

Off-flavours in water: hydroxyketones and β -ionone derivatives as new odour compounds of freshwater cyanobacteria

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ABSTRACT: Cyanobacteria essentially contribute to the off-flavours of drinking water and freshwater fish. They are primary nuisance microorganisms and their control in lakes and ponds used for the production of drinking water and fish farming is expensive. We describe new odour compounds of freshwater cyanobacteria that are fermentation products and norcarotenoids. These may contribute to the off-flavours frequently observed with cyanobacteria. The fermentation products were represented by 2,3-pentandione, 2,3-hexandione, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone and 2,3-hexandiol. These compounds are important for odour quality in microbially-processed dairy food, caused by the metabolism of fungi and heterotrophic bacteria. Their cyanobacterial synthesis was shown by ^{13}C -labelling, growth kinetics and biotransformation experiments. The labelled mass spectra of the hydroxyketones showed unequivocally the cyanobacterial rather than bacterial origin of the compounds. Norcarotenoids of the β -ionone-type (β -ionol, β -ionone-5,6-epoxide, dihydro- β -ionone, dihydro- β -ionol, tetrahydroionone, 4-oxo- β -ionone and 2,4,4-trimethyl-3-(3-oxobutyl)-2-cyclohexen-1-one) were another important group of compounds that were found in axenic cyanobacterial cultures and a monoxenic culture of *Phormidium* sp. The results implicate the presence of further oxygenases in cyanobacteria. Except for tetrahydroionone, the syntheses of the β -ionone derivatives were established by biotransformation experiments applying the precursor β -ionone. The norcarotenoid 6-methyl-5-hepten-2-one, which is regarded as a precursor compound of 1,3,3-trimethyl-2,7-dioxabicyclo(2,2,1)heptane (TDH), was ^{13}C -labelled, while TDH did not show any labelling. There is evidence that TDH arises from the analytical procedure and is an artefact rather than a biogenic compound produced by the cyanobacteria. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: drinking water; fish; off-flavour; cyanobacteria; volatile organic compounds; 2,3-diketones; hydroxyketones; norcarotenoids; β -ionone derivatives; 1,3,3-trimethyl-2,7-dioxabicyclo(2,2,1)heptane

Introduction

Cyanobacteria have been found to be major sources of off-flavours in drinking water and fish from aquaculture.¹ The irregular monoterpene 2-methylisoborneol (2-MIB) and the irregular sesquiterpene geosmin are primarily responsible for imparting earthy-musty odours to water and fish. Consumers can easily detect both compounds in the pico- to nanomolar concentration range because of their particularly low odour threshold concentrations.² Most consumer complaints about drinking water are caused by these compounds³ and channel catfish that bioaccumulate these compounds become unacceptable to consumers, which causes severe economic losses.^{4,5}

Effective management of surface waters that are sources of drinking water and are used for aquaculture is necessary to control the development of cyanobacteria. Costly

precautions are necessary to reduce the bloom formation of planktonic cyanobacteria⁶ and the formation of cyanobacteria biofilms in the littoral zone of lakes⁷ and exposed canals.⁸

Many off-flavours other than geosmin and 2-MIB have been reported to be produced by cyanobacteria. Lipooxygenase products, such as oct-1-en-3-one and oct-1-en-3-ol, cause a mushroom-like odour; unsaturated aldehydes exhibit a strong, unpleasant rancid odour; and norcarotenoids, such as β -cyclocitral and β -ionone, give a more pleasant odour.⁹ Another group of off-flavour compounds that can be characterized as catabolites of amino acids (4-cresol, indol, skatol) has been reported in natural samples of cyanobacteria,¹⁰ but since these collected samples also contained heterotrophic bacteria, which organism is actually producing these well known bacterial fermentation products is still open to question. Axenic cultures of cyanobacteria and the application of tracers are both necessary to prove unequivocally the sources of these compounds.

In this study we describe the formation of new fermentation products and report on new flavour active norcarotenoids of cyanobacteria.

