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HIGHLIGHT

Production and role of volatile halogenated compounds from marine algae†

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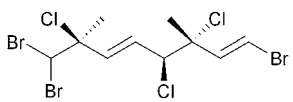
Marine organisms are an important source of volatile halogenated natural products. Marine algae, in particular, contribute significantly to the global budget of halogenated hydrocarbons that play an important role in climate functioning. However, despite the large amounts of halogenated metabolites from algae, we know relatively little about their function in the producing organism. In this *Highlight*, we discuss the current knowledge of volatile halogenated compounds from algae, with a focus on biosynthesis, algal physiology and chemical ecology. We also briefly discuss geochemical aspects arising from the release of halogenated natural products from micro- and macroalgae.

1 Introduction

Ever since the pioneering work by R. E. Moore, volatile halogenated products from marine algae have attracted the interest of marine researchers from multiple disciplines.[1] It was recognized early on that a single algal species could be responsible for a highly diverse assortment of volatile halogenated metabolites. For example, the edible odoriferous red seaweed *Asparagopsis taxiformis* releases a complex mixture of more than 120 halogenated metabolites containing less than five carbons in the longest chain.[2–4] Although relatively simple volatile terpenes have been recognized as halogenated algal natural products, halogenated monoterpenes such as the polyhalogenated **1** were first discovered in the digestive gland of the sea hare *Aplysia californica*.[5] Shortly afterwards, it was identified that the red alga *Plocamium pacificum* was the true source of **1** and other related polyhalogenated metabolites, thereby suggesting that the sea hare sequesters these halogenated metabolites from the algae for its own chemical defense.[6]



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The aim of this *Highlight* is to discuss aspects of biosynthesis, algal physiology, and chemical ecology of algal metabolites together with geochemical aspects. We have excluded cyanobacteria-derived metabolites, which are often of high molecular weight, but instead refer the reader to several comprehensive reviews.[7–10] We aim to provide a picture of the function of volatile halogenated metabolites for the producing alga and their role as bulk metabolites in the oceans. For further reading on specific topics we can recommend overviews on the biosynthesis of halogenated natural products,[11–13] on mechanistic aspects of halogenating enzymes[14] and on general aspects of marine natural products of environmental relevance,[15] as well as reviews on halogenated metabolites from brown algae[16] and red algae.[2]

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2 Occurrence and biosynthesis of volatile halogenated metabolites from algae

The production of halogenated metabolites is a common feature of marine micro- and macroalgae. Brown, green, and red seaweeds produce a variety of halogenated metabolites from different pathways including halomethanes, short-chain hydrocarbons, terpenes, and phenols.[17] Out of all the marine seaweeds, red algae (Rhodophyceae) possess the highest abundance of unique biosynthetic pathways for organohalogen production.[2] In contrast, microalgae are predominant producers of halomethanes, and only few structurally more complex halogenated metabolites have been reported from phytoplankton. In this section, we give an overview of the most important classes of volatile halogenated metabolites from algae, while more comprehensive compilations of halogenated algal metabolites can be found in dedicated reviews.[2,16,18]

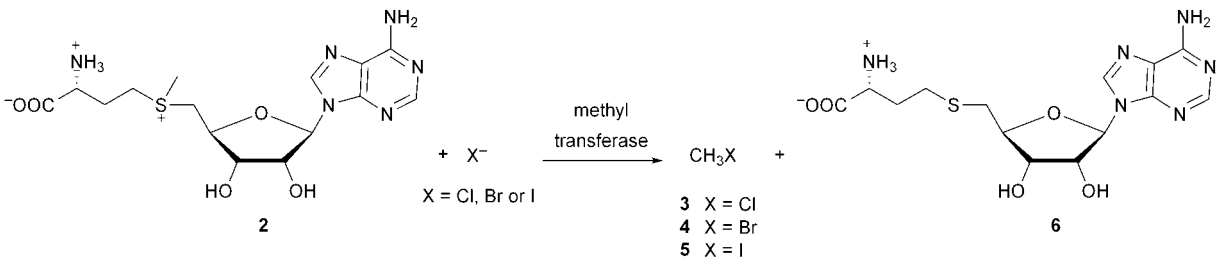
2.1 Halomethanes

Halomethane production is ubiquitously observed in marine algae. Chlorinated, brominated and iodinated halomethanes, as well as mixed structures, have been reported in screenings. In addition, the degree of halogenation is varied, with many reported examples of methyl halides, dihalomethanes, haloforms and carbon tetrahalides.[1,17–20] Besides the more complex halogenated metabolites, single species of red algae often produce highly

diverse mixtures of volatile halomethanes that include $CHBr_3$, $CHBr_2I$, $CHBrI_2$, CHI_3, $CHBr_2Cl$, $CHClBrI$, CH_2Br_2, CH_2BrI, CH_2I_2, and CBr_4.[1,2] In a survey of Arctic macroalgae, it was observed that brown and green algae generally exhibited a higher release of these organohalogens compared to red alge.[21] Bromoform was the predominant metabolite isolated from brown and green algae, reaching production rates of up to four μg per g of algal biomass per day.[20] Microalgae are also effective producers of this metabolite, and the subsurface maximum of bromoform observed in the tropical eastern Atlantic Ocean can be attributed to a phytoplanktonic source.[22,23] In general, the ability to produce halocarbons is widespread in unicellular algae. A recent survey of phytoplankton cultures consisting of three different classes revealed that diatoms, coccolithophorids and a chlorophyte all produce chloromethane, bromomethane and bromoform in differing quantities.[24] Marine plankton belonging to the Cryptophyceae, Dinophyceae, Prasinophyceae and Prymnesiophyceae, as well as cyanobacteria, have also been reported as halomethane producers.[25–27]

