

A Novel Vanadyl Alkylporphyrins from Geological Samples: A Possible Derivative of Divinylchlorophylls or Bacteriochlorophyll *a*?

Yuichiro Kashiya,^{*1,2} Motoo Shiro,³ Ryuji Tada,² and Naohiko Ohkouchi¹

¹*Institute for Research on Earth Evolution, Japan Agency for Marine-Earth Science and Technology, Yokosuka 237-0061*

²*Department of Earth and Planetary Science, The University of Tokyo, Tokyo 133-0033*

³*X-ray Research Laboratory, Rigaku Co., Akishima 196-8666*

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A novel C₃₀ vanadyl alkylporphyrins, 13,15-ethano-3-ethyl-2,7,12,18-tetramethylporphyrin (i.e., 8-nor-deoxyphylloerythroetioporphyrin; 8-nor-DPEP) has been isolated from a Middle Miocene siliceous shale. The structure of intact vanadyl porphyrins was determined by X-ray crystallography. The structure suggests it to be originated from specific chloropigments such as chlorophylls *a*₂, *b*₂, *c*₂, and *c*₃ or bacteriochlorophyll *a*, hence potentially being a biomarker of specific photoautotrophs.

Porphyrins complexed with vanadium(IV) (as vanadyl oxide) are the most common components of metalloalkylporphyrins extracted from geological samples (other components are complexed with nickel(II), copper(II), or iron(III)). Their origins are mostly assigned to various chlorophylls or bacteriochlorophylls from the past phototrophic organisms.¹ Significantly, origins of some alkylporphyrins have been assigned to chlorophylls *c* (specific for some groups of algae)² and bacteriochlorophylls *d* and *e* (specific for anaerobic green sulfur bacteria)³ based on their structural grounds, hence being important indicator in the molecule-based paleobiological and paleoenvironmental studies.⁴

In the previous works, the structures of vanadyl porphyrins have been determined by ¹H NMR after removal of paramagnetic vanadium atom.⁵ However, the demetallation procedure of vanadyl porphyrins (MeSO₃H, 100 °C; 4 h) generally suffers from considerably low recovery (less than 75%)⁶ or results in near complete loss for some specific varieties.⁴ Consequently, it requires isolation of a considerable quantity of vanadyl porphyrins, which is often hardly achieved for minor components of geological samples. Instead, recent improvements of instruments for X-ray diffraction analysis allow determination of molecular structures of intact vanadyl porphyrins with only a hundred micrograms or less quantity of samples. Thus, in the present report, we describe full structural determination of a novel vanadyl alkylporphyrin from a geological sample by X-ray crystallography as well as a suggestion for the precursory chloropigments based on the structural characteristics.

The bituminous mixture extracted from the pulverized siliceous shale (Middle Miocene Onnagawa Formation)⁴ was separated into several fractions by silica-gel open column chromatography. Individual vanadyl alkylporphyrins with structural varieties in alkyl side chains were isolated by a multi-step HPLC preparative method with both reversed- and normal-phase conditions (Figure 1).⁷ Crude characterizations of the isolated compounds were made simultaneously in HPLC by UV-vis absorption spectra with the on-line photodiode array detector

and by mass spectra with the mass selective detector connected through an atmospheric pressure chemical ionization interface.⁸ The UV-vis spectrum of the newly isolated compound (compound 1, Figure 1) exhibits the typical characteristics of vanadyl alkylporphyrins ($\lambda_{\text{max}} = 408, 532, 572 \text{ nm}$; rel. int. 100:5.9:6.9; Figure 2a). The molecular ion by mass spectrometric analysis indicated a C₃₀ skeleton $\{m/2[M+1]^+514\}$ with an additional degree of unsaturation suggesting presence of a cycloalkano side chain or a reduced side chain (Figure 2b).

The molecular structure of the newly isolated compound was determined as 13,15-ethano-3-ethyl-2,7,12,18-tetramethylporphyrin (compound 1; Figure 3) by the single-crystal X-ray

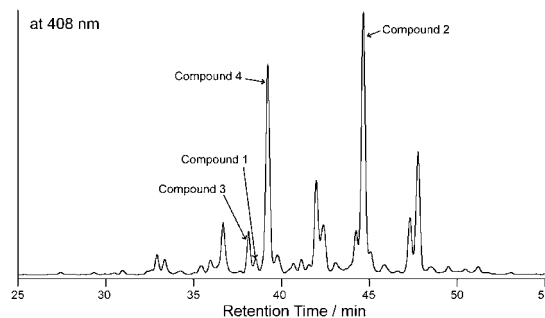


Figure 1. Reversed phase HPLC chromatogram (UV-vis at 408 nm) of vanadyl porphyrin fraction showing compound 1.⁸

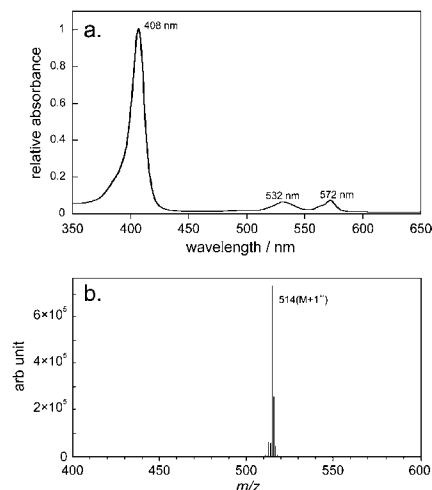


Figure 2. a) UV-vis spectrum of compound 1 obtained by HPLC on-line photodiode array detector; b) mass spectrum of compound 1 obtained by a mass selective detector connected through an atmospheric pressure chemical ionization interface.