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Materials and Methods

Origin and Cultivation of Cyanobacteria

The origin, cultivation conditions and ^{13}C -labelling of the axenic cyanobacterial strains *Calothrix parietina* (PCC 6303), *Calothrix* sp. (PCC 7507), *Plectonema notatum* (PCC 6306) and *Plectonema* sp. (PCC 7410) have been described recently.¹¹ The ^{13}C -labelled cultures were analysed for volatile organic compounds (VOCs) after growth periods of 2 (PCC 7507), 3 (PCC 6306 and 7410) and 4 months (PCC 6303). The monoxenic cultures represented by *Phormidium* sp., *Rivularia* sp. and *Tolypothrix distorta* were isolated from cyanobacterial biofilms from Lake Zurich (Switzerland). Unlike the axenic cultures, *Rivularia* sp. was cultivated in BG 11 medium with a Na_2CO_3 concentration of $3.8\ \mu\text{M}$ at $24\ ^\circ\text{C}$ and $5\ \mu\text{mol/m}^2/\text{s}$ irradiance from fluorescent tubes.

Analysis of VOC

Closed-loop stripping, thermodesorption and analysis by gas chromatography–mass spectrometry (GC–MS) (Fison Instruments, GC 8000 Top, MD 800) were performed as described.¹² For separation and identification of the VOCs, a chemically bound fused silica capillary column (DB-1301, $30\ \text{m} \times 0.32\ \text{mm}$ i.d., film thickness $0.25\ \mu\text{m}$; J&W Scientific, Folsom, USA) was used. The pressure of the carrier gas helium was $50\ \text{kPa}$ and the gas flow of the split $15\ \text{ml/min}$. The GC temperature programme was $4\ \text{min}$ at $0\ ^\circ\text{C}$ and then $5\ ^\circ\text{C/min}$ to $250\ ^\circ\text{C}$. Electron impact mass spectra (EI–MS) were recorded in the range $m/z\ 29$ – 550 . For positive chemical ionization (PCI), the electron beam of the MS was set at $350\ \text{eV}$, the detector voltage at $400\ \text{V}$, and isobutane was used as the reactant gas. 2-Hydroxy-3-hexanone and 3-hydroxy-2-hexanone were separated on a capillary column DB-FFAP ($30\ \text{m} \times 0.32\ \text{mm}$ i.d., film thickness $0.25\ \mu\text{m}$; J&W Scientific, Folsom, USA) according to Schröder and co-workers.¹³

Production of Hydroxyhexanones by Biotransformation

Biotransformation of 2,3-hexandione was used to synthesize the corresponding hydroxyhexanones. Standing cultures of *Plectonema* sp. were supplemented with $0.34\ \text{mM}$ 2,3-hexandione. After 4 months the cultures were harvested, filtered and the hydroxyketones were extracted with *tert*-butylmethyl ether (tBME). The ether phase was dried with anhydrous Na_2SO_4 and the solvent removed with a vacuum rotary evaporator at $25\ ^\circ\text{C}$. The residue was dissolved in tBME, diluted 1:100 in MeOH and purified on a HPLC system [Shimadzu 10AVP system, with a photodiode array detector and a reversed-phase

column (C-18 Grom-Sil 120 ODS-4 HE, $4.6 \times 250\ \text{mm}$, $5\ \mu\text{m}$ particle size; Stagroma, Germany)]. The flow rate of the solvent was $1\ \text{ml/min}$. The applied solvent gradient was a linear increase from 50% to 100% MeOH in 15 min. The hydroxyhexanone fraction was eluted using a retention time of 5.2–5.7 min.

Biotransformation of 2,3-Pentandione and β -Ionone

2,3-Pentandione ($0.4\ \text{mM}$) was added to a standing culture of *Plectonema* sp. The VOC analysis by GC–MS was performed after 4 months. To determine the hydroxypentanones, the ions $m/z\ 45$ and $m/z\ 59$ were extracted; 3-hexanone ($m/z\ 57$) served as internal standard. For the biotransformation of β -ionone, a $0.093\ \text{mM}$ solution was used in a standing culture of *Phormidium* sp. For analysis of the VOC, $0.5\ \text{ml}$ of the culture medium was analysed by GC–MS. To calculate the increase or decrease of the compounds in the analysed media, the fragment ions $m/z\ 177$ (β -ionone), $m/z\ 119$ (β -ionol), $m/z\ 43$ (dihydro- β -ionone), $m/z\ 57$ (dihydro- β -ionol) and $m/z\ 163$ (4-oxo- β -ionone) were extracted and integrated; 2-ethylhexanol ($m/z\ 57$) served as internal standard.

Growth Kinetics of the Hydroxyhexanones

The formation of 2,3-hexandione and hydroxyhexanones were determined in a standing culture of *Plectonema* sp. The cyanobacterium was cultivated in a 2 l Erlenmeyer flask containing 1 l of cyanobacterial suspension: $50\ \text{ml}$ of the suspension was removed weekly and analysed by closed-loop stripping and GC–MS.

