PB	39.0	1.9	25.0	34.1	0	0	Caudales *et al.* 1993
	ANT	Ross Ice Shelf (Antarctica)	MA	33.7	0	42.4	23.9	0	0	Potts *et al.* 1987
	Albufera	Lagoon Albufera de Valencia (Spain)	MA	51.8	0	6.1	42.1	0	0	Vargas *et al.* 1998
	Caquena	a lake (Chile)	FW	36.0	0	40.5	23.5	0	0	*ditto*
	Chile	*ditto*	FW	44.3	0	19.2	36.5	0	0	*ditto*

Chucula	Caquena River (Chile)	FW	32.2	0	32.0	35.8	0	0	*ditto*
FACHB713	*Hong Kong University*, collection	TE	22.6	0	46.7	30.7	0	0	Liu *et al.* 2003
FACHB599	*ditto*	TE	26.2	0	45.8	28.0	0	0	*ditto*
HUN	Hunan Province (China)	TE	27.1	1.1	43.3	28.5	0	0	Potts *et al.* 1987
Llaita	Caquena River (Chile)	FW	32.4	0	31.6	36.0	0	0	Vargas *et al.* 1998
Loa	Loa River (Chile)	FW	31.5	0	35.0	33.5	0	0	*ditto*
REICH	Reichenau, Constance (Germany)	TE	31.2	1.4	29.5	37.9	0	0	Potts *et al.* 1987
UTEX1544	*Hong Kong University*, collection	TE	27.2	0	44.7	28.1	0	0	Liu *et al.* 2003
ATCC 27895	*Rutgers University*	FW	29.8	2.2	31.6	36.4	0	0	Caudales and Wells 1992
	Houston University, collection	TE	41.6	0	29.8	28.6	0	0	Schneider *et al.* 1970
152	Lake Saaskjarvi (Finland)	FW	27.2	4.1	22.7	46.0	0	0	Gugger *et al.* 2002
PCC73102	Root section (Australia)	TE	26.9	1.2	25.7	46.2	0	0	*ditto*

[a]FW – freshwater, MA – marine, PB – photobiont, TE – terrestrial.

Table III. Relative monosaccharide[a] composition (molar % ± SE) of polysaccharides from three *Nostoc* species[b]

Species	Sample	Ara	Rha	Fuc	Xyl	Man	Gal	Glc	GlcA
N. commune	field	–	–	–	27.0 ± 0.8	1.1 ± 0.1	21.8 ± 0.3	50.2 ± 0.5	–
	culture	6.3 ± 0.2	2.1 ± 0.1	4.9 ± 0.1	43.5 ± 0.6	<1	21.2 ± 0.2	7.1 ± 0.9	14.9 ± 0.4
	culture-N[c]	12.8 ± 0.5	2.9 ± 0.1	5.2 ± 0.2	35.1 ± 0.7	<1	20.2 ± 0.6	14.0 ± 0.9	9.7 ± 0.3
	EPS	9.5 ± 0.1	–	5.8 ± 0.1	40.1 ± 0.6	<1	18.8 ± 0.8	12.5 ± 0.3	13.3 ± 0.1
	EPS-N[c]	25.7 ± 0.3	–	–	12.0 ± 0.1	1.7 ± 0.1	11.7 ± 0.1	48.9 ± 0.5	–
N. flagelliforme	field	3.3 ± 0.1	–	–	23.2 ± 0.3	7.3 ± 0.3	21.0 ± 1.0	45.7 ± 0.8	–
	culture	–	–	–	–	15.2 ± 1.2	21.6 ± 0.5	40.0 ± 1.6	23.3 ± 0.2
	culture-N[c]	–	–	–	–	20.8 ± 1.2	27.7 ± 0.3	28.7 ± 0.9	22.8 ± 0.6
	EPS	–	–	–	–	19.7 ± 0.4	27.8 ± 0.7	52.5 ± 1.2	–
	EPS-N[c]	–	–	–	4.0 ± 0.5	19.3 ± 0.5	24.6 ± 0.4	31.4 ± 1.1	20.8 ± 0.4
N. sphaeroides[d]	field[e]	–	–	–	20.5 ± 0.4	14.8 ± 0.3	21.1 ± 0.4	43.6 ± 0.1	–
	culture	–	8.8 ± 0.8	18.7 ± 0.7	16.2 ± 0.2	9.4 ± 0.1	6.1 ± 0.5	40.8 ± 1.1	–
	culture-N[c]	–	4.6 ± 0.6	18.1 ± 0.6	16.6 ± 0.1	9.6 ± 0.1	8.2 ± 0.6	42.9 ± 0.8	–

[a]Ara L-arabinose Rha L-rhamnose Fuc L-fucose Xyl D-xylose Man D-mannose Gal D-galactose Glc D-glucose GlcA D-glucuronic acid

[b]Compiled from papers of Rao (1994), Singh *et al.* (2001), Takenaka *et al.* (1998), Huang *et al.* (1998), Hokputsa *et al.* (2003), and Hu *et al.* (2003).

[c]From nitrogen-free medium.

[d]The EPS contained very low total saccharide levels (<10 %).

[e]From 100 °C water extract.

FA of cyanobacteria have been investigated for their content, composition types, metabolism, and biotechnological applications (Ahlgren *et al.* 1992; Murata *et al.* 1992; Vargas *et al.* 1998; Liu *et al.* 2003). In terms of chemotaxonomy by FA composition, Li and Watanabe (2001) indicated that there should be a separation between 24 axenic strains of planktonic *Anabaena* sp. with straight trichomes, in the genus *Anabaena* based on the difference in their FA composition. Caudales and Wells (1992) as well as Kruger *et al.* (1995) and Kerwin (1994) also demonstrate the importance of FA composition in the taxonomy of cyanobacteria at the genus and subgenus levels. Our previous study shows that hydrocarbon and FA composition can be used in chemotaxonomy of cyanobacteria even at the species level based on examination of many strains of cyanobacteria (Dembitsky and Srebnik 2002; Řezanka *et al.* 2003). A large variation in individual FA contents determined by statistical analysis according to species, season, and location belonging to the genus *Nostoc* was reported (Temina *et al.* 2006).

From literature data, it can be concluded that some filamentous nitrogen-fixing cyanobacteria represent a source of essential FA that are of commercial interest, including linoleic, and - and -linolenic acids, among others. The presence of -linolenic acid in *Nostoc* sp. from Chile is an interesting finding, because this FA had not previously been reported to be present in strains belonging to these genera (Vargas *et al.* 1998). Investigation of different *Nostoc* species collected from marine, freshwater and terrestrial ecosystems, and also in symbiotic species showed a large biodiversity of >80 FA.

4 DERIVATIVES OF AMINO ACIDS

Few alkaloids were found in *Nostoc* species. The indole-3-acetic acid (**12**), an auxin phytohormone that promotes cell growth and elongation and influences rooting, is produced by plants (Sheldrake 1973) and plant-associated bacteria (Patten and Glick 1996; Costacurta and Vanderleyden 1995). The ability of different strains of *Nostoc* isolated from the angiosperm *Gunnera*, liverwort, hornwort or cycad to produce the phytohormone indole-3-acetic acid was reported (Sergeeva *et al.* 2002).

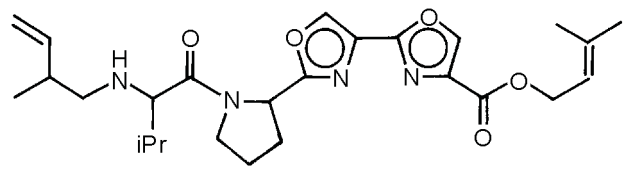
A freshwater *N. spongiaeforme* produced and excreted a novel violet pigment, nostocine A (**13**), which had a broad spectrum of growth-inhibiting activity (Hirata *et al.* 1996*a*). Nostocine A also inhibited the growth of some typical strains of microorganisms, algae, cultured plants, and established animal cell lines (Hirata *et al.* 1996*b*). Hirata *et al.* (2003) studied the bioactivity of nostocine A of several model organisms breeding with *N. spongiaeforme* in the natural environment. To microalgae, nostocine A exhibited a growth-inhibiting activity comparable to paraquat, and the activity tended to be stronger to green algae than to cyanobacteria. Nostocine A also exhibited strong inhibitory activity to root elongation of barnyard grass, strong counter-feeding activity to cotton ballworm, and acute toxicity to mice resulting in its classification as a dangerous poison. The results suggest that nostocine A may act as a toxin or an allelochemical to breeding organisms in nature and that its adverse effects on various organisms may be related to its function in generating toxic reactive oxygen species, which occurs in the cells of target organisms (Hirata *et al.* 2003). Treatment of *Chlamydomonas reinhardtii* in the light with **13** accelerated the generation of ROS in the green alga. The reduction potential indicated that **13** and O_2 can easily exchange electrons depending on the mass balance between their oxidized and reduced forms. The mechanism of generation of intracellular ROS into the target cells is reduced specifically by intracellular reductants such as NAD(P)H. This similar intracellular ROS generation mechanism to that of paraquat may cause the cytotoxicity (Hirata *et al.* 2004).

