THE LIFECYCLE OF DIAMONDOID CAGE HYDROCARBONS IN NATURE

Zhibin WEI[1], J. Michael MOLDOWAN[1], Jeremy DAHL[1], Dan JARVIE[2] and Ron HILL[3]

1. Department of Geological and Environmental Sciences, Stanford University, CA 94305-2115, USA
2. Humble Geochemical Services, Division of Humble Instruments & Services, Inc., P. O. Box 789, Humble, Texas 77347, USA
3. U.S. Geological Survey, Box 25046, Denver Federal Center, MS 939, Denver, CO 80225, USA

Diamondoids, also called "nanodiamonds", can be regarded as extraordinary small fragments of a diamond lattice. They naturally occur in virtually all petroleum and are composed of fused cyclohexane rings all in chair conformation forming a cage structure. These nanodiamonds have shown potential in many research fields, e.g., nanotechnology, pharmacology, petroleum geochemistry. Although lower diamondoids (adamantanes, diamantanes and triamantanes) have been formed in laboratory experiments by super-acid rearrangement of a wide variety of isomeric precursor molecules, the origin of diamondoids in nature has puzzled organic geochemists for several decades.

In this study, we attempt to ascertain the organic precursors, formation mechanisms, and fate of diamondoids, which may shed light on the lifecycle of diamondoids in nature. The present work has clearly demonstrated that diamondoids in nature originate from petroleum or petroleum precursor molecules through molecular rearrangements involving numerous carbonium ion intermediates in the presence of acidic clays. The pattern of diamondoid formation follows carbonium ion mechanisms rather than free radical mechanisms, as supported by the findings that acidic clays largely facilitate the generation of diamondoids from kerogen compared with S^0 and other minerals (Wei et al., 2006a). Diamondoid analysis was performed on a large set of oil and condensate samples. Our results indicate that diamondoids become enriched in oil as oil cracking increases (Dahl et al., 1999). Despite their high stability, diamondoids are perishable in nature. Compelling evidence can be provided by thermal destruction of diamantane at high temperatures in the laboratory, by a dramatic drop in the abundance of diamondoids in the extracts of highly mature coals and sedimentary rocks (Wei et al., 2006b), and by the biodegradability of adamantane in petroleum reservoirs where microbial activities are evident. Therefore, diamondoid cage compounds have their lifecycle in nature: birth, enrichment and demise (Figure 1).

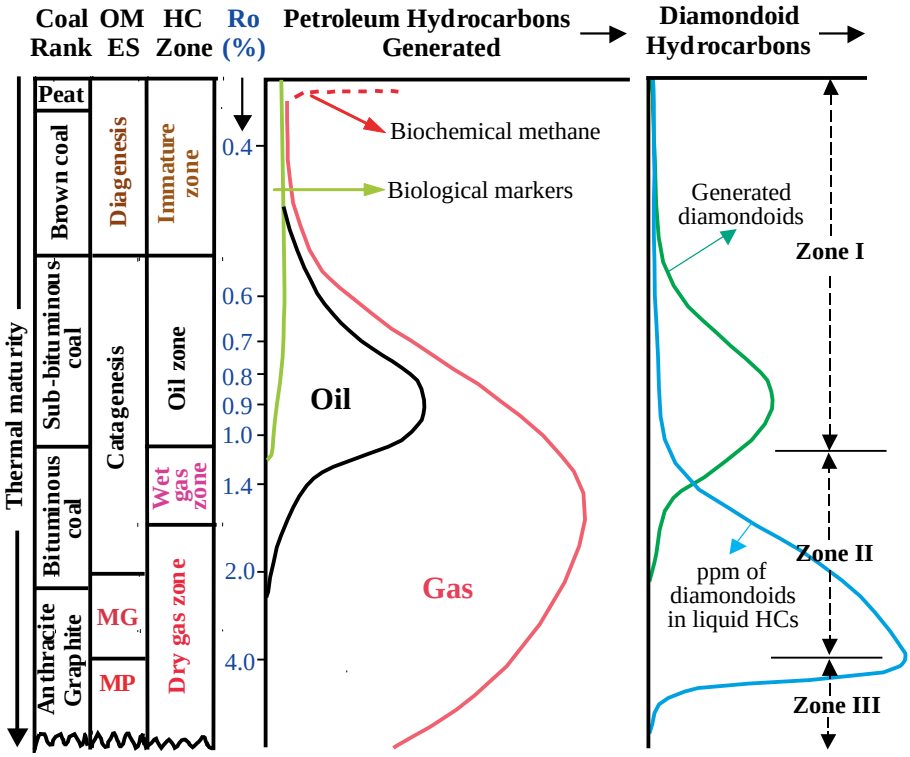

Figure 1. General schemes for formation of petroleum hydrocarbons and diamondoids, concentrations of diamondoids in liquid hydrocarbons, and stages of coal rank as a function of thermal maturity of the source rock, showing the "lifecycle" of diamondoids in nature. Coal Rank = stages of coal rank; OM ES = main stages of evolution of organic matter; HC Zone = main zones of hydrocarbon generation; MG = metagenesis; MP = metamorphism; Zone I = diamondoid birth/generation; Zone II = diamondoid generation and enrichment; Zone III = diamondoid destruction.

REFERENCES

Dahl, J.E., Moldowan, J.M., Peters, K., Claypool, G., Rooney, M., Michael, G., Mello, M. and Kohnen, M. (1999) Diamondoid hydrocarbons as indicators of oil cracking. *Nature* **399**, 54-66.

Wei, Z., Moldowan, J.M., Goldstein, T. and Jarvie, D. (2006a) The catalytic effects of minerals on the formation of diamondoids from kerogen macromolecules. *Organic Geochemistry* **37**, 1421-1436.