Reference Compounds

Standard compounds of β -ionol and dihydro- β -ionol were obtained upon reduction of β -ionone and dihydro- β -ionone, respectively, with NaBH_4 in ethanol. 4-Oxo- β -ionone was synthesized after the method of Becher and co-workers,¹⁴ and the tetrahydroionones were synthesized by hydrogenation of β -ionone with hydrogen over palladium. 1,3,3-Trimethyl-2,7-dioxabicyclo(2,2,1)heptane (TDH) and dihydro- β -ionone were gifts from Givaudan (Dübendorf, Switzerland). The sources of the other compounds have been described previously.¹⁵

Results

The axenic cyanobacteria *Plectonema* and *Calothrix* formed dense biofilms on the bottom of Erlenmeyer flasks when grown without shaking. The VOC produced

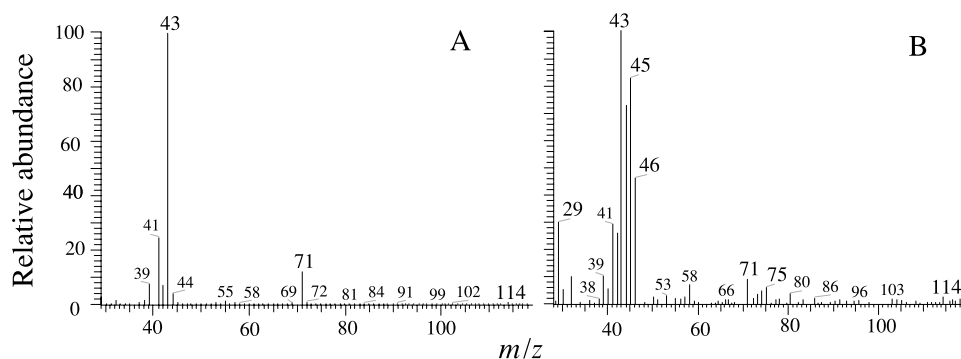


Figure 1. EI-MS of 2,3-hexandione isolated from an unlabelled (A) and a ^{13}C -labelled (B) culture of *Plectonema* sp.

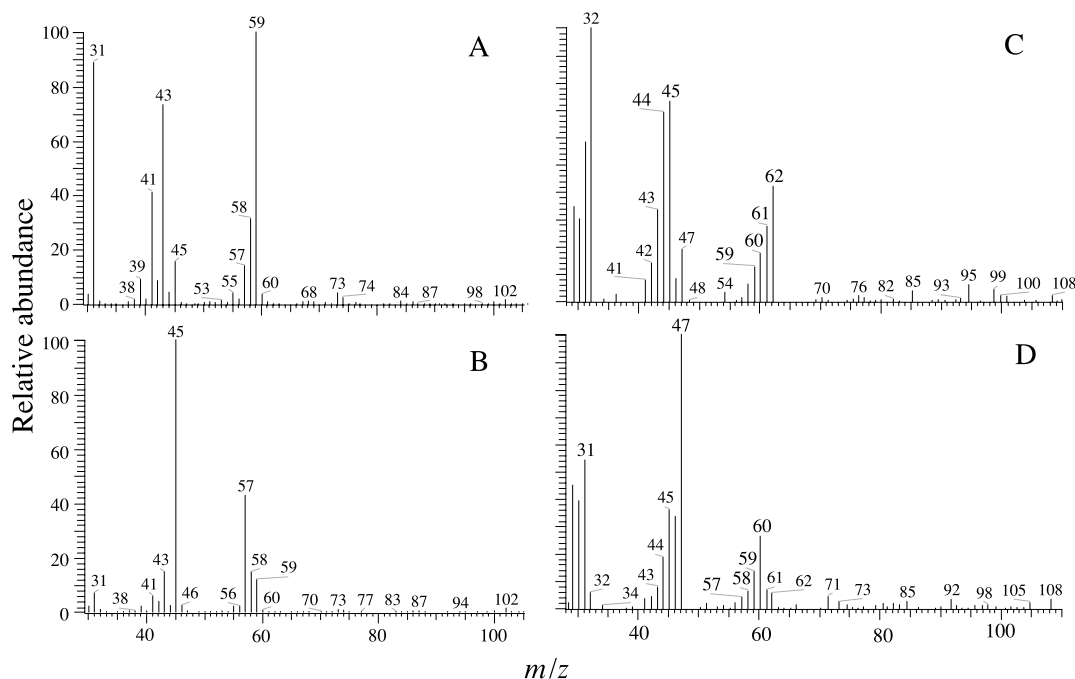


Figure 2. EI-MS of 3-hydroxy-2-pentanone (A, C) and 2-hydroxy-3-pentanone (B, D) isolated from an unlabelled and ^{13}C -labelled (C, D) culture of *Plectonema* sp.