Indolo[2,3-*a*]-3-methoxy-4-cyanocarbazole (**14a**) and *N*-methylindolo[2,3-*a*]-3-methoxy-4-cyanocarbazole (**14b**) are responsible for most of the cytotoxicity and antiviral activity associated with the blue-green alga *N. sphaericum* EX-5-1 (Knubel *et al.* 1990).

Anatoxin A (**15a**), the neuromuscular blocking agent was first isolated from the freshwater cyanobacterium *Anabaena flos-aquae* (Devlin *et al.* 1977); later it has been isolated from other cyanobacterial species including *N. muscorum* (Carmichael 1997). Its pharmacological properties have been investigated and compared with that of synthetic anatoxin A which was derived from l-cocaine. Anatoxin A is a potent depolarizing neuromuscular blocking agent possessing both muscarinic and nicotinic activity; it acts as a potent agonist of the secretory response of chromaffin cells with an EC_{50} of 1–2 mol/L, compared with an EC_{50} of 4–5 mol/L for nicotine. The cells responded to anatoxin A and nicotine with bell-shaped concentration-response curves consistent with desensitization at anatoxin A concentration of >5 mol/L and of nicotine >20 mol/L (Molloy *et al.* 1995). The natural analogs homoanatoxin A (**15b**) and 4-hydroxyhomoanatoxin A (**15c**) have been isolated from *Raphidiopsis mediterranea* (Namikoshi *et al.* 2003).

A novel oligopeptide alkaloid, muscoride A (**16**), was isolated from terrestrial freshwater *N. muscorum*. Muscoride A was the first compound possessing *N*-(2-methylbut-3-en-2-yl)valine and two contiguous

methyloxazoles (Nagatsu *et al.* 1995). Muscoride A showed weak antibacterial activity against *Bacillus subtilis* and *Escherichia coli* (Hammerschmidt *et al.* 1993).



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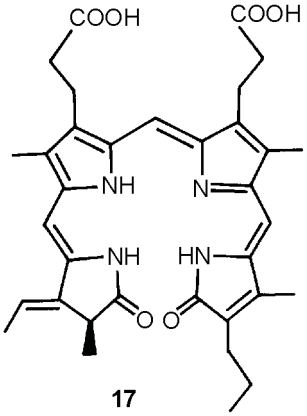

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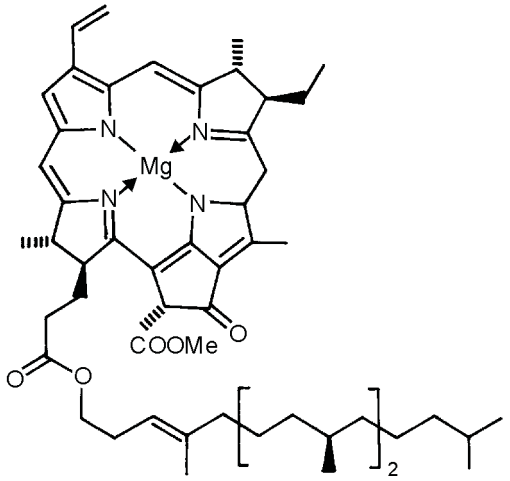

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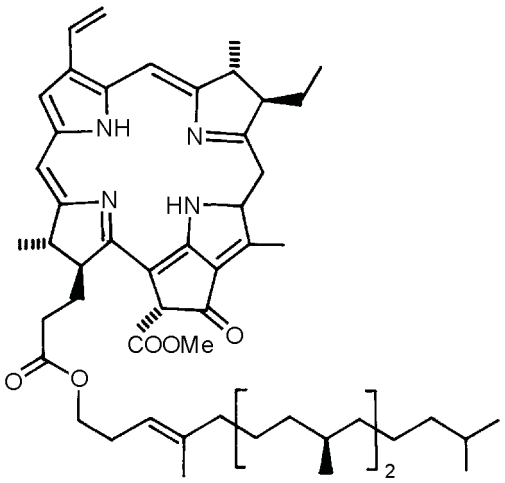

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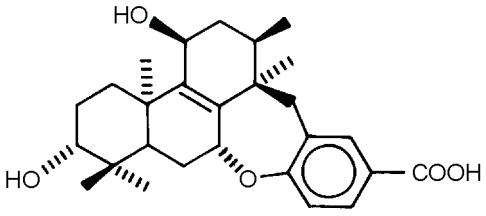

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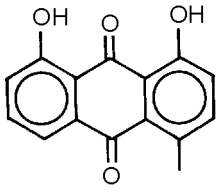

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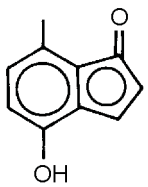

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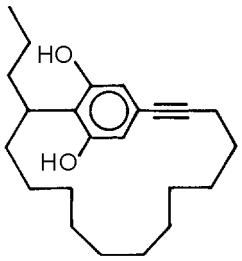

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Phycocyanobilin (**17**) is a blue pigment, with a maximum absorbance at 680 nm, found in some cyanobacterial species, being thermostable but dissociating at acidic or basic pH.

Two pigments having common structure, chlorophyll A (**18**) and pheophytin A (**19**) have been detected in all investigated *Nostoc* species (Potts *et al.* 1987).

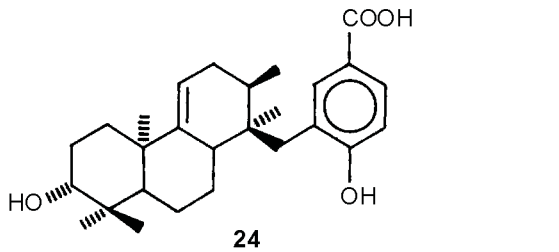
5 TERPENOIDS AND AROMATIC COMPOUNDS

Two new compounds, a diterpenoid and an anthraquinone, as well as an indane derivative, which was reported as a natural product for the first time, have been isolated from *N. commune* (Jaki *et al.* 2000*a*). In a continuation of this investigation the isolation, structure elucidation, and biological activity of further

three compounds, *i.e.* a derivative of dodecahydrophenanthrene (**20**), 4-methylchrysazin (**21**), and 4-hydroxy-7-methylindan-1-one (**22**), from the cells were reported. Compound **20** displays a selective potent antibacterial activity against *Staphylococcus epidermidis* equal to chloramphenicol. Moderate antibacterial activity against *S. epidermidis* could be also detected for compounds **21** and **22** and against *Bacillus cereus* for all 3 compounds (Jaki *et al.* 2000*b*).

A novel acetylene-containing acetylenic (1,4)-cyclophane, nostocyclyne A (**23**), possessing antimicrobial activity, is the major active metabolite of the natural bloom of the *Nostoc* sp. (Ploutno and Carmeli 2000). Nostocyclyne A shows a weak inhibition of photosynthesis (5 % at 100 g/mL) of green algae (Ben-Haim *et al.* 1999).

The diterpenoid compound, noscomin (**24**), has been isolated from the culture medium of the terrestrial *N. commune* (Jaki *et al.* 1999). Noscomin showed antibacterial activity against *Bacillus cereus* (MIC 32 ppm), *Staphylococcus epidermidis* (8 ppm), and *Escherichia coli* (128 ppm). These values are comparable with those obtained for the standards chloramphenicol (*B. cereus*, MIC 8 ppm; *S. epidermidis*, 4 ppm) and tetracycline (*E. coli*, 64 ppm) (Jaki *et al.* 1999).



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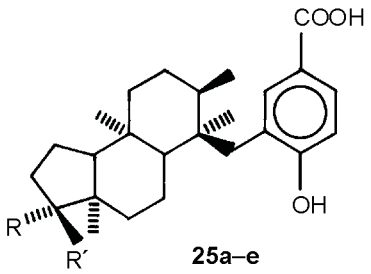
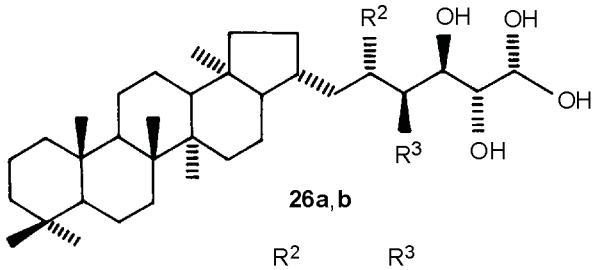


25a–e

	R	R´
25a	CH_2OH	Me
25b	CHO	Me
25c	COOH	Me
25d	$CH(OMe)_2$	Me
25e	Ac	H



26a,b

	R^2	R^3
26a	H	OH
26b	OH	H

Five novel extracellular metabolites with an unprecedented diterpenoid skeleton, named as comnostin A (**25a**), B (**25b**), C (**25c**), D (**25d**), and E (**25e**) have been isolated from *N. commune* (Jaki *et al.* 2000*a*). All comnostins A–E showed antibacterial activity (Table II). Additionally, cytotoxic and molluscicidal activities were found for comnostin B.

Table II. Biological activity of comnostins (Jaki *et al.* 2000*a*)

Compound	Antibacterial activity[a]			Molluscicidal activity[a]	Cytotoxicity[b]	
	B. cereus	*S. epidermdis*	*E. coli*	*B. glabrata*	KB cells	Caco-2 cells
Comnostin A (**25a**)	32	16	128			
Comnostin B (**25b**)	32	16	128	20	0.40	0.18
Comnostin C (**25c**)	32	16	64			
Comnostin D (**25d**)	16	32	128			
Comnostin E (**25e**)	128	4				
Chloramphenicol	8	4				
Tetracycline			64			
$CuSO_4$				100		
Podophyllotoxin					0.01	0.02

[a]MIC, ppm. [b]ED_{50}, ppm.

