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Production of chlorinated hydrocarbons and methyl iodide by the red microalga *Porphyridium purpureum*

Abstract—Two experiments were performed using axenic batch cultures of the red microalga *Porphyridium purpureum.* The cultures were grown in sealed 5-liter glass vessels under a high-purity artificial atmosphere and analyzed for the production of several halocarbons, including chloroform ($CHCl_3$), methylene chloride (CH_2Cl_2), methyl iodide (CH_3I), trichloroethylene (C_2HCl_3), and tetrachloroethylene (C_2Cl_4). Two cultures and a control were used in each experiment. The first experiment followed the system for 17 d at low light intensity (20 μmol quanta m^{-2} s^{-1}), while the second was comprised of 7 d at low light followed by 24 h at high light intensity (800 μmol quanta m^{-2} s^{-1}). In both experiments, only chloroform and methyl iodide were produced in measurable quantities. This is the first report of chloroform production in a microalgal species. The chlorophyll *a* (Chl *a*)-normalized production rate of chloroform in the various cultures ranged between 1.3×10^{-7} and 7.8×10^{-7} mol (g Chl *a*)$^{-1}$ d^{-1} (15–93 μg [g Chl *a*]$^{-1}$ d^{-1}). Chloroform production peaked during the logarithmic growth phase. Methyl iodide production ranged between 2.0×10^{-7} and 1.2×10^{-6} mol (g Chl *a*)$^{-1}$ d^{-1} (28–170 μg [g Chl *a*]$^{-1}$ d^{-1}). Exposure of the cultures to high irradiance (800 μmol quanta m^{-2} s^{-1}) did not stimulate the production of any of the compounds. In contrast to an earlier published account involving this species of algae, the production of tri- and tetrachloroethylene was not detected in any of the cultures under either low or high irradiance.

In recent years, there has been considerable interest in the natural production of organic chlorine compounds (Gribble 1996), particularly those that are volatile and enter the atmosphere. Work has been directed at identifying sources and fluxes of compounds that have known anthropogenic and possible natural sources. Abrahamsson et al. (1995) reported that the compounds tri- and tetrachloroethylene, normally regarded as anthropogenic, have a natural source in a number of species of marine algae. It is this significant report that the work presented here was designed to corroborate.

Information on natural production of C_2Cl_4 is significant because it has a bearing on our understanding of the importance of Cl atom chemistry in the atmosphere (Singh et al. 1996). While the hydroxyl radical is of prime importance in many atmospheric oxidation reactions, there has been recent interest in the possible involvement of Cl atoms as oxidants (e.g., Graedel and Keene 1995). Tetrachloroethylene is significant because its loss rate through reaction with Cl atoms is relatively high (ca. 300 times faster than with OH). Therefore, a constraint on the importance of Cl atom chemistry is that the total destruction rate of C_2Cl_4 must not, on average, exceed its rate of supply to the atmosphere. It follows that any increase in estimated release rates of C_2Cl_4 to the atmosphere potentially makes room for greater importance of Cl radical chemistry, because increased supply would have to be balanced by a higher destruction rate for a steady state to exist.

Various species of marine algae including both microalgae and macroalgae have been identified as producers of halogenated hydrocarbons. Methyl halides are known to be produced by giant kelp (Manley and Dastoor 1987, 1988) and by several species of phytoplankton (Tait and Moore 1995; Scarratt and Moore 1996, 1998; Sæmundsdóttir and Matrai 1998). In addition, bromoform and other polyhalogenated methanes have been observed from phytoplankton and ice algae (Sturges et al. 1992, 1993; Tokarczyk and Moore 1994; Moore et al. 1996*b*). The red marine microalga *P. purpureum* is an epiphytic organism frequently found in coastal waters. It exists as single spherical cells of approximately 5–8 μm in diameter. In laboratory cultures, it has been associated with the production of C_2HCl_3 and C_2Cl_4 (Abrahamsson et al. 1995), as well as small amounts of methyl bromide (CH_3Br) and methyl chloride (CH_3Cl) (Scarratt and Moore 1998). This paper reports the results of experiments directed at confirming and quantifying the production of C_2HCl_3 and C_2Cl_4 as well as other halocarbons including CH_3I in *P. purpureum* using gas chromatography-mass spectrometry (GC-MS).