The biosynthesis of methyl chloride, methyl bromide and methyl iodide **3–5** is generally carried out with methyl transferases. These enzymes methylate nucleophilic halide anions by employing *S*-adenosyl-L-methionine **2** (SAM) as the methyl donor (Scheme 1).[28–30] The crystal structure of a plant SAM-dependent halide methyltransferase has given further insight into the halogenation mechanism in *Arabidopsis thaliana*.[31] A model for substrate/nucleophile binding and reaction at the active site has revealed that the reactive sulfonium methyl group orients into a large cavity. This seems to be the reason for the observed promiscuous nature with respect to a variety of nucleophiles. The enzyme promotes reaction most efficiently with thiocyanate, compared to the halides.[31] Experiments with a partially purified methyl transferase from the microalga *Pavlova pinguis* revealed that a single enzyme can transfer chloride, bromide and iodide to SAM.[32] Methyl halide transferases have a surprisingly low affinity towards halides, which is reflected by their high Michaelis–Menten constants.[30,33] However, this might be compensated by relatively high halide concentrations in algal tissue compared to low concentrations of SAM. It also has to be noted that relative amounts of the respective halomethanes detected in seawater and in the atmosphere do not necessarily correlate with the total number of biosynthesized molecules, because abiotic transformations such as nucleophilic substitution reactions can readily transform methyl halides.[34]

The biosynthesis of polyhalomethanes such as $CHCl_3$ or CH_2Br_2 is catalysed by haloperoxidases (HPOs).[35] These enzymes can contain iron or vanadate as co-factors, and are categorized on the basis of the most electronegative halide that can be oxidized. Thus, chloroperoxidases (ClPO) oxidize chloride, bromide and iodide, while iodoperoxidases (IPO) only



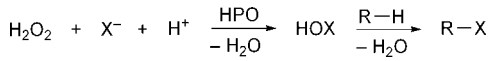
Scheme 1 Biosynthesis of methyl halides employing a methyl halide transferase.

Carsten Paul

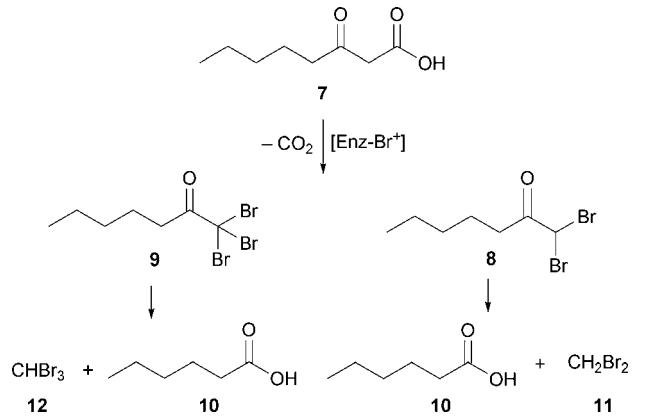
Carsten Paul (born 1983) graduated in 2008 from the Friedrich Schiller University in Jena with a diploma in chemistry (environmental chemistry). After a short-term internship with Dr. Paul Jensen at Scripps Institution of Oceanography in La Jolla, USA, he returned to Jena where he was awarded a graduate fellowship by the Jena School for Microbial Communication (JSMC). He is currently completing his Ph.D. studies under the guidance of Professor Pohnert. His research interests are chemically mediated interactions of microalgae with bacteria and other microalgae.

Georg Pohnert

Georg Pohnert obtained his Ph.D. at the University of Bonn on the pheromone chemistry of brown algae. In 1997 he started as a postdoc at Cornell University, where he studied the E. coli *phenylalanine receptor site. He then moved to the Max-Planck-Institute for Chemical Ecology, where he started his independent research on algal defense reactions. In 2005, he was appointed as professor at the Ecole Polytechnique Fédérale de Lausanne. In 2007 he accepted a Lichtenberg professorship and chair of bioorganic analytics at the Friedrich Schiller University in Jena. Currently his research interests focus on the chemical ecology of micro- and macroalgal chemical communication and the oxylipin chemistry of defense reactions.*



Scheme 2 Simplified haloperoxidase reaction with organic substrates.



Scheme 3 Biosynthesis of polyhalomethanes.

oxidize iodide. Only the central aspects of haloperoxidase chemistry are mentioned herein, but there are several reviews dealing in detail with enzymatic properties, reaction mechanisms and distribution of haloperoxidases.[13,14,35,36] The most dominant class of haloperoxidases among marine algae are bromoperoxidases (BrPO), which are mostly vanadium-dependent, and have been found in all types of algae including Chlorophyta,[37,38] Rhodophyta,[39,40] Phaeophyta,[41–43] and Bacillariophyta.[44] Vanadium- and iron-dependent ClPO, on the other hand, are most commonly found in fungi,[45] while no vanadium-dependent ClPO has yet been characterized from algae. Recently it was shown that marine prokaryotes also have the genetic potential for vanadium-dependent ClPO production.[46] During the haloperoxidase reaction, H_2O_2 is used to oxidize halide anions by a two-electron oxidation yielding the corresponding hypohalous acid. This reactive X^+ intermediate can then be used to halogenate electron-rich organic substrates (Scheme 2). In algae, the formation of dibromomethane **11** and bromoform **12** proceed through the precursor 3-ketooctanoic acid **7** (Scheme 3). Following its decarboxylation, bromination yields the intermediates 1,1-di- **8** and 1,1,1-tribromo-2-heptanone **9**. These are then subjected to non-enzymatic hydrolysis, yielding the respective halomethanes and hexanoic acid **10**.[40,47] The release of oxidized halogen species by extracellular BrPO, and subsequent reaction with dissolved organic matter in seawater, may represent an alternative mechanism for the formation of polyhalomethanes.[33]

