Two Possible Pathways of Formation of Pristane and Phytane from Chlorophylls on Diagenesis: Carbon Isotopic Results from Laboratory Heating of Chlorophyll-a

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Carbon isotopic ratios of C_{20} and C_{19} isoprenoid hydrocarbons (ISOPs) produced on laboratory heating (250 °C to 350 °C) of chlorophyll-a (Chl-a) were measured to understand the linkage between the phytyl chain of Chl-a and the ISOPs found in petroleum and geological samples. About 1 ‰ difference in carbon isotopic ratio was observed between the C20 and the C_{19} ISOPs. This difference almost agrees with that observed by Trindade et al. for the ISOPs in several petroleum mixtures.

In petroleum and bitumen (organic solvent extract of sedimentary rocks), there are a number of organic compounds whose carbon structure is essentially formed by living organisms, which are called biomarkers. The investigation of origin and formation pathway of biomarkers is helpful for clarifying the formation process of petroleum, which is indispensable for development of oil exploration technique. Pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) are important biomarkers because they are commonly present in petroleum and bitumen in a relatively large amount.

A most likely precursor of these ISOPs is Chl-a (Figure 1).[1] Chl-a has a C_{20} isoprenoid chain called a phytyl group (a in Figure), and abundance of Chl-a in nature is by far larger than the compounds that have an isoprenoid chain longer than C_{20}.

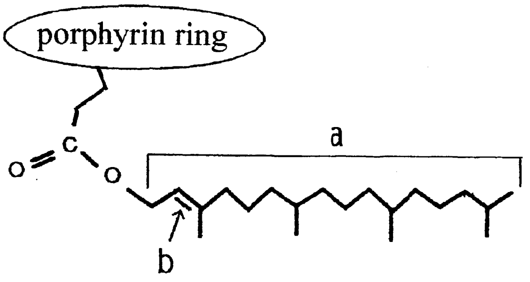

Figure 1. Phytyl chain of chlorophyll-a and its peripheral groups.

However, the pathway of formation of these ISOPs has not been fully clarified: Recent studies[2] have revealed that petroleum is the decomposition product of kerogen (insoluble organic matter in sedimentary rocks)[3,4] which is formed from organic compounds in living matter. Thus, the ISOPs in petroleum would be a decomposition products of kerogen. However, the prominent ISOP product upon flash pyrolysis of kerogen is prist-1-ene ($C_{19}H_{38}$)[5] which is a minor product of Chl-a.[6-8] On the other hand, phytenes ($C_{20}H_{20}$) and phytadienes ($C_{20}H_{18}$) are major ISOP products upon flash pyrolysis of Chl-a, although kerogen hardly produces them.

In a previous study[9] we found that when Chl-a was heated at a temperature ranging from 150 to 340 °C for several hours under vacuum, a notable amount of solid product hardly soluble in many organic solvents was obtained accompanied with formation of phytenes and phytadienes. Upon flash pyrolysis (470 °C, 15 s), the solid product produced prist-1-ene, prist-2-ene (tentatively assigned) and several ISOPs smaller than C_{19}, which are generally found in kerogen pyrolyzate. Several C_{20} ISOPs were also produced upon the flash pyrolysis of the solid product, while the total yields of them were considerably reduced in comparison with those obtained from unheated Chl-a. These results point out that "an artificial proto-type kerogen" is formed by heating Chl-a at low temperatures (below 340 °C). Since a thermogravimetry[9] revealed that the total yield of phytenes and phytadienes was about 30 % (mole/mole) when Chl-a was heated up to 340 °C, a considerable part of the phytyl chain would be incorporated into the "artificial kerogen". Thus, elucidation of the reaction of the phytyl chain is one of the key points to unveil the formation process of the ISOPs in petroleum.

