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SUBJECT: Final Technical Report
ONR Award No. N00014-97-1-0345
PI: Dr. Ralf Goericke

Enclosed for your records is the final technical report for the above referenced grant.

Sincerely,

Ann F. Dunbar
Contract & Grant Assistant
SIO, UCSD

Cc: Ms. Nancy Wilson
Dr. Ralf Goericke

REPORT DOCUMENTATION PAGE

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13. ABSTRACT (Maximum 200 words)
An LC/MS system consisting of the Finnigan LCQ ion-trap mass-spectrometer and a Shimadzu HPLC system was purchased. The system was installed successfully and has been operational since September 1997. We have developed a number of LC/MS methods for the analysis of plant and sedimentary pigments. The instruments MS(n) capabilities, i.e. perform fragmentation experiments on fragments, are far beyond expectations, MS(10) experiments are possbile with some molecules. Using the instrument, we have discovered novel classes of chlorophyll a degradation products in marine sediments. One of these are carotenol chlorin esters that might be uniquely derived from diatoms and may be used as tracers for carbon derived from diatoms. Another class of compounds are cyclic pheophorbides. One of these, 13(2), 17(3)-cyclopheophorbide a enol had been found before in marine sediments. We have discovered analogous compounds derived from divinyl-chlorophyll a and b as well as chlorophylls c1 and c2. We have also found an unusual carotenoid, parasiloxanthin, in the cyanobacterium Prochlorococcus marinus, a carotenoid that has to date only been found in the Common Japanese Catfish. The system is also heavily used by other gorups at Scripps; it has become an instrument essential to the thesis work of some graduate students.

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FINAL REPORT

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TITLE:	DURIP: Acquisition of a liquid-chromatography / Mass-spectrometry system
PI	Dr. Ralf Goericke, Assistant Research Oceanographer
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ABSTRACT: An LC/MS system consisting of the Finnigan LCQ ion-trap mass-spectrometer and a Shimadzu HPLC system was purchased. The system was installed successfully and has been operational since September 1997. We have developed a number of LC/MS methods for the analysis of plant and sedimentary pigments. The instruments MS(n) capabilities, i.e. perform fragmentation experiments on fragments, are far beyond expectations, MS(10) experiments are possible with some molecules. Using the instrument, we have discovered novel classes of chlorophyll a degradation products in marine sediments. One of these are carotenol chlorin esters that might be uniquely derived from diatoms and may be used as tracers for carbon derived from diatoms. Another class of compounds are cyclic pheophorbides. One of these, 13(2), 17(3)-cyclopheophorbide a enol had been found before in marine sediments. We have discovered analogous compounds derived from divinyl-chlorophyll a and b as well as chlorophylls c1 and c2. We have also found an unusual carotenoid, parasiloxanthin, in the cyanobacterium Prochlorococcus marinus, a carotenoid that has to date only been found in the Common Japanese Catfish. The system is also heavily used by other groups at Scripps; it has become an instrument essential to the thesis work of some graduate students.

TASKS COMPLETED:

An LC/MS system has been purchased and installed at the Scripps Institution of Oceanography with funds provided by the DURIP program and the SIO director's office. The MS is a Finnigan LCQ, an ion-trap instrument capable of MS and MS^n that uses either electrospray or atmospheric pressure chemical ionization to generate ions for MS analysis. The LC is a Shimadzu dual-pump system with a UV/Vis detector capable of microcolumn use. The system has been fully operational since September 1997. The MS can also be used as a stand-alone instrument by infusing solutions with a syringe pump. Infusing samples, we have been able to perform MS^{10} experiments on pheophytin *a*, i.e. take off almost every functionality off the macrocycle, giving us structural information that is otherwise only obtainable from NMR. Using the MS in conjunction with the LC allows us to perform alternating MS and MS^2 experiments, thus not only characterizing compounds based on their molecular weight but also based on their fragmentation patterns.

