12.158 Lecture 5

- Hopanoids and other cyclic terpenoids
  - Structures, biosynthesis, diagenesis
  - Hopanoid hydrocarbons; stereochemistry vs maturity
  - Hopanoids as process and environment indicators

Hopanoids

- First recognised as a class of C_{30} pentacyclic triterpenes found in ferns, mosses and dammar resins

- 'Hopane' named after the Dipterocarp plant genus *Hopea*, itself after botanist John Hope

- Biosynthetic kinship to sterols, tetrahymanol & oleanoids, via squalene recognised in 60's

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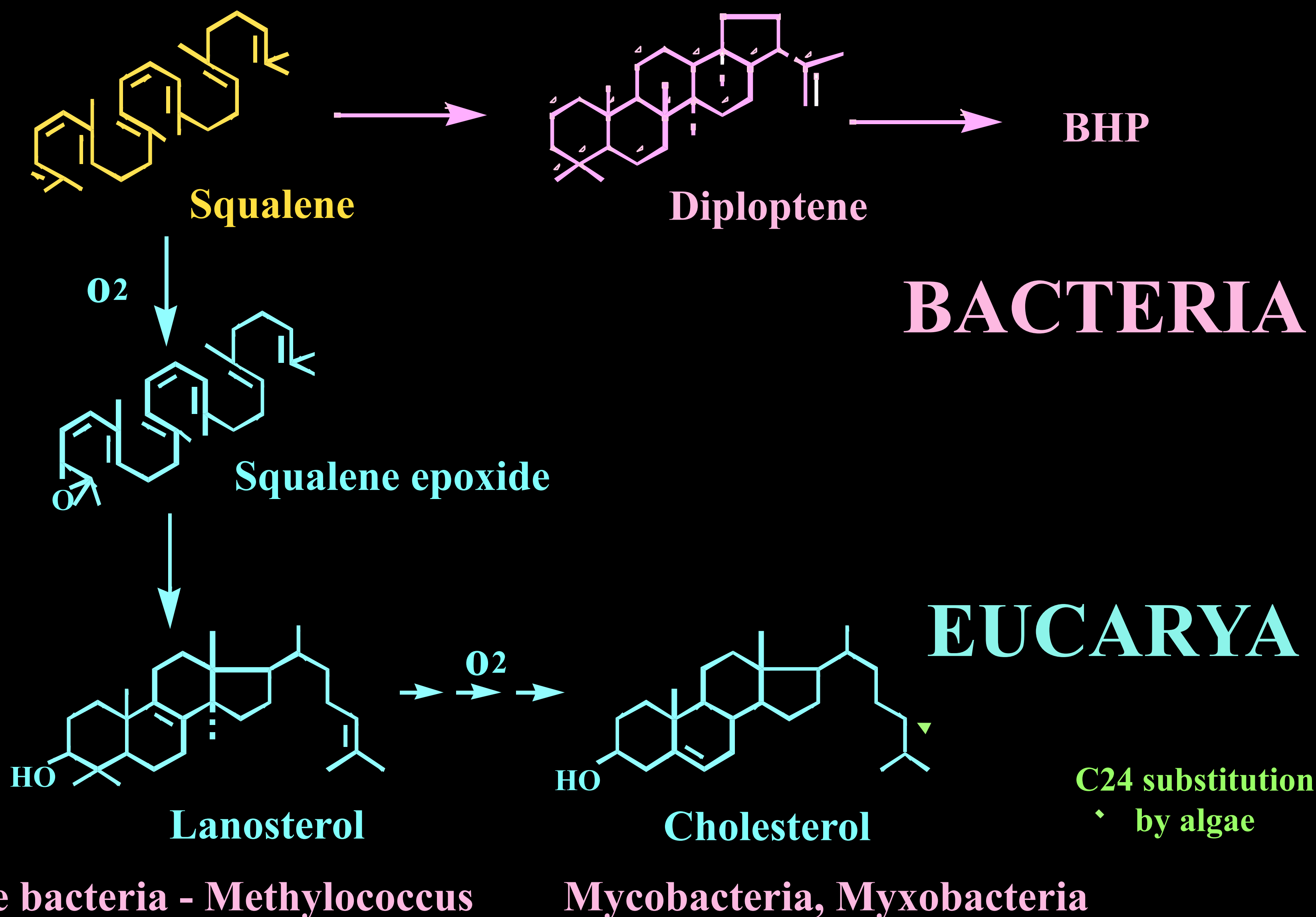
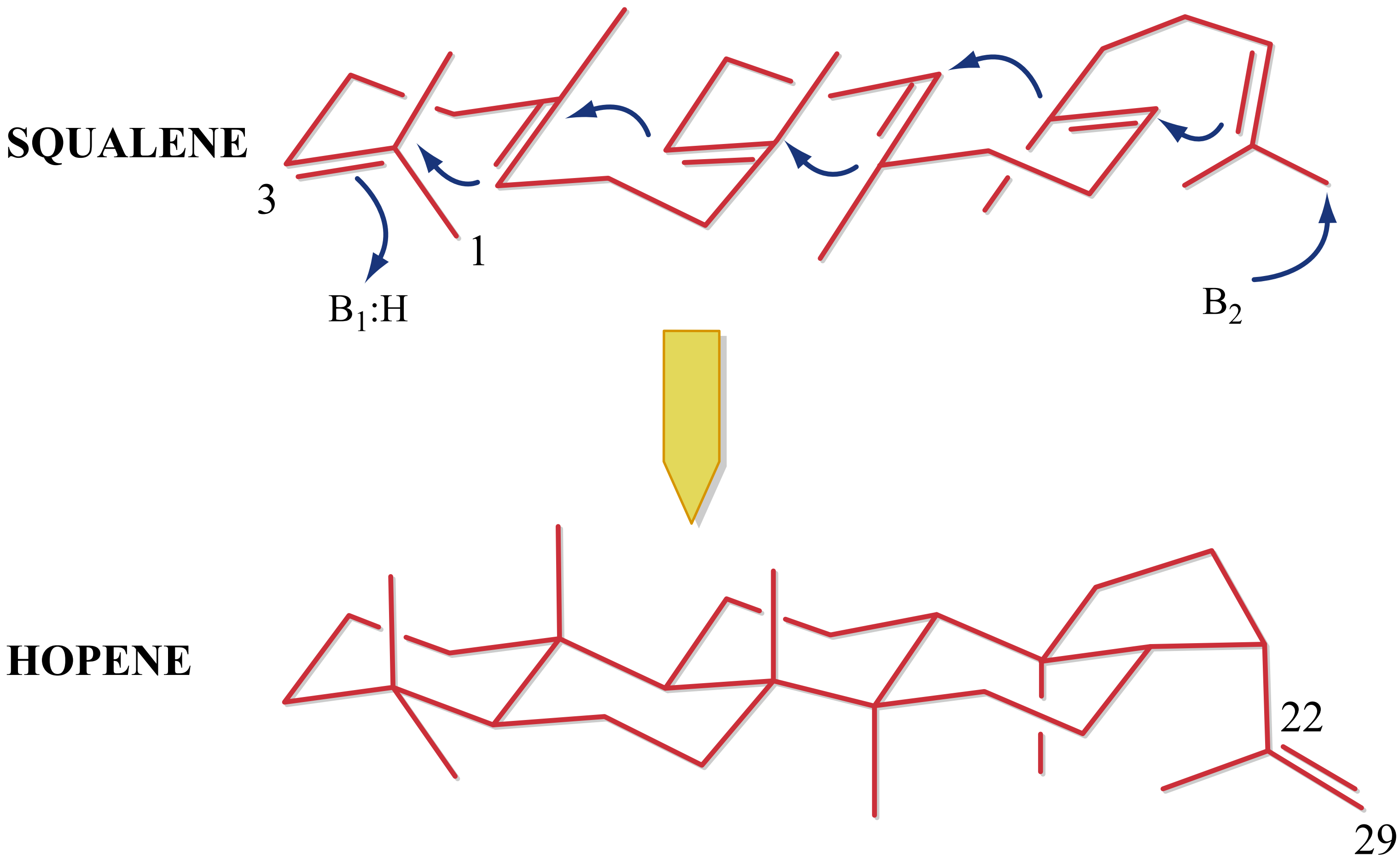
Evolution of Hopane & Sterol Bioynthesis



