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Brominated anisoles and cresols in the red alga *Polysiphonia sphaerocarpa*

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Abstract

The red marine alga *Polysiphonia sphaerocarpa* was extracted by a simultaneous steam distillation-solvent extraction technique and several brominated compounds were identified by gas chromatography–mass spectrometry. The compounds detected were 2,4-dibromoanisole, 2,4,6-tribromoanisole, 3-bromocresol, 3,5-dibromocresol, 3-bromo-4- hydroxybenzaldehyde, 3,5-dibromo-4-hydroxybenzaldehyde, 2-bromophenol, 4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol and 2,4,6-tribromophenol. This is the first time brominated anisoles and cresols have been detected in marine algae.

Keywords: *Polysiphonia sphaerocarpa*; Rhodophyta; Algae; Bromophenol; Bromocresol; Bromoanisole; Bromohydroxybenzaldehyde; Natural; Gas chromatography; Mass spectrometry

1. Introduction

Brominated compounds are naturally present in a large number of marine organisms, including marine algae (Gribble, 1996). Several species of marine algae are known to contain brominated compounds, from simple bromomethanes to complex bromophenols (Neidleman & Geigert, 1986; Gribble, 1996). They are also known to release large amounts of volatile halocarbons such as bromoform ($CHBr_3$), chloromethane (CH_3Cl), bromomethane (CH_3Br) and iodomethane (CH_3I) into the atmosphere (Gribble, 1996). Bromoanisoles have also been identified in the atmosphere (Wittlinger & Ballschmiter, 1990; Führer & Ballschmiter, 1998) and it has been speculated that these compounds could also be derived from the marine environment (Führer & Ballschmiter, 1998). As part of our study of the occurrence (Whitfield, Helidoniotis & Drew, 1997; Whitfield, Helidoniotis, Shaw & Svoronos, 1999) and biosynthesis (Flodin, Helidoniotis & Whitfield, 1999; Flodin & Whitfield, 1999) of bromophenols in marine algae, we undertook to search for bromoanisoles in these plants. This paper reports the identification of two bromoanisoles and two bromocresols together with several other brominated compounds in the red alga *Polysiphonia sphaerocarpa*.

2. Results and discussion

The red marine alga *P. sphaerocarpa*, obtained from exposed rocks at low tide, was analysed for brominated compounds. The compounds detected are presented in Table 1 and comprise brominated phenols, anisoles, cresols and 4-hydroxybenzaldehydes. To the best of our knowledge, this is the first time brominated anisoles and brominated cresols have been detected in marine algae.

Both brominated phenols and brominated 4-hydroxybenzaldehydes have previously been found in other marine algae or marine animals. Mono-, di- and tribromophenols are present in a large number of red, green and brown marine algae (Whitfield et al., 1999), whereas mono- and dibrominated 4-hydroxybenzalde-

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Table 1
Brominated compounds detected in the red alga *P. sphaerocarpa* collected on various occasions at Turimetta Head, Sydney, Australia

Date	Amounts of brominated compounds[a] (ng/g fresh wt.)										
	2-BP	4-BP	2,4-DBP	2,6-DBP	2,4,6-TBP	2,4-DBA	2,4,6-TBA	3-BC	3,5-DBC	3-BH	3,5-DBH
1.5.97	1	8	6	4	14	0.3	0.2	3	0.8	–[b]	+[c]
30.5.97	0.6	1	2	3	7	0.1	0.3	4	1	+	+
26.11.97	0.2	1	1	5	16	0.2	0.7	6	1	+	+
25.2.98	0.4	3	2	12	7	0.1	0.2	2	0.7	+	+

[a] Abbreviations: BP, bromophenol; DBP, dibromophenol; TBP, tribromophenol; DBA, dibromoanisole; TBA, tribromoanisole; BC, bromocresol; DBC, dibromocresol; BH, bromo-4-hydroxybenzaldehyde; DBH, dibromo-4-hydroxybenzaldehyde.
[b] Not detected.
[c] Detected but not quantified.

hyde have previously only been detected in the red marine algae *Rhodomela larix* (Suzuki, Kowata & Kurosawa, 1980), *Prionitis lyallii* (Phillips & Towers, 1981) and *Polysiphonia urceolata* (Kurata & Amiya, 1980). In marine animals, 3,5-dibromo-4-hydroxybenzaldehyde has been found in the polychaeta *Lanice conchilega* (Goerke & Weber, 1990) and the marine annelid *Thelepus setosus* (Higa & Scheuer, 1974). *L. conchilega* also contains 3,5-dibromocresol (Weber & Ernst, 1978; Goerke & Weber, 1990).

Polysiphonia as a genus is rich in various brominated compounds (Gribble, 1996). Species of *Polysiphonia* have been shown to contain brominated 3,4-dihydroxybenzaldehydes, 4-hydroxybenzyl alcohols, and 3,4-dihydroxybenzyl alcohols, including lanosol (5,6-dibromo-3,4-dihydroxybenzyl alcohol). However, *P. sphaerocarpa* was not included in any of these studies and, in the present study, none of these compounds were detected in this alga.

The levels of bromophenols in *P. sphaerocarpa* were comparably low. A survey of a large number of marine algae has shown that 2,4,6-tribromophenol can be present in marine algae in concentrations up to 1900 ng/g (Whitfield et al., 1999). Furthermore, a seasonal variation in 2,4,6-tribromophenol content has been demonstrated in the green alga *Ulva lactuca* (Flodin et al., 1999). However, in *P. sphaerocarpa* no such variation was revealed for any of the compounds detected (Table 1). The concentrations of 3-bromo- and 3,5-dibromo-4-hydroxybenzaldehyde in this alga were found to be similar to those of the other brominated compounds.

