

## Identification of novel sulfur-containing derivatives of chlorophyll *a* in a Recent sediment†

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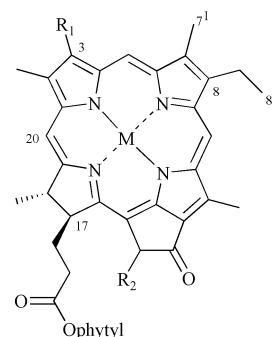
**Novel transformation products of chlorophyll *a* incorporating a methyl sulfide group in the substituent at the C-3 position have been identified in Recent sediments from an Antarctic lake.**

Sequestration *via* sulfur crosslinking of small molecules by the geopolymer kerogen is an important mechanism in the preservation of organic residues in the natural environment.<sup>1,2</sup> Organosulfur compounds derived from biolipids occur in Recent sediments both bound to solvent insoluble residues and in solvent extractable fractions. In the latter they can occur as oligomeric and dimeric moieties with intermolecular sulfur binding, and as monomers following intramolecular sulfur incorporation.<sup>3,4</sup> The differing reactivities of various functionalities towards sulfur species can result in their concentration in, or absence from, particular sulfur-bound fractions; for example the number of double bonds and their relative positions in highly branched isoprenoids is thought to influence the extent of sulfur incorporation in these biomarker lipids.<sup>3</sup> This selective preservation has clear implications when considering the significance of biomarker distributions in palaeoenvironmental assessment.<sup>5</sup>

The recognition of sulfur-bound porphyrins as a substantial fraction of the total porphyrins present in an ancient sediment<sup>6</sup> demonstrates the potential importance of crosslinking in the selective preservation of these markers of the primary producer community. There are, however, no reports of the incorporation of sulfur into solvent extractable monomer porphyrins or their precursor chlorins. Thus, little is known about the mechanism, its timing and the relative importance of sulfurisation in relation to sedimentary tetrapyrroles. During the course of analysing a suite of Recent sediments, we have identified derivatives that provide insight into the early stages of sulfur incorporation with respect to porphyrins.

Reversed-phase high performance liquid chromatography (RP-HPLC)<sup>7</sup> analysis of acetone extracts of sediments from Pup Lagoon, a coastal Antarctic lake deposited under marine basinal conditions,<sup>8</sup> revealed distributions containing abundant carotenoids and tetrapyrroles. Chlorophyll *a* (**1a**) and its transformation products, present in low relative abundance, and a number of xanthophylls provide evidence of oxygenic photoautotrophy at the time of sediment deposition. Bacteriochlorophyll *c* and *d* derivatives indicate the presence of the obligate anaerobic green sulfur bacteria (Chlorobiaceae),<sup>9</sup> indicating partial water column anoxia. A previously unidentified free base chlorin eluting just prior to phaeophytin *a* (**1b**) and exhibiting an identical online UV/vis spectrum ( $\lambda_{\max} = 406, 660$  nm) was observed in several samples, having a maximum relative abundance nine times that of phaeophytin *a*, and corresponding to 1.1 mg gTOC<sup>-1</sup> in the sediment in which its content was greatest. Atmospheric pressure chemical ionisation liquid chromatography–mass spectrometry (APCI LC-MS) revealed a protonated molecule  $[M + H]^+$  at  $m/z$  919, a difference in mass from phaeophytin *a* of 48 Da. MS/MS of  $m/z$  919 yielded product ions in MS<sup>2</sup> at  $m/z$  641 (100%) and 581 (56%). The losses are

analogous to those observed for phaeophytin *a*:  $-278$  Da corresponds to loss of phytene from the C-17 ester and  $-338$  Da to concerted loss of phytene and the carboxymethyl group from C-13<sup>2</sup>. An additional loss of 326 Da ( $m/z$  593; 36%) corresponds to concerted loss of phytene and a further 48 Da. The main loss from  $m/z$  641 in MS<sup>3</sup> was 47 Da. The losses of 48 and 47 Da can be rationalised as loss of a neutral molecule with hydrogen abstraction ( $-48$  Da) and loss of the same group as a radical. Notably, these losses do not occur during MS/MS of phaeophytin *a* or other derivatives reported to-date.<sup>7</sup> The MS<sup>4</sup> and MS<sup>5</sup> spectra only contained ions that are significant in phaeophytin *a*. The similarity of the UV/vis and mass spectra of the unknown chlorin and those of phaeophytin *a* suggests a strong structural similarity, with a single structural feature accounting for the 48 Da difference in mass. This mass corresponds to the empirical formula CH<sub>4</sub>S, incorporation of which into phaeophytin *a* gives a calculated accurate mass of 918.56929 Da. The putative sulfur-containing chlorin was isolated by preparative HPLC. High-resolution fast atom bombardment MS gave a molecular ion ( $[M]^+$ ) at  $m/z$  918.56891, corresponding to C<sub>56</sub>H<sub>78</sub>N<sub>4</sub>O<sub>5</sub>S<sub>1</sub>, consistent with the incorporation of CH<sub>4</sub>S into phaeophytin *a* and reduction of a double bond.