2.2 C_2–C_9-halogenated hydrocarbons

In macroalgae, the production of longer chain-length halogenated hydrocarbons is typically observed. Halogenated alcohols, acetaldehydes and acetones as well as mono- and polyhalogenated ethanes, propanes, butanes and pentanes have been reported from red and brown algae.[48–50] The red alga *Asparagopsis taxiformis* is a prolific resource for these metabolites as well as for the production of unsaturated and otherwise functionalized hydrocarbons.[2] A selection illustrating the high structural variability is given in Fig. 1.[1–4] However, to date, no

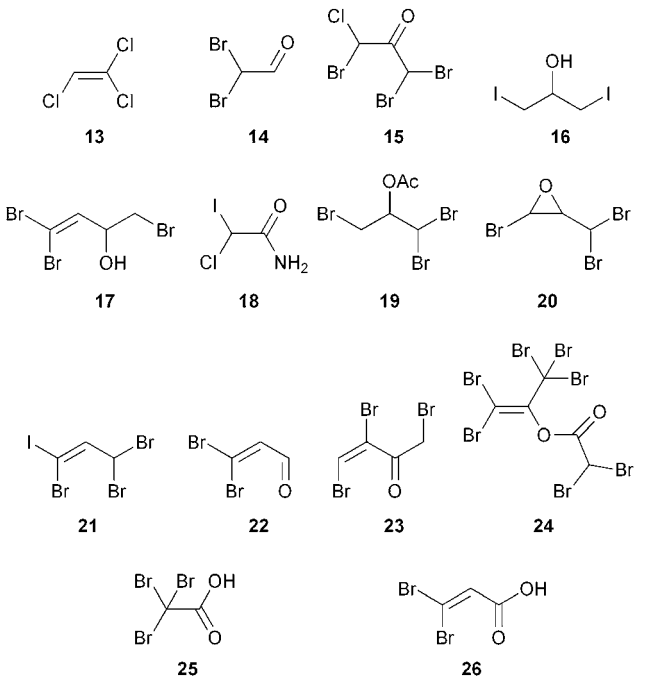
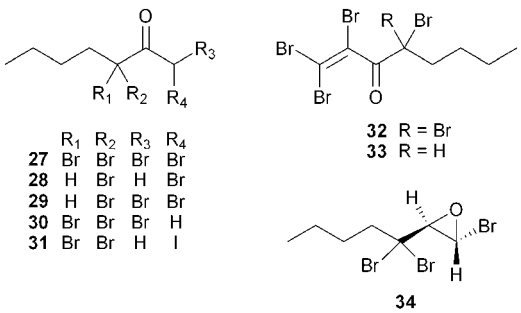


Fig. 1 Short-chained algal halocarbons representing a high variability of chemical functionalities.

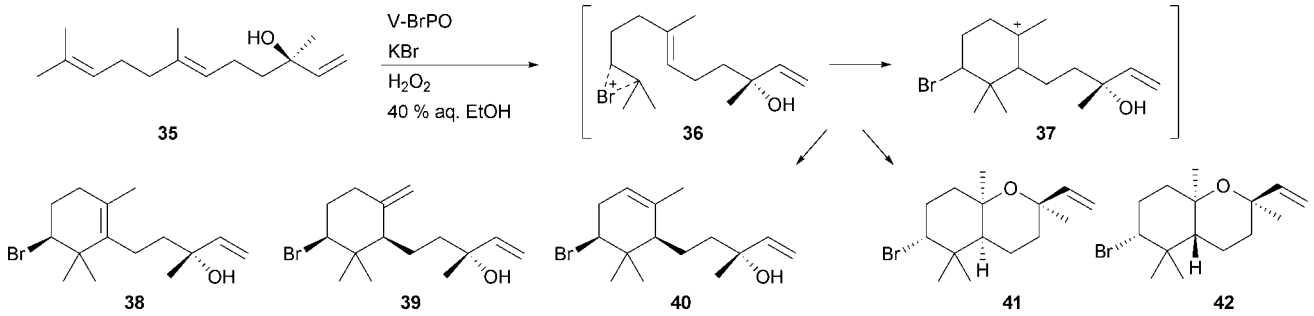
experiments on the biosynthesis of these metabolites have been reported, but it can be rationalized that rather unspecific HPOs are involved in key steps leading to this high structural diversity.

Besides these small molecules, functionalized hydrocarbons with longer chain length are also produced by macroalgae. A well-studied example are the red algae of the genus *Bonnemaisonia*, which produce polyhalogenated heptan-2-ones **27–31**, 1-octen-3-ones **32** and **33**, and the halogenated epoxide **34**.[51] Labeling experiments showed that these metabolites are derived from acetate and that labeled palmitate is also incorporated with a high percentage rate.[52] Due to the ambiguous results, however, only a tentative connection to fatty-acid biochemistry can be drawn, because it cannot be clarified whether anabolic or catabolic processes are involved in the formation of heptanone and heptenone derivatives. Polyhalogenated octenones have been detected from another Bonnemaisoniaceae species, *Delisea fimbriata*, which is also a source of halogenated lactones (see Section 4.2).[53]



2.3 Halogenated terpenes

Many red algae are producers of halogenated terpenes, and only a few of these metabolites have been reported from other groups

