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Analysis of dimethyl sulfonium compounds in marine algae

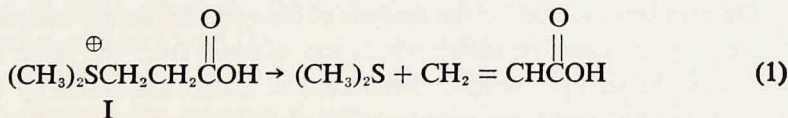
by Robert H. White¹

ABSTRACT

A method is described for the quantitation of the dimethyl sulfide which is released from marine algae by treatment with alkali. Results show that this dimethyl sulfide originates from at least two separate pools of dimethyl sulfonium compounds present in the algae. One of the pools is rapidly decomposed (< 5 min) at 23°C with 1M NaOH and is believed to consist of dimethyl- β -propiothetin. A second pool which completely loses its dimethyl sulfide only after heating at 100°C for 2 hr may be S-methylmethionine. The quantitation of these sulfonium compounds in 16 species of algae is presented along with a discussion of the importance of these sulfonium compounds to the natural sulfur cycle and the biological production of the methyl halides.

1. Introduction

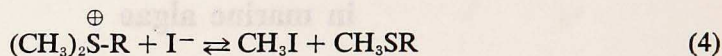
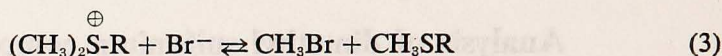
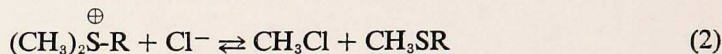
Dimethyl sulfide (DMS) has been found to occur ubiquitously throughout the *et al.*, 1978; Schwarzenbach *et al.*, 1978) and in marine organisms (Challenger *et al.*, 1957; Andreae *et al.*, 1981). It is very likely the compound most responsible for 1957; Andreae *et al.*, 1981). It is very likely the compound most responsible for the characteristic "odor of the sea." Although its origin in these systems is not definitely known, it most likely results from the cleavage of dimethyl sulfonium compounds which are present in marine algae and phytoplankton. The best characterized of these compounds is dimethylpropiothetin (DMPT) (I) which was first shown to be present in algae by Challenger *et al.* (1957). Cantoni and Anderson (1956) subsequently found that an enzyme present in algae caused the cleavage of this thetin to DMS as shown in Eq. (1).



The decomposition of sulfonium salts as described in (1), however, is only one of

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several possible reaction routes. A second route, the one with which this work is concerned, is the well known reversible reaction of methyl sulfonium ions with halides to produce the methyl halides, as outlined by (2)-(4) (Oae, 1977).



It is proposed that these reactions which occur between the dimethyl sulfonium compounds present in the algae and the halides concentrated by the algae may be responsible for the methyl halides which have been found to occur in seawater and in the atmosphere (Lovelock *et al.*, 1973; Lovelock, 1975; Penkett *et al.*, 1980; Rasmussen *et al.*, 1980) and in marine organisms (Dickson and Riley, 1976). They are believed to be a result of algal metabolism.

As a first step in establishing the importance of these reactions to the natural origin of the methyl halides, I have developed a method for the analysis of dimethyl sulfonium compounds in algae. This method is described herein along with some of the analytical results on several macroscopic algae.

2. Materials and methods

Dimethyl- β -propiothetin bromide was prepared as described by Challenger and Simpson (1948) from 3-bromopropionic acid and dimethyl sulfide. The final compound, after recrystallization from warm ethanol, had a m.p. of 112-114°C. DL-Methionine-S-methyl sulfonium chloride was obtained from Sigma Chemical Co. It will be referred to herein as S-methylmethionine.

Algae were collected from both Atlantic and Pacific waters. The Atlantic algae were collected and subsequently identified by Dr. H. G. Marshall, Old Dominion University, Norfolk, Virginia in the waters around Norfolk on July 29, 1981. The Pacific algae were collected by the author on August 21, 1981 in La Jolla, California and identified by Dr. William Fenical and Vallrie J. Paul, Scripps Institute of Oceanography, La Jolla, California. All algae were subjected to alkaline treatment within 2 hr of collection.