Structure and Function of a Squalene Cyclase

K. Ulrich Wendt, Karl Poralla, Georg E. Schulz *

The crystal structure of squalene-hopene cyclase from *Alicyclobacillus acidocaldarius* was determined at 2.9 angstrom resolution. The mechanism and sequence of this cyclase are closely related to those of 2,3-oxidosqualene cyclases that catalyze the cyclization step in cholesterol biosynthesis. The structure reveals a membrane protein with membrane-binding characteristics similar to those of prostaglandin-H_2 synthase, the only other reported protein of this type. The active site of the enzyme is located in a large central cavity that is of suitable size to bind squalene in its required conformation and that is lined by aromatic residues. The structure supports a mechanism in which the acid starting the reaction by protonating a carbon-carbon$^{\alpha}$double bond is an aspartate that is coupled to a histidine. Numerous surface   helices are connected by characteristic QW-motifs (Q is glutamine and W is tryptophan) that tighten the protein structure, possibly for absorbing the reaction energy without structural damage.



The proposed reaction steps in squalene-hopene cyclases involving carbocationic intermediates. The general acid B_1:H protonates (H) squalene at C_3, whereas the general base B_2 deprotonates at C_{29} of the hopenyl cation. In a side reaction, the cation is hydroxylated forming hopan-22-ol (i.e. diplopterol)

Image by MIT OpenCourseWare.

This image has been removed due to copyright restrictions.

Figure 5. The color-coded surface representations (30) with nonpolar (yellow), positive (blue), and negative (red) areas. (**A**) View similar to Fig. 2 but rotated around a vertical axis and sliced. The cutting plane (checked) opens the large internal cavity with the bound inhibitor LDAO. The nonpolar channel runs to the left, opening into a nonpolar plateau. The channel constriction (C) appears closed, but it is mobile enough to be readily opened. At the upper left, hopane (two views) is shown at scale. (**B**) View similar to Fig. 2 directly onto the 1600 Å2 nonpolar plateau with the channel entrance (E) at its center and two nonpolar side chains pointing to the outside. This is the only large nonpolar region on the surface

Discovery of Geohopanoids

- Hopane identified in Green River Shale by Burlingame, Haug, Belksky & Calvin, PNAS, 1965.

- C_{27}-C_{31} Triterpanes in optically active petroleum distillates, Hills & Whitehead, Nature 1966

- Homohopane in Green River, Ensminger Maxwell (Bristol & Strasbourg, '72)

- Extended hopane series to C_{35} ubiquitous in the geosphere incl. petroleum, soils and diverse sediments

Ensminger, van Dorsselaer, Spyckerelle, Albrecht, Ourisson (Strasbourg) and Eglinton, Maxwell, Kimble, Philp & Brooks (Bristol) 1973-4

Hills & Whitehead (BP) speculated there was a C_{35} precursor

Hopanoids in Bacteria & Rocks?

- Diploptene (C_{30}) identified in bacteria & cyanonbacteria, de Rosa (1971) incl. *Methylococcus capsulatus* , Bird et al., (1971)

•ββ-hopane and ββ-homohopane in *Bacillus acidocaldarius,* de Rosa 1973

- Fossil hopanoids exhibit αβ, βα and ββ stereochemistry with 22S+R; ββ and βα recognized as less stable than αβ

- C_{30} C-3 oxygenated triterpane alcohols and ketones in Messel, but not equivalent C_{30} hydrocarbons suggest many hopane hydrocarbons entered as 3-desoxy components

- Mystery 'almost' solved when Forster, Biemann et al characterized a C_{35} tetrahydroxy triterpenoid in *Acetobacter xylinum* (1973)