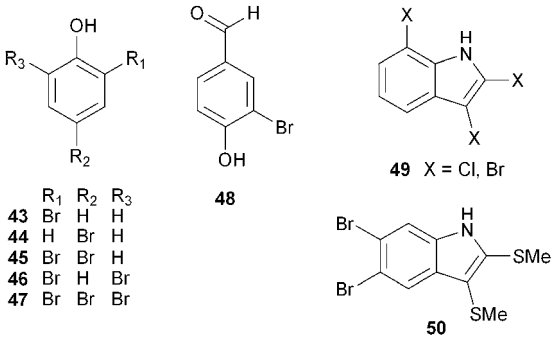


Scheme 4 Proposed bromonium-initiated cyclization in terpene biosynthesis.[55,56]

of algae. The structures of most algae-derived halogenated terpenes can be rationalized on the basis of well-known principles of terpene biosynthesis with additional steps involving halogen peroxidases as formal cation donors.[35,54] Acyclic terpenes can be formed by electrophilic reaction of X^+ with electron-rich carbon centers of the isoprene subunits (Scheme 4). The resulting cations can then react with additional halogen anions to give multiply-halogenated products. X^+ ions can also initiate the biosynthesis of cyclic terpenes, which involves additional enzymatic or non-enzymatic internal cyclization reactions of cationic intermediates (Scheme 4).[55,56] This principle has been demonstrated using purified BrPOs from the red algae *Corallina officinalis*, *Laurencia pacifica*, and *Plocamium cartilagineum*.[56] When (*E*)-(+)-nerolidol **35** reacts with BrPO in the presence of bromide and hydrogen peroxide, a mixture of bromoether, bromoalcohol, bromohydrin, and epoxide species is produced. Among them are the natural snyderols **38–40** and 3β-bromo-8-*epi*-caparrapi oxides **41** and **42** (Scheme 4). The application of these simple biosynthetic principles allows the formal construction of hundreds of cyclic and noncyclic chlorinated and brominated terpenes found in the red algae.

2.4 Halogenated aromatic metabolites

Macroalgae are also known for their production of halogenated aromatic metabolites. Due to the electrophilic character of the chemical equivalent of X^+, which is generated by XPO, these enzymes play the central role in the bromination of aromatic metabolites. Simple halogenated aromatic metabolites are very common, which was illustrated by a screening of 49 species of marine red, green and brown macroalgae for the key seafood flavor components 2- and 4-bromophenol **43** and **44**, 2,4- and 2,6-dibromophenol **45** and **46**, and 2,4,6-tribromophenol **47**. All five bromophenols were found in 62% of samples, four in 32% of the samples, and three in the remaining 6% of samples. In most cases, **47** was found as the dominant metabolite.[57] The biosynthesis of these bromophenols was addressed using the green alga *Ulva lactuca*. Crude cell extract containing BrPO transformed 4-hydroxybenzoic acid to **47**.[58] 3-Bromo-4-hydroxybenzaldehyde **48** is produced from tyrosine in a chloroplast-enriched fraction of the red alga *Odonthalia floccosa*.[59] Halogenated indoles **49** and **50** with strong antifungal activity have been detected in several red algae as well.[60,61] Most other halogenated aromatic metabolites from algae, like diiodotyrosine or halogenated phloroglucinols and phenols, are of higher molecular weight and are not within the scope of this review.[16]



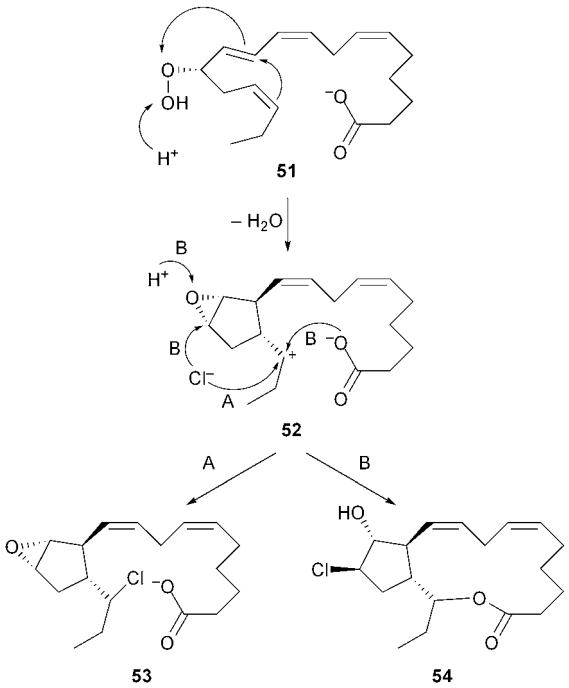
	R_1	R_2	R_3
43	Br	H	H
44	H	Br	H
45	Br	Br	H
46	Br	H	Br
47	Br	Br	Br

2.5 Oxylipins

Halogenated oxylipins are rarely found in marine algae. These metabolites arise from the initial oxidation of unsaturated fatty-acids by lipoxygenases. The resulting intermediate fatty-acid hydroperoxides may be transformed in reactions involving a nucleophilic attack of a halide. Chlorinated fatty-acid-derived metabolites named egregiachlorids A–C (*e.g.*, egregiachlorid A **53**) were isolated from the brown alga *Egregia menziesii*.[62] Even though the biosynthesis was not explored in detail, a pathway was hypothesized that involves the initial oxidation of stearidonic acid to a hydroperoxy fatty-acid **51** by a 13-lipoxygenase, cyclization to the cyclopentyl cation **52** and subsequent nucleophilic attack by a chloride anion yielding the chlorinated oxylipins **53** and **54** (Scheme 5). A related pathway was later suggested for several other C_{18} oxylipins from the brown alga *Eisenia bicyclis*.[63] Recently, a new enzymatic halogenation mechanism *via* a hydroperoxide halolyase was established in the marine diatom *Stephanopyxis turris* (Scheme 6).[64] This diatom can transform C_{20} fatty-acids such as eicosapentaenoic acid **55** with a lipoxygenase to form hydroperoxide intermediates such as **56**. Cleavage of the intermediate is presumably assisted by a nucleophilic attack of a chloride anion, yielding chlorinated octadienes **57** and **58** and (5*Z*,8*Z*,10*E*)-12-oxo-5,8,10-dodecatrienoic acid **59**. Evidence for enzyme participation is given by the high enantiomeric excess of the optically active chlorinated product **57**.[64]