The standard method for the analysis of dimethyl sulfonium compounds consisted of assaying the dimethyl sulfide which was released from the algae after treatment with base. Fresh algae samples were freed of excess salt water by blotting with a paper towel and one-gram samples were placed in each of two 3-dram vials. One vial was dried at 110°C for 3 hr to obtain the dry weight of the algae. The other sample was sealed in the vial using a Pierce Tuf-Bond™ Teflon®-silicone cap liner held in place by the vial's original cap. This cap had been modified by removal of

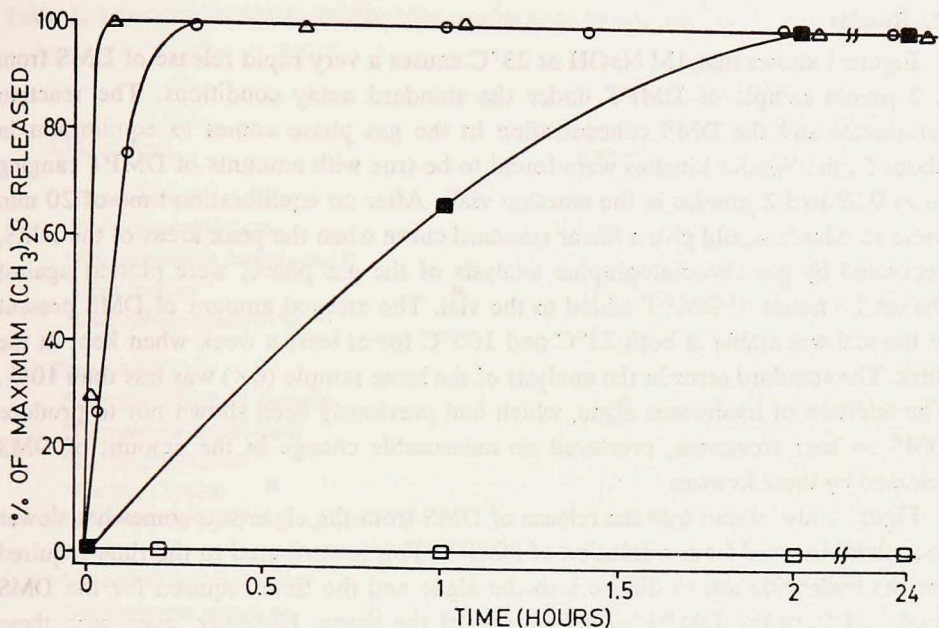


Figure 1. Release of DMS from dimethyl sulfonium compounds and algae by base. Samples were assayed as follows: For the dimethylpropiothetin (Δ) and S-methylmethionine (\square , \blacksquare) samples, 10 μ l of a 0.2 M solution of each sulfonium salt was injected into a vial containing 2 ml of 1 M NaOH at 23°C. For the algae sample (o), 2 ml of 1 M NaOH was added to one gram of *Ulva lautuca* in a vial kept at 23°C. The S-methylmethionine sample (\blacksquare) was kept at 100°C. Samples of gas were removed and assayed in each vial for DMS at the indicated times.

its liner and the drilling of a small hole (~ 2 mm) through its top in order that gas samples could be removed. Three ml of air were then removed from the vial followed by the addition of 2 ml of 1M NaOH. (The removal of the 3 ml volume of air prior to the injection of base leaves a slight negative pressure in the vial which prevents escape of any DMS produced.)

Samples were stored at room temperature for at least 24 hr after which a portion of the head-space gas was assayed for DMS by gas chromatography. The DMS was separated on a 1/8" x 6' glass column packed with 60/80 mesh Chromosorb 101 and was detected by flame ionization. With a helium flow rate of 30 ml/min and a column temperature of 150°C the DMS had a retention time of ~ 90 sec. DMS was the only significant peak detected in any of the samples. Its identity was confirmed by mass spectrometry on several samples. Quantitation was obtained by comparing peak areas to a series of known samples generated by adding 2 ml of 1M NaOH to 1 ml solutions containing known amounts of dimethylpropiothetin in vials of the same volume as the unknowns.

