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VOLATILE COMPOUNDS OF THE CYANOPHYCEAE — A REVIEW

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ABSTRACT

A relationship between blue-green algae and off-flavours in water was reported as early as 1883. Continuing research has shown that two metabolites, geosmin and methylisoborneol are major contributors to unpalatable flavours in water and aquatic organisms. Many instances of the co-occurrence of these two compounds and dense blooms of blue-green algae have been recorded. Cultures of Anabaena, Lyngbya, Oscillatoria, and Symploca species have been shown to produce geosmin or methylisoborneol while blooms of Aphanizomenon, Anabaena, Microcystis, Oscillatoria, and Gomphosphaeria have been found in water containing geosmin or the odour of this compound. Actinomycetes have also been shown to produce these two compounds.

In addition to geosmin and methylisoborneol, there is evidence that several other blue-green algal metabolites contribute to aquatic taste and odour problems. Among them is β -cyclocitral which has a distinctive tobacco flavour. Blue-green algae produce a variety of organic compounds including hydrocarbons, fatty acids, aromatics, ketones, terpenoids, amines and sulfides which could contribute to the over-all flavour of water and aquatic organisms.

KEYWORDS

Blue-green algae, geosmin, methylisoborneol, β -cyclocitral, hydrocarbons, aromatics, fatty acids, amines, sulfur compounds, terpenoids, esters, tastes, odours, water.

INTRODUCTION

Tastes and odours in water are a worldwide problem which probably existed well before reports began appearing in the scientific literature. Farlow (1883) recognized that blue-green algae were responsible for tastes and odours in water. Since then many other reports (cf. Persson, 1981) have been presented which relate the occurrence of unpalatable flavours in water and fish with the presence of blue-green algae. The odour of water contaminated by blue-green algae is generally described as "muddy" or "earthy". However, other odours produced by several species of blue-green algae have been described (Table 1). In addition,

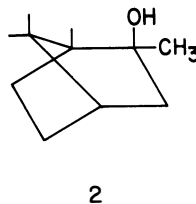
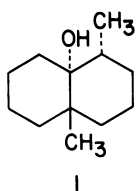
odours described as "grassy", "geranium", "vile", "moldy", "sewage", and "nasturtium" have been reported for members of the genera *Anabaena*, *Aphanizomenon* (Tracy and Leonard, 1980; Montiel, 1979), *Cyclidospermum*, *Gomphosphaeria*, *Microcystis*, *Nostoc*, *Oscillatoria*, and *Rivularia* (Montiel, 1979). It has not been demonstrated that these odours are characteristic of either individual species or entire genera of these blue-green algae.

TABLE 1 Odours Produced by Blue-Green Algae¹

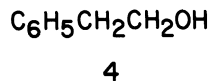
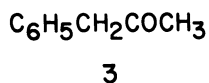
ALGA	ODOUR
<i>Oscillatoria</i> cf. <i>acutissima</i> Kufferath	Mild Cabbage
<i>O. salina</i> Biswas	Grass
<i>Lyngbya</i> cf. <i>martensiana</i> Meneghini	Aldehyde-Ketone
<i>Aphanizomenon</i> cf. <i>flos-aquae</i> (Linnaeus) Ralfs	Mild odour
<i>Diplocystis aeruginosa</i> (Kutzing) Trevis	Grass
<i>L.</i> cf. <i>martensiana</i> Schkovaalov	Cabbage

¹ Tabachek and Yurkowski, 1976.

The two compounds most frequently identified as causing off-flavours in water are geosmin (1) and 2-methylisoborneol (2). These two compounds are produced by actinomycetes (Gerber, 1968, 1969) and blue-green algae (Table 2) and are so potent [threshold odour concentration 10-200 ng/l, Persson (1980)] that very little is required to cause a problem.



In addition to taste and odour problems caused by geosmin (1) and methylisoborneol (2) it is possible that the combination of various metabolic or degradation products of blue-green algae may interact to impair water quality. For example, phenylacetone (3) and 2-phenylethanol (4) are considered to add to the muddy taste of water in the presence of geosmin or 2-methylisoborneol (Montiel, 1979).