Hopanoids

Biochem. J. (1973) **135**, 133-143
Printed in Great Britain

The Structure of Novel C_{35} Pentacyclic Terpenes from *Acetobacter xylinum*

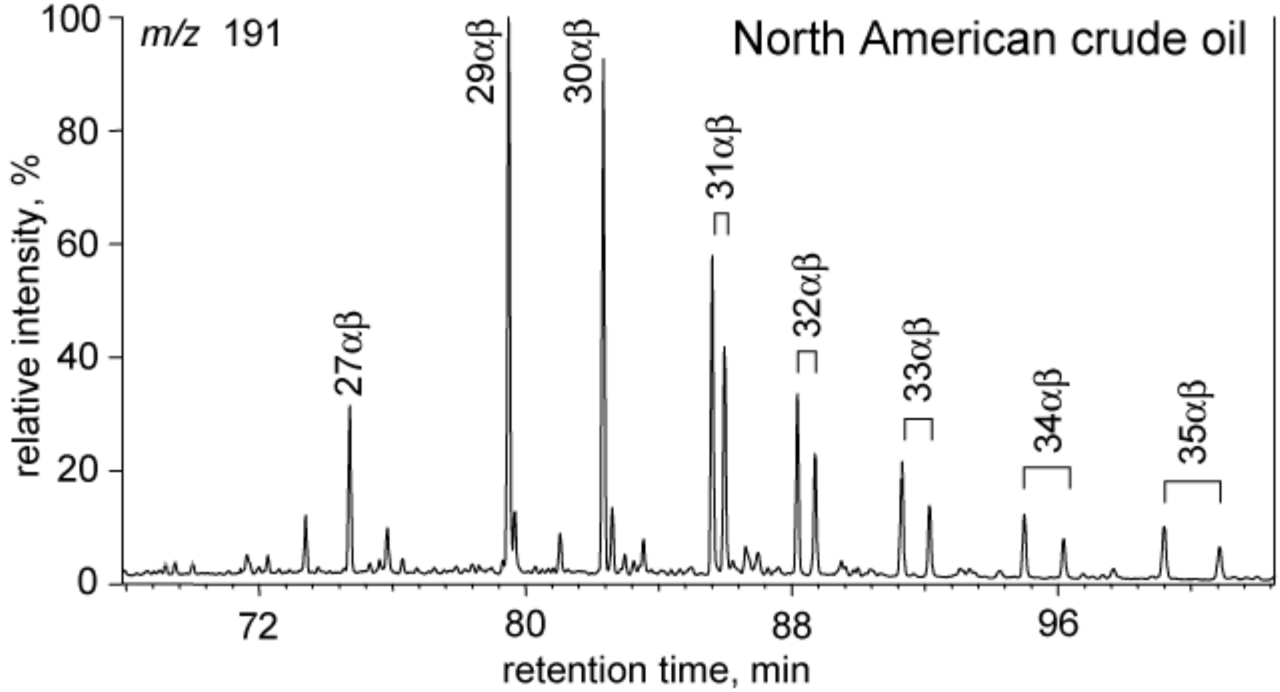
By HANS J FŎRSTER * and KLAUS BEIMANN
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass. 02139, U.S.A.

And W.GEOFFREY HAIGH
Biomedical Research Laboratory, Dow Corning Corporation, Midland, Mich, 48640, U.S.A.

And NEIL. H.TATTRIE and J.ROSS COLVIN,
Division of Biological Sciences, National Research Council of Canada, Ottawa K1A OR6, Canada

(Received 14 February 1973)

Patterns of Geohopanoids in GC-MS



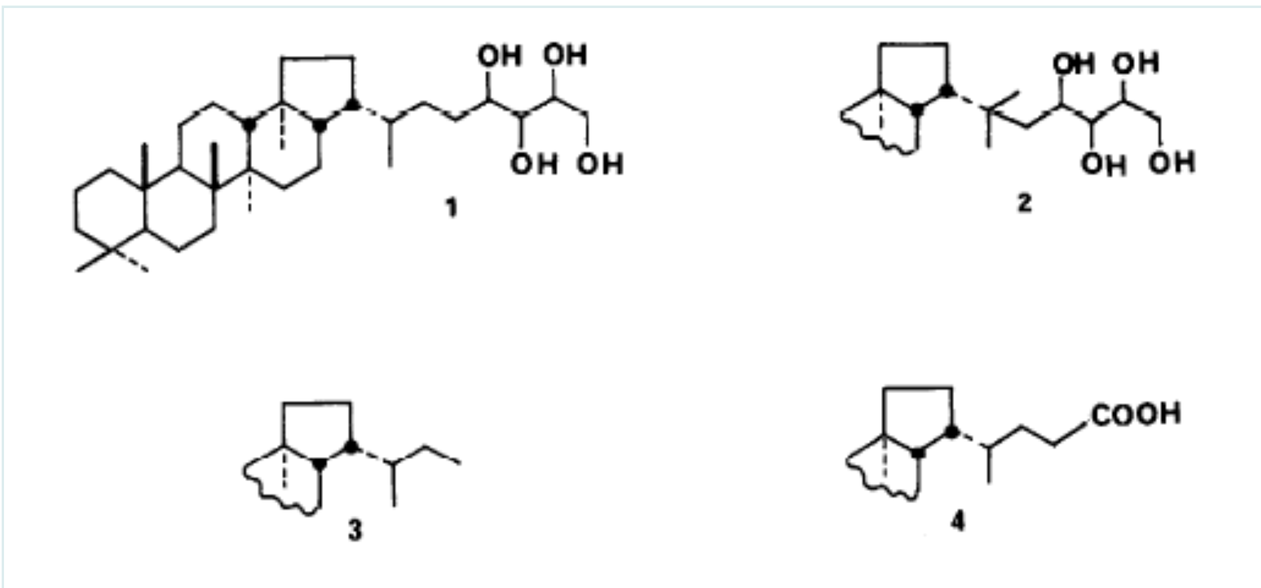
Geochemists figured that the absence of C_{28} homologue and pairs of peaks from C_{31}-C_{35} are informative about side-chain structure

Hopanoids

Tetrahedron Letters No. 40, pp 3633 - 3636, 1976. Pergamon Press. Printed in Great Britain.