by these biofilms were determined by GC-MS and compared with reference compounds. In the medium of both *Plectonema* strains and *C. parietina*, the fermentation products 2,3-pentandione, 2,3-hexandione (Figure 1), 2-hydroxy-3-pentanone (Figure 2A), 3-hydroxy-2-pentanone (Figure 2B), 2-hydroxy-3-hexanone and 3-hydroxy-2-hexanone were found (Table 1). The 2-hydroxy-3-pentanone and 3-hydroxy-2-pentanone were eluted with retention times of 13.52 min and 13.86 min, respectively, and their mass spectra were identical to those reported in the literature.^{16,17} In contrast to the hydroxypentanones, the hydroxyhexanones eluted as a single peak on the capillary column DB-1301. However, a base peak separation was achieved on a DB-FFAP capillary column.¹³ Under EI-ionization mode, the molecular ions were not visible, but under PCI-ionization quasi-

molecular ions at m/z 117 were obtained. The EI-MS were identical to those of 2-hydroxy-3-hexanone and 3-hydroxy-2-hexanone, published recently.¹³

The time course of 2,3-hexandione, 2-hydroxy-3-hexanone and 3-hydroxy-2-hexanone formation in a standing culture of *Plectonema* sp. is presented in Figure 3. In freshly inoculated cultures the concentrations of VOCs were very low, but after an incubation time of 30 days 2,3-hexandione and hydroxyhexanones were formed. The capacity of *Plectonema* sp. to biotransform 2,3-diones to the corresponding hydroxyketones in a mineral medium was used to produce the latter compounds, which are commercially unavailable. The hydroxyhexanones obtained by the biotransformation experiment were identical to the hydroxyhexanones observed in the untreated cultures. The low volatility of

Table 1. Fermentation products and norcarotenoids of filamentous cyanobacteria

VOC	M	Rt (min)	PCC 6303	PCC 7507	PCC 6306	PCC 7410	<i>Phormidium</i> sp.	<i>T.</i> <i>distorta</i>	<i>Rivularia</i> sp.
Acetate pathway									
2-Butanone	72	6.27	–	–	–	+	–	–	–
2-Pentanone	86	8.72	–	–	+	+	–	×	–
2-Pentanol	88	9.88	–	–	+	+	–	×	–
2,3-Pentandione	100	9.24	+	–	+	+	–	–	–
3-Hydroxy-2-pentanone	102	13.52	+	–	+	+	–	–	–
2-Hydroxy-3-pentanone	102	13.86	+	–	+	+	–	–	–
2,3-Hexandione	114	11.90	+	–	+	+	–	–	–
3-Hydroxy-2-hexanone	116	16.34	+	–	+	+	–	–	–
2-Hydroxy-3-hexanone	116	16.34	+	–	+	+	–	–	–
2,3-Hexandiol	118	20.17				×			
Isoprenoid pathway									
6-Methyl-5-hepten-2-one	126	18.69	+		+	+	×	×	×
6-Methyl-5-hepten-2-ol	128	19.26	+		+	+	–	–	–
2,6,6-Trimethylcyclohexanone	140	19.77	+	–	+	+	–	–	×
TDH	142	15.41	0	0	0	0	×	–	–
β -Cyclocitral	152	26.45	+	–	+	+	–	–	×
β -Cyclogeraniol	154	26.12	–	–	+	+	–	–	–
2-Hydroxy-2,6,6-trimethylcyclo-hexan-1-one	156	23.31	+	–	+	+	×	–	×
Dihydroactinidiolide	180	36.69	+	–	+	+	×	×	×
β -Ionone	192	33.97	+	–	+	+	×	×	×
Dihydro- β -ionone	194	32.61	+	–	+	+	×	–	×
Dihydro- β -ionol	196	32.76	–	–	×	×	×	–	–
Tetrahydroionone	196	32.52	–	–	–	–	×	–	–
β -Ionone-5,6-epoxide	208	34.36	+	+	+	+	×	×	×
4-Oxo- β -ionone	206	39.69	–	–	×	×	×	–	–
2,4,4-Trimethyl-3-(3-oxobutyl)-2-cyclohexen-1-one	208	40.10	–	–	–	–	×	–	–

The axenic cultures (*Calothrix*, PCC 6303, PCC 7507; and *Plectonema*, PCC 7410, PCC 6306) were ^{13}C -labelled. VOCs showing ^{13}C -labelling are indicated by (+) and no labelling by (0). For monoxenic cultures of *Phormidium* sp., *Rivularia* sp. and *T. distorta* and unlabelled axenic cultures, VOCs are indicated by an (×). If VOCs were not detected, this is indicated by (–). M, molecular mass; Rt, retention time on a DB-1301 capillary column.