ALIPHATIC HYDROCARBONS

Several hydrocarbons ranging from C_{14} to C_{20} were isolated from cells of *Anacystis nidulans* grown in laboratory cultures (Oro and colleagues, 1967). The main

Table 2 Blue-Green Algae as Sources of Geosmin and 2-Methylisoborneol

Geosmin	2-Methylisoborneol
<u>Symploca muscorum</u> ^{1,2}	
<u>Oscillatoria tenuis</u> ^{2,3}	<u>Oscillatoria tenuis</u> ⁴
<u>O. prolifica</u> ³	<u>O. curviceps</u> ⁴
<u>O. cortiana</u> ³	<u>Lyngbya cryptovaginata</u> ³
<u>O. variabilis</u> ³	
<u>O. agardhii</u> ³	
<u>O. splendida</u> ³	
<u>Lyngbya aestuarii</u> ³	
<u>Anabaena scheremetievi</u> ⁴	
<u>A. circinalis</u> ⁵	
<u>Schizothrix muelleri</u> ⁶	

- ¹ Safferman and coworkers, 1967. ² Medsker and coworkers, 1968.
³ Tabachek and Yurkowski, 1976. ⁴ Izaguirre and coworkers, 1982.
⁵ Narayan and Nunez, 1974. ⁶ Kikuchi and coworkers, 1974. (This bloom was slightly contaminated with actinomycetes and bacteria.)

components of the hydrocarbon fraction were *n*-heptadecane, *n*-pentadecane and a monounsaturated heptadecane although minor amounts of *n*-hexadecane, *n*-octadecane, and branched hydrocarbons (2,6,10,14-tetramethylpentadecane and 2,6,10,14-tetramethylhexadecane) were also detected. Han and coworkers (1968a) investigated the hydrocarbon fraction from the blue-green algae species Nostoc muscorum and Anacystis nidulans and found essentially the same compounds in the Anacystis nidulans as did Oro and coworkers (1967). However, Nostoc muscorum yielded a significant amount of two branched C₁₈ hydrocarbons which were identified (Han and coworkers, 1968b) as a 1:1 mixture of 7- and 8-methylheptadecanes (species producing this mixture are indicated in Table 2). These workers extended their investigations to include two other species, Phormidium luridum and Chlorogloea fritschii. Their results also indicated that *n*-heptadecane was the major component of the hydrocarbon fraction, and in addition they identified another branched hydrocarbon, 4-methylheptadecane, which has also been found in cultures of Microcystis aeruginosa and M. wesenbergii (Jüttner, 1976). These results are of biological interest since methyl-branched alkanes have not been found in photo-synthetic or non-photosynthetic bacteria (Han and coworkers, 1968b) and have been reported for only one species (Scenedesmus quadricauda) of green algae (Stransky and coworkers, 1968).

The predominance of *n*-heptadecane in the hydrocarbon fraction appears to be a characteristic of the Cyanophyceae since this compound is the main hydrocarbon produced by the majority of these organisms which have been specifically investigated for this class of compound (Table 3). Only three of the species thus examined did not have *n*-heptadecane as the main component. These were Coccochloris elabens and Agmenellum quadruplicatum (Winters and coworkers, 1969) and Anacystis montana (Gelpi and coworkers, 1970) for which the main hydrocarbons were C₁₉:1 and C₂₇:1, respectively.

Table 3 Blue-green Algae Producing n-Heptadecane as the Main Component of the Hydrocarbon Fraction

<u>Anacystis cyanea</u> ^{1,6}	<u>Microcystis wesenbergii</u> ⁵
<u>Anacystis nidulans</u> ^{1,2,3}	<u>Nostoc muscorum</u> ^{1,2,4,6}
<u>Chlorogloea fritschii</u> ⁴	<u>Oscillatoria williamsii</u> ²
<u>Chroococcus turgidus</u> ^{1,6}	<u>Phormidium luridum</u> ⁴
<u>Lyngbya aestuarii</u> ^{1,6}	<u>Plectonema terebrans</u> ²
<u>Lyngbya lagerheimii</u> ^{2,6}	<u>Spirulina platensis</u> ¹
<u>Microcoleus chthonoplastes</u> ²	<u>Trichodesmium erythaeum</u> ²
<u>Microcystis aeruginosa</u> ⁵	

¹ Gelpi and coworkers, 1970; Nostoc sp. not identified. ² Winters and coworkers, 1969. ³ Oro and coworkers, 1967. ⁴ Han and coworkers, 1968b. ⁵ Jüttner, 1976. ⁶ These species also produce 8-38% of the hydrocarbon fraction as 7- and 8-methylheptadecane.

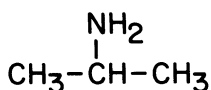
None of the foregoing investigations indicated hydrocarbons smaller than C₁₄, however, n-decane, n-undecane, n-tridecane and n-pentadecane have been isolated from cultures of Cyanidium caldarium (Jüttner and Wurster, 1979). As the hydrocarbons isolated from blue-green algae do not have pronounced tastes or odours, their effect on water quality is probably not significant.

