

The First Experimental Demonstration of Side Chain Extension of Geoporphyrins in Sediments

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To investigate the formation process of high carbon number (>C₃₂) sedimentary porphyrins, heating experiments of several porphyrins were performed. Chromic acid oxidation of the heating products of protoporphyrin IX dimethyl ester afforded 2-methyl-3-*n*-propylmaleimide as the predominant product among the side-chain extension products formed. On the other hand, saturated substituents of etioporphyrin were also extended on heating to slowly form normal and branched homologs. These results may suggest that the transalkylation of porphyrin side chains proceeds mainly by a regioselective mechanism involving alkyl radical addition to a vinyl group of chlorophylls or their diagenetic products.

Alkyl porphyrins in sediments and petroleum occur as complex mixtures¹ (Chart 1). They are suggested to be mostly derived from defunctionalization of chlorophylls and hence have the potential to provide information about the original chlorophylls, depositional conditions, and geochemical transformations.² During the past 30 years, the molecular structures of more than 80 porphyrins obtained from sedimentary organic matter have been determined using HPLC, MS, and NMR techniques. Oxidative degradation of porphyrins into maleimides and subsequent analysis by GC-MS have also been applied to obtain information regarding the nature of the side chains of geoporphyrins.³ Geoporphyrins with a skeleton of 32 carbons are accounted for by a mechanism involving decarboxylation of chlorophylls, but the presence has been shown of geoporphyrins with carbon numbers greater than 32.⁴ It was proposed that these high carbon number porphyrins were derived from bacteriochlorophylls (Chart 1) or from a random transalkylation at the porphyrin side chains in sediments. On the other hand, it has been reported that 2-*n*-alkyl-3-methylmaleimides (**1**) with *n*-alkyl side

chains extending to C₁₁ were obtained by chromic acid oxidation of geoporphyrins from Cretaceous crude oil, together with branched alkylmaleimides as minor components (Scheme 1).⁵ Similar results have been reported, in which the maleimides containing *n*-alkyl side chains up to C₅ predominated in the oxidation products of geoporphyrins from Permian sedimentary rocks, and 2-methyl-3-*n*-propyl- and 2-isobutyl-3-methylmaleimide were appreciated as a biomarker of photosynthetic bacteria utilizing bacteriochlorophylls, and further as an indicator molecule of photic-zone euxinia (i.e., a seawater containing free H₂S) in connection with the massive extinction of organisms.⁶ In order to assess the origins of these high carbon number geoporphyrins as well as the maleimides that possess long side chains or specific alkyl groups related to bacteriochlorophylls, it is important to elucidate the extension modes of porphyrin side chains by transalkylation in sediments. For this purpose, we performed heating experiments of protoporphyrin IX dimethyl ester (**2**) and etioporphyrin I (**3**, Chart 2). Porphyrin **2** was employed to elucidate side chain extension from a vinyl group at the early stage of organic maturation in sediments and porphyrin **3**, which possesses saturated alkyl substituents, was used as a model substrate of thermally matured geoporphyrins.

Porphyrins **2** and **3** (ca. 0.2 mg) were heated at 300 and 350 °C for up to 360 h in a degassed sealed tube. The products were oxidized with 10% CrO₃ in 25% H₂SO₄ (1 mL) at 0 °C for 1 h and at room temperature for 1 h to afford a mixture of maleimides and phthalimides that originated from benzoporphyrins formed during the heating.⁷ These products were then extracted with benzene and analyzed by GC-MS equipped with an FFAP capillary column (60 m × 0.25 mm i.d.). Identification and quantification of the products were performed by comparison of the GC-MS data with those of authentic samples.