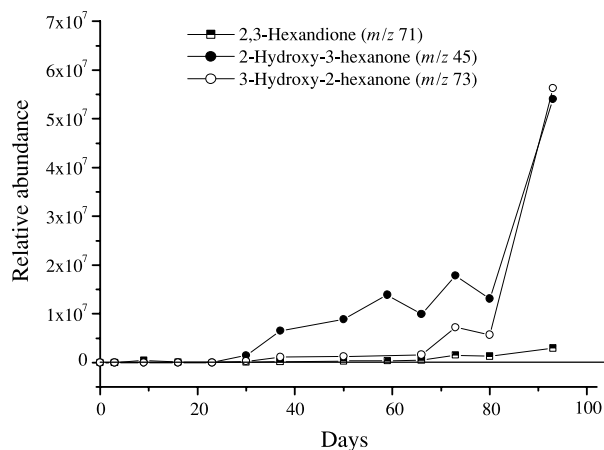


Figure 3. Formation of 2,3-hexandione (m/z 71), 2-hydroxy-3-hexanone (m/z 45) and 3-hydroxy-2-hexanone (m/z 73) in a standing culture of *Plectonema* sp.

the diols did not allow the separation of 2,3-hexandiol by closed-loop stripping, but this compound was found in the tBME extract of the medium of the biotransformed culture. A similar biotransformation experiment demonstrated the formation of both ketols from 2,3-pentandione.

After an incubation time of 4 months, *Plectonema* sp. had produced 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone. Interestingly, the fermentation product 2-butanone was found, but 2-butanol and 2,3-butandione were missing (Table 1).

Phormidium sp. isolated from a cyanobacterial biofilm in the littoral zone of Lake Zurich was a particularly rich source of norcarotenoids. Strains of *Plectonema* and *C. parietina* produced a variety of β -ionone derivatives (Table 1), as did *Phormidium* sp. Reference and synthesized compounds were used for confirmation of the structures. A typical gas chromatogram, which was obtained from a culture of *Phormidium* sp., is presented in Figure 4. Dihydro- β -ionol, tetrahydroionone and 4-oxo- β -ionone were detected by their mass spectra and were confirmed with reference compounds. In contrast to the compound obtained by chemical synthesis, only one stereochemical form of tetrahydroionone was found in the cultures. 2,4,4-Trimethyl-3-(3-oxobutyl)-2-cyclohexen-1-one was tentatively identified by its similarity to EI-MS data published by Pasqual and co-workers.¹⁸

Phormidium sp. was used as a biocatalyst to transform β -ionone into different norcarotenoids (Figure 5). During growth, the β -ionone concentrations in the culture steadily decreased, while the concentrations of dihydro- β -ionone

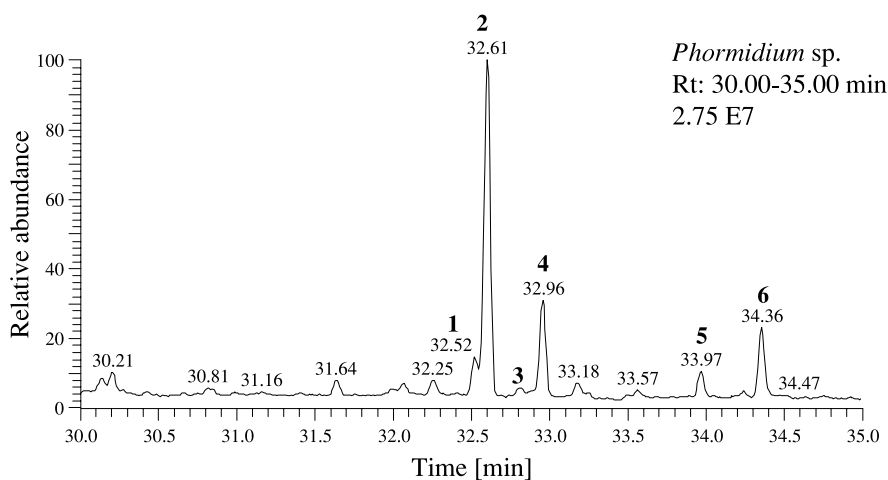


Figure 4. Gas chromatogram of VOCs isolated from a culture of *Phormidium* sp. (1, tetrahydroionone; 2, dihydro- β -ionone; 3, dihydro- β -ionol; 4, *E*-geranylacetone; 5, β -ionone; 6, β -ionone-5,6-epoxide)