Axenic cultures of *P. purpureum* (strain CCAP 1380/3) were obtained from the Culture Collection of Algae and Protozoa, Dunstaffnage Marine Laboratory, Oban, Scotland. This is the same clone of *P. purpureum* as that used by Abrahamsson et al. (1995) in their study. Axenicity was verified at the beginning and end of each experiment by epifluorescence microscopy using acridine orange stain. No indication of bacterial contamination during the experiments was observed. The culture medium consisted of filtered, autoclaved seawater enriched with f/8 nutrients (Guillard and Ryther 1962) supplemented with 0.4 g liter^{-1} $NaHCO_3$. The culture vessels were 5-liter round glass flasks with a custom-made glass insert sealed into the neck with a threaded Teflon nut and ferrule (Fig. 1). The inserts were fitted with two ports, which allowed the flasks to be slightly pressurized with air (zero grade), while water samples (40 ml) were withdrawn through a long stainless steel needle into a glass syringe. In this fashion, contamination of the cultures with halocarbons from the ambient atmosphere could be avoided. Before inoculation, each culture vessel was purged of residual halocarbons by bubbling the medium with zero-grade air containing 2,000 ppm CO_2 (flow rate = 50 ml min^{-1} for 24 h). The air was further purified by passage through a molecular sieve trap prior to addition of the CO_2. Duplicate culture vessels were inoculated with 10 ml of stationary-phase *P. purpureum* culture and incubated on an orbital shaker table (50 rpm) under cool-white fluorescent lights (20 μmol quanta m^{-2} s^{-1}, 17 : 7 light : dark [LD] 22°C). A third vessel was not inoculated and served as a control. Halocarbons were extracted from the water samples by purging and cryotrapping followed by GC-MS analysis in the manner

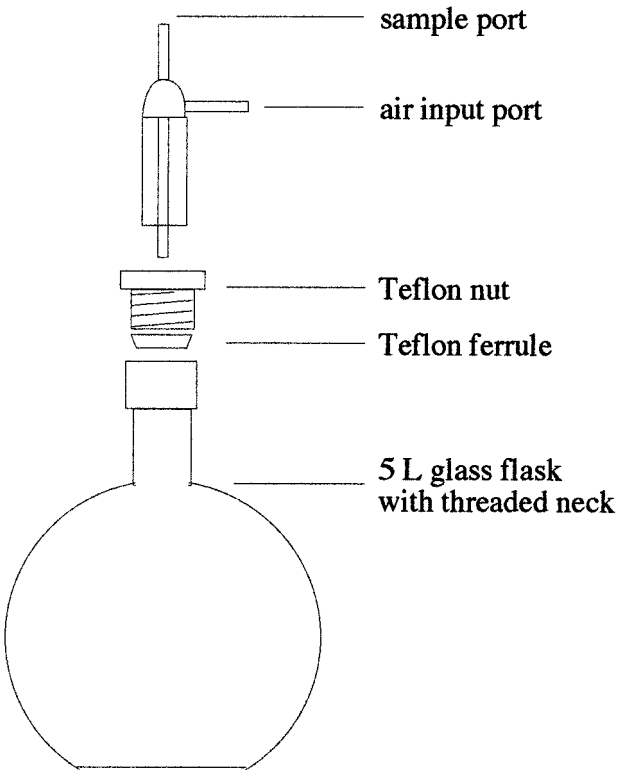

Fig. 1. Expanded view of culture apparatus showing 5-liter round flask with sample port insert and Teflon nut/ferrule seal.

described by Moore et al. (1996*a*). Single samples were taken from each vessel at each time point. Tests of the sampling method show that halocarbon analyses such as these have a precision of better than 10%. The GC-MS instrument was calibrated by injecting microliter amounts of a standard mixture of the compounds of interest directly into the cryotrap apparatus. The samples were analyzed for a suite of halocarbons including C_2HCl_3, C_2Cl_4, $CHCl_3$, CH_2Cl_2, and CH_3I. Carbon disulfide (CS_2) was also monitored, and the results are reported elsewhere (Xie et al. in press). All halocarbon analyses are reported as aqueous concentrations (pM). Detection limits for all compounds were calculated as three times the standard deviation of the blank determinations. They are as follows: C_2HCl_3 = 1.7 pM (0.22 ng liter^{-1}); C_2Cl_4 = 1.6 pM (0.27 ng liter^{-1}); $CHCl_3$ = 1.1 pM (0.13 ng liter^{-1}); CH_2Cl_2 = 1.4 pM (0.12 ng liter^{-1}); and CH_3I = 0.97 pM (0.14 ng liter^{-1}). The production rates of halocarbons were calculated by dividing the slope of the concentration curve during the period of fastest production by the average Chl *a* concentration during the same period. The results are expressed as moles of halocarbon per gram of Chl *a* per day. To facilitate comparison with other published works, the equivalent values in micrograms per gram of Chl *a* per day are given in parentheses.