FATTY ACIDS

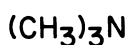
Saponification of the lipid fraction of Anacystis nidulans Drout gave hexadecanoic acid (47%) and hexadecenoic acid (44%) as the main fatty acid components with octadecenoic acid (2-10%) as a minor constituent (Holton and coworkers, 1964). The location of the double bond in these and other unsaturated fatty acids was not specified. This data was very similar to that reported by Oro and coworkers (1967) for Anacystis nidulans, but as more species of Cyanophyceae were examined, it was found that although the C_{16:0} and C_{16:1} acids were the main components, a number of organisms contained appreciable quantities (up to 26%) of di- and tri-unsaturated C₁₈ acids (Parker and coworkers, 1967; Holton and coworkers, 1968; Schneider and coworkers, 1970). The major fatty acids of these organisms range from C_{16:0} to C_{18:3}, however, small amounts of C_{12:0}, C_{14:0}, C_{14:1} acids have been obtained from several species of blue-green algae (Parker and Leo, 1965; Parker and coworkers, 1967; Holton and coworkers, 1964) and in the case of one bloom of Trichodesmium erythaeum, 50% of the fatty acid fraction comprised decanoic acid (Parker and coworkers, 1967). Earlier work by Mazur and Clarke (1942) had indicated that 20% of the saturated, and 26% of the unsaturated fatty acids were present in Gloeotrichia echinulata in the free form. However, it was suggested that partial hydrolysis of the lipid fraction had occurred during air drying of the cells. Although these bound fatty acids are not immediately available to affect water quality, they would tend to be released as the algae decomposed. Some of the lower fatty acids, such as butyric and hexanoic acid, have very disagreeable tastes and odours and while the higher fatty acids may not be sufficiently volatile to influence the smell of water, it is possible that they could affect the taste.

AMINES

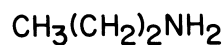
The only species of blue-green algae which has been investigated specifically for amines is *Microcystis aeruginosa* (Herrmann and Juttner, 1977). Only 2-aminopropane (5) was identified although trimethylamine (6), 1-aminopropane (7), and ethanolamine (8) were found in a culture of the golden-brown alga *Ochromonas danica*, and butanolamine (9) was detected in water contaminated with a species of the green alga *Chlamydomonas* (Herrmann and Juttner, 1977).



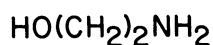
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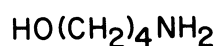
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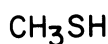
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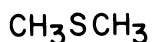
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SULFUR CONTAINING COMPOUNDS

Methyl mercaptan (10), dimethylsulfide (11) [which has a definite fishy odour (Tracy and Leonard, 1980)], and dimethyldisulfide (12) have been detected only in old cultures of *Oscillatoria chalybea* and *Microcystis flos-aquae* (Jenkins and coworkers, 1967). These compounds were considered to be formed during bacterial degradation of the algae, an opinion that was supported by the formation of small amounts of dimethylsulfide on treatment of *Oscillatoria agardhii* Gom, *Aphanizomenon flos-aquae* Rolfs, and *Nodularia spumigena* Merritt with alkali (Granroth and Hattula, 1976). However, an actively growing fresh culture of *Microcystis flos-aquae* did produce isopropyl mercaptan (13), but this seems to be a unique case (Jenkins and coworkers, 1967).



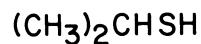
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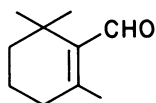
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TERPENOID COMPOUNDS

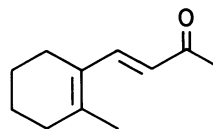
Terpene formation by natural populations of *Aphanizomenon flos-aquae*, *Phormidium uncinatum* and *Microcystis aeruginosa* and pure cultures of *M. aeruginosa* was reported by Sirenko and Sakevich (1967) although no compounds were identified. However, the monoterpene β -cyclocitral (14), which has a tobacco-like odour, was isolated from a bloom of *Microcystis wesenbergii* and a laboratory culture of *M. aeruginosa* (Juttner, 1976). Other terpenoid compounds from a culture of *Cyanidium caldarium* include 6-methylhept-5-en-2-one (15), β -ionone (16), geranylacetone (17), 1,2-dihydro-1,1,6-trimethylnaphthalene (18), and 6-(but-2-enylidene) trimethylcyclohex-1-ene (19) (Juttner, 1979). The structural similarities of compounds 14, 16, 18, 19 and 15, 17 suggest common precursors for these algal products.



14



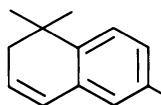
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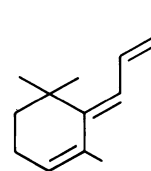
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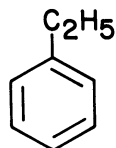
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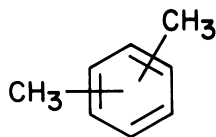
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AROMATIC COMPOUNDS

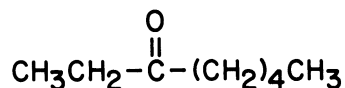
Further examination of volatiles from *Cyanidium caldarium* indicated that in addition to compounds 15-19 the following were also produced: ethylbenzene (20), *o*-, *m*-, *p*-xylene (21), *p*-dichlorobenzene (23), and naphthalene (24) as well as octan-3-one (22) (Juttner, and Wurster 1979). Compounds 20-24 are relatively volatile and would be expected to contribute to tastes and odours in water and aquatic life.