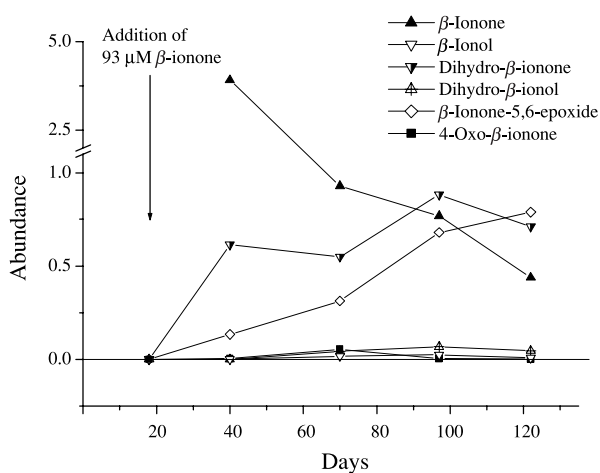


Figure 5. Kinetics of β -ionone and its biotransformation products in a culture of *Phormidium* sp.

and β -ionone-5,6-epoxide increased. β -Ionol, dihydro- β -ionol, 4-oxo- β -ionone and 2,4,4-trimethyl-3-(3-oxobutyl)-2-cyclohexen-1-one were present, but did not accumulate to higher concentrations.

The gas chromatograms obtained from the analysed cyanobacterial cultures indicated that, in addition to cyanobacterial VOCs, compounds that were typical contaminants and artefacts were present. To demonstrate the biogenic origin of the compounds, ^{13}C -labelling experiments were performed. Cyanobacteria take up hydrogen carbonate ($\text{H}^{13}\text{CO}_3^-$) by photosynthesis, incorporate the ^{13}C into their metabolites and release ^{13}C -labelled VOCs. Except for the natural ^{13}C -ratio, compounds of anthropogenic sources should be free of any labelling. Strongly ^{13}C -labelled VOCs (Table 1), such as the 2,3-diketones (Figure 1), the hydroxyketones (Figure 2) and the norcarotenoids (Figure 6), were obtained but TDH exhibited no labelling (Figure 6).

TDH was present in varying amounts in the standing culture of *Plectonema* sp., while the concentrations of 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol (data not shown) increased. This was in contrast to the results observed in the biotransformation experiment with the *Phormidium* sp. cultures. Neither TDH nor 6-methyl-5-hepten-2-one accumulated. In addition, the corresponding alcohol, 6-methyl-5-hepten-2-ol, was not present. In the ^{13}C -labelling experiment of the *Calothrix* sp. culture, 6-methyl-5-hepten-2-one was not labelled. This indicates that this compound may not necessarily be of biogenic origin but could also be introduced from unknown anthropogenic sources (Table 1).

Discussion

In this study we focused on the VOC analysis of different strains of filamentous cyanobacteria forming biofilms in the littoral zone of freshwater lakes. We describe for the first time fermentation products, e.g. hydroxyketones, and a number of norcarotenoids as new compounds in cyanobacteria. Hydroxyketones have been reported previously in the metabolism of bacteria and fungi. Using labelled axenic cultures of cyanobacteria, we proved unequivocally that these photoautotrophic microorganisms are producers of hydroxyketones. Heterotrophic microorganisms, which are frequently regarded as potential sources of such fermentation products, are ruled out by these experiments. This is ecologically important because of the occurrence of heterotrophic microorganisms in natural cyanobacterial biofilms. Further, we demonstrated the capacity of cyanobacteria to synthesize hydroxyketones in the light. We assume their formation is an analogue reaction (α -acetolactic acid to acetoin), as described for lactic acid bacteria.¹⁹ In our investigations the cyanobacteria released hydroxyketones with a chain