Two experiments were performed. In the first, the cultures were grown under fluorescent light, and halocarbon production was monitored for 17 d. In the second experiment, the cultures were grown under fluorescent lights for 7 d until they had achieved a reasonable biomass. They were then

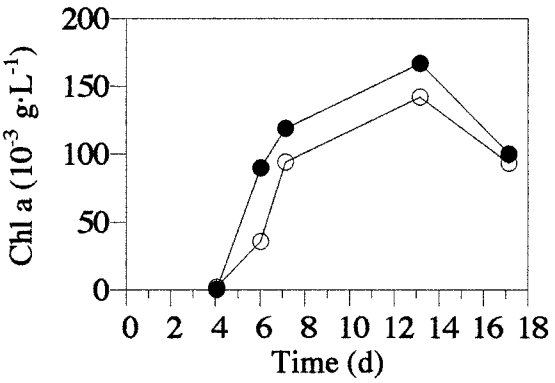

Fig. 2. Chl *a* concentrations in two cultures of *P. purpureum* from the first experiment. Culture 1 (open circles); culture 2 (filled circles).

exposed to a high-intensity quartz-halogen lamp (800 μmol quanta m^{-2} s^{-1}). Halocarbon determinations were performed three times in the initial 5 h of high irradiance and again after 24 h, when the experiment was terminated. This was an attempt to determine if high light stress could stimulate the production of halocarbons and approximately replicate the conditions used by Abrahamsson et al. (1995).

The two experiments produced very similar results, and for brevity, only those from the first one are shown here. The second experiment indicated a very similar pattern of halocarbon production. Figure 2 shows the Chl *a* concentration in the culture vessels throughout the course of the first experiment. The cultures displayed rapid growth after day 4, before leveling off and declining slightly at the end of the experiment. Note that due to a sampling error, Chl *a* was not measured on day 10, so it is possible that the maximum Chl *a* concentrations were higher than those observed on day 13. However, for the purposes of calculation, the values for day 13 will be taken as the maxima.

Figure 3 shows the aqueous concentrations of C_2HCl_3, C_2Cl_4, $CHCl_3$, CH_2Cl_2, and CH_3I in the two cultures and the control from the first experiment. Neither C_2HCl_3 nor C_2Cl_4 shows any accumulation during the experiment. The concentrations in the culture vessels remained close to the detection limit and were indistinguishable from the control. Similarly, CH_2Cl_2 also showed no accumulation, although the slightly elevated values on day 4 suggest that there may have been some production early in the life of the culture. However, the culture did not start growing rapidly until after day 4, and the Chl *a* concentration prior to this point was extremely low. It therefore seems more likely that the elevated CH_2Cl_2 concentrations on day 4 are artifacts. The $CHCl_3$ data are strikingly different. After the cultures entered log-phase growth, there was a rapid increase in the concentration of $CHCl_3$, which continued for $>$10 d, tailing off only as the cultures became senescent. Chloroform reached a maximum concentration of nearly 60 pM and was still increasing when the experiment was terminated. Chloroform concentrations in the control vessel remained low throughout.