- 1a:** M = Mg; R<sub>1</sub> = vinyl; R<sub>2</sub> = CO<sub>2</sub>Me  
**1b:** M = 2H; R<sub>1</sub> = vinyl; R<sub>2</sub> = CO<sub>2</sub>Me  
**1c:** M = 2H; R<sub>1</sub> = ethyl; R<sub>2</sub> = CO<sub>2</sub>Me  
**1d:** M = 2H; R<sub>1</sub> = CH(Me)SMe; R<sub>2</sub> = CO<sub>2</sub>Me  
**1e:** M = 2H; R<sub>1</sub> = CH(Me)SMe; R<sub>2</sub> = H

The <sup>1</sup>H NMR spectrum (Table 1) closely resembles that of phaeophytin *a* with the exception of resonances due to substituents at the C-3 position: the vinyl resonances present in phaeophytin *a*<sup>10</sup> are not observed, indicating reduction of the C-3 vinyl group (*cf.* mesophaeophytin *a*; **1c**). Resonances are observed at  $\delta$  2.18, 2.28 and 5.65 that are not present in the spectrum of phaeophytin *a*. Cross peaks in the <sup>1</sup>H<sup>1</sup>H COSY show coupling between the proton at  $\delta$  5.65 and the methyl group at  $\delta$  2.28. Observed NOE enhancements demonstrate spatial connectivity of the substituent groups around the tetrapyrrole ring between the C-20 and C-7<sup>1</sup> positions. This provides confirmation that the aforementioned proton and methyl group are at positions C-3<sup>1</sup> and C-3<sup>2</sup> respectively, and shows that the isolated methyl group at  $\delta$  2.18 corresponds to a CH<sub>3</sub>S group at position C-3<sup>1</sup>. Thus, the MS and NMR results provide strong evidence for a phaeophytin *a* transformation product with the addition of CH<sub>3</sub>S- to the C-3 substituent at a position  $\alpha$  to the tetrapyrrole macrocycle (proposed structure **1d**).

In addition to the phaeophytin *a* derivative, the counterparts derived from chlorophyll *a* ( $\lambda_{\max} = 429, 660$  nm; M<sub>w</sub> 941 detected as  $m/z$  919<sup>12</sup>) and pyropheophytin *a* ( $\lambda_{\max} = 408, 660$  nm;  $m/z$  861; **1e**) were also identified.

† Electronic supplementary information (ESI) available: <sup>1</sup>H NMR data. See <http://www.rsc.org/suppdata/cc/b2/b212243j/>

**Table 1** Partial <sup>1</sup>H NMR data for **1b**, **1c** and **1d**

Proton <sup>c</sup>	Phaeophytin <i>a</i> <sup>10a</sup> ( <b>1b</b> )	Mesophaeophytin <i>a</i> <sup>11a</sup> ( <b>1c</b> )	Methyl sulfide phaeophytin <i>a</i> <sup>a,b</sup> ( <b>1d</b> )		
			<sup>1</sup> H	<sup>1</sup> H <sup>1</sup> H COSY cross peaks	NOE enhancements
CH <sub>3</sub> -2 <sup>1</sup>			3.50 s		5.65, 8.89
H-3 <sup>1</sup>	7.94 dd 12,18		5.65 q 7	2.28	2.18, 2.28, 3.50, 9.89
CH <sub>2</sub> -3 <sup>1</sup>		3.84 q 7			
CH <sub>3</sub> S-3 <sup>1</sup>			2.18 s		
H-3 <sup>2</sup>	6.20 dd 2,18				
H-3 <sup>2'</sup>	6.07 dd 2,12				
CH <sub>3</sub> -3 <sup>2</sup>		1.71 t 7	2.28 d 7	5.65	5.65, 9.89
H-5			9.89 s		3.30, 5.65
CH <sub>3</sub> -7 <sup>1</sup>			3.30 s		3.78, 9.89
CH <sub>2</sub> -8 <sup>1</sup>	3.42 q 7	3.62 q 7	3.78 q 8	1.70	
CH <sub>3</sub> -8 <sup>2</sup>	1.59 t 7	1.62 t 7	1.70 t 8	3.78	
H-18			4.63 m	1.83	
H-20			8.87 s		3.50, 4.63

<sup>a</sup> Values quoted report  $\delta$  in ppm in (CD<sub>3</sub>)<sub>2</sub>CO, multiplicity and *J* in Hz. <sup>b</sup> All spectra acquired in (CD<sub>3</sub>)<sub>2</sub>CO at 500 MHz. <sup>c</sup> <sup>1</sup>H NMR data for the C-8 ethyl group, the only other two carbon substituents present on the macrocycle, are included for comparison.

The occurrence of C-3 ethyl derivatives of chlorophyll *a* in immature sediments indicates that the reduction of the C-3 vinyl group occurs early during diagenesis.<sup>13,14</sup> The presence of a C-3 reduced methyl sulfide analogue of the Mg-containing precursor, chlorophyll *a*, indicates a mechanism whereby this reduction process can occur at the earliest stages in the degradation process. The formation of C-3-reduced chlorins has been demonstrated in algal degradation studies in a modified sea water medium and in the presence of anaerobic sulfate reducing bacteria. It was suggested that the bacteria were involved in the reduction of the C-3 vinyl substituent of chlorophyll *a*.<sup>15</sup> It is noteworthy, therefore, that the sulfur-containing derivatives of chlorophyll *a* identified here occur in marine sediments where biomarkers of anaerobic phototrophic bacteria indicate an appreciable production of H<sub>2</sub>S, the product of sulfate reduction.

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