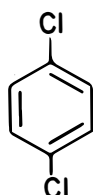
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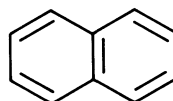
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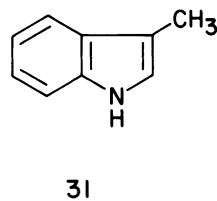
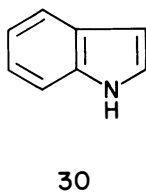
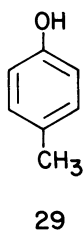
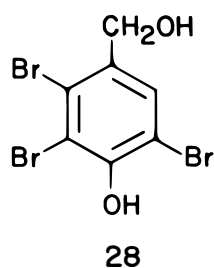
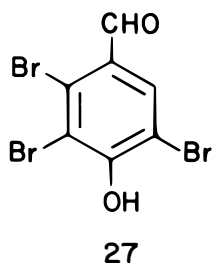
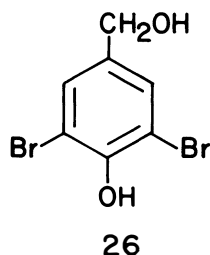
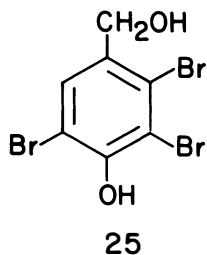


23



24

p-Dichlorobenzene appears to be the only chlorinated compound detected in a culture of blue-green algae. However, other halogenated metabolites such as the bromophenols (25-28) have been isolated from a culture of *Calothrix brevis* (Pedersen and Da Silva, 1973). *p*-Cresol (29), indole (30) and 3-methylindole (31) together with geosmin and methylisoborneol have been isolated from river water contaminated with an *Oscillatoria* sp. (Tsuchiya and coworkers, 1980). (This information was obtained from an abstract of the original Japanese report and it is not clear if these compound were also produced by a pure culture of the alga).



ESTERS

Two new products of blue-green algae which have been identified as attractants for beetles of the family Carabidae are the methyl esters of hexadecanoic acid and 9-octadecenoic acid (Evans, 1982). These compounds were isolated from mats of *Oscillatoria animalis* Agardh and *O. subbrevis* Schmidle growing on the beach of a saline lake in western Saskatchewan (Canada). The two esters were described as having a "strong odour".

ETIOLOGY OF OFF-FLAVOURS

The muddy flavour in rainbow trout (*Salmo gairdneri*) in several lakes in western Canada was traced to the presence of geosmin (Yurkowski and Tabachek, 1974). However, a similar taste problem in several fish varieties in Cedar Lake, Manitoba

was found to be caused by both geosmin and methylisoborneol (Yurkowski and Tabachek, 1980). In neither of the foregoing instances was the organism producing these compounds identified. Since most water bodies contain a variety of microorganisms it is difficult, if not impossible, to decide which is causing a problem. However, there have been many reports over the years (cf. Persson, 1981) that unpalatable tastes in fish and water appear shortly after the formation of dense blooms of blue-green algae. In a study of the muddy flavour of bream (Abramis brama) Persson (1979) established that there was a correlation between the increase in the biomass of Oscillatoria agardhii and the detectability of the muddy taste in fish. This was again substantiated by a later study (Persson, 1980, 1981) which also indicated no relationship between the flavour of fish and the quantity of actinomycetes. A similar correlation between the presence of geosmin in water and blooms of Anabaena, Aphanizomenon, Microcystis, Oscillatoria and Gomphosphaeria has also been reported by Cees and coworkers (1974).

CONCLUSION

The main contributors to the undesirable qualities of water and aquatic life are considered to be geosmin and methylisoborneol because of their potency and probably because they are target compounds in any investigation of off-flavours. However, it is clear that the Cyanophyceae produce a variety of volatile organic compounds many of which (for example, β -cyclocitral) could contribute to the deterioration of water quality. There is ample evidence to implicate the blue-green algae as important sources of tastes and odours in water and aquatic life. In addition, as stated by Tracey and Leonard (1980) "when environmental conditions are right, Anabaena and Aphanizomenon can produce surface covering blooms in a matter of several days" and quickly reduce the palatability of water. The ability of many species of blue-green algae to undergo this rapid proliferation with concurrent release of the very potent compounds geosmin and methylisoborneol, as well as many other odourous compounds, would explain why these organisms have such a devastating effect on water and aquatic species.

ACKNOWLEDGEMENT

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