Wei, Z., Moldowan, J.M., Jarvie, D. and Hill, R. (2006b) The fate of diamondoids in coals and sedimentary rocks. *Geology* **34**, 1013-1016.

ASSESSING THE ROLE OF STEROL METHYLTRANSFERASE HOMOLOGUES IN THE METHYLHOPANOID SYNTHESIS PATHWAY OF *METHYLOBACTERIUM EXTORQUENS* AM-1

Alexander S. BRADLEY[1], Christopher J. MARX[2] and Roger E. SUMMONS[1]

1. Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 USA.
2. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138 USA

Hopanoids are polycyclic triterpenoids that are abundant in the geological record and common in some bacteria. Among the classes of hopanoids, methylhopanoids have been of particular utility to organic geochemists. In this study we test the hypothesis that the gene responsible for hopanoid A-ring methylation is a bacterial homologue of eukaryotic S-adenosyl-L-methionine (SAM)-dependent sterol methyltransferase.

Several cyanobacteria and proteobacteria produce hopanoids methylated at the C-2 position (Summons *et al.*, 1999). In ancient sediments, the detection of 2α-methylhomohopanes (derived from 2β-methyl-bacteriohopanepolyols) in high amounts relative to desmethylhopanes is usually attributed to ancient cyanobacterial productivity. These compounds have been detected in rocks 2.7 billion years old; this has been interpreted as the earliest evidence for the evolution of oxygenic photosynthesis (Brocks *et al.*, 1999). Similarly, 3β-methylhopanoids are produced by aerobic type I methanotrophs such as *Methylococcus* (Summons & Jahnke, 1992). The detection of their hydrocarbon derivatives in ancient rocks is usually interpreted as a proxy for aerobic methane cycling and used to infer paleoenvironmental conditions (e.g. Brocks *et al.*, 2005).

Despite the importance of these molecules to organic geochemists, very little is known regarding either the genetic basis for hopanoid methylation or the physiological function of hopanoids. Identification of the gene or genes responsible for hopanoid methylation would provide a basis with which to rapidly examine the potential distribution of methylhopanoids in microbes (via screening of genomic databases). Comparative sequence analysis of these genes may shed light on the evolution of this ancient pathway. Understanding the physiological role of these molecules would be a boon to investigations that make paleoenvironmental interpretations upon their detection.

The methyl group on the hopanoid A-ring is known to be derived from L-methionine, likely via SAM (Zundel & Rohmer, 1985). SAM is a methyl donor for a variety of methyltransferase enzymes, including the sterol methyltransferases (SMTs) that operate on the sterol side chain in some eukaryotes. Examination of genomic databases revealed the

presence of SMT homologues in the genomes of several methylhopanoid-synthesizing bacteria that lack sterols, including the cyanobacteria *Synechococcus* and *Nostoc*, the methanotroph *Methylococcus*, and the methylotroph *Methylobacterium*, among others. The translated amino acid sequences for these genes contain a highly conserved region that corresponds to the SAM-binding domain in SMT, but lack the sterol-binding region that is unique to SMT. Due to i) the presence of these genes in a wide variety of methylhopanoid synthesizing organisms, ii) the fact that these genes are homologous to SMT, which operates on triterpenoids, and iii) the likelihood that these genes are SAM-dependent, it has been hypothesized that these genes encode enzymes operating to methylate hopanoid A-rings (Summons *et al.*, 2006).

The α-proteobacterium *Methylobacterium extorqens* AM1 is a genetically tractable methylhopanoid producer in which we are conducting experiments to test this hypothesis. When grown on succinate, *Methylobacterium* produces C_{30}-methylhopanols. Its genome contains a SMT homologue with 35% identity to SMT1 of *Arabidopsis* and a conserved SAM-binding region. We are creating several mutant strains of *Methylobacterium*, each of which lacks a gene we suspect may be involved in hopanoid synthesis (such as squalene-hopene cyclase) or methylation. Examination of the hopanoid content of the mutant strain lacking the SMT homologue will test our hypothesis that this gene participates in A-ring methylation. Physiological characteristics of this and other mutant strains will be compared to wild-type *Methylobacterium*, in an attempt to gain insight into the physiological function of hopanoids and methylhopanoids.

REFERENCES

Brocks, J.J., Logan, G.A., Buick, R., Summons, R.E., (1999) Archean molecular fossils and the early rise of eukaryotes. *Science (Washington, D. C.)*, 285(5430), 1033-1036.

Brocks, J.J., Love, G.D., Summons, R.E., Knoll, A.H., Logan, G.A., Bowden, S.A., (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. *Nature (London, United Kingdom)*, 437(7060), 866-870.

Summons, R.E., Bradley, A.S., Jahnke, L.L., Waldbauer, J.R., (2006) Steroids, triterpenoids and molecular oxygen. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, 361(1470), 951-968.

Summons, R.E., Jahnke, L.L., (1992) Hopenes and hopanes methylated in Ring-A: Correlation of the hopanoids from extant methylotrophic bacteria with their fossil analogues. In: J.M. Moldowan, P. Albrecht, R.P. Philp (Eds.), *Biological Markers in Sediments and Petroleum*, pp. 182-200. Prentice Hall, Englewood Cliffs, New Jersey.

Summons, R.E., Jahnke, L.L., Hope, J.M., Logan, G.A., (1999) 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature (London)*, 400(6744), 554-556.

Zundel, M., Rohmer, M., (1985) Prokaryotic triterpenoids. 1. 3 beta-Methylhopanoids from Acetobacter species and Methylococcus capsulatus. *European journal of biochemistry / FEBS*, 150(1), 23-7.