STRUCTURE DES BACTÉRIOHOPANETÉTROLS D'ACETOBACTER XYLINUM

M. ROHMER* et G. OURISSON**



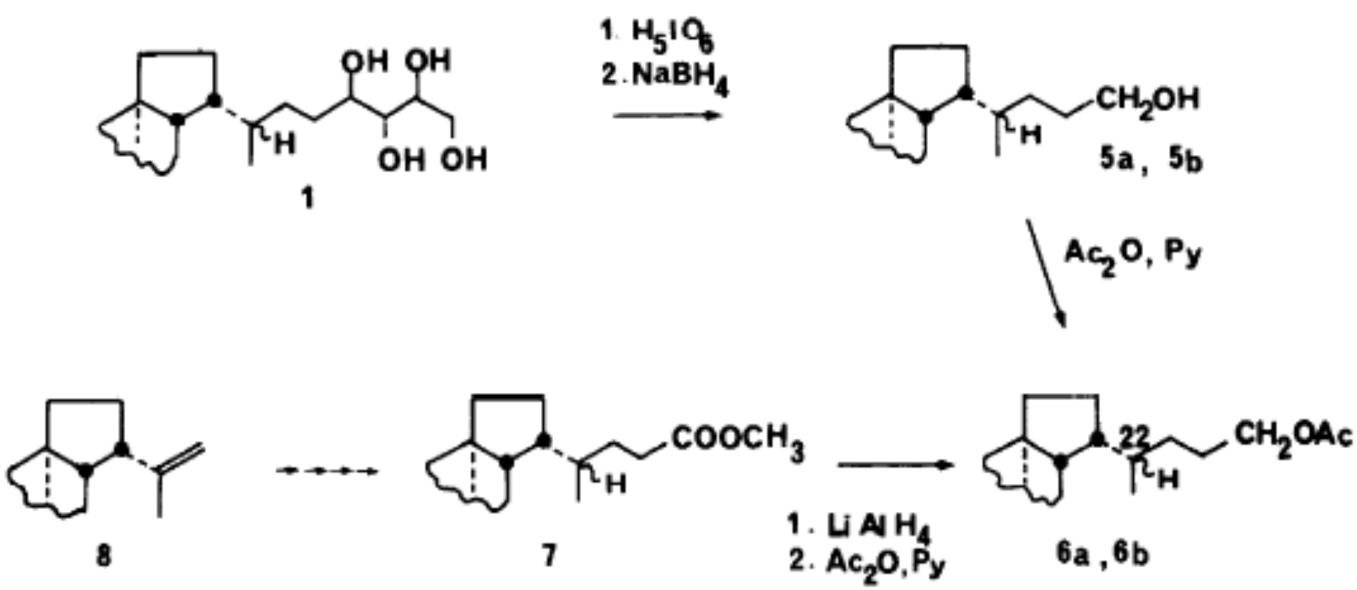
Courtesy Elsevier, Inc., http://www.sciencedirect.com. Used with permission.

Tetrahedron Letters No. 40, pp 3633 - 3636, 1976. Pergamon Press. Printed in Great Britain.

STRUCTURE DES BACTÉRIOHOPANETÉTROLS D'ACETOBACTER XYLINUM

M. ROHMER* et G. OURISSON**

La corrélation de dérivés du bactériohopane avec le diploptène 8 montre donc sans ambigüité que le squelette triterpénique est bien celui du hopane, et que la chaîne linéaire à cinq atomes de carbone supplémentaires est insérée en C_{29} sur la chaîne latérale. D'après leur structure, ces dérivés du bactériohopane pourraient donc être les précurseurs des géolipides hopaniques à plus de trente atomes de carbone.



Hopanoids

Biochem J. (1973) 135, 133–143 133
Printed in Great Britain

The Structure of novel C_{35} pentacyclic terpenes from *Acetobacter xylinum*

By HANS J. FÖRSTER* and KLAUS BIEMANN
Department of Chemistry, Massachusetts Institute of Technology,
Cambridge, Mass. 02139, U.S.A.
and W. GEOFFREY HAIGH
Biomedical Research Laboratory, Dow Corning Corporation, Midland,
Mich. 48640, U.S.A.
and NEIL H. TATTRIE and J. ROSS COLVIN
Division of Biological Sciences, National Research Council of Canada,
Ottawa K1A OR6, Canada

(Received 14 February 1973)

A novel C35 terpene and its monounsaturated analogue were isolated from cultures of Acetobacter xylinum, together with traces of their C36 homologues. These substances were found to be hopane derivatives substituted by a five-carbon chain bearing four vicinal hydroxyl groups. For the parent hydrocarbon the term bacteriohopane is proposed. The elucidation of the structures utilized high-resolution mass spectrometry of the terpenes, degradation to C32 hydrocarbons and detailed mass-spectrometric comparison of these with C32 hydrocarbons synthesized from known pentacyclic triterpenes. High-resolution mass-spectral data of the terpenes are presented. N.m.r. data are in agreement with the proposed structures, which are further supported by the isolation from the same organism of 22-hydroxyhopane and derivative hopene(s).

Hopanoid quotes

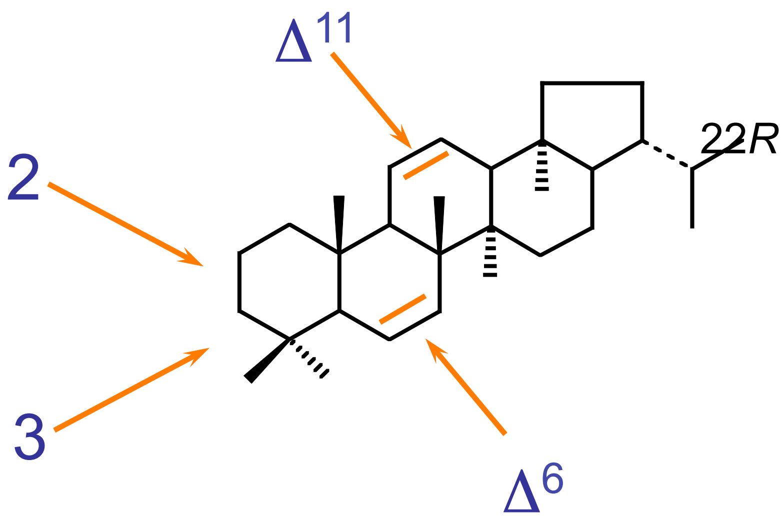
- The formation of the more stable $\alpha\beta$ hopane epimers could constitute , in a given environment, a geochemical clock unless they happen to be still unrecognized constituents of living organisms

  …………. Ensminger et al., Advances in OG..1973

- The total amount of geohopanoids is estimated to be ~10^{12} tons and same order as total mass of organic carbon in all living organisms

  …………. Ourisson and Albrecht, Geohopanoids, the most abundant natural products on Earth, Acc Chem Res 1992

Ring Variations

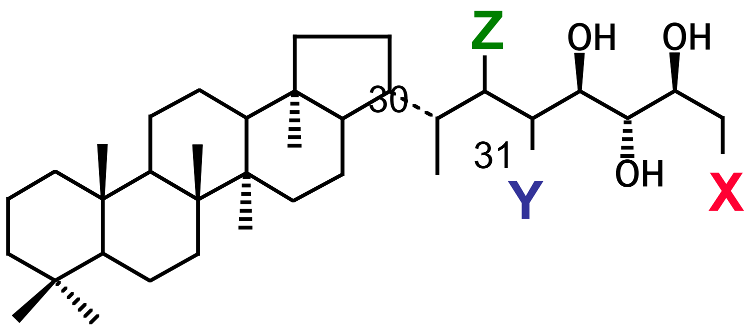


C-2 Me
CYANOBACTERIA
Summons et al., 2000

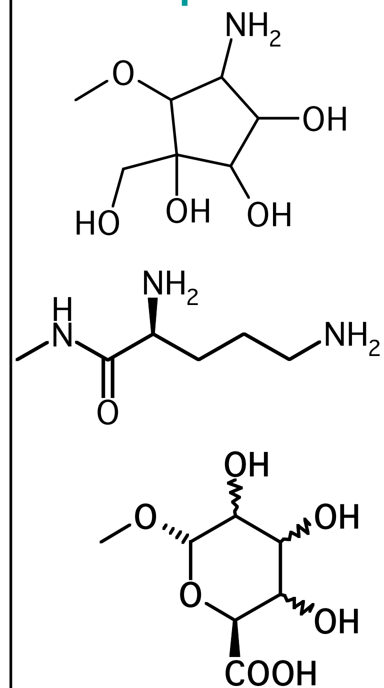
C-3 Me
METHANOTROPHS
(Acetic Acid bacteria)