Recently, $^{13}C/^{12}C$ isotopic ratios ($\delta^{13}C$ values) have been measured for the pristane and the phytane in petroleum or geological samples by use of GC/C/IRMS (gas chromatography/combustion/ isotope ratio mass spectrometry).[1,10] Hayes et al.[1] measured $\delta^{13}C$ values of pristane, phytane and geoporphyrins from some bitumens. The results are: the $\delta^{13}C$ values of these two ISOPs are almost the same, and the difference between the $\delta^{13}C$ values of these ISOPs and those of geoporphyrins is almost the same as that between the $\delta^{13}C$ value of the phythol and the porphyrin ring which are obtained by hydrolysis of Chl-a. On the basis of a hypothesis that the ratio of stable carbon isotopes of organic compounds is mostly preserved upon biogeochemical processes when substrates are large, Hayes et al.[1] concluded that these ISOPs in bitumen are formed from the same source, probably Chl-a.

However, Trindade et al.[10] measured $\delta^{13}C$ values of pristane and phytane in several petroleum mixtures and found that the $\delta^{13}C$ values of the former are about 1.5‰ lighter than those of the latter. Thus, a new question arose: whether $\delta^{13}C$ values of pristane and phytane from Chl-a obtained experimentally are same or not.

We heated Chl-a at a temperature ranging from 250 to 350 °C under vacuum in a sealed Pyrex glass tube, and determined the $\delta^{13}C$ values of the C_{19} and C_{20} ISOPs produced by the heating. About 10 mg chlorophyll-a (Wako Pure Chemical Industries Ltd., spirulina) was placed in a Pyrex glass ample (450 mm x 9 mm i.d.) and sealed under vacuum. The sample was heated in a muffle oven at a temperature ranging from 250 to 350 °C for one hour. Small amounts of transparent liquid adhering the glass surface and greenish black lump were observed when the ample was cooled to room temperature. The liquid was dissolved in n-hexane (n-hexane solution). The lump was washed with n-hexane and the washing was added to the n-hexane solution, thus n-hexane soluble fraction (HS) was obtained. The HS was concentrated and analyzed by gas chromatography (GC) and/or gas

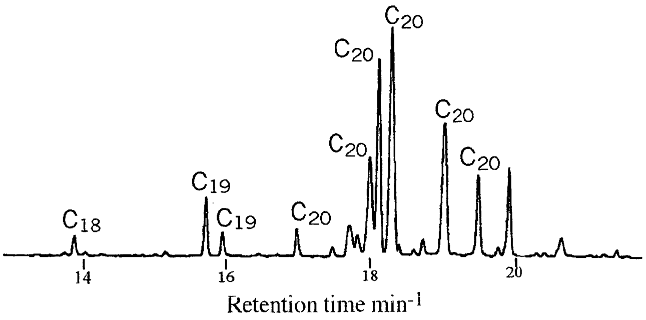

Figure 2. A gas chromatogram of n-hexane soluble fraction of heated chlorophyll-a.

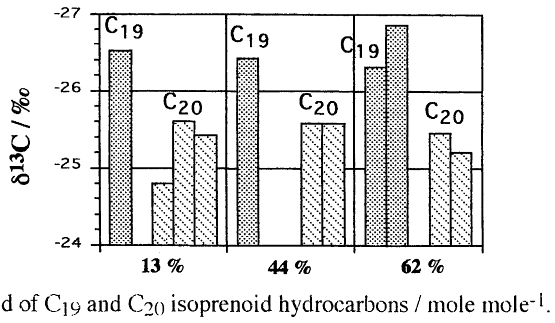

Figure 3. Carbon isotopic ratio of isoprenoid compounds obtained by pyrolysis of chlorophyll-a.

chromatography-mass spectrometry (GC-MS) under the conditions reported previously.[9] Figure 2 shows a gas chromatogram of an HS. Prist-1-ene, prist-2-ene (tentatively assigned), phytenes and phytadienes were identified from their mass spectral interpretation.