Two bacteriohopanepentols **26a** and **26b** which differ from one another by the presence of 1,2- or of mixed 1,2/1,3-pentahydroxy-type side chains have been isolated from the genus *Nostoc* (Zhao *et al.* 1996). Pentacyclic triterpenoids of the hopane series are commonly found in bacteria where they play an important role in maintaining membrane stability (Ourisson and Rohmer 1992). The most common compounds found to date are the C_{35} bacteriohopanepolyols in which an additional sugar-derived acyclic C_5 unit is linked to the isopropyl group of the hopane framework (Rohmer 1993). Whereas hydroxy side-chains with free primary amino groups are quite common, free hydroxy compounds have been rarely reported. The main free hydroxy compounds, tetrols from *Acetobacter* species, have been isolated (Peiseler and Rohmer 1992).

6 CAROTENOIDS

Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen. Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotenoids from their diet. Within animals, carotenoids provide bright coloration, serve as antioxidants, and can be a source for vitamin A activity (Ong and Tee 1992; Britton *et al.* 1995).

Carotenoids are responsible for many of the red, orange, and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds, insects, fish, and crustaceans. Some familiar examples of carotenoid coloration is the orange of carrots and citrus fruits, the red of peppers and tomatoes, and the pink of flamingoes and salmon (Pfander 1992). Some 600 different carotenoids are known to occur naturally (Ong and Tee 1992), and new carotenoids are continuously being identified.

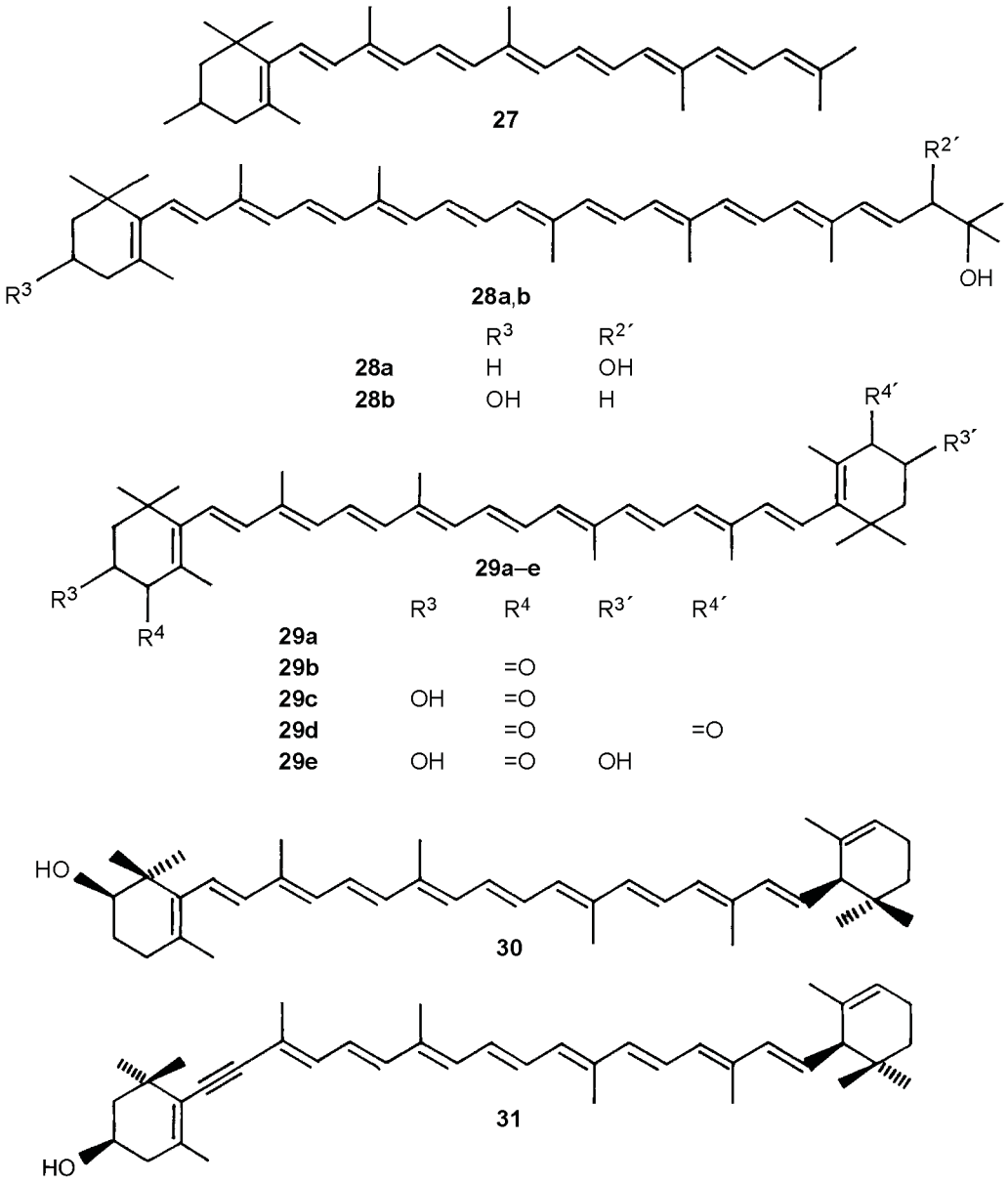
Most cyanobacteria have a mucilaginous sheath, or coating, which is often deeply pigmented (Paerl 1984). The colors of the sheaths in different species include light gold, yellow, brown, red, green, blue, violet, and blue-black; they impart color to individual cells and colonies as well as to "blooms" of cyanobacteria in aquatic environments (Hirschberg and Chamovitz 1994).

Several carotenoid compounds (**27**–**31**), in desiccated *N. commune* collected from China, Germany, and Antarctica and in axenic cultures of the desiccation-tolerant strains *N. commune* and *Hydrocoleum* sp. have been reported (Potts *et al.* 1987). *Nostoc* species contained (in contrast to the axenic strains) significant amounts of apocarotenoids and a P384 pigment which, upon reduction with $NaBH_4$, yielded a mixture of a chlorophyll derivative and a compound with an absorption maximum of 451 nm. A clear distinction can be made between the carotenoid contents of the axenic cultures and the desiccated field materials. In the former, -carotene and echinenone, canthaxanthin and the , series of carotenoids were found.

Specific growth rates and carotenoid contents of 3 Antarctic and tropical strains of cyanobacteria, *viz. Nostoc* sp., *Anabaena* sp. and *Phormidium* sp., were studied and compared in batch and mass cultures to assess the biopotential of Antarctic strains for cost-effective carotenoid production. Antarctic strains exhibited slightly lower specific growth rates but contained higher carotenoid contents (per dry mass) than the tropical ones. Modification of normal composition of BG-11 culture medium, by altering nitrogen and carbon sources resulted in 25–38 % increase in carotenoid content in both types of geographic strains. The observations suggest that Antarctic cyanobacteria may have potential as superior strains for maximizing the yield of carotenoids (Shukla and Kashyap 2003).

7 BORON-CONTAINING MACROCYCLES

Borophycin (**32**, as sodium salt) is the potent cytotoxin in the lipophilic extract of a marine strain of the cyanobacterium *N. linckia* (Dembitsky *et al.* 2002; Hemscheidt *et al.* 1994). Borophycin is built from two almost identical moieties with an overall structure reminiscent of the ionophoric antibiotics boromycin and aplasmomycin. All three compounds are acetate-derived oligoketides that utilize a C_3 precursor as a starter unit and methionine for the methyl branches of the oligoketide chain. Whereas 3-phosphoglycerate or phosphoenolpyruvate has been suggested to be the C_3 starter unit in the biosynthesis of boromycin and aplasmomycin, the C_3 starter unit for the biosynthesis of **32** is derived from acetate and methionine, but not propionate (Hemscheidt *et al.* 1994). Borophycin was also isolated from the methanol extract of *N. spongiaeforme* var. *tenue* (Banker and Carmeli 1998).

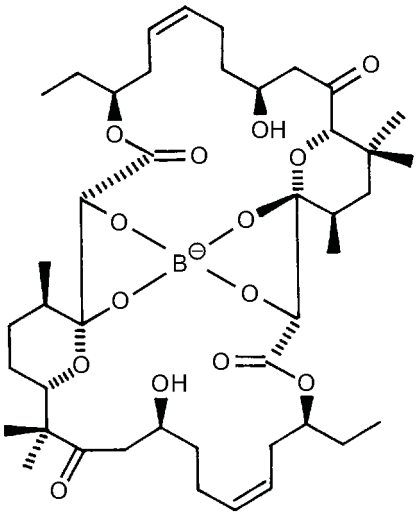


8 ARSENOLIPIDS

Recent investigation showed that the arsenic compounds in terrestrial and aquatic plants, lichens, fungi, and algal species can be divided into water-soluble and lipid-soluble compounds (Dembitsky and Řezanka 2003). Recently, a freshwater *Nostoc* sp. was found to contain a 3 ppm concentration of arsenic and only ⅓ was extractable. The extract representing 1 ppm of arsenic contained one arsenosugar (**33**), the rest being dimethylarsenic acid (Lai *et al.* 1997).