Δ^{6} and/or Δ^{11}
ACETIC ACID BACTERIA
(Methanotroph)

Side Chain Variations



Composite



TETRA: X=OH, NH_2, composite; Y = Z = H

PENTA: X = OH, NH_2, composite; Y = OH, Z = H
X = OH, Y = H, Z = OH

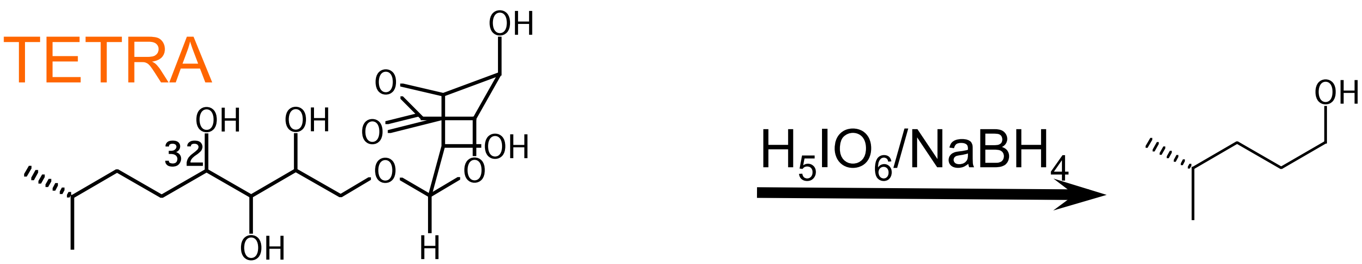
HEXA: X = NH2; Y = Z = OH

Analysis Of Bbiohopanoids

- Highly functionalised, amphiphillic
- Not amenable to conventional GC-MS
- Side chain cleavage (Rohmer et al., 1984)
  - Periodic acid/sodium borohydride
  - Product structure directly related to number and position of functional groups in side chain
- Specific nature of functional groups lost

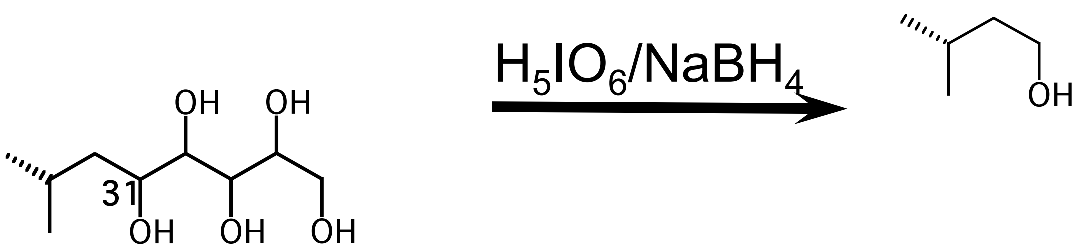
Periodic Acid Oxidation

TETRA



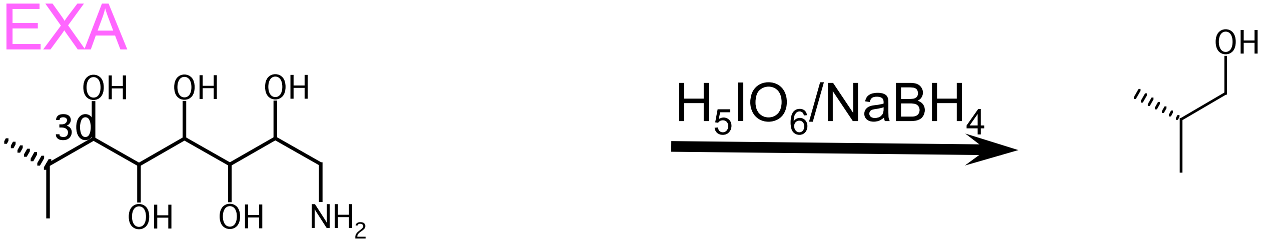
32-hopanol

PENTA



31-hopanol

HEXA



30-hopanol

Hopanoids

The effect of thermal stress on source-rock quality as measured by hopane stereochemistry

WOLFGANG K. SEIFERT, J. MICHAEL MOLDOWAN

Chevron Oil Field Research Company, P.O. Box 1627, Richmond, California 94802, U.S.A.