The rate of production of $CHCl_3$ by each culture peaked

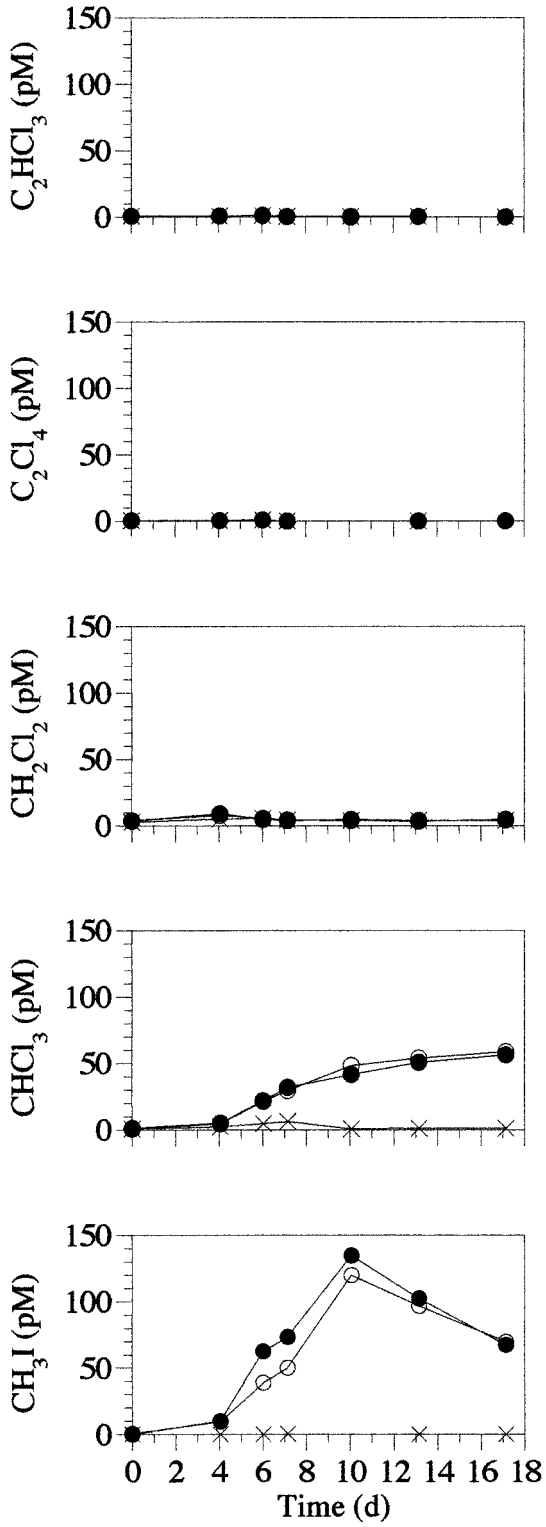

Fig. 3. Aqueous concentrations of five halocarbons (C_2HCl_3, C_2Cl_4, CH_2Cl_2, $CHCl_3$, and CH_3I) in *P. purpureum* cultures and control vessel from the first experiment. The same vertical scale is used in all panels to show relative concentrations. Control (crosses); culture 1 (open circles); culture 2 (filled circles).

Table 1. Production rates of $CHCl_3$ and CH_3I in *P. purpureum* for four cultures in two experiments (E1 and E2).

Culture	Chl *a* (μg liter^{-1})	$CHCl_3$ (mol[g Chl *a*]$^{-1}$ d^{-1})	CH_3I (mol[g Chl *a*]$^{-1}$ d^{-1})
E1 C1	44.0	1.9×10^{-7}	2.5×10^{-7}
E1 C2	69.8	1.3×10^{-7}	2.0×10^{-7}
E2 C1	40.5	7.8×10^{-7}	1.2×10^{-6}
E2 C2	62.2	3.9×10^{-7}	4.8×10^{-7}

between days 4 and 7, and Chl *a*-based production rates were calculated over this interval. While culture 1 showed a slightly higher production rate on a Chl *a* basis than did culture 2, note that their $CHCl_3$ concentrations in Fig. 3 are virtually identical throughout the time series. The difference in the calculated production rates is explained by the lower Chl *a* content of culture 1. However, a large part of this difference can be explained by the variability of the Chl *a* measurements, which showed a scatter of 10–20% between replicates. Therefore, the difference in production rates between cultures may not be significant. Chloroform production rates on a Chl *a* basis were 1.9 and 1.3×10^{-7} mol (g Chl *a*)$^{-1}$ d^{-1} (22.2 and 14.9 μg [g Chl *a*]$^{-1}$ d^{-1}) for cultures 1 and 2, respectively (*see Table 1*). Production rates were not calculated for C_2HCl_3, C_2Cl_4, and CH_2Cl_2 because their concentrations did not increase during the experiment.