9 MYCOSPORINE-LIKE AMINO ACIDS

Mycosporine-like AA have been identified in taxonomically diverse organisms, including a marine heterotrophic bacterium (Arai *et al.* 1992), cyanobacteria (Řezanka *et al.* 2004; Garcia-Pichel and Castenholtz 1993; Karsten and Garcia-Pichel 1996), microalgae (Okaichi and Tokumura 1980; Carreto *et al.* 1990; Vernet and Whitehead 1996; Shashar *et al.* 1997), and macroalgae (Dunlap and Shick 1998). Two mycosporine-like AA such as shinorine (**34**) and scytonemin (**35**) have been isolated from some *Nostoc* species (Řezanka *et al.* 2004).



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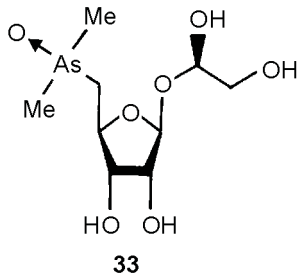

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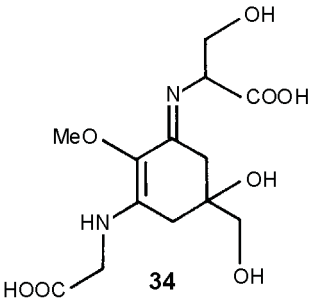

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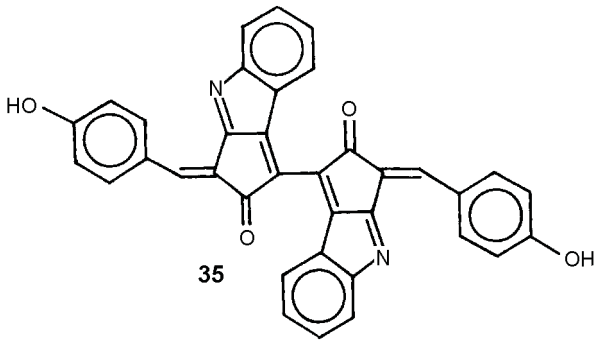

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10 MONOSACCHARIDES AND POLYSACCHARIDES

Many cyanobacteria colonize arid and semiarid areas and produce superabundant sheath or capsular jelly, which aggregates soil particles and is important for moisture retention (Flaibani *et al.* 1989). Strains with cells embedded in a mucilaginous sheath, *e.g.*, *N. commune*, have been reported to be more tolerant to UV irradiation than those without such coverings, *e.g.*, *Anabaena* sp. (Sinha *et al.* 1995). Some cyanobacteria are able to remain desiccated for months or years and recover metabolic activity after rehydration (Dodds *et al.* 1995). These properties may relate to the polysaccharides or glycoconjugates produced by cyanobacteria, as is the case of many other microorganisms. Compared to many macroalgal and microbial polysaccharides, cyanobacterial polysaccharides are less well characterized, and reports in the literature focus mainly on their monosaccharide composition; their structures and properties have been described in only a few reports (Bertocchi *et al.* 1990).

Nostoc, one of the most widespread genera of nitrogen-fixing filamentous cyanobacteria, is able to form macroscopic or microscopic colonies (Dodds *et al.* 1995). Some special characteristics in the colony formation and life cycle of *N. commune* are well known and are quite different from those of other cyanobacteria. The morphological aspects concerning the polymorphic life cycle and colony development of *N. commune* are well documented (Vagnoli *et al.* 1992; Abdelahad and Bazzichelli 1989), and metabolic differences in strains and life cycle stages leading to different shapes of colonies have been reported (Doods 1989; Bilger *et al.* 1994). However, there has not been a systematic investigation of the biochemistry of the colony-supporting matrix, *i.e.* the main gelatinous substances surrounding the trichomes.

Traditionally, some species of *N. flagelliforme* have been used as a food source (Gao 1998), and some have been used as medicine to treat cancer and gout. A broad spectrum of antimicrobial compounds has been found in both cellular extracts and extracellular products of some *Nostoc* spp. (*e.g.*, Rao 1994; Singh *et al.* 2001). *N. commune* has been reported to significantly depress serum cholesterol levels in rats (Hori *et al.* 1994). This cholesterol lowering activity may be due to "dietary fiber", according to the authors, but no details on this viscous product exist. A hot water extract of *N. flagelliforme* has been reported to have antitumor activity; this effect may be due to polysaccharides (Takenaka *et al.* 1998; Huang *et al.* 1998), but no characterization has been done. Hokputsa *et al.* (2003) and Hu *et al.* (2003) studied an EPS from 5 desert cyanobacteria, including *Nostoc* sp. The authors show that polysaccharides contain up to 6 sugars (in %): L-rhamnose 3.5, D-xylose 20.9, D-mannose 1.6, D-galactose 21.5, D-glucose 44.0, and 2-*O*-methyl-D-glucose 8.6. De Philippis *et al.* (2000) studied the exocellular polysaccharides released during the photoautotrophic growth by 25 *Nostoc* strains belonging to the PCC. The results are shown in Tables III (*see* p. 371) and IV.

N. commune produces quite complex EPS, and oligosaccharide fractions that were isolated. The structures of the oligosaccharides were determined, and two different series that can originate from two repeating pentamers were identified:

GlcA-1→4/6-GlcA-1→4-Gal-1→4-Glc-1→4-Xyl and
GlcA-1→4/6-Glc-1→4-Gal-1→4-Glc-1→4-Xyl (Brull *et al.* 2000).

Table IV. Monosaccharide composition (molar ratios[a]) of the polysaccharides released by *Nostoc* PCC strains[b]

Strain	GlcA	GalA	Gal	Man	Ara	Xyl	Rib	Fuc	Rha
6302	+	–	0.10	0.45	2.30	0.05	–	0.80	0.40
6310	+	+	0.27	0.10	–	0.23	0.07	0.10	0.23
6705	+	–	1.87	0.40	0.76	0.29	0.10	0.21	0.13
6719	+	+	2.42	0.05	1.40	0.02	–	0.02	0.06
6720	+	+	1.61	2.09	–	3.13	–	0.96	0.13
7107	+	–	0.30	0.34	0.38	0.31	0.10	0.57	0.10
7119	–	+	2.69	–	2.00	–	–	0.02	0.10
7413	–	+	2.24	0.45	1.58	0.14	0.10	0.23	0.18
7416	+	–	0.18	0.16	0.18	0.19	–	0.25	0.05
7422	–	+	0.33	2.43	0.05	0.29	–	1.33	0.24
7423	+	–	0.42	0.17	–	0.40	–	0.05	0.01
7706	+	–	7.75	5.47	–	3.83	–	1.75	0.12
7803	–	+	0.50	0.02	–	0.81	0.62	0.03	0.08
7807	+	+	–	0.37	–	–	0.01	0.03	0.01
7906	–	+	0.42	1.12	0.31	0.96	–	0.58	0.19
7933	–	+	1.22	1.05	0.27	0.46	–	0.53	0.26
7936	+	–	1.10	0.80	–	–	–	0.06	–
7937	–	–	2.07	0.11	0.87	0.07	–	0.03	0.09
8009	–	+	1.35	1.18	0.29	1.35	–	0.29	0.12
8109	+	–	1.04	0.29	–	0.39	–	0.17	0.13
8112	+	+	0.43	0.27	0.27	0.66	–	0.39	0.14
8113	–	+	12.5	1.25	–	20.0	18.0	11.5	4.25
8306	–	+	0.33	0.19	–	0.11	–	0.44	0.08
9202	+	+	0.41	0.36	0.20	0.30	–	0.06	0.02
9305	–	+	0.12	0.09	0.22	0.21	–	0.07	0.06

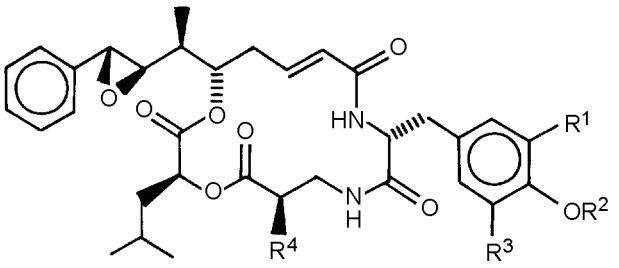
[a]For glucose is 1. [b](+) present, (–) not detectable (De Philippis 2000).

11 PEPTIDES AND LIPOPEPTIDES

The cyanobacteria and predominantly the genus *Nostoc* are known as producers of dozens pharmacologically active compounds, composed of unusual AA (Carmichael 1992; Patterson *et al.* 1994).