To reduce the error which is caused by the GC peak overlap on GC/C/IRMS, HS was hydrogenated in n-hexane in the presence of PtO_2 catalyst. The hydrogenation of pristenes, phytenes and phytadienes was confirmed by GC and GC-MS. GC/C/IRMS of hydrogenated HS was carried out under the following conditions. GC/C/IRMS system: an HP Model 5890 GC connected to a Finnnigan MAT delta-S mass spectrometer *via* a combustion interface, Sample size: 1μl of n-hexane solution, GC column: DB-5 /fused silica capillary column (60 m x 0.32 mm i.d.), Carrier gas: He, GC column temperature: 50 -120 °C/30 °C/min, 120 - 310 °C/5 °C/min and at 310 °C for 25 min, isotopic internal standard: n-$C_{24}D_{50}$. Carbon isotopic ratios were expressed in unit of $\delta^{13}C$ relative to the PDB standard (a belemnite from the Cretaceous Pee Dee formation, South Carolina).

Figure 3 shows the $\delta^{13}C$ values of pristane and phytane obtained under various decomposition conditions. The number of bar represents the number of times of analysis. Figure 3 reveals that the $\delta^{13}C$ values for pristane and phytane are practically constant (-26.5±0.2 and -25.4±0.3‰ in average, respectively) regardless of the yield (mole/mole) and that the $\delta^{13}C$ value for pristane is 1.1‰ lower than that for phytane in average.

It is highly noteworthy that the difference in $\delta^{13}C$ value between pristane and phytane almost agrees with that observed by Trindade et al.[10] This agreement suggests that the ISOPs in the petroleum examined by Trindade et al. derived from Chl-a, and that isotopic fractionation occurred *via* a reaction similar to that occurs upon heating Chl-a.

The bond involved in the formation of pristenes is located at next to the C-C double bond (b in Figure 1). Since the α-cleavage of double bond is highly unfavorable, reaction rate of pristene formation would be significantly small. Thus, light carbon would react preferentially for the formation of pristenes.

Our result does not agree with that obtained by Hayes et al.[1] Possibly, the pristane in the bitumens examined by Hayes et al.[1] was formed through a reaction in which significant isotopic fractionation did not occur. One possible reaction is: the C-C double bond of the phytyl chain was hydrogenated under reducing sedimentary circumstance and then the C-C single bond was cleaved. This assumption is under investigation.

References and Notes

1 J. M.Hayes, K. H. Freeman, B. N. Popp, and Christpher H. Hoham, *Org. Geochem.*, **16**, 1115 (1990).
2 B. P. Tissot and D. H. Welte, "Petroleum Formation and Occurrence, Second Revised and Enlarged Edition", Springer-Verlag: Berlin, Heidelberg, New York, Tokyo (1984), Part II, Chap. 5.
3 J. P. Forsman and J. M. Hunt, in "Habitat of Oil," ed by L. G.Weeks, Am. Assoc. Pet. Geologists, Tulsa (1958), p.747.
4 B. Durand, in "KEROGEN Insoluble Organic Matter from Sedimentary Rocks," ed by B. Durand, Editions Technip, Paris (1980), p.13.
5 S. R. Larter, H. Solli., A. G. Douglas, F. de Lange, and J.W. de Leeuw, *Nature* **279**, 405 (1979).
6 M. Ishiwatari, R. Ishiwatari, H. Sakashita, T. Tatsumi, and H. Tominaga, *Chem. Lett.*, **1990**, 875.
7 M. Ishiwatari, R. Ishiwatari, H. Sakashita, T. Tatsumi, and H. Tominaga, *J. Anal. Appli Pyrolysis* , **18**, 207 (1991).
8 D. van de Meent, J. W. de Leeuw, and P.A. Schenck, in "Advances in Organic Geochemistry 1979," ed by J.R. Maxwell and A.G. Douglas, Pergamon, Oxford (1980), p 469.
9 M. Ishiwatari, R. Ishiwatari, H. Sakashita, and T. Tatsumi, *J. Anal. Appl. Pyrolysis,* **35**, 237 (1995).
10 L.A.F. Trindade and S. C. B. Brassel, *Revista Latino-Americana de Geochimica Organica,* **1**, 1 (1995).