The results also clearly show a high rate of CH_3I production in this phytoplankton species. Little increase in CH_3I was observed during the first few days after inoculation. However, after day 4, the CH_3I concentrations in both cultures increased dramatically, coinciding with an increase in the Chl *a* concentration. After day 10, the CH_3I concentration began to decline. Table 1 gives CH_3I production rates calculated over the interval of days 4–10. Culture 1 yielded 2.5×10^{-7} mol CH_3I (g Chl *a*)$^{-1}$ d^{-1} (35.5 μg [g Chl *a*]$^{-1}$ d^{-1}), while culture 2 produced 2.0×10^{-7} mol CH_3I (g Chl *a*)$^{-1}$ d^{-1} (28.1 μg [g Chl *a*]$^{-1}$ d^{-1}).

The second experiment produced qualitatively similar results, except that the concentrations of halocarbons observed were higher, as were the production rates (*see Table 1*). The Chl *a*-normalized production rate of $CHCl_3$ ranged from 3.9 to 7.8×10^{-7} mol (g Chl *a*)$^{-1}$ d^{-1} (93 μg [g Chl *a*]$^{-1}$ d^{-1}), while that for CH_3I ranged from 4.8×10^{-7} to 1.2×10^{-6} mol (g Chl *a*)$^{-1}$ d^{-1} (173 μg [g Chl *a*]$^{-1}$ d^{-1}). These rates are four- to fivefold higher than in the first experiment. It is difficult to draw a conclusion from this given the small number of replicates, but ranges of similar magnitude have been observed in the production rates of CH_3Cl and CH_3Br in the diatom *Phaeodactylum tricornutum* under similar culture conditions (Scarratt and Moore 1998). As with the first experiment, no accumulation of C_2HCl_3, C_2Cl_4, or CH_2Cl_2 was measured. Exposing the cultures to high light intensities (800 μmol quanta m^{-2} s^{-1}) did not stimulate the production of any of the compounds measured, including C_2HCl_3 and C_2Cl_4. Production of CH_3I actually appeared to drop during the 24-h high-irradiance period.

The results from both experiments show that no CH_2Cl_2, C_2HCl_3, or C_2Cl_4 were produced by *P. purpureum* under the

conditions employed. The concentrations of all three compounds remained low throughout the experiments in both the cultures and the controls. By contrast, the production of both $CHCl_3$ and CH_3I was rapid in all cultures. The $CHCl_3$ and CH_3I curves followed a pattern similar to the Chl *a* curve, with their maximum production rates coinciding with the logarithmic growth phase. Similarly, Moore et al. (1995) observed bromoform ($CHBr_3$) production in the diatom *Nitzschia* sp. coinciding with the logarithmic growth phase. This suggests that $CHBr_3$ and $CHCl_3$ may share a common synthetic pathway whose action is a product of normal cell metabolism. This is different from the pattern observed for the methyl halides CH_3Cl and CH_3Br, where production peaks during the stationary phase, suggesting they could be autolytic products (Scarratt and Moore 1996). There are two types of halogenating enzymes known in algae—haloperoxidases and methyl transferases. The former have been identified in phytoplankton (Moore et al. 1996*b*) and associated with the production of $CHBr_3$, while the latter have been shown to exist in red macroalgae, where they are associated with the production of CH_3Cl (Wuosmaa and Hager 1990), and it is reasonable to suppose they might exist in *P. purpureum* and other taxa also. While it would seem logical that CH_3I should be produced by the same mechanism as CH_3Cl and CH_3Br, the results here seem to suggest otherwise. However, it should be noted that CH_3I readily undergoes substitution by Cl^- (Elliott and Rowland 1993), which could explain its early and rapid decline in the cultures compared with CH_3Cl and CH_3Br. This is not inconsistent with a common synthetic pathway for all three methyl halides.