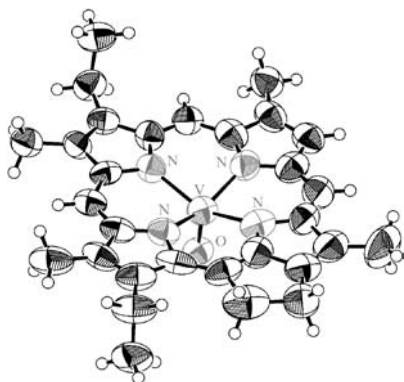


Figure 3. X-ray crystal structure of compound 1.

diffraction analysis.⁹ Dark red block crystals of $C_{30}H_{30}N_4O_2V$ were grown by vapor diffusion (methanol into $CHCl_3$ solution), prepared from 102 μg of the isolated compound. A single crystal having approximate dimensions of $0.1 \times 0.1 \times 0.04 \text{ mm}^3$ was mounted on a glass fiber. The measurement was made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated $Cu K\alpha$ radiation. The structure is 8-nor-equivalent of deoxophylloerythroetioporphyrin (DPEP; **2**) that is the most commonly observed structure among sedimentary porphyrins,^{1,10} hence hereafter called as 8-nor-DPEP.

Absences of alkyl side chain in 3-nor- and 17-nor-DPEPs (**3** and **4**, respectively) have been attributed to defunctionalizations of the vinyl group at C-3 position of various chlorophylls and the carboxyvinyl group at C-17 positions of chlorophylls c_1 , c_2 , and c_3 (**7a–7c**), respectively, during early stage of the degradation.⁴ Stable isotopic analyses of carbon and nitrogen among those molecules confirmed that the 17-nor-DPEP indeed originates in a unique biological source that is most likely to be chlorophylls c -producing algae.⁴ Similarly, absence of alkyl side chain in 8-nor-DPEP can be attributed to devinylation at C-8 position of chlorophylls a_2 , b_2 , c_2 , and c_3 (**5b**, **6b**, **7b**, and **7c**, respectively). (Chart 1) Alternatively, 8-nor-DPEP could be one of diagenetic products of bacteriochlorophyll a , because the ethyl side chain at C-8 position attached to the pyrrole ring of the bacteriochlorin structure are probably more or less easily eliminated compared to side chains attached to the pyrrole ring

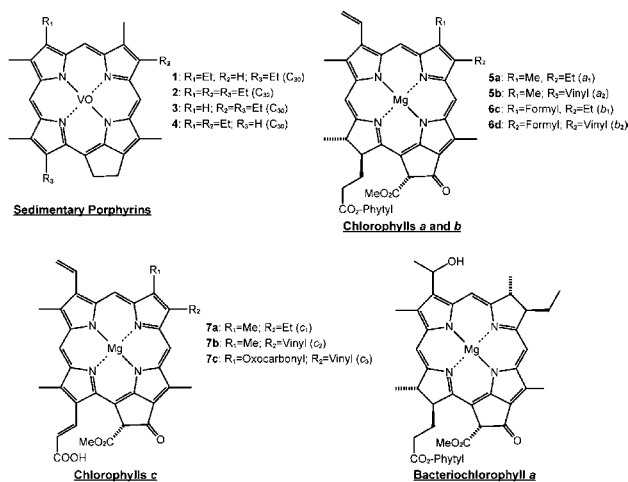


Chart 1.

of the chlorin structure of chlorophylls. All other chloropigments including chlorophyll a_1 (**5a**), which is the most abundant pigment in the natural environment, possess saturated alkyl side chains and are less likely to lose the function in low-temperature environment, since the thermal maturity of the sample is apparently minimal considering the composition of porphyrins.⁴ Stable isotopic characterization of 8-nor-DPEP relative to that of DPEP also suggested its occurrence from a rather unique biological source.

In conclusion, this novel alkylporphyrin could be a biomarker of specific photoautotrophs which existed in the past environments. The method for isolation and structural determination of the trace component of sedimentary porphyrins used in this study would contribute to future progresses in the porphyrin chemistry.

References and Notes

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- HPLC conditions: isocratic mobile phase of acetonitrile/ H_2O /acetic acid/pyridine (90:10:0.5:0.5, v/v); three analytical-scale C-18 columns ($4.6 \times 250 \text{ mm}$; $5 \mu m$ silica particle size) connected in series; thermal gradient using column oven (0 min-40 °C; 10 min-40 °C; 50 min-80 °C; 55 min-80 °C).
- Crystal data for compound **1**: $C_{30}H_{30}N_4O_2V$, $M_r = 513.53$, monoclinic, space group $C2/c$ (#15), $a = 24.904(4)$, $b = 13.7017(17)$, $c = 15.5939(16)$ Å, $\beta = 113.926(6)^\circ$, $V = 4863.8(10)$ Å³, $Z = 8$, $Cu K\alpha$ radiation ($\lambda = 1.54187$ Å), $D_{\text{calcd}} = 1.403 \text{ g cm}^{-3}$, $\mu(Cu K\alpha) = 36.580 \text{ cm}^{-1}$, 21674 measured reflections, 4441 unique reflections [$R_{\text{int}} = 0.109$; $I > -3\sigma(I)$], 4441 reflections included in the refinement, $R = 0.108$ [1529 reflections with $I > 2\sigma(I)$], $wR = 0.371$ (all reflections). Crystallographic data for the structural analysis of compound **1** has been deposited with the Cambridge Crystallographic Data Centre (CCDC No. 627948). Copies of this information can be obtained free of charge via, www.ccdc.cam.ac.uk/conts/retrieving.html.
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