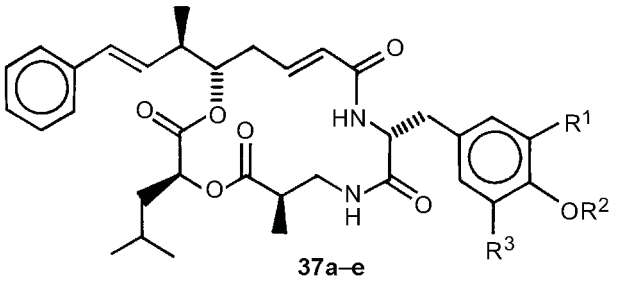
Cryptophycin-1 (**36b**) was found in the extracts of *Nostoc* sp. (Schwartz *et al.* 1990). Later, it was identified in the crude lipophilic extract; it was cytotoxic against the KB human nasopharyngeal carcinoma cell line (0.24 ng/mL) and against the human colorectal adenocarcinoma cell line (6 ng/mL) (Trimurtulu *et al.* 1994). Cryptophycin-1 is an effective inhibitor of tubulin polymerization, causes tubulin to aggregate, and depolymerizes microtubules to linear polymers somewhat similar to the spiral-like structures produced by the *Vinca* alkaloids. Cryptophycin-1 also inhibits vinblastine binding to tubulin but not colchicine binding (Kerksiek *et al.* 1995). The major naturally occurring representative of this class of cyclic depsipeptides, cryptophycin-1, shows excellent activity against a broad spectrum of solid tumors, including drug-resistant ones, implanted in mice (Golakoti *et al.* 1995; Moore *et al.* 1996; Subbaraju *et al.* 1997).

Cryptophycin-46 (**37b**), -175 (**37a**), and -176 (**37e**) have been identified as three new trace constituents of *Nostoc* sp. (Trimurtulu *et al.* 1994). Cryptophycin-46 is to date the only naturally occurring analog having the *S* configuration at C^{10}. Cryptophycin-175 and -176 also differ in unit B where **37c** is the *O*-methyl analog of **37d** and **36c** is the *O*-demethyl analog of cryptophycin-21 (**36d**). Five minor cryptophycins B–F, (**36c**,**g**, **37c**–**e**) have also been isolated from *Nostoc* sp. and their total structure and cytotoxicity were determined. Two types of cryptophycins were present in this cyanobacterium, the major series possessing a monochlorinated *O*-methyl-L-tyrosine unit and the minor series possessing a nonchlorinated *O*-methyl-D-tyrosine unit.



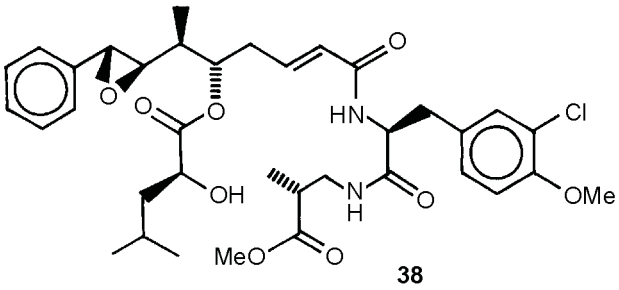
	R^1	R^2	R^3	R^4
36a		Me		Me
36b	Cl	Me		Me
36c	Cl			
36d	Cl	Me		
36e	Cl			Me
36f	Cl		Cl	Me
36g	Cl	Me	Cl	Me

36a–g



	R^1	R^2	R^3
37a		Me	
37b	Cl		
37c	Cl	Me	
37d	Cl		Cl
37e	Cl	Me	Cl

37a–e



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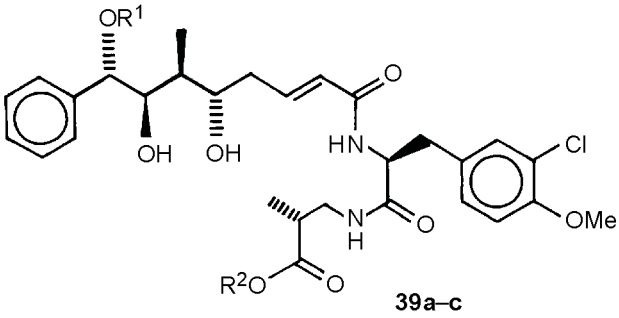


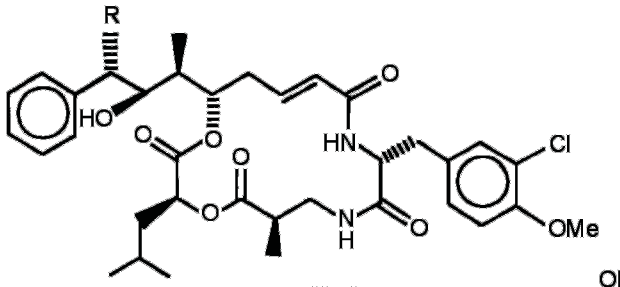
	R^1	R^2
39a		
39b		Me
39c	Me	Me

39a–c

The *Nostoc* sp. grow in large scale to procure adequate minor metabolites of **36b** and the cryptophycins **36a**, **37a**,**b** and **38** for further *in vivo* evaluation and semisynthesis of analogs for structure–activity relationship studies. As a consequence of this expanded investigation, authors identified eight new cryptophycins **39**–**43** as minor constituents (Golakoti *et al.* 1995).

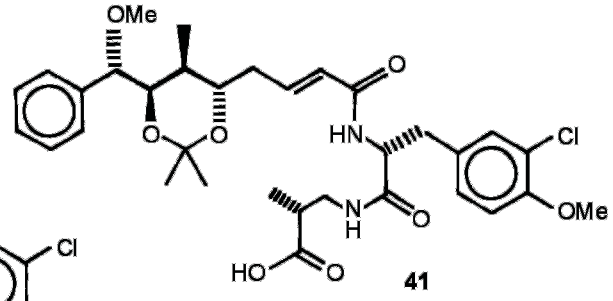
A novel lipopeptide, nostofungicidine (**44**), was isolated from the methanolic extract of a field-grown terrestrial *N. commune* (Kajiyama *et al.* 1998). Nostofungicidine contains a novel -AA, *viz.* 3-amino-6-hydroxystearic acid. Nostofungicidine showed potent antifungal activity against *Aspergillus candidus* (MIC 1.6 g/mL) and cytotoxicity against NSF-60 cell (IC_{50} 1.5 mol/L).

The isolation and total structure determinations of nostopeptolides A_1 (**45b**), A_2 (**45a**) and A_3 (**45c**) were described by Golakoti *et al.* (2000). These cyclic depsipeptides, which are devoid of cytotoxic, antifungal and inhibition of peptidase activity, are characteristic constituents of the cryptophycin-producing *Nostoc* sp. None of the nostopeptolides showed significant cytotoxicity against KB and LoVo ($IC_{50} < 1$ mol/L), antifungal activity against *Candida albicans* (25 g per disc), antibacterial activity against *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* (>25 g per disc), or inhibition of peptidase activity against trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), papain (EC 3.4.22.2), thrombin (EC 3.4.21.5), plasmin (EC 3.4.21.7) ($IC_{50} < 40$ mol/L) and against leukocyte elastase (EC 3.4.21.37) ($IC_{50} < 20$ mol/L).



	R
40a	Cl
40b	OMe

40a,b



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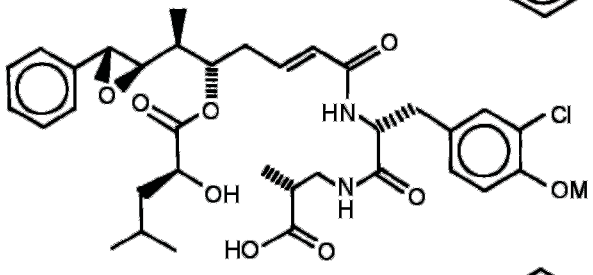

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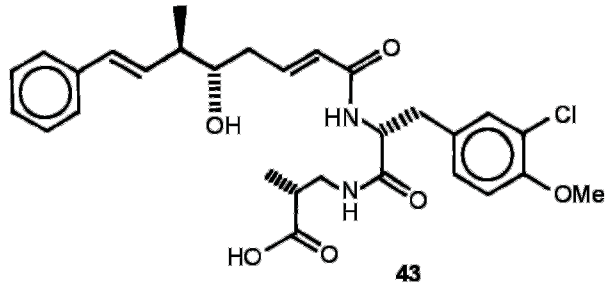

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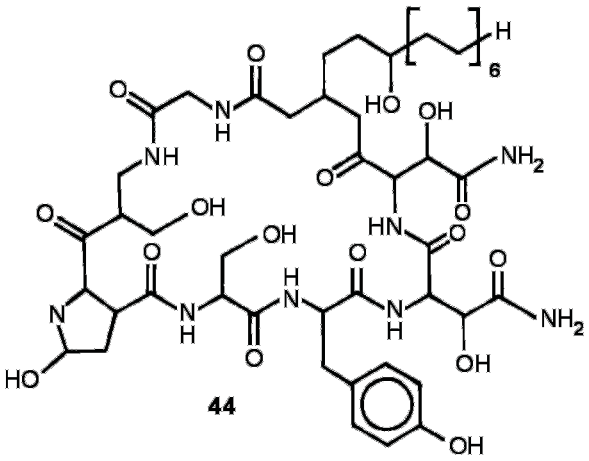