Figure 1 shows typical gas chromatograms obtained by the heating experiment. Porphyrin **2** afforded insoluble polymeric

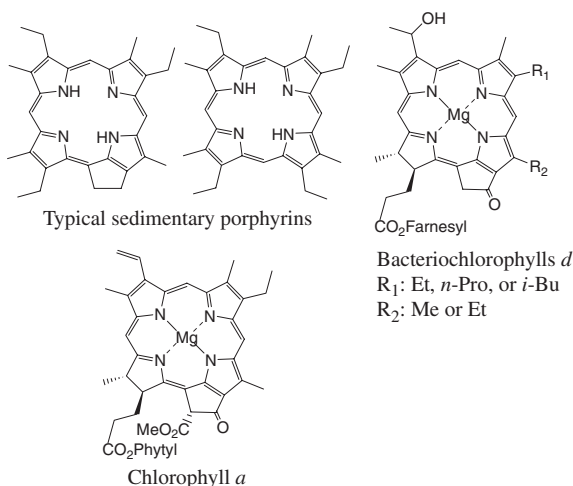
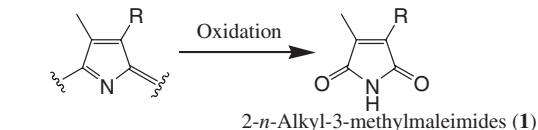


Chart 1.



Scheme 1.

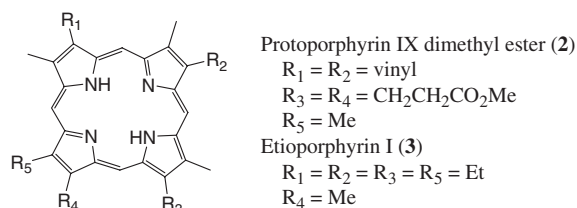


Chart 2.

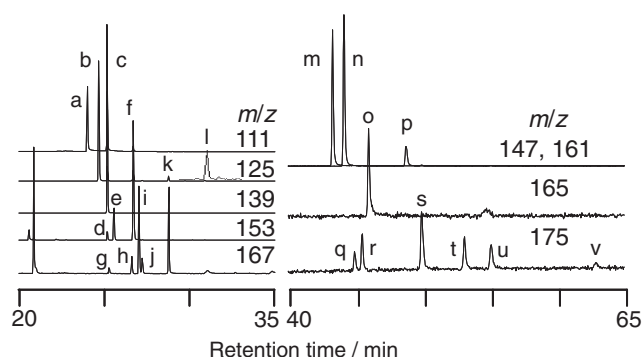


Figure 1. Mass fragmentograms of maleimides and phthalimides obtained by oxidation of protoporphyrin IX dimethyl ester heated at 300 °C for 360 h. a: MMMi, b: DMMi, c: EMMi, d: MiPMi, e: DEMi, f: MnPMi, g: EiPMi, h: MsecBMi, i: EnPMi, j: MiBMi, k: MnBMi, l: MnPenMi, m: Pi, n: 3-MPi, o: MceMMi, p: 4-MPi, q: 3,6-DMPi, r: 3-EPI, s: 3,5-DMPi, t: 4-EPI, u: 3,4-DMPi, v: 4,5-DMPi.

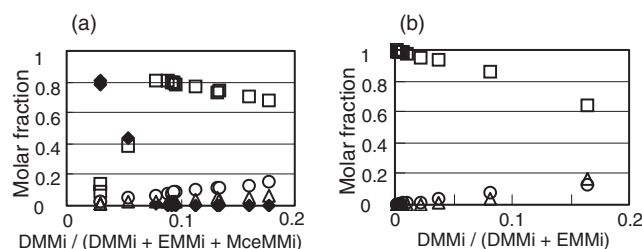


Figure 2. Plots of the molar fractions of C₅ to C₇ maleimides and MceMMi formed from heated protoporphyrin IX dimethyl ester (a) and etioporphyrin I (b) against the maturity index. ◆: MceMMi, □: EMMi, ○: DMMi, △: MMMi.

products by heating, but maleimides and phthalimides were detected after oxidation of the products. 2-Methyl-3-vinylmaleimide that should have originated from the starting material was not observed, possibly due to decomposition of the vinyl group during the chromic acid oxidation. The determined alkyl maleimides possessing extended alkyl side chains are three C₈ (DEMi, MnPMi, and MiPMi), five C₉ maleimides (EiPMi, EnPMi, MsecBMi, MiBMi, and MnBMi), and a C₁₀ maleimide (MnPenMi). MnPenMi was not formed from **3**.⁸