The CH_3I production rate in these cultures greatly exceeds anything previously observed in phytoplankton. If the production rates are normalized to cell numbers, they are about 40 times higher than rates reported from similar experiments with *Porosira glacialis* (Manley and de la Cuesta 1997). Thus, it appears that *P. purpureum* produces CH_3I at a very high rate compared with other species of microalgae that have been tested. It must be pointed out that all the production rates measured here are net rates, not gross rates. As noted earlier, CH_3I is subject to removal by the nucleophilic substitution of chloride. The decrease in CH_3I concentrations observed at the end of the experiments is probably due to the removal of CH_3I from the water by this process. By contrast, the chlorinated compounds ($CHCl_3$, CH_2Cl_2, C_2HCl_3, and C_2Cl_4) are not subject to this mechanism, and their removal rates in seawater, while unknown at present, are likely to be extremely low. However, in spite of the high production rates observed in this experiment, *P. purpureum* is unlikely to be a major source of either CH_3I or $CHCl_3$ on a global scale because its distribution in nature is limited primarily to the intertidal and subtidal zones, and its global biomass will therefore be small. The detection of $CHCl_3$ is nevertheless significant in that it has not previously been observed in microalgae. However, Keene et al. (in press) stated that the oceans are a substantial source of $CHCl_3$ to the atmosphere, which suggests that perhaps other species of microalgae also produce this compound.

The lack of production of C_2HCl_3 and C_2Cl_4 contrasts with the results of Abrahamsson et al. (1995), who reported production rates of 67 and 35 μg (g Chl *a*)$^{-1}$ h^{-1}, respectively, in the same clone of *P. purpureum.* However, these production rates were not sustained, appearing to tail off after little more than 1 h. This is unlike the production curves for any other halocarbons in microalgae, including those in this study, which tend to show sustained rates persisting for at least several days (Tokarczyk and Moore 1994; Tait and Moore 1995; Scarratt and Moore 1996, 1998; Manley and de la Cuesta 1997; Sæmundsdóttir and Matrai 1998). This suggests that if the production of C_2HCl_3 and C_2Cl_4 in the cultures of Abrahamsson et al. (1995) was indeed biogenic, then the mechanism must be quite different from that seen for other halogenated compounds.

It must be noted that the culture apparatus and methodologies employed in the two studies are very different. Abrahamsson et al. (1995) harvested the algal cells from a mature culture by centrifugation and resuspended them in fresh medium prior to analysis, whereas in this study, the cultures themselves were analyzed along with a parallel control. Our method was designed to avoid any possible contamination of the cultures with halocarbons from the ambient atmosphere. A further substantial difference is the time scale of the assays. The Abrahamsson et al. (1995) experiments lasted for 3 h, whereas this study followed the cultures for 17 d. We estimate that the method employed in this paper should allow the detection of a change in the concentrations of C_2HCl_3 and C_2Cl_4 as low as 5 pM over 10 d, yielding a production rate of approximately 5×10^{-9} mol (g Chl *a*)$^{-1}$ d^{-1} or 2.8×10^{-2} μg (g Chl *a*)$^{-1}$ h^{-1} for C_2HCl_3. This is more than three orders of magnitude lower than the rates reported by Abrahamsson et al. (1995). Accordingly, it is unlikely that our cultures were merely producing the compounds at reduced rates. It is safe to conclude that we did not detect any production of C_2HCl_3 and C_2Cl_4 in *P. purpureum,* even after attempting to replicate the high-irradiance stress conditions employed by Abrahamsson et al. (1995). In this context, it is worth noting that Marshall et al. (unpubl. data) reported no detectable production of C_2HCl_3 and C_2Cl_4 in the macrophyte *Falkenbergia hillebrandii,* a species also reported by Abrahamsson et al. (1995) to be a prolific producer of these compounds. Furthermore, Nightingale et al. (1995) found no evidence for production of C_2HCl_3 and C_2Cl_4 in intertidal pools containing natural assemblages of algae, but they found substantial production of other halocarbons, including $CHCl_3$ and CH_3I. Marshall et al. (unpubl. data) discussed the possibility that tri- and tetrachloroethylene could be formed abiotically from 1,1,2,2-tetrachloroethane and pentachloroethane, but this raises the question of why the precursor compounds would be present in the culture experiments.

The results of this study show no evidence for biotic production of C_2HCl_3, C_2Cl_4, or CH_2Cl_2 by *P. purpureum.* This finding contrasts with that of Abrahamsson et al. (1995), who measured substantial production of both C_2HCl_3 and C_2Cl_4. If there is no biogenic source of these compounds in the ocean, then the atmospheric budget is limited by anthropogenic production. This restriction on the rate of tetrachloroethylene production reasserts a limit on the potential for substantial Cl atom chemistry in the atmosphere as a whole. In contrast to the above compounds, substantial production

of CH_3I and $CHCl_3$ was observed. This is the first report of $CHCl_3$ production in a microalgal species.

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