3 Geochemical impact of halogenated metabolites from algae

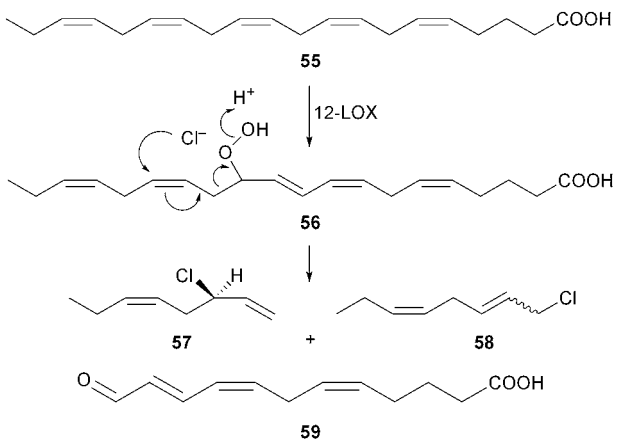
Natural and anthropogenic emissions of halogenated compounds have attracted much attention due to their role in atmospheric chemistry. Halocarbons or inorganic halogens such as iodine influence the radiation budget of the earth and the



Scheme 5 Postulated pathway for fatty-acid-derived halogenated oxylipins.[63]

oxidation power of the atmosphere. The major impact of these compounds on atmospheric chemistry can be seen in their contribution to tropospheric and stratospheric ozone depletion.[65] In the polar regions, the impact of natural halogenated volatiles may be more significant than in other regions of the world due to their close proximity to the major zone of ozone destruction. In consequence a lot of research activity focused on investigation of algal halocarbon emissions from Arctic and Antarctic regions.[66,67]

Brown algae belonging to the order Laminariales are strong accumulators of inorganic iodide. These algae represent a major pump in the global biogeochemical cycle of iodine, and are among the major source of iodocarbons in the coastal atmosphere.[68]



Scheme 6 Halolyase-mediated transformation of eicosapentanoic acid to chlorinated octadienes.[64]

Certain volatile halocarbons can be clearly attributed to natural production while others are exclusively produced from anthropogenic activity, but several compounds are produced by both biogenic and anthropogenic sources. It is proposed that the most dominant natural halogenated metabolite is methyl chloride, which is released by marine algae, as well as some higher plants and fungi (Table 1).[69] As far as the ocean flux of halocarbons to the atmosphere is concerned, then estimated values vary greatly – the source strength for methyl chloride of the open oceans is estimated between 380 and 650 kilotonnes per year.[70,71] However, from the viewpoint of stratospheric ozone depletion, methyl bromide, with its high ozone chemistry potential, is the more detrimental metabolite. This compound is not only released from natural sources but is also produced and widely used as an industrial fumigant. The estimation of the natural production and release rates of this marine halomethane is also speculative (Table 1). It has been suggested that the combined emissions from micro- and macroalgae contribute substantially to the amounts of bromine in the global cycle; perhaps in the same order of magnitude as anthropogenic sources.[72] Methyl bromide emission of the oceans have been estimated around 35 kilotonnes per year by Khalli *et al.*,[73] whereas Lobert *et al.* claim that there is no general supersaturation of methyl bromide in seawater and that the oceans may thus be a net sink (rather than a source) of methyl bromide.[74] Bromide emissions from the oceans are only indirectly related to algal productivity because other sources and sinks for this metabolite have to be considered such as methyl bromide release by sediments as well as bacterial or abiotic degradation.[75] Other shorter-lived halocarbons from algae are significant in tropospheric and potentially stratospheric chemistry as well. For example, $CHBr_3$, CH_2Br_2, and CH_3I are highly supersaturated in the ocean. It can therefore be speculated that because of their massive contribution to halocarbons in ocean waters, algae are also significant contributors to halometabolites found in the atmosphere.[76]

Both micro- and macroalgae are considered as relevant producers of halogenated methanes and structurally related climate relevant metabolites. Since macroalgae are restricted to the seashore, their production influences the concentration of halocarbons in seawater mainly close to the coast. Their halogenated metabolites may have a substantial impact locally, but on the global scale, only a minor contribution to the overall monohalomethanes is estimated from kelp and other macroalgae.[77] However, the production of polyhalogenated bromomethanes, which are predominantly produced by macroalgae, contribute significantly to the global budget.[78] In contrast, microalgae belonging to the phytoplankton are not restricted to

Table 1 Estimated release rates of CH_3Cl and CH_3Br from biotic and anthropogenic sources (calculated after ref. 69)

	Estimate (kilotonnes/year)	
Source	CH_3Cl	CH_3Br
Oceanic	650[70]	35[73]
Macroalgae	0.14	0.06
Total biotic	3350	83
Anthropogenic	386	48

the coastal zone and are responsible for a significantly higher share of global primary production compared to macroalgae. This means that phytoplankton have greater importance for the global fluxes of halocarbons, even though their net production rates may be considerably lower compared to macroalgae.[25]

Correlation of halocarbon concentration patterns in air and seawater with other parameters can be used to estimate the impact of algae on halocarbon production.[79] An exemplary study that correlated the halogenated compounds iodoethane, 1-iodobutane, 1-chlorobutane, 2-chlorobutane, dichloromethane, chloroform, and tetrachloroethene with pigments, which are indicative of phytoplankton blooms, was conducted in Menai Strait, UK.[80] Partial least-squares modeling of the complex data set highlighted the importance of microalgae on the signature of halogenated metabolites during their spring blooms, whereas macroalgae and sediments dominated as sources for these volatile compounds during non-bloom conditions.[80] Ship-borne measurements during a crossing of the Southern Indian Ocean was also used to correlate organohalogen production to chlorophyll concentrations, thereby supporting the significance of phytoplankton from the open ocean as a halomethane source.[81] Such observations match well with results from numerous field and laboratory studies on single macroalgal species or microalgal cultures.