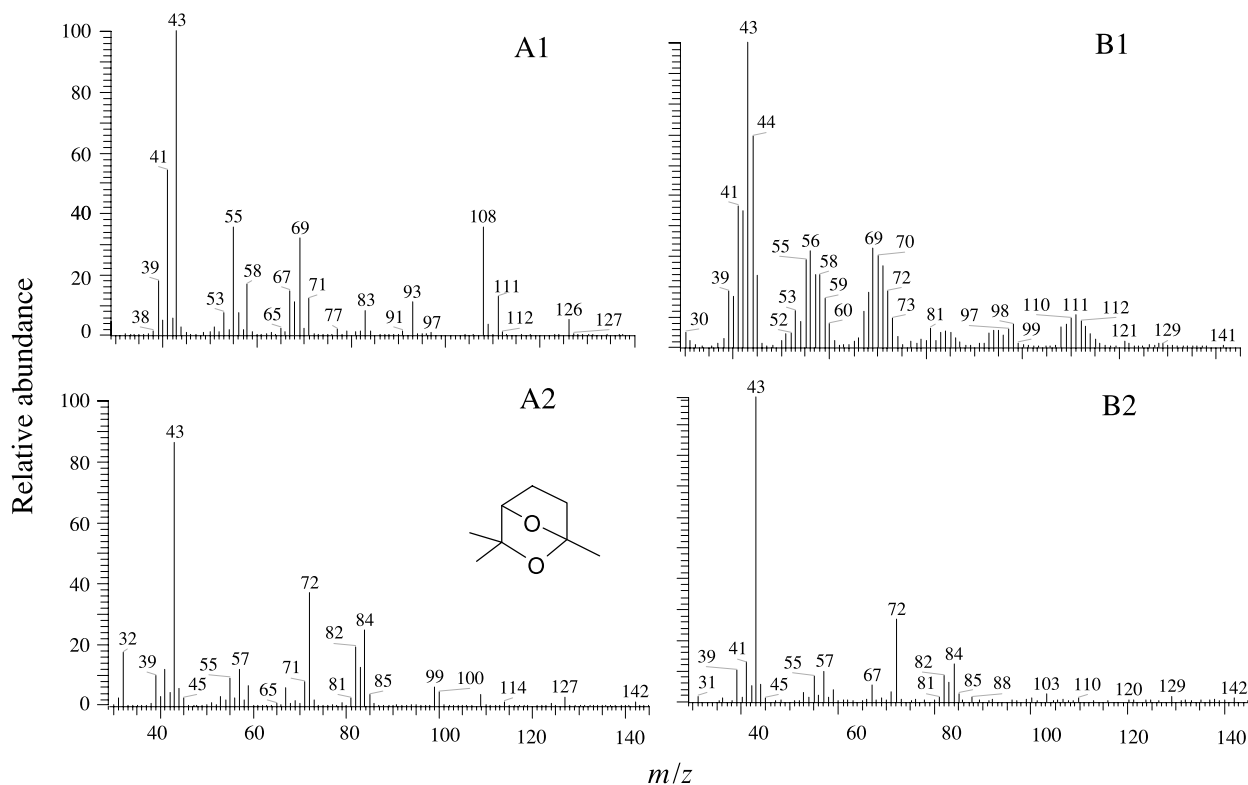


Figure 6. EI-MS of 6-methyl-5-hepten-2-one (A1, B1) and TDH (A2, B2). EI-MS of A1 and A2 are isolated from the same unlabelled and B1 and B2 from the ^{13}C -labelled culture of *Plectonema* sp.

length of five and six carbon atoms in their growth medium, but interestingly, acetoin was not found. Hydroxyketones have been analysed in the flavours of fermented food, particularly, in wine,²⁰ beer,²¹ yoghurt,²² etc. and contribute essentially to the odour quality of dairy food. For example, hydroxypentanones smell similar to butter, caramel and hay, while hydroxyhexanones exhibit earthy, mushroom- and hay-like odour qualities.²³

In dairy products, hydroxyketones are accompanied by their corresponding 2,3-diketones, which contribute to the flavour. In the cyanobacterial cultures investigated, we also found these compounds but there was no accumulation of the 2,3-diketones. This can be explained by the results of the biotransformation experiment, which showed an efficient reduction of 2,3-diketones to the corresponding hydroxyketones in the presence of light. In fungi and microorganisms this process is catalysed by alcohol dehydrogenase²⁴ and/or diacetyl reductase.²⁵ Alcohol dehydrogenase is thought to exist in cyanobacteria.²⁶ A further reduction to 2,3-hexandiol was also found in the biotransformation experiments. However, the reduction can not be catalysed by either of the two enzymes mentioned above. A glycerol dehydrogenase was found to be responsible for such a transformation in *Saccharomyces cerevisiae*²⁴ and, therefore, a similar enzyme may exist in cyanobacteria.