3. Results

Figure 1 shows that 1M NaOH at 23°C causes a very rapid release of DMS from a 2 μ mole sample of DMPT under the standard assay conditions. The reaction terminates and the DMS concentration in the gas phase comes to equilibrium in about 5 min. Similar kinetics were found to be true with amounts of DMPT ranging from 0.02 to 3.2 μ moles in the reaction vials. After an equilibration time of 20 min these standards would give a linear standard curve when the peak areas of the DMS, generated by gas chromatographic analysis of the gas phase, were plotted against the total amount of DMPT added to the vial. The assayed amount of DMS present in the vial was stable at both 23°C and 100°C for at least a week when kept in the dark. The standard error in the analysis of the same sample (6 \times) was less than 10%. The addition of freshwater algae, which had previously been shown not to produce DMS on base treatment, produced no measurable change in the amount of DMS released by these knowns.

Figure 1 also shows that the release of DMS from the algae was somewhat slower than that observed from a solution of DMPT. This is attributed to the time required for the hydroxide ion to diffuse into the algae and the time required for the DMS produced from the DMPT to diffuse out of the tissue. However, even with these factors operating, the release of DMS from the algae was found to be complete in 20 min and to remain constant for periods of up to one week, the longest tested.

This rapid release of DMS from the algae and the DMPT solutions is in contrast to that observed for S-methylmethionine when treated with base at 23°C. The release of DMS from this material was complete only after heating at 100°C for 2 hr. Furthermore, quantitation of the total amount of DMS produced showed it to be only $13.4 \pm 1.8\%$ as much DMS as was produced by an equivalent amount of DMPT. This difference in the reactivity of these two different dimethyl sulfonium compounds is consistent with their chemical structures. The DMPT has an acidic proton β to the sulfonium group which facilitates the elimination of DMS, the S-methylmethionine does not. DMPT is decomposed completely by the elimination of DMS whereas the S-methylmethionine is decomposed more readily by the nucleophilic attack of the hydroxide ion on the anionic sulfonium methyl group (as opposed to DMS elimination). Since the nucleophilic reaction destroys the dimethyl sulfide portion of the molecule by converting one of its methyl groups to methanol, this explains the reduced amount of DMS produced.

The fact that these two sulfonium compounds have such different rates of decomposition allows for the quantitation of their occurrence by measuring the amount of DMS released from the algae at 23°C and 100°C. The amount of DMS released at 23°C will reflect the amount of DMPT in the algae and 1/.13 or 7.7 \times the difference between that produced at 23°C and 100°C for 2 hr will give the amount of dimethyl sulfonium compound not having an acidic β proton.

Table 1. Liberation of dimethylsulfide from marine algae by alkaline.

	Treatment	
	23°C, 24 hr <u>μmoles DMS</u> g dwt	100°C, 2 hr <u>μmoles DMS</u> g dwt
Chlorophyta		
<i>Enteromorpha minima</i>	108	108
<i>Enteromorpha linza</i>	72	72
* <i>Enteromorpha intestinatis</i> (?)	59.6	79.5
<i>Ulva lactuca</i>	71	71
<i>Cladophora laetevirens</i>	11	11
Phaeophyta		
* <i>Egrecia menziesii</i> (young)	3.0	4.4
* <i>Egrecia menziesii</i> (old)	2.8	3.2
* <i>Egrecia menziesii</i>	1.0	1.6
* <i>Halidrys dioica</i>	0.2	0.34
* <i>Macrocystis pyrifera</i> (top growth)	0.021	0.15
* <i>Macrocystis pyrifera</i> (bottom growth)	0.011	0.21
* <i>Endarachne binghamiae</i>	0.06	0.32
* <i>Dictyopteris undulata</i>	0.14	0.26
Rhodophyta		
<i>Gracilaria verrucosa</i>	1.9	1.9
<i>Solieria tenera</i>	0.67	0.67
* <i>Gelidium</i> sp.	0.23	0.27
* <i>Corallina pinnatifolia</i>	0.20	0.20
* <i>Plocamium cartilagineum</i>	0.17	0.17
blue-green bacteria		
<i>Microcoleus lyngbyaceus</i>	0.36	0.36
grasses		
* <i>Zostera</i> sp.	12.0	12.0