4 Function of halogenated metabolites from algae

4.1 Algal physiology

Despite the massive production rates, the physiological and ecological roles of methyl halides are largely unknown. Based on observations of high methyl halide production rates of the salt marsh plant *Batis maritima*, it was proposed that methyl halide release can be a means to regulate the halide concentration of the plant tissue.[28] However, estimations of methyl halide production in the kelp *Macrocystis pyrifera* suggest that only a small proportion can be excreted *via* this pathway, and this would most likely not be sufficient for the regulation of intra-algal chloride concentrations.[77] The idea of a "metabolic accident" in which ubiquitous free halide ions are non-specifically methylated by methyl transferases that fulfill other metabolic purposes has also been brought forward.[33] A distinct metabolic function of CH_3Cl could be shown in fungi. There, the biosynthesis of methyl esters is linked to CH_3Cl as the methyl donor. These findings are supported by the fact that the formation of both esters and CH_3Cl were inhibited by SCN^-.[82,83] However, no similar mechanism is reported for algae. This brief overview shows that much additional work is needed to elucidate the role of methyl halides. Perhaps in the near future, the emerging tool of gene deletion experiments in combination with metabolomic techniques and bioassays could provide the first answers regarding the specificity and role of methyl transferases in algae.

In contrast to the nucleophilic substitution reactions involving free halide anions, the haloperoxidase reaction consumes H_2O_2 (Scheme 2). This can be shown by the external addition of H_2O_2 that leads to an increased production of polyhalogenated methanes in macroalgae.[84,85] The halocarbon metabolism is thus closely coupled to oxidative processes, with several physiological consequences for the producer. Pedersen *et al.* suggested that halocarbons are side products in the breakdown of surplus hydrogen peroxide in algal cells under oxidative stress.[85] Several observations support this hypothesis. Release of $CHBr_3$ and CH_2Br_2 by the kelp *Macrocystis pyrifera* was reduced in the darkness. H_2O_2 is produced by photoautotrophs during the Mehler reaction in the chloroplasts and BrPO, consequently, might serve as a way to rid the cells of this harmful product. Since H_2O_2 additions partially restored the ability to produce these metabolites during darkness, this halocarbon production is apparently limited by oxidant supply.[72] Furthermore, the addition of the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea, which causes a reduced electron flux and consequently less H_2O_2, also diminishes the release of $CHBr_3$.[86]

Haloperoxidases, in particular IPO, also play an essential role in the iodine metabolism of *Laminaria digitata*. This kelp is an effective iodide accumulator and was harvested for the retrieval of this element.[87] IPO is known to play a crucial role in the uptake of iodine by oxidizing iodide from the seawater to HOI with the aid of H_2O_2.[87] In the cells, HOI is reduced to iodide, where it presumably serves the organism as an inorganic antioxidant.[68] In accordance with this hypothesis, gene expression analysis revealed the induction of two IPO coding genes after induction of *L. digitata* by oligoguluronates that are known to trigger signaling cascades involving reactive oxygen species (ROS).[88] The brown alga *Ectocarpus siliculosus* (for which the genome was recently sequenced) also accumulates halides, although to a significantly lower level.[89] This difference was reflected in the genome where only one bromoperoxidase was detected. In contrast, large families of haloperoxidases are found in kelps. Interestingly, the *Ectocarpus* genome encodes 21 putative dehalogenases, which may serve to protect the alga against halogenated compounds produced by co-occuring kelps.[89]

In contrast to these bulk chemicals released by algae, the physiological reasons for the production of other halogenated metabolites are often less clear. It was suggested that BrPO-mediated oxidation of phenolic polymers in the brown alga *Fucus serratus* is involved in adhesive formation and cell-wall strengthening.[90] If model oxidation reactions involving BrPO, polyphenols and H_2O_2 were carried out in the presence of bromide or iodide, differing polymer properties resulted.[91] It is, however, not clear how the halogen influences polymer formation. Two mechanisms could be envisaged involving either the bromination of phenols followed by their oxidation and polymerization, or the enzymatic generation of oxidized bromine species, which can further react with the nucleophilic phloroglucinol repeating unit.[90] A majority of the other halogenated secondary metabolites observed in macroalgae may perhaps be involved in the interaction of the alga with its environment, as summarized in the next section.

4.2 Chemical ecology

Halogenated metabolites attracted the early interest of natural product chemists from the perspective of pharmacological significance. This was motivated by the observation that halide substitutions of natural products often result in an alteration of their pharmacological properties.[13,92,93] This modulation of activities may also play a significant role in the function of halogenated metabolites in an ecological context.

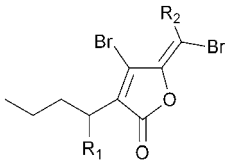
Besides its function in the regulation of reactive oxygen species in intact algae, bromoform might also contribute towards the alga's chemical defense. The observation that the surface of dead cells of the red alga *Corallina pilulifera* were covered with diatoms, while bromoform-producing cells were not, motivated further studies in this direction.[94] Indeed, bromoform inhibits diatom growth at concentrations somewhat higher than the bromoform emissions by the algae.[94] However, *C. pilulifera* produces additional halogenated methanes such as CH_2Br_2 and $CHBr_2Cl$ that might have additional effects and could explain the experimental outcome. This observation is of great relevance for general antifouling mechanisms of algae, since production of the halomethanes is widespread among micro- and macroalgae.