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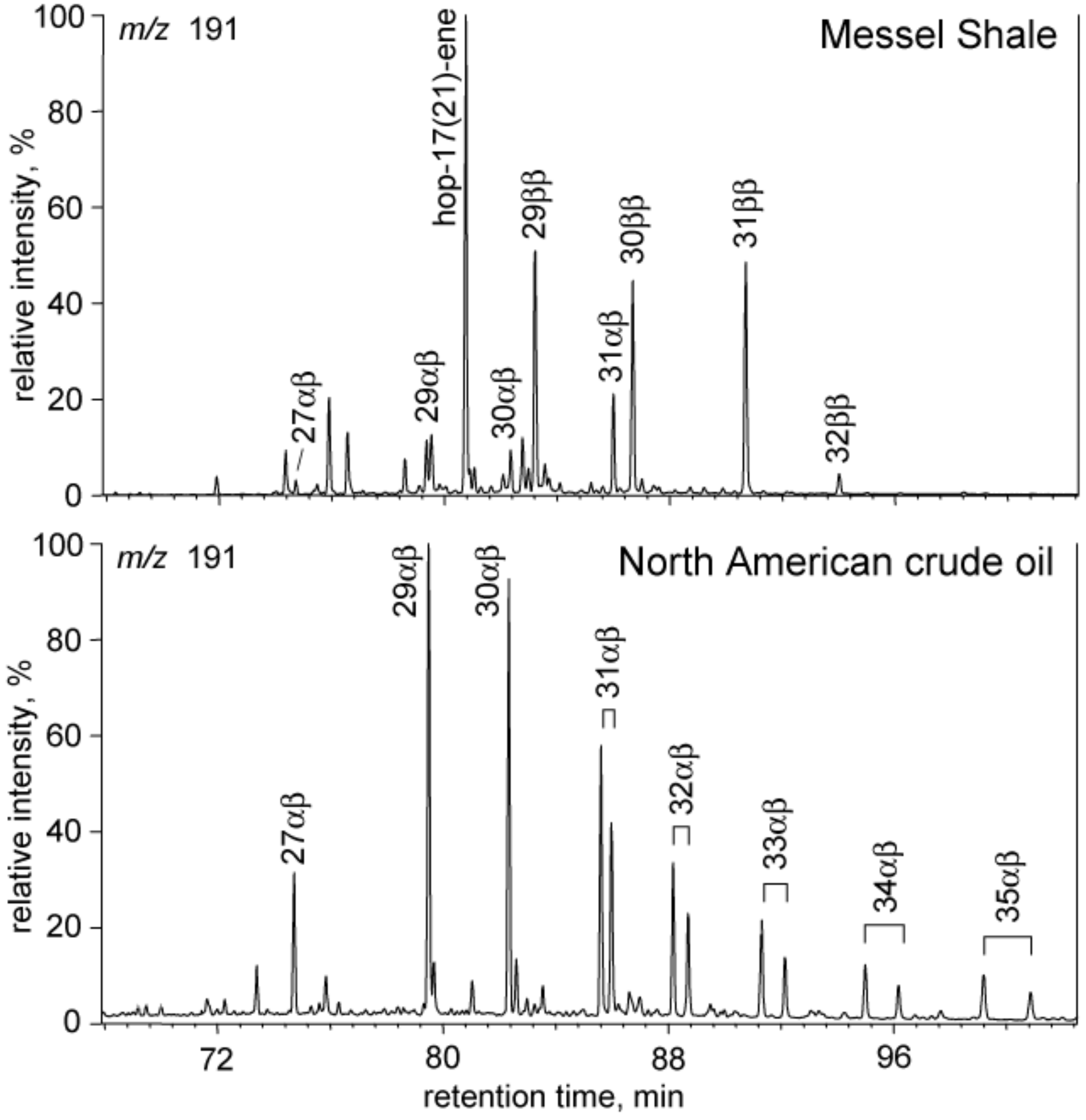
In: A.G. Douglas and J.R. Maxwell, Editors, *Advances in Organic Geochemistry*, Pergamon Press, Oxford (1980), pp. 229–237

Hopanoids & Thermal Maturity

Hopane stereoisomers in an immature rock and a crude oil



Structural Diversity of Hopanoids

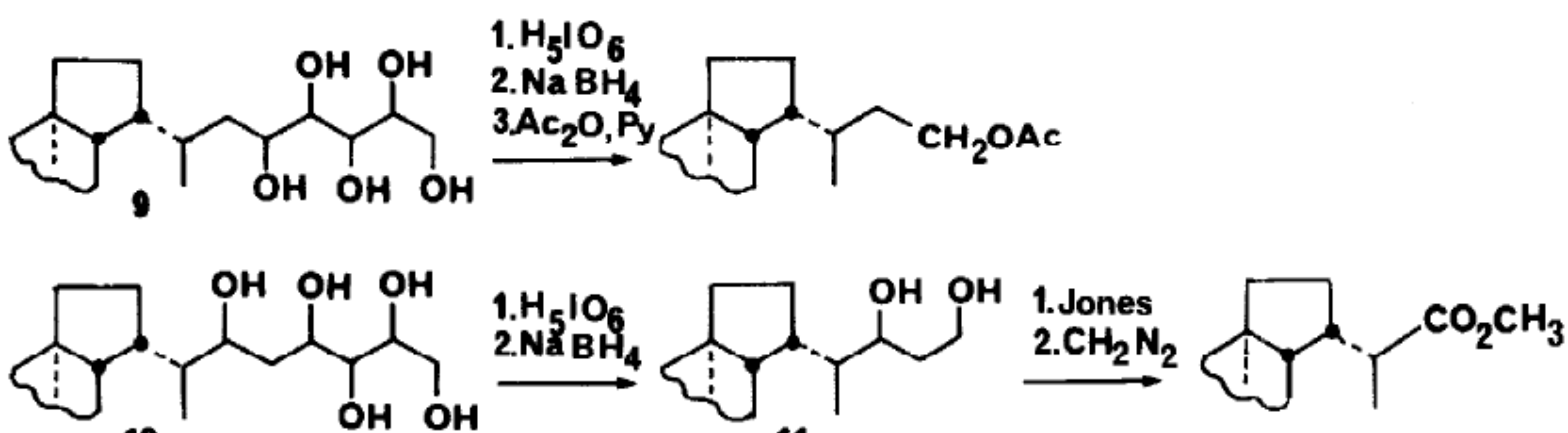
Tetrahedron Letters No. 40, pp 3637 - 3640, 1976. Pergamon Press. Printed in Great Britain.

DÉRIVÉS DU BACTÉRIOHOPANE : VARIATIONS STRUCTURALES ET RÉPARTITION

M. ROHMER* et G. OURISSON**

* Institut de Botanique, Laboratoire de Biochimie Végétale, 28 rue Goethe, 67083 Strasbourg-Cedex, France

** Institut de Chimie, Laboratoire de Chimie Organique des Substances Naturelles, 1 rue Blaise Pascal, 67008 Strasbourg-Cedex, France.



Hopanoids and Physiology

Distribution of hopanoid triterpenes in prokaryotes

Rohmer, M. , Bouvier-Nave, P. , Ourisson, G.
Ecole Nationale Superieure de Chimie de Mulhouse, Universite de Haute Alsace, 68093 Mulhouse Cedex, France

Present in 50% of some 100 strains across diverse taxa

In almost all cyanobacteria, obligate methylotrophs , purple non-sulfur bacteria and diverse chemoheterotrophs

Absent from purple sulfur bacteria, archaebacteria

Not detected in any anaerobe

Bacteriohopane tetrol most common

Journal of General Microbiology Volume 130, Issue 5, 1984 1137-1150

Functional Role of Hopanoids?