* = collected at La Jolla, CA. Remainder collected at Norfolk, VA.

The results of these analyses are shown in Table 1. It is evident that the Chlorophyta contain the highest level of dimethyl sulfonium compounds, the Phaeophyta and Rhodophyta having values generally 10× lower. Although one member each of the green and red algae released more DMS upon heating, the brown algae clearly dominate with all species showing an increase in DMS. Considering that base treatment at 23°C released the major portion of the DMS from all of the algae, and that DMPT has been identified in several algae (Challenger *et al.*, 1957), it is logical to assume that DMPT is the major source of this portion of the DMS. The reason for the additional release of DMS at 100°C is, at present, unknown, but could con-

ceivably be due to either free S-methylmethionine or protein-bound S-methylmethionine present in the algae. With the exception of DMPT, S-methylmethionine is the only dimethyl sulfonium compound known to occur naturally. It, however, has only been found to occur in cabbage and parsley (McRorie *et al.*, 1954).

If we assume that the compound releasing DMS at 100°C produces DMS with the same efficiency as S-methylmethionine, then we can calculate, using the data in Table 1, that the average concentration of DMS bound as dimethyl sulfonium compounds in these algae is $28 \frac{\mu\text{moles}}{\text{g dwt}}$. Assuming this to be representative for all marine macrophytes containing a total plant biomass of 2×10^9 tons of carbon (Smith, 1981), we can calculate that, at any one time on a global scale, there is a standing stock of $\sim 10^{13}$ g of DMS bound in macrophytes. If we consider an active metabolism whereby the algae could produce at least twice this amount of DMS in one year, then we have a biological source for at least 20% of the sulfur of biological origin which is required to balance the sulfur cycle (Kellogg *et al.*, 1972; Lovelock *et al.*, 1972).

The remainder of this sulfur could possibly be supplied by the DMS produced by marine phytoplankton. This is supported by the positive correlation found between the vertical distribution of phytoplankton and the concentration of DMS in the marine water column and by the demonstration that axenic algal cultures release DMS into the medium (Andreae *et al.*, 1981). The idea that marine phytoplankton are a significant source of DMS is strengthened both by the observation that many of these organisms contain high concentrations of DMPT (Ackman *et al.*, 1966) and the fact that in their natural environment they have such very high turnover rates (Smith, 1981).

Competing with decomposition of these dimethyl sulfonium compounds to produce DMS would be the transfer of one of the sulfonium methyl groups to various nucleophiles (Coward and Sweet, 1971). Considering the ability of some algae to concentrate large amounts of iodide (Shaw, 1962) and the relative nucleophilic reactivity of iodide ion (Swain and Scott, 1953), it is very likely that iodide ion could be the most reactive nucleophile in some algae. The resulting methyl iodide could then react either intercellularly or extracellularly with chloride and bromide to produce the other methyl halides (Zafirou, 1975). Whether or not these reactions are responsible for the methyl halides found in the sea and air around marine algae (Lovelock, 1975; Chameides and Davis, 1980) is, at present, unknown. It is known, however, that methyl chloride and methyl bromide are produced by the Basidiomycete *Phellinus pomaceus* from the methyl group of methionine and the respective halide without the involvement of iodide (White, 1982). This would indicate that reactions (2) and (3) may lead to the direct production of methyl chloride and methyl bromide. This is supported by the recent observation that reaction (3) is

catalyzed by homogenates of the green alga *Ulva lactuca* (White, 1981, unpublished results).

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