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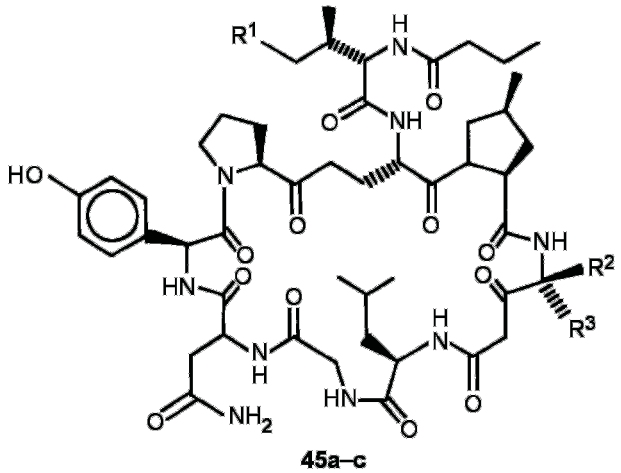
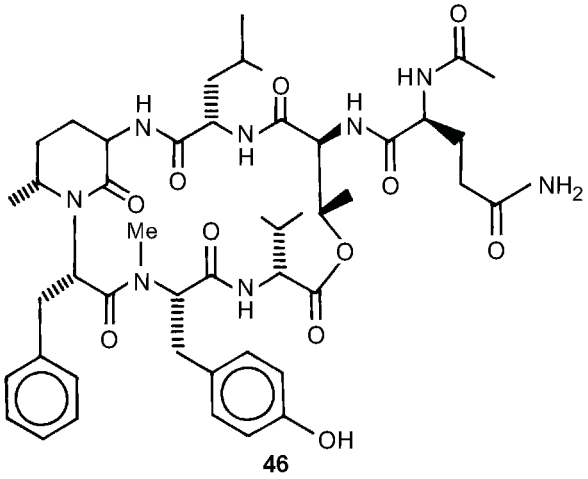


	R^1	R^2	R^3
45a		iBu	
45b	Me	iBu	
45c	Me		iBu

45a–c

Six new metabolites, *viz.* nostopeptin BN920 (**46**), nostoginin BN741 (**47b**), nostoginin BN578 (**47a**), banyascyclamide A (**48**), banyascyclamide B (**49a**) and banyascyclamide C (**49b**) were isolated from the hydrophilic extract of a *Nostoc* sp. (Ploutno and Carmeli 2002). Compound 46 inhibits the serine endopeptidase chymotrypsin, while compound **47b** inhibits aminopeptidases (EC 3.4.11). The inhibitory activity of nostopeptin (**46**) was determined against two enzymes, the serine endopeptidases trypsin and chymotrypsin. In addition, compound **46** inhibits chymotrypsin with IC_{50} 0.11 mol/L, but not trypsin at 45.0 g/mL. Nostoginin BN741 (**47b**) inhibits a bovine aminopeptidase with IC_{90} 1.3 mol/L, but does not inhibit bovine neutral endopeptidase and peptidyl-dipeptidase A (angiotensin converting enzyme; EC 3.4.15.1) at 48 g/mL (Ploutno and Carmeli 2002).



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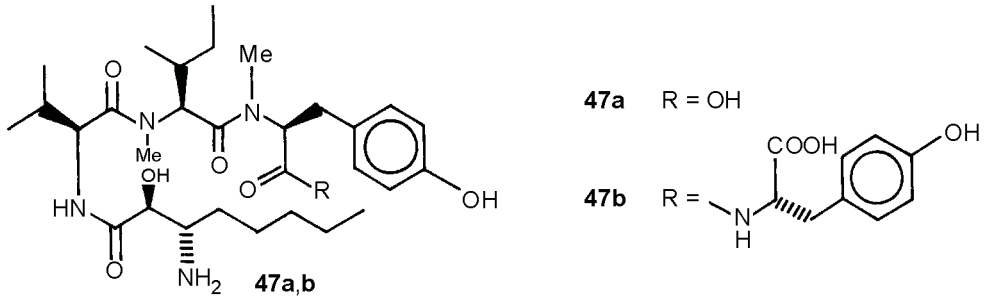
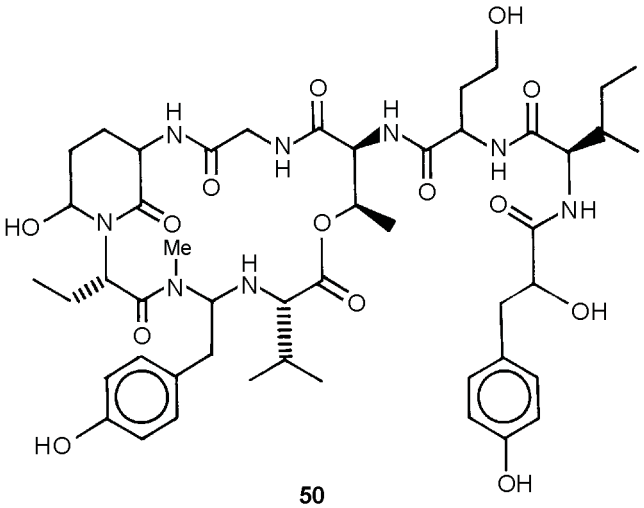


47a,b

Nostocyclin (**50**), a novel 3-amino-6-hydroxy-2-piperidone-containing cyclic peptide was isolated from a hepatotoxic strain of cyanobacterium *Nostoc* sp. (Kaya *et al.* 1996). Nostocyclin is nontoxic in acute *in vivo* bioassays but inhibits phosphoprotein phosphatase (EC 3.1.3.16) activity at high concentration *in vitro*. Nostocyclin was not toxic in an intraperitoneal mouse bioassay at up to 2500 g/kg body mass or in a brine shrimp bioassay at up to 5 mol/L. *In vitro* phosphoprotein phosphatase inhibition assay, inhibition by nostocyclin was found, although with a relatively high IC_{50} of 64 mol/L (1280× higher than microcystin-LR).



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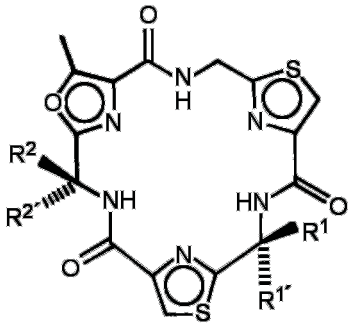
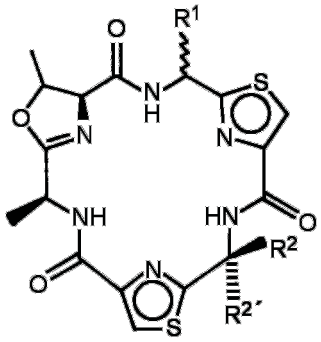


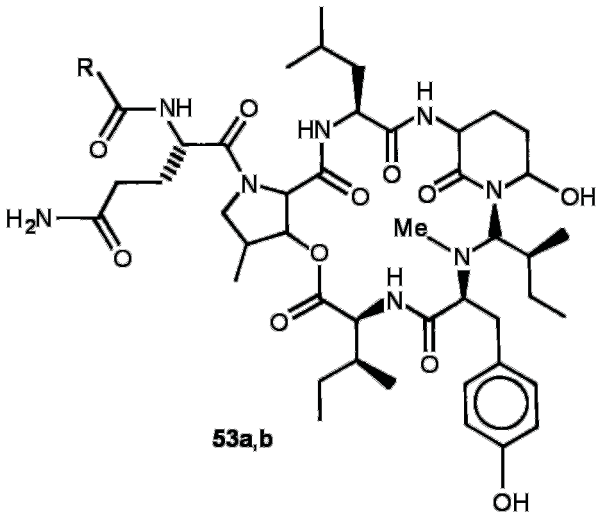
51a,b

	R^1	$R^{1'}$	R^2	$R^{2'}$
51a		iPr	Me	
51b	$-CH_2CH_2SMe$			Me



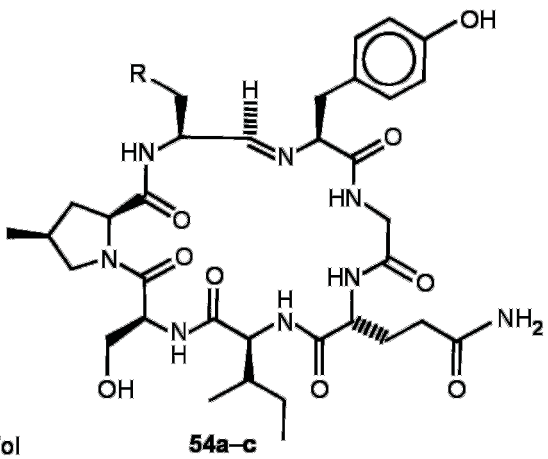
52a–d

	R^1	R^2	$R^{2'}$
52a	Me	Me *or* H	H *or* Me
52b	Me	H *or* Me	Me *or* H
52c		$-CH_2CH_2SMe$	
52d		$-CH_2CH_2SMe$ ↓ O	