SCIENTIFIC RESULTS:

We have used the instrument for a variety of projects. In each case exciting and important results have emerged

1. Access to this instrument allowed us to obtain a grant from ONR-Biology for the study of chromophores associated with marine detrital matter. Work on this grant has been very successful. Some of the results described below were obtained with funds from that grant.

2. Carotenol chlorin esters (CCEs) associated with sediments and copepod fecal pellets were characterized. We were able to show that the carotenols are isofucoxanthin-5'-dehydrate and isofucoxanthinol-5'-dehydrate, compounds uniquely derived from fucoxanthin, a biomarker for

diatoms. It is possible that CCEs could be used as a biomarker for diatoms in the marine environment. Based on this research a paper was submitted to Geochim. Cosmochim. Acta and a grant proposal was submitted to NSF.

3. The pigments of the marine cyanobacterium *Prochlorococcus marinus* were studied in more detail. It was found that the chl c-like pigment is identical to 2, 4 divinyl Mg-pheophorphyrin a5 methyl ester, as we had speculated. Interestingly, we found a carotenoid, parasiloxanthin, associated with deep populations of Prochlorococcus that has previously only been found in the Common Japanese Catfish. This pigment is derived from zeaxanthin and might be used by *Prochlorococcus* to fine-tune membrane fluidity with varying environmental temperature. A paper based on this work and the discovery of populations of *Prochlorococcus* in the suboxic zone of the Arabian Sea and the Eastern Tropical North Pacific is in preparation.

4. The pigment 13^2, 17^3-cyclopheophorbide a enol (CCPA517) has only recently been found in marine sediments (Ocampo et al., 1999), where it contributes up to 60% of all solvent extractable chl a degradation products. We have developed an analytical system for its quantification in sediments and discovered a whole new series of cyclic pheophorbides based on divinyl chlorophylls a and b and on chlorophyll c1 and c2. We (primarily a summer student fellow from MIT) were able to show that even though CPPA517 is very labile in organic solvents it is fairly stable when associated with sedimentary particles that are suspended in oxygenated seawater. It is probable that CPPA517 associated with sediments is stabilized by complexation with metals. We found that CPPA517 is produced by all marine herbivores we have studied to date, i.e., diverse protozoans, the copepod *Calanus pacificus*, the euphausid *Euphausia* sp. and salps. Analyzes of CPPAs in material collected in sediment traps in the Eastern Tropical North Pacific allowed us to deduce that *Prochlorococcus* contributes significantly to export fluxes of pigments, and by implication carbon, in that environment. A manuscript based on this work will be submitted within five weeks.

5. We characterized the reactions of fucoxanthin in acid and base and found a convenient method for the synthesis of the common fucoxanthin degradation products found in sediments, in particular isofucoxanthin-5'-dehydrate and isofucoxanthinol-5'-dehydrate, whose previous synthesis had been very difficult.

In addition to the work described above the instrument has been used extensively by the natural products chemists at Scripps (Faulkner's and Fennical's groups) and by students from F. Azam's and G. Mitchel's lab.

To summarize, the LC/MS system has produced astounding results - e.g. the discovery of new chromophores associated with detrital material in the ocean - that may give us new insights into how optical fields in the ocean are affected by detrital matter and how carbon is cycled in the marine environment. In addition the LC/MS system represents an important resource for the graduate students at Scripps that significantly enhances their work.

PERSONNEL / EDUCATION:

The LC/MS system has been used over the last year extensively by graduate students and post-docs at Scripps. In some cases it has become for these students an essential instrument for the work of these students. The instrument was also used for the work performed by summer student fellow - confronting them with the use of cutting edge technology in biological oceanography.

PUBLICATIONS AND PRESENTATIONS:

Goericke R., A. Shankle, D. J. Repeta, 1999, Novel carotenol chlorin esters from marine sediments and water column particulate matter, Subm. to Geochim. Cosmochim. Acta.

Goericke R., S. Strom, M. Bell, Distribution and sources of cyclic pheophorbides in the marine environment, in prep. for Limnol. & Oceanogr.