In Figures 2 and 3, the ratios of each maleimide to all the maleimides and phthalimides obtained during the heating of porphyrins **2** and **3** are plotted against DMMi/(DMMi + EMMi + MceMMi) and DMMi/(DMMi + EMMi), respectively, which represent thermal maturity of the porphyrins.^{3b} MceMMi that originates from the starting porphyrin rapidly decreased at the initial stage of the heating by demethoxycarbonylation, producing EMMi as shown in Figure 2a. The main products formed from an ethylmethylpyrrole unit by the heating of **2** and **3** were DMMi and MMMi, which increased with increasing thermal maturation (Figure 2). When these porphyrins were heated with naphthalene at 400 °C for 24 h and the products were extracted and analyzed, the alkyl reactive species released from the porphyrins were successfully captured as alkylnaphthalenes; the ratios of methyl-, ethyl-, and propylnaphthalenes from **2** and **3** were 85:13:2 and 76:22:2, respectively. These results may indicate that these alkyl reactive species affected the extension of the porphyrins.

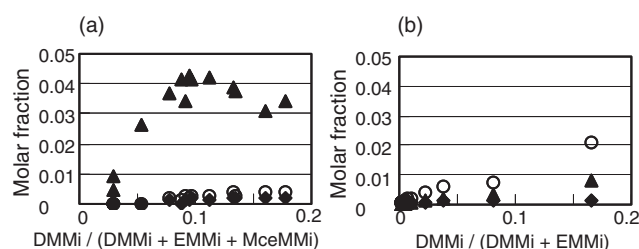
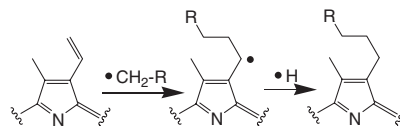
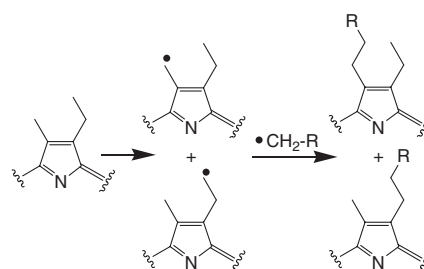


Figure 3. Plots of the molar fractions of C₈ maleimides formed from heated protoporphyrin IX dimethyl ester (a) and etioporphyrin I (b) against the maturity index. ▲: MnPMi, ○: DEMi, ◆: MiPMi.



Scheme 2.



Scheme 3.

Heating of **2** and **3** respectively afforded a characteristic profile of C₈ maleimide formation, as shown in Figure 3. MnPMi obtained during the heating of **2** rapidly increased in the region of the maturity index up to ca. 0.08, while all the C₈ maleimides from **3** gradually increased with increasing maturity. The amount of MnPMi formed from **2** was about 4% of all the products at its maximum. It is possible to consider that a methyl radical generated in the reaction system selectively added itself to a terminal carbon of a vinyl group of **2** to form a methyl-*n*-propylpyrrole structure (Scheme 2). The rapid formation of MnPMi may be attributed to the high reactivity of a vinyl group to radicals, resulting in complete consumption of the vinyl group until the maturity index of ca. 0.08.

Another remarkable difference was observed in the isomer distribution of the C₈ maleimides formed. Thus, the major product from **3** was DEMi, which can be formed by one-carbon extension at a methyl group of an ethylmethylpyrrole unit. Formation of MnPMi exceeded that of MiPMi to some extent (Scheme 3). The tendency for a saturated side chain to extend into a straight chain was confirmed by separate heating experiments using octaethylporphyrin, which afforded EnPMi and EiPMi in the ratio of 4 to 1 in the range of the maturity index (EMMi/(EMMi + DEMi)) up to 0.4.