It has also been observed that other fouling organisms are affected by volatile halogenated metabolites. Paul *et al.* measured the antimicrobial activity of bromoform and dibromoacetic acid from the red algae *Asparagopsis armata*.[95] These metabolites were active against six bacterial strains, including two marine *Vibrio* species. Further evidence for the role of brominated metabolites as antifouling agents was achieved by omitting bromide from the culture medium, which suppressed the metabolic capacity to produce brominated compounds. Algae that lacked brominated secondary metabolites exhibited increased bacterial colonization compared to algae cultured in a bromide-containing medium.[95] Microscopic investigation of *A. armata* revealed that halogenated metabolites are stored in specialized gland cells that maintain a physical connection with the outer cell wall. These structures could be responsible for the observed release of bromoform and dibromoacetic acid into the surrounding environment.[95] Dibromomethane, which is for example produced by the green alga *Ulvella lens* and by the red alga *Lithophyllum* sp., induces larval metamorphosis in the sea urchin *Strongylocentrotus nudus*.[96,97] The toxicity of bromoform on marine mussles, shrimp and fish was tested by Gibson *et al.*, who found LC_{50} values of 7–50 ppm in short-term toxicity assays, and also observed altered behavior of shrimp and fish upon exposure to bromoform.[98]

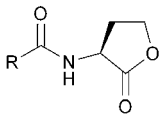
Compared to the extensive literature on antifouling activities of halomethanes from macroalgae, little is known about activities in plankton interactions. However, because phytoplankton cells are also challenged by bacteria and other microorganisms, as well as by competing phytoplankton, similar processes might be expected.

Antibacterial activity has additionally been observed from extracts of *Bonnemaisonia hamifera*. In the field, this filamentous red alga exhibits lower epibacterial abundance than other co-existing algal species. The surface extract, which is obtained by briefly dipping the alga in hexane, inhibits bacterial growth of several marine bacteria.[99] Additional bioassay-guided fractionation revealed 1,1,3,3-tetrabromo-2-heptanone **27** as the active compound that inhibits the growth of ecologically relevant fouling bacteria isolated from algal surfaces at natural concentrations. Furthermore, the biofouling on artificial surfaces that were treated with **27** was significantly reduced.[100]

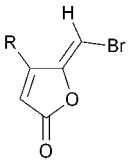
A completely different strategy that does not rely on antibiotic activity of natural products for the defense against bacteria was established with the red alga *Delisea pulchra*. This alga is capable of interfering with bacterial communication using brominated furanones **60–63**, which suppress bacterial development on its surface. Initial observations indicated that variations in furanone concentration are inversely correlated with bacterial abundance of the alga. Nevertheless, ecologically relevant concentrations of the metabolites did not have antibiotic activity. However, these metabolites affected mobility and attachment of bacteria, which pointed towards the disruption of bacterial communication.[101]



60 R_1 = H; R_2 = Br
61 R_1 = H; R_2 = H
62 R_1 = OAc; R_2 = H
63 R_1 =OH; R_2 = H



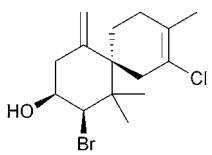
R = alkyl, 2-oxoalkyl...
64



65 R = H
66 R = Br

Gram-negative bacteria use excreted acylated homoserine lactones (AHLs) **64** for decision-making in a process termed quorum sensing.[102] The concentration of AHL in the environment of bacterial cells regulates their metabolic activity and behavior and thereby influences the settlement success of the fouling organisms. An essential protein in this communication mechanism is LuxR. When AHL reaches a threshold concentration, it becomes bound to LuxR, and this complex activates transcription of operons encoding relevant enzymes for metabolic reactions.[102] The red alga *D. pulchra* produces **60–63** that are structurally related to the quorum-sensing regulator AHL. These furanones inhibit bacterial colonisation by preventing binding of AHL to LuxR or by displacing bound AHL from the LuxR complex.[103] This leads to an accelerated degradation of the LuxR-like protein and a disruption in quorum-sensing mechanisms.[104,105] Since AHL-based quorum sensing is a widely distributed mechanism not only limited to marine fouling bacteria, these metabolites also have the potential for the treatment of infectious diseases.[106] Meanwhile, synthetic derivatives **65** and **66** of the *Delisea* furanones with enhanced quorum-sensing inhibitory properties are available and in use for medicinal purposes.[107] Further investigations of this well-studied model alga revealed multiple ecological functions for the polybrominated furanones. These metabolites also inhibit the settlement of four epiphytic algae that are representative of the fouling community in the environment of *D. pulchra*.[108] Further studies gave evidence that these furanones also deter feeding of local herbivores,[109] completing the broad spectrum of their biological activities.

Besides bacteria and epiphytes, algal surfaces are also challenged by settlement of larvae, and halogenated secondary metabolites are also active against these organisms. Barnacle larval settlement was inhibited by polyhalogenated monoterpenes isolated from the red alga *Plocamium costatum*,[110] and the brominated and chlorinated sesquiterpene elatol **67** from *Laurencia rigida* is active against other invertebrate larvae.[111]