Even though the amounts of brominated compounds in *P. sphaerocarpa* were relatively low, the presence of naturally occurring brominated anisoles is of special interest. As previously indicated, bromoanisoles have been found in the atmosphere and they have also been identified as contaminants in some marine animals (Watanabe, Kashimoto & Tatsukawa, 1983). But despite the wide spread occurrence of both brominated and chlorinated anisoles in the environment, the specific sources of these compounds remains largely unknown. In general, haloanisoles are not produced or used industrially in technical quantities. However, these compounds can be formed in nature by *O*-biomethylation of the corresponding halogenated phenols. Organisms that have been shown to be capable of *O*-methylation include bacteria (Neilson, Allard, Hynning, Remberger, & Landner, 1983; Allard, Remberger & Neilson, 1987), fungi (Cserjesi & Johnson, 1972; Curtis et al., 1974; Tindale, Whitfield, Levingston & Nguyen, 1989) and, possibly, plants (Weiss, Moza, Scheunert, Haque & Korte, 1982). It is not known if marine algae are capable of *O*-methylation, although, some marine algae have been shown to be able to methylate mercury and lead (Pongratz & Heumann, 1998). Furthermore, the biosynthesis of dimethylsulfide by marine algae involves the methylation of 4-methylthio-2-hydroxybutyrate to give the corresponding dimethylated product, 4-dimethylsulphonio-2-hydroxybutyrate (Gage et al., 1997).

The samples of *P. sphaerocarpa* were not cultivated under axenic conditions. Thus, it is highly likely that these plants were contaminated with bacteria and/or fungi, which are capable of *O*-biomethylation. Consequently, although it is known that marine algae biosynthesise bromophenols (Flodin & Whitfield, 1999), it is not possible to claim, at this stage, that these plants are also capable of *O*-methylating these compounds. However, the results of the present study do indicate that algae, with or without the assistance of *O*-biomethylating fungi or bacteria, can be a contributing source of bromoanisoles detected in the atmosphere.

After the completion of this study, samples of the green algae *U. lactuca* and *Enteromorpha intestinalis*, collected from the same site, were analysed. This revealed that these algae also contained 2,4-dibromo- and 2,4,6-tribromoanisole. The concentrations of these compounds in *E. intestinalis* were similar to that of *P. sphaerocarpa*, whereas the concentrations in *U. lactuca* were about ten times lower. Brominated cresols were

not detected in *E. intestinalis*, however, low concentrations of 3,5-dibromocresol (0.02–0.1 ng/g) were detected in *U. lactuca*.

3. Experimental

3.1. Collection of P. sphaerocarpa

The red marine alga *P. sphaerocarpa* was collected at Turimetta Head, just north of Sydney on the eastern coast of Australia. It was collected in the intertidal zone at low tide on four occasions during 1997 and 1998. The alga was frequently growing closely together with other species of red algae within the genera of *Ceramium* and *Centroceras*. However, *P. sphaerocarpa* was collected only when and where it was growing by itself. The alga was transported on ice to the laboratory, identified as *P. sphaerocarpa* by microscopic examination and stored at −20°C. The initial identification was made by Dr A. J. K. Millar at the Royal Botanic Gardens, Sydney, Australia.

3.2. Extraction of brominated compounds from algae

The alga was thawed and excess water was removed by patting the alga between paper tissues. Thirty grams of the alga was extracted by a simultaneous steam distillation-solvent extraction method described in detail by Whitfield, Shaw and Svoronos (1994). The brominated compounds were identified and quantified by GC–MS.

3.3. GC–MS parameters

The gas chromatograph was equipped with a non-polar bonded phase capillary column (HP-5 Trace Analysis (5% Ph 95% Me Siloxane) 25 m × 0.2 mm × 0.33 μm film thickness). Helium was used as the carrier gas at a constant velocity of 30 cm/s. Injection temp. was 250°C and one μl of sample was injected with a split ratio of 1:20. The column temp. was initially held at 40°C for 2 min, then programmed from 40°C to 280°C at 20°C/min before holding this temp. for 20 minutes. The MS detector was operating in the EI mode (ionisation 70 eV at 170°C).

All brominated compounds, except the hydroxybenzaldehydes, were identified and quantified in the selected ion monitoring mode against calibration curves of each compound with 2,6-dibromophenol-d_3 as internal standard. Positive identifications were based on matching retention times with that of authentic compounds and appearance of the correct isotopic ratios of the selected ions. Target ions were as follows: 2,6-dibromophenol-d_3, m/z 253, 255, 257; monobromoanisoles, m/z 143, 145, 186, 188; dibromoanisoles, m/z 249, 251, 253, 264, 266, 268; tribromoanisoles, m/z 329, 331, 344, 346; monobromocresols, m/z 186, 188; dibromocresols, m/z 264, 266, 268; monobromophenols, m/z 172, 174; dibromophenols, m/z 250, 252, 254; tribromophenols, m/z 330, 332.

3-Bromo-4-hydroxybenzaldehyde and 3,5-dibromo-4-hydoxybenzaldehyde were tentatively identified by interpretation of their mass spectra obtained from GC–MS in the full scan mode. The characteristic ions in the spectra were m/z 199, 200, 201 and 202 for 3-bromo-4-hydroxybenzaldehyde and m/z 277, 278, 279, 280, 281 and 282 for 3,5-dibromo-4-hydroxybenzadehyde (Higa & Scheuer, 1975).

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