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 847-851, February 1979
Evolution

Molecular evolution of biomembranes: Structural equivalents and phylogenetic precursors of sterols

(triterpenes/tetraterpenes/prokaryotes/pre-aerobic evolution)

MICHEL ROHMER, PIERRETTE BOUVIER, AND GUY OURISSON

Evolution: Rohmer *et al.*

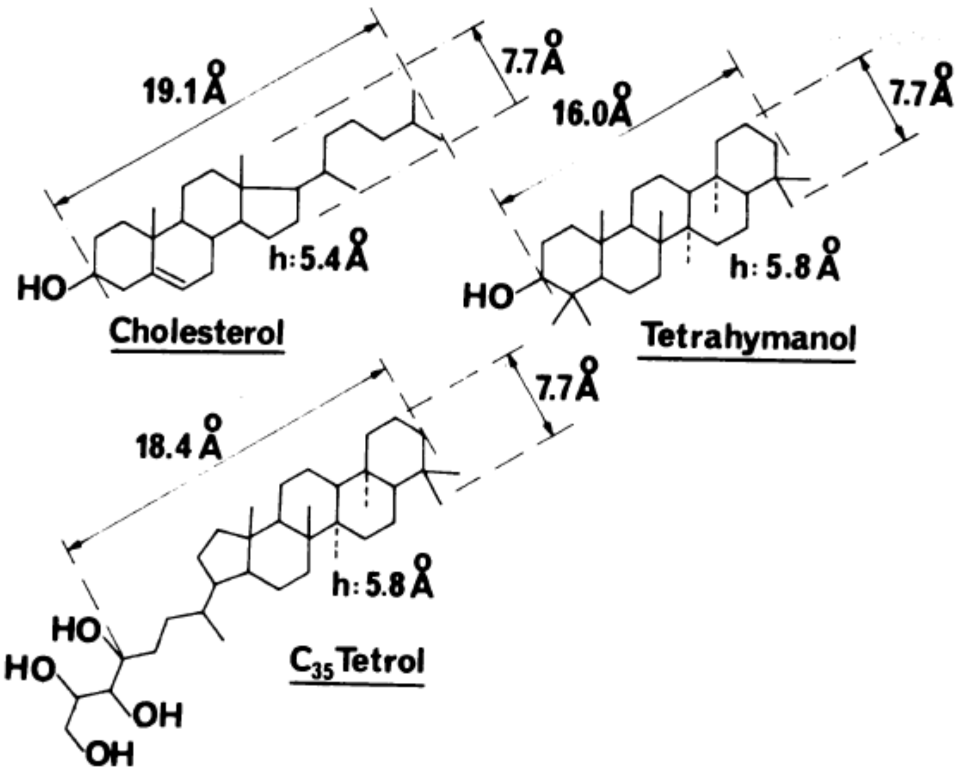


FIG. 2. Comparison of the dimensions of sterols and structural equivalents.

Rohmer & Ourisson, 1976

Rohmer et al., 1979

Kannenberg & Poralla, 1980

Many lines of evidence show an association of BHP with cellular membranes

Image courtesy of Michel Rohmer.
Used with permission.

First Report of 3-Methyl Hopanoids

Tetrahedron Letters No. 40, pp 3641 - 3644, 1976. Pergamon Press. Printed in Great Britain.

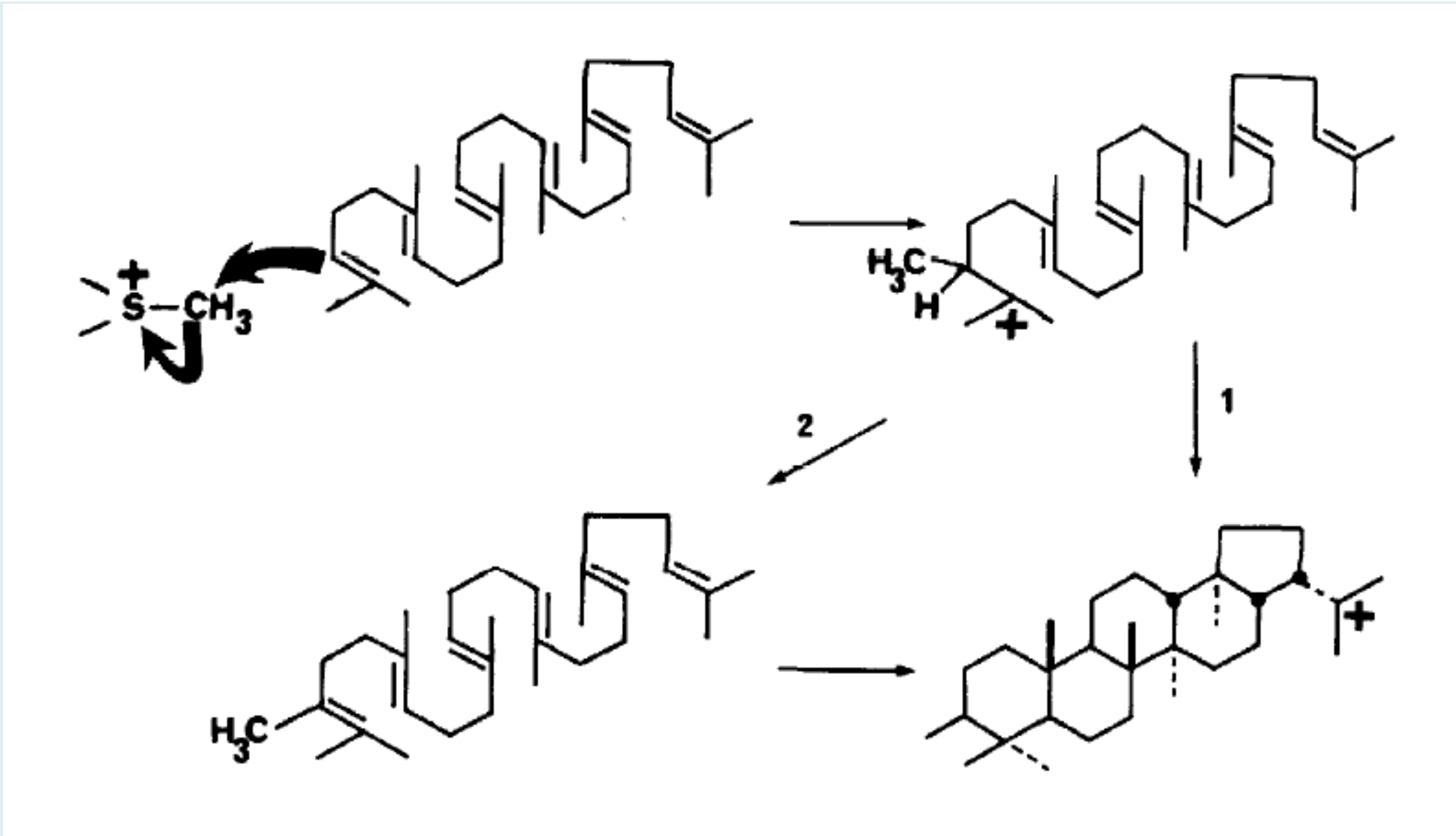
MÉTHYL-HOPANES D'*ACETOBACTER XYLINUM* ET D'*ACETOBACTER RANCENS*: UNE NOUVELLE FAMILLE DE COMPOSÉS TRITERPÉNIQUES

M. ROHMER* et G. OURISSON**

* Institut de Botanique, Laboratoire de Biochimie Végétale, 28 rue Goethe, 67083 Strasbourg-Cedex, France

** Institut de Chimie, Laboratoire de Chimie Organique des Substances Naturelles, 1 rue Blaise Pascal, 67008 Strasbourg-Cedex, France.