53a,b

	R
53a	Me
53b	Pr



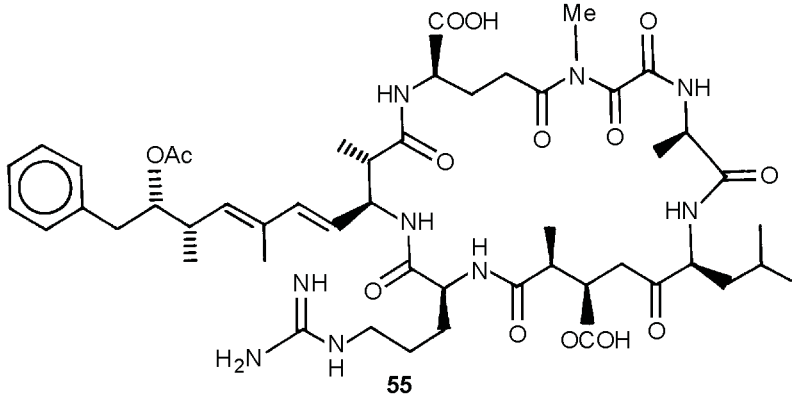
54a–c

	R
54a	iPr
54b	Ph
54c	*p*-Tol

A new anticyanobacterial and antialgal secondary metabolite, nostocyclamide (**51a**), has been isolated as a major component from the *Nostoc* sp. (Todorova *et al.* 1995). Inhibitory activity was quantitatively determined against the diatom *Navicula minima*; it was also toxic against *Brachionus calyciflorus*. A cyclic peptide nostocyclamide M (**51b**), containing thiazole and oxazole moieties, was isolated from a freshwater *Nostoc* sp. (Jüttner *et al.* 2001). Compound **51b** exhibited grazer toxicity and allelopathy against related cyanobacteria.

Four modified cyclic hexapeptides, tenuecyclamides A–D (**52**), were isolated along with the known antibiotic, borophycin (**32**), from the methanol extract of *N. spongiaeforme* var. *tenue* (Banker and Carmeli 1998).

Nostopeptins A (**53b**) and B (**53a**) were isolated from the cultured freshwater *N. minutum* (Okino *et al.* 1997). These cyclic depsipeptides containing 3-amino-6-hydroxy-2-piperidone inhibited elastase and chymotrypsin. Nostopeptins A and B inhibited elastase (IC_{50} 1.3 and 11.0 g/mL, respectively) and chymotrypsin (IC_{50} 1.4 and 1.6 g/mL, respectively), while neither compound inhibited papain, trypsin, thrombin, or plasmin, even at 100 g/mL.



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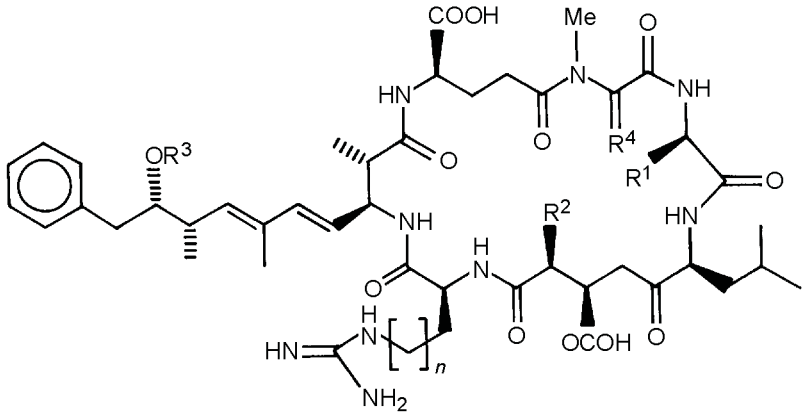

56a–h

	R^1	R^2	R^3	R^4	*n*
56a	Me	Me		CH_2	2
56b	Me	Me	Me	CH_2	2
56c	Me		Ac	CH_2	2
56d	Me		Ac	CH_2	3
56e	Me	Me	Ac	CH_2	2
56f	Me	Me	Ac	CH_2	3
56g	Me	Me	Ac	H, CH_2OH	2
56h	CH_2OH	Me	Ac	CH_2	2

Cyclic heptapeptides, *viz.* nostocyclopeptides A_1 (**54a**), A_2 (**54b**), and A_3 (**54c**), possessing a unique imino linkage in the macrocyclic ring, are characteristic constituents of the cryptophycin-producing cyanobacterium *Nostoc* sp. (Golakoti *et al.* 2001). Studies were carried out on the biosynthesis and on the bio-

logical activity of these cyclic peptides. The nostocyclopeptides **54a** and **54b** showed weak cytotoxicity (IC_{50} 1 mol/L) against human nasopharyngeal carcinoma and human colorectal adenocarcinoma cell lines and were devoid of antifungal activity (*Candida albicans* at 25 g per disc) and antibacterial activity (*Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* at >25 g per disc). None of the compounds displayed significant inhibition of peptidase activity against trypsin, thrombin, and plasmin (IC_{50} <50 mol/L) or against chymotrypsin, elastase, and papain (IC_{50} <25 mol/L) (Golakoti *et al.* 2001).

Some genera of fresh water and brackish cyanobacteria produce potent hepatotoxic cyclic peptides and have been known for more than 20 years (Carmichael 1988; Rinehart *et al.* 1988). Microcystins are hepatotoxic heptapeptides and general tumor promoters produced by several species of the genera *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc*, of which *Microcystis* is the most harmful freshwater bloom-forming cyanobacterium (Kuiper-Goodman *et al.* 1999).

A novel cyclic peptide, nostophycin (**55**), possessing a weakly cytotoxic activity, was isolated together with microcystins from the toxic *Nostoc* sp. (Fujii *et al.* 1999). Nostophycin is composed of six AA, *viz.* D-glutamine, glycine, L-phenylalanine, D-*allo*-isoleucine, 2 mol of L-proline, and a novel -AA, (2*S*,3*R*,5*R*)-3-amino-2,5-dihydroxy-8-phenyloctanoic acid. These results suggest that nostophycins are biosynthetically related to the microcystins, because they have a -AA and two D-AA in common.

Eight cyclic heptapeptide hepatotoxins (**56**) including thee new, *viz.* D-Ser^1,APD^5-microcystin-LR (**56h**), D-Asp^3,APD^5-microcystin-LHar (56d), and APD^5,MeSer^7-microcystin-LR (**56g**) (where APD is 9-acetoxy-3-amino-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, and Har L-homoarginine), were isolated from the *Nostoc* sp., together with four known microcystins (Namikoshi *et al.* 1990). All three new toxins contained 9-acetoxy-3-amino-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid instead of the corresponding 9-methoxy derivative, while compound **56a** contains the corresponding 9-hydroxy analog (Sivonen *et al.* 1992). Compound **56h** is the first microcystin reported that contains D-serine in lieu of the D-alanine unit, which was thought to be an invariable AA component of the microcystins. Compound **56d** has L-homoarginine instead of L-arginine in compound **56c** and D-aspartic acid instead of D-*erythro*-3-methylaspartic acid in compound **56f**. Compound **56g**, the *N*-methylserine variant of the *N*-methyldehydroalanine unit in hepatotoxin (**56g**), would be a biosynthetic precursor of compound **56e**.

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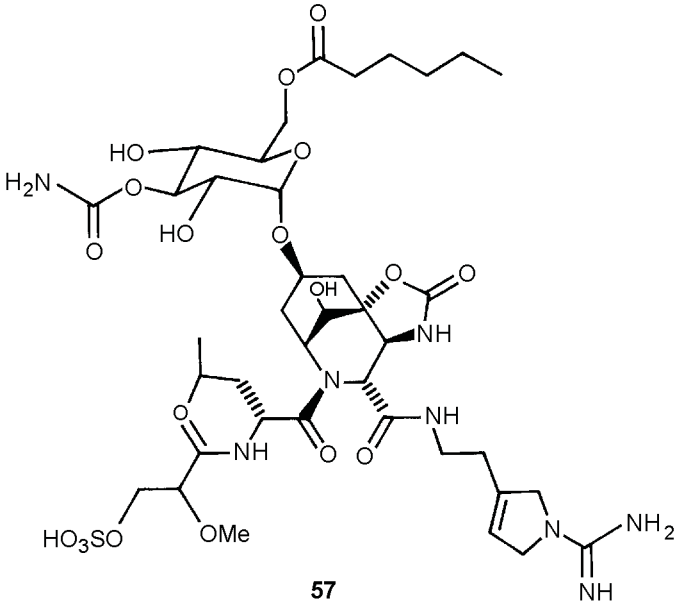
Note added in proof

The identification of the molecular structures of carotenoids was described in some *Nostoc* species (Takaichi *et al*. 2005). The myxoxanthophyll and ketomyxoxanthophyll in *Nostoc* sp. PCC 7120, *N. punctiforme* PCC 73102 and *Nostoc* sp. HK-01 were (3*R*,2′*S*)-myxol 2′-fucoside and (3*S*,2′*S*)-4-ketomyxol 2′-fucoside, respectively. The glycoside moiety of the pigments was fucose, not rhamnose. The major carotenoids were -carotene and echinenone, and the minor ones were -cryptoxanthin, zeaxanthin, canthaxanthin and 3′-hydroxyechinenone. Based on the identification of the carotenoids and the completion of the entire nucleotide sequence of the genome in *N. punctiforme* PCC 73102, we proposed a biosynthetic pathway for the carotenoids and the corresponding genes and enzymes. Since only -carotene ketolase from *N. punctiforme* PCC 73102 have been functionally identified, the other genes were searched by sequence homology only from the functionally confirmed genes. Finally, a phylogenetic relationship was described among some *Nostoc* species, including some newly isolated species.