Recently, the ecological role of hydroxyketones as infochemicals has been established. Sakai and co-workers²⁷ described for the first time 2-(*S*)-hydroxy-3-octanone and 2-(*S*)-3-(*R*)-octandiol as components of the sexual pheromone of the grape borer (family Cerambycidae). 3-(*R*)-Hydroxy-2-hexanone was discovered as a male sexual pheromone in the old house borer, *Hylotrupes bajulus*,^{13,28} and the longhorn beetle, *Anaglyptus subfasciatus*.²⁹ Further components of the sexual pheromones of these beetles were 2,3-hexandione and 2-(*S*)-3-(*R*)-hexandiol.^{12,27} Since insect larvae populate the cyanobacterial biofilms in high densities under natural conditions, these compounds may be responsible for the observed attractions of mosquitos to cyanobacterial biofilms.³⁰

Norcarotenoids are another important group of odour compounds released by cyanobacteria. They are degradation products of carotenes and carotenoids, and carotene oxygenases are responsible for their formation. Cyanobacteria have been shown to be a rich source of these norcarotenoids.¹¹ The oxygenase reaction was first described in the cyanobacterium *Microcystis*. The enzyme catalysed the cleavage reaction of β -carotene to β -cyclocitral and crocetindial.³¹ The present study extends the spectrum of norcarotenoids that cyanobacteria can synthesize. We particularly observed derivatives of β -ionone by combinations of different oxygenation

reactions. Recently, the existence of β -ionone oxygenase was shown in higher plants also.³² Our results demonstrate the biological synthesis of norcarotenoids, in contrast to their abiotic formation induced by heat and light.³³ In general, ionones exhibit pleasant odours and they are found in many flowers, such as *Osmanthus fragrans* and species of *Rosa* and *Viola*.³⁴ Therefore, they are important and widely used in industrial production. However, their ecological role has not been well established.

We studied the origin of the 6-methyl-5-hepten-2-one and TDH in more detail. Both norcarotenoids are derived from oxygenation reactions of straight-chain carotenes. 6-Methyl-5-hepten-2-one was first described as a metabolite in fungi, but was subsequently detected in nearly every VOC analysis of plant matter. In addition, it was also found in the pheromones of insects. TDH was first described as a mandibular gland constituent in ants.³⁵ Subsequently it was described as a constituent of the Granny Smith apple aroma, and 6-methyl-5-hepten-2-one was proposed as a potential precursor.³⁶ Since TDH was never ¹³C-labelled in our labelling experiments while 6-methyl-5-hepten-2-one was labelled, we regard this compound as an artefact of the analytical procedure, rather than as a biogenic compound. The occurrence of organic compounds with biogenic structures in biological matter does not demonstrate a biogenic origin *per se*.

Odour problems in drinking water frequently occur in episodes which, in most cases when biogenic sources are involved, coincide with the mass development of cyanobacteria in the water body from which the raw water is taken. In many cases sensory and chemical analyses have demonstrated a close connection between consumer complaints and the occurrence of geosmin.³⁷ Other odour compounds with different odour qualities have also been described and were applied to an odour wheel to facilitate the description of odours observed in water.³⁸ While derivatives and isomers of geosmin and 2-MIB have not been reported to date, these other odour compounds occur in complex mixtures representing different homologues, isomers and enantiomers. The large number of compounds observed in the chromatograms do not allow a particular compound to be traced readily as the source of the odour quality observed.

The same is valid for freshwater fish, which can take up excreted cyanobacterial odour compounds from the water or incorporate them as biomass-bound odour compounds through bioaccumulation. The basic investigation by Yurkowski and Tabachek³⁹ demonstrated the importance of cyanobacteria for the musty-earthy odour of freshwater fish.⁴⁰ In aquaculture particularly, where high densities of phytoplankton are observed, off-flavours have been reported to taint channel catfish,⁴ trout⁴¹ and Nile tilapia.⁴² Frequently, geosmin and 2-MIB were determined to be the causative agents, but other, as yet

unknown compounds were also obviously present in the fish flesh.

The odour compounds of cyanobacteria described for the first time in this investigation can behave in a similar way to geosmin and can enter the bioaccumulation pathway. There are reports that fish become tainted under various environmental conditions, but the responsible compounds have yet to be determined. The new odour compounds of this investigation should be included in such a search for the causative agents.

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