MnBMi was formed as the major product among the C₉ maleimides during the heating of **2**, and the amount was about a tenth of that of MnPMi. Since an ethyl reactive species can be generated from the porphyrin as stated above, MnBMi obtained was likely formed by addition of an ethyl radical to a vinyl group of a methylvinylpyrrole unit. The superior production of MnBMi

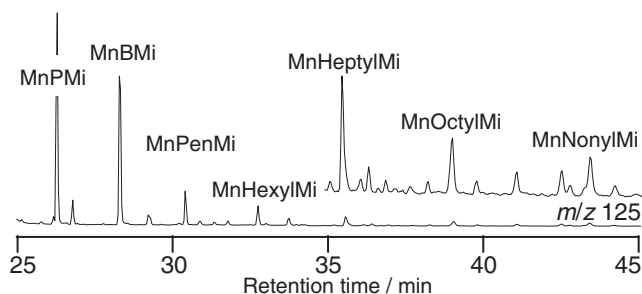


Figure 4. Mass fragmentograms of maleimides obtained by oxidation of protoporphyrin IX dimethyl ester heated at 350 °C for 6 h in the presence of *n*-decane.

and EnPMi to branched C₉ maleimides was observed in the heating of **3**, indicating a straight chain-forming tendency for the side-chain extension of alkylporphyrins. MiBmi, which can potentially be used as a biological marker for bacteriochlorophylls, was formed from both **2** and **3**, though in small amounts.

The 2-*n*-alkyl-3-methylmaleimides generated by the heating were MnPMi, MnBmi, and MnPenMi from porphyrin **2** and MnPMi and MnBmi from **3**, but those with even longer substituents have been reported to exist in sediments.⁵ We then performed heating experiments of the porphyrins in the presence of *n*-alkane to explore the possibility of transalkylation from coexisting carbon sources to porphyrin side chains. Porphyrins **2** and **3** were heated at 350 °C for 6 h with a large excess of *n*-decane and the products were oxidized and analyzed. A major homologous series of methylmaleimides with *n*-alkyl side chains extending to C₉ were observed in the heating products of **2** (Figure 4), showing that fragments of *n*-decane were introduced to the porphyrin side chains without suffering any rearrangement. Compared with the heating of **2**, the superiority of 2-*n*-alkyl-3-methylmaleimides was rather ambiguous in the heating products of porphyrin **3**, possibly because 2-*n*-alkyl-3-ethylmaleimides were also produced.

Maleimides were then identified in the oxidative extracts of natural sediments, such as the Cretaceous/Tertiary stratigraphic sequence of sediments at Soumaoki, Japan. Thus, after washing of powdered sediment (ca. 5 g) with a mixture (10 mL) of benzene and methanol (5:1, v/v) by sonication over five cycles, the sample was oxidized with 10% CrO₃ in 25% H₂SO₄ (10 mL) at 0 °C for 2 h and at room temperature for 2 h. The suspension was extracted five times with benzene (10 mL), and the extracts were combined and concentrated carefully to 100 μL under a nitrogen flow and analyzed as stated above. The total concentrations of all the maleimides and phthalimides in the sediments were 3–70 nmol/g-rock and those of the C₈ and the C₉ to C₁₀ maleimides were 40–580 and 40–220 pmol/g-rock, respectively. The molar fractions of maleimides possessing extended side chains in all the maleimides and phthalimides determined in the sediments were comparable to those obtained during the heating of the porphyrins. The isomer compositions of the C₈ maleimides were in accord with the results of the heating experiments of protoporphyrin throughout the sequence, with MnPMi being the predominant isomer (Figure 5a). This fact may indicate that a methyl-*n*-propylpyrrole unit of sedimentary porphyrins had been produced mainly from a methylvinylpyrrole unit of tetrapyrrole pigments in the early stage of geochemical transformation and preserved in the sediments. Alkyl radical addition could account

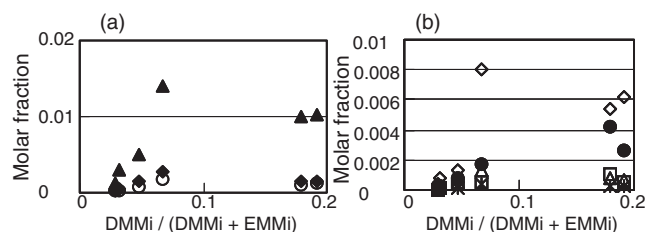


Figure 5. Plots of the molar fractions of C₈ (a) and, C₉ and C₁₀ maleimides (b) extracted from the stratigraphic sequence of sediments at Soumaoki against the maturity index. ▲: MnPMi, ○: DEMi, ◆: MiPMi, ◇: MnBmi, ●: MnPenMi, □: MiBmi, △: MsecBmi, *: EnPMi.