The fatty acid profile of *Nostoc flagelliforme* (strains FACHB838 and CCAP1453/33), an edible terrestrial cyanobacterium, at different temperatures (*i.e.*, 15, 20, 25 and 30 °C) and developmental stages (hormogonia, filaments, seriate and aseriate) in liquid suspension culture was investigated (Liu *et al*. 2005). The cyanobacterial species could be classified according to the existing taxonomic system based on fatty-acid profiling, due to the presence of 18:3 and the absence of 16:2 and 16:3 fatty acids. Within the temperature range investigated, the content of 18:3 increased at the expense of 18:2 as temperature decreased, while the fatty acid suites remained unchanged. The degree of fatty acid unsaturation also increased with decreasing temperature, with the highest being 1.28 and 1.37 at 15 °C for the strains FACHB838 and CCAP1453/33, respectively. With respect to the effect of developmental stages, there was a slight variation in fatty acid composition and contents in the two strains without changing the fatty acid suite. At the aseriate stage at 25 °C, the highest C_{18} fatty acid proportion amounted to 63.2 and 65.1 % and the degree of fatty acid unsaturation peaked at 1.32 and 1.31 in the strains FACHB838 and CCAP1453/33, respectively, indicating that more long-chain unsaturated fatty acids were accumulated at this stage.

Three new modified peptides, *viz*. banyasin A, banyaside A (**57**) and banyaside B, were isolated from the hydrophilic extract of a natural bloom of the cyanobacterium *Nostoc* sp. (Ploutno and Carmeli 2005). Banyasides A and B are structurally closely related to the cyanobacterial metabolite, suomlide and to the sponge-derived dysinosins. Banyaside A and B were found to be trypsin and thrombin inhibitors.



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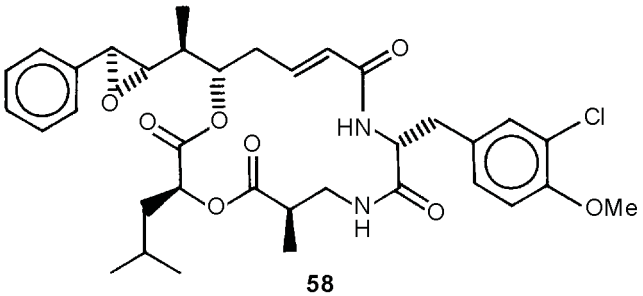
A terrestrial cyanobacterium *Nostoc* sp. strain IO-102-I, producer of the six different microcystins, was isolated from a lichen association (Oksanen *et al*. 2004). The dominant microcystin produced by *Nostoc* sp. strain IO-102-I was the highly toxic [ADMAdda(5)]microcystin-LR, which accounted for *c*. 80 % of the total microcystins. The structure of [DMAdda(5)]microcystin-LR and [D-Asp(3),ADmAdda(5)] microcystin-LR and a partial structure of three new [ADMAdda(5)]-XR type of microcystin variants was assigned. Interestingly, *Nostoc* spp. strains IO-102-I and 152 synthesized only the rare ADMAdda and DMAdda subfamilies of microcystin variants. Phylogenetic analysis demonstrated congruence between genes involved directly in microcystin biosynthesis and the 16S rRNA and *rpoC1* genes of *Nostoc* sp. strain IO-102-I. *Nostoc* sp. strains 152 and IO-102-I are distantly related, revealing a sporadic distribution of toxin production in the genus *Nostoc*. Strain IO-102-I is closely related to *Nostoc punctiforme* PCC 73102 and other symbiotic *Nostoc* strains and most likely belongs to this species. Taken together, this suggests that other terrestrial and aquatic strains of the genus *Nostoc* may have retained the genes necessary for microcystin biosynthesis.

Microcystins produced by cyanobacterial 'blooms' in reservoirs and lakes pose significant public health problems because they are highly toxic due to potent inhibition of protein serine/threonine phosphatases (PPP) (Hastie *et al*. 2005). A dehydrobutyrine (Dhb)-containing microcystin variant [Asp(3), ADMAdda(5), Dhb(7)]microcystin-HtyR isolated from *Nostoc* sp. was found to potently inhibit PP1, PP2A, PPP4 and PPP5 with IC_{50} values similar to those of microcystin-LR. However, in contrast to microcystin-LR, which forms a covalent bond with a cysteine residue in these protein phosphatases, Asp, ADMAdda,

Dhb-microcystin-HtyR did not form any covalent interaction with PP2A. Since the LD_{50} for Asp, ADMAdda, Dhb-microcystin-HtyR was 100 g/kg compared to 50 g/kg for microcystin-LR, the data indicate that the noncovalent inhibition of protein phosphatases accounts for most of the harmful effects of microcystins *in vivo*. A 3-amino-6-hydroxy-2-piperidone-containing cyclic peptide, nostocyclin, also isolated from *Nostoc* sp., was nontoxic and exhibited more than 500-fold less inhibitory potency towards PP1, PP2A, PPP4 and PPP5, consistent with the conclusion that potent inhibition of one or more of these protein phosphatases underlies the toxicity of microcystins, both lacking and containing Dhb.

Cryptophycin-38 (**58**), -326 (**59**), and -327 (**60**) are three new trace constituents of the terrestrial cyanobacterium *Nostoc* sp. GSV 224 (Chaganty *et al.* 2004). Cryptophycin-38 is a stereoisomer of crypto-



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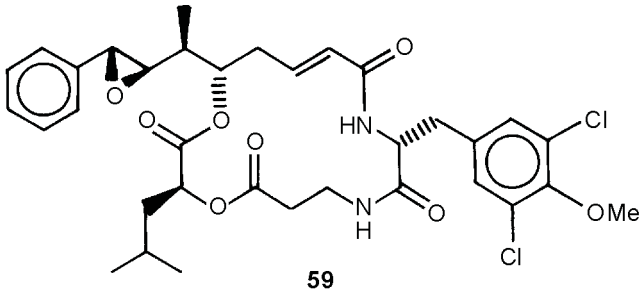

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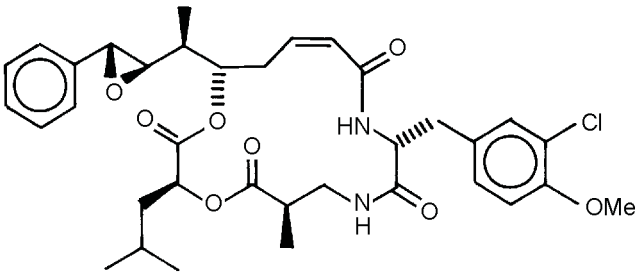

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phycin-1 and to date is the only naturally occurring analogue that possesses a *SS* epoxide group in unit A. Cryptophycin-327 is a geometric isomer that differs from cryptophycin-1 in having a *Z* ^{2}-double bond in unit A. Cryptophycin-326 is related to cryptophycin-21, but has two chlorine atoms *ortho* to the methoxy group in unit B. All three new analogues are weaker cytotoxins than cryptophycin-1 against the human tumor cell line KB (nasopharyngeal).

Compound	Oxirane ring	Double bond
Cry-38 (**58**)	*S*,*S*	*E*
Cry-326 (**59**)	*R*,*R*	*E*
Cry-327 (**60**)	*R*,*R*	*Z*

A novel acidic polysaccharide, nostoflan, was isolated from a terrestrial cyanobacterium, *Nostoc flagelliforme* (Kanekiyo *et al.* 2005). Nostoflan exhibited a potent anti-herpes simplex virus type 1 (HSV-1) activity with a selectivity index (50 % cytotoxic concentration and 50 % inhibitory concentration against viral replication) of 13 000. Sugar composition and methylation analyses revealed that it was mainly composed of →4)-D-Glc*p*-(1→, →6,4)-D-Glc*p*-(1→, →4)-D-Gal*p*-(1→, →4)-D-Xyl*p*-(1→, D-GlcA*p*-(1→, D-Man*p*-(1→ with a ratio of *c.* 1 : 1 : 1 : 1 : 0.8 : 0.2. Two pyridylaminated oligosaccharides were prepared by partial acid hydrolysis and pyridylamination. On the basis of MS and NMR analyses, they were found to

be -D-Glc*p*-(1→4)-D-Xyl-PA and -D-GlcA*p*-(1→6)- -D-Glc*p*-(1→4)-D-Gal-PA. From these results, nostoflan might be mainly composed of the following two types of sugar sequence:

→4)- -D-Glc*p*-(1→4)-D-Xyl*p*-(1→ and →4)-[-D-GlcA*p*-(1→6)]- -D-Glc*p*-(1→4)-D-Gal*p*-(1→.

Besides anti-HSV-1 activity, nostoflan showed potent antiviral activities against HSV-2, human cytomegalovirus, and influenza A virus, but no activity against adenovirus and coxsackie virus was observed. Therefore, nostoflan has a broad antiviral spectrum against enveloped viruses whose cellular receptors are saccharides. Furthermore, nostoflan showed no antithrombin activity, unlike sulfated polysaccharides.

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