67

The localization and release mechanisms of active compounds that comprise an ecological function in surface interactions of algae with epibionts are crucial. Early studies were conducted on the brominated sesquiterpene β-snyderol, the major terpene from the red alga *Laurencia snyderae*, which binds 77% of the organic bromine of the alga. Dispersive X-ray fluorescence spectroscopy was used for the selective detection of Br-containing species, which can be localized using this technique. It has been observed that intracellular vesicles called *corps en cerise* contain elevated amounts of this metabolite.[112] A recent study demonstrated that these halogenated terpenoid-containing vesicles can travel to the cell-wall region of the alga.[113] Interestingly, this process, which delivers the terpenoids to the algal surface, can be induced by the presence of fouling bacteria, which is the first report of an induced exocytosis of secondary metabolites from algae.[113] Such induced mechanisms might help to reduce the costs affiliated with secondary metabolite production, thereby increasing the performance of the algae.[114] Related storage systems are also reported for other halogenated metabolites, such as brominated furanones **60–63** from *D. pulchra* or the halogenated heptan-2-ones **27–31** from *B. hamifera*.[53,115] These algae localize the brominated metabolites in specialized gland cells that mediate the release of bioactive molecules onto the surface of the alga (Fig. 2).[116] Such gland cells have also been identified in the red algae *Asparagopsis* (Falkenbergia stage). Light-microscopy techniques revealed that a threshold bromide concentration in the medium is necessary to form and maintain these vesicle cells.[117] Further studies established stalk-like structures connecting gland cells with the outer wall of the pericentral cells, and might provide a mechanism for the transfer of metabolites to the algal surface.[118] Such storage-and-release systems have so far only been reported from red algae.

A critical point in chemical ecology is the determination of the ecologically relevant concentration that is encountered by organisms co-occurring with the producer. In antifouling assays, the surface concentration is considered to be the relevant factor. Establishing this concentration is not trivial, and offers challenges for analytical chemists. Some methods are based on the extraction with an organic solvent such as hexane,[119] but these methods are limited to low-polarity solvents, since the algal cells should not be destroyed by the solvent treatment. Recent studies employed desorption electrospray ionization mass spectrometry imaging (DESI-MS) techniques to demonstrate a surface-associated chemical defense of the red algae *Callophycus serratus* against the fungal pathogen *Linda thalassiae*.[120] On the surface of this alga, a patchy distribution of bromophycolides was observed with concentrations that were sufficient to maintain a chemical defense.[120] The ability of DESI-MS to reveal spatial distribution patterns of bioactive molecules at micrometer resolution has a definite potential to improve our understanding of chemical interactions on algal surfaces.

The activity of halogenated metabolites from algae is, however, not limited to fouling and defense against pathogens. Herbivory, which can have a major impact on algal performance, is also influenced by this compound class.[121] The involvement of halogenated metabolites, in particular brominated secondary metabolites, in chemical defense of red algae have also been brought forward by Paul *et al.*[122] The authors tested the consumption rate of different algae using the generalist *Hyale nigra*. They compared the consumption rate of filamentous algae with cellular inclusions containing halogenated metabolites to algae without gland cells, and found a higher potential of chemical defense in algae with inclusions. The removal of bromide from the media of *A. armata*, an alga with cellular inclusions, resulted in a clearly reduced chemical defense activity against the herbivore *H. nigra*, thereby suggesting the involvement of brominated compounds in the defense of this species. The specific activity of secondary metabolites is demonstrated by numerous bioassays using volatile halogenated compounds and a broad spectrum of marine herbivores. Macroalgae are capable of deterring herbivore feeding with halogenated terpenoids including monoterpenes,[123–125] sesquiterpenes[121,126,127] and brominated hydroquinones.[121]

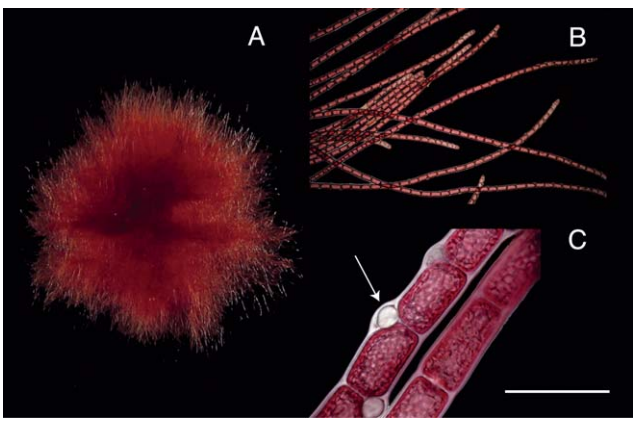


Fig. 2 *Bonnemaisonia hamifera*: (A) in the tetrasporophytic phase; (B) filaments; and (C) a one-layer-thick filament. The arrow in panel C points to a gland cell that stores bioactive halogenated compounds. Scale bars: A = 1 cm, B = 700 μm, C = 60 μm. Re-printed with permission from *Marine Ecology Progress Series*.[116]

5 Conclusion

Marine algae are a rich source of volatile halogenated metabolites, in particular, halomethanes which make algae a substantial contributor to the global budget of these molecules. Nevertheless, little is known about their physiological function and their roles in ecological interactions. Unfortunately, it is very difficult to address such aspects for metabolites that are ubiquitous in organisms and their environment. This is especially true for phytoplankton where their overall concentrations in the water column might be relevant, as well as locally elevated amounts of the metabolites in the immediate vicinity of the producing cells. In some cases, results will be difficult to rationalise by the accepted models of plankton ecology. New concepts involving both physiological consequences as well as considerations of chemical ecology will be required to produce new explanations for the potential costs and functions for the producer. In the near future, the availability of whole-genome sequences for several micro- and macroalgae, coupled with emerging transformation and silencing techniques, will open new routes to an in-depth exploration. In strong contrast to simple methyl halides from micro- and macroalgae, considerably more is known about the elaborate halogenated natural products found in many

macroalgae for which functions in chemical ecology can often be clearly defined. Due to the presence of halogen atoms, these metabolites often have exceptionally high biological activities and can aid in chemical defense or act as antifouling agents for the producing organism. Often these biologically active metabolites are produced in high quantities. Especially in dense kelp forests or in dense red algal populations, these metabolites can be very abundant, and might not only play a role for the producer itself but also cause cascading effects. Such complex interactions influencing whole ecosystems still await exploration.

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