for the formation of MnBmi and MnPenMi, both of which were found in the sediments in larger amounts than the other C₉ maleimides (Figure 5b). It is worth noting that the oxidative extracts of the sediments contained MnPMi and MiBmi in the amounts comparable to those produced during the heating of protoporphyrin, showing that the major portions of these maleimides are likely not of bacteriochlorophyll origin but produced by geochemical transformation in sediments.

This study has established the chemical background for interpreting the formation of high carbon number porphyrins in sediments for the first time. Thus, the transalkylation of porphyrin side chains has been demonstrated to proceed mainly by a regioselective mechanism involving alkyl radical addition to a vinyl group of chlorophylls or their transformation products, which could explain the molecular distribution of 2-*n*-alkyl-3-methylmaleimides in oxidative extracts from sedimentary rocks. This process was accompanied by reactions that extend saturated substituents of etioporphyrin to produce its normal and branched homologs, with normal ones being major products. The results of this study also implicate the role of diagenesis as an important control on the occurrence of structurally altered biomarker molecules in matured sediments.

References and Notes

- 1 a) A. Treibs, *Angew. Chem.* **1936**, *49*, 682. b) E. W. Baker, *J. Am. Chem. Soc.* **1966**, *88*, 2311.
- 2 B. Huseby, R. Ocampo, C. Bauder, H. J. Callot, K. Rist, T. Barth, *Org. Geochem.* **1996**, *24*, 691.
- 3 a) A. Shimoyama, K. Kozono, H. Mita, S. Nomoto, *Geochem. J.* **2001**, *35*, 365. b) M. Kozono, S. Nomoto, A. Shimoyama, *Chem. Lett.* **2002**, *470*. c) S. Nomoto, H. Kigoshi, *Res. Org. Geochem.* **2005**, *20*, 31.
- 4 a) R. Ocampo, H. J. Callot, P. Albrecht, *J. Chem. Soc., Chem. Commun.* **1985**, 200. b) J. Verne-Misner, R. Ocampo, H. J. Callot, P. Albrecht, *Tetrahedron Lett.* **1986**, *27*, 5257. c) R. Gibbison, T. M. Peakman, J. R. Maxwell, *Tetrahedron Lett.* **1995**, *36*, 9057. d) D. H. Mawson, J. S. Walker, B. J. Keely, *Org. Geochem.* **2004**, *35*, 1229.
- 5 J. Martin, E. Quirke, G. R. Shaw, P. D. Soper, J. R. Maxwell, *Tetrahedron* **1980**, *36*, 3261.
- 6 R. D. Pancost, N. Crawford, J. R. Maxwell, *Chem. Geol.* **2002**, *188*, 217.
- 7 S. Nomoto, M. Satou, T. Yoshida, H. Mita, G. Kumagai, K. Nomoto, H. Kigoshi, Y. Kashiya, *Chem. Lett.* **2008**, *37*, 490.
- 8 Abbreviations of maleimides (Mis) and phthalimides (Pis) are as follows; MMi: 3-methylMi, DMMi: 2,3-dimethylMi, EMMi: 2-ethyl-3-methylMi, DEMi: 2,3-diethylMi, MnPMi: 2-methyl-3-*n*-propylMi, MiPMi: 2-methyl-3-isopropylMi, EnPMi: 2-ethyl-3-*n*-propylMi, EiPMi: 2-ethyl-3-isopropylMi, MnBmi: 2-*n*-butyl-3-methylMi, MiBmi: 2-isobutyl-3-methylMi, MsecBmi: 2-*sec*-butyl-3-methylMi, MnPenMi: 2-methyl-3-*n*-pentylMi, MceMMi: 2-methoxycarbonyl-ethyl-3-methylMi, MPi: methylPi, EPI: ethylPi, DMPi: dimethylPi.