Discovery of Methylated Hopanoids



Acetobacter BHP hypothesized to be 3-methylhopanoids on biosynthetic grounds

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Discovery of Methylated Hopanoids

Proof of structure of 3-methyl BHT by correlation with synthetic 3-methyl bishomohopane

Stereochemistry at C3 not assigned until 1985

3β by nmr &
comparison with 3β-methyldiplopterol & 3β-methylhopan-29-01 synthesized from 22-hydroxyhopan-3-one.

3β-methylhopanoid content of *A. pasteurianus ssp. pasteurianus* could be increased up to 60% of the total hopanoid content by addition of L-methionine, the actual methyl donor, to the culture medium.

Discovery of 2β-Me Hopanoids

Prokaryotic triterpenoids
2.2ß-Methylhopanoidsfrom *Methyfobacterium organophilum* and *Nostoc muscorum*, a new series of prokaryotic triterpenoids

Philippe BISSERET, Magali ZUNDEL and Michel ROHMER

Ecole Nationale Suptrieure de Chimie de Mulhouse

Eur. J. Biochem. *150*, 29-34 (1985)

2β-Me Hopanoids

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2β by nmr & direct comparison with 2β-methyldiplopterol synthesized from 22-hydroxyhopan-3-one.

2α-Me Hopanoids

Tetrahedron Vol 47, No. 34, pp 7081-7090, 1991
Printed in Great Britain

2 α-Methylhopanoids: First Recognition in the Bacterium Methylobacterium organophilum and Obtention via Sulfur Induced Isomerization of 2ß-Methylhopanoids.
An account for their presence in sediments.

P Stampf. D Herrmann. P Bisseret. M Rohmer

c. 5% methyldiplopterols 2α by ^{13}C nmr & direct comparison with 2α-methyldiplopterol synthesized from 22-hydroxyhopan-3-one.

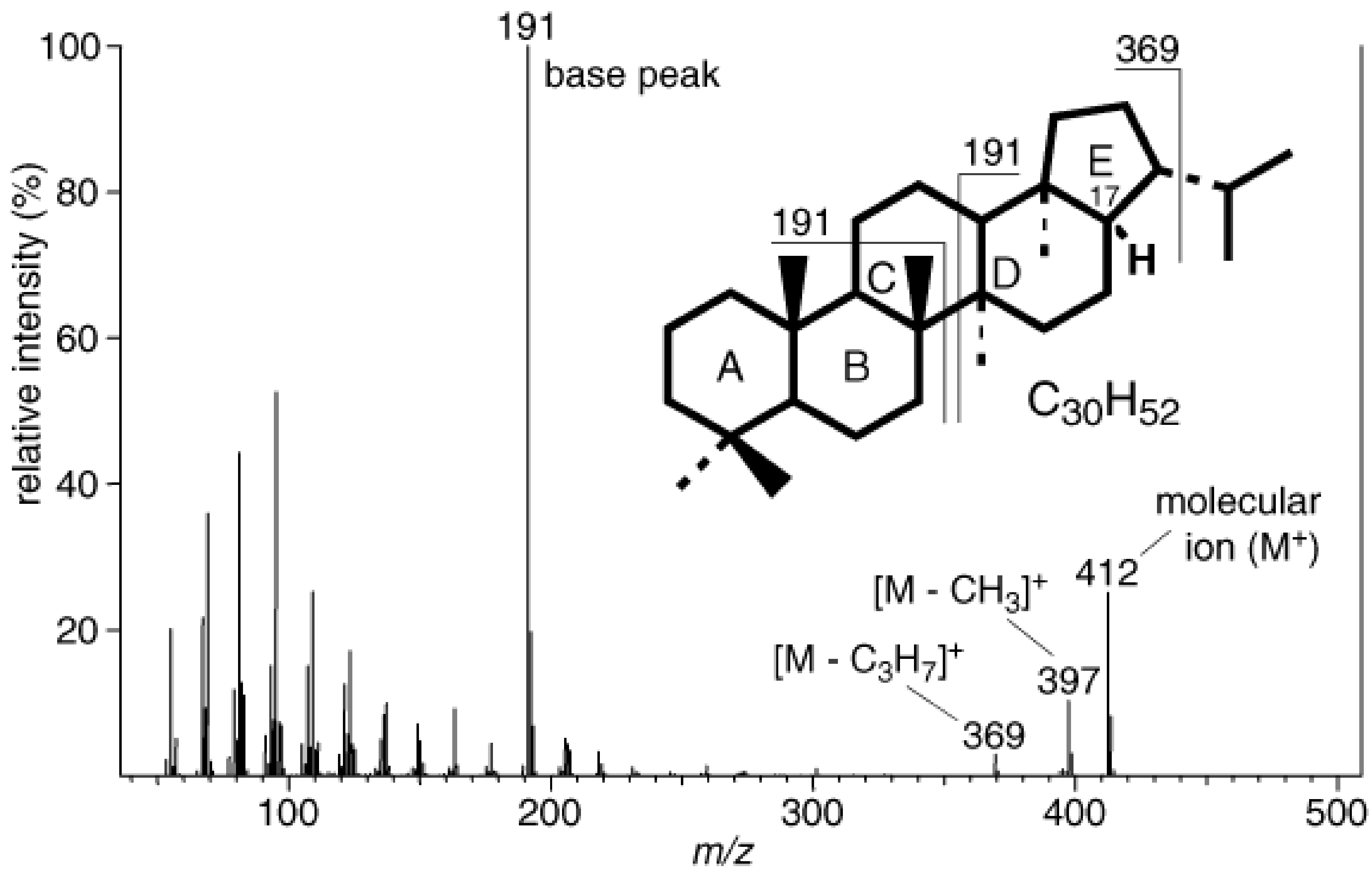
Sloppy methylase? Any 2α-methyl in *Nostoc* or *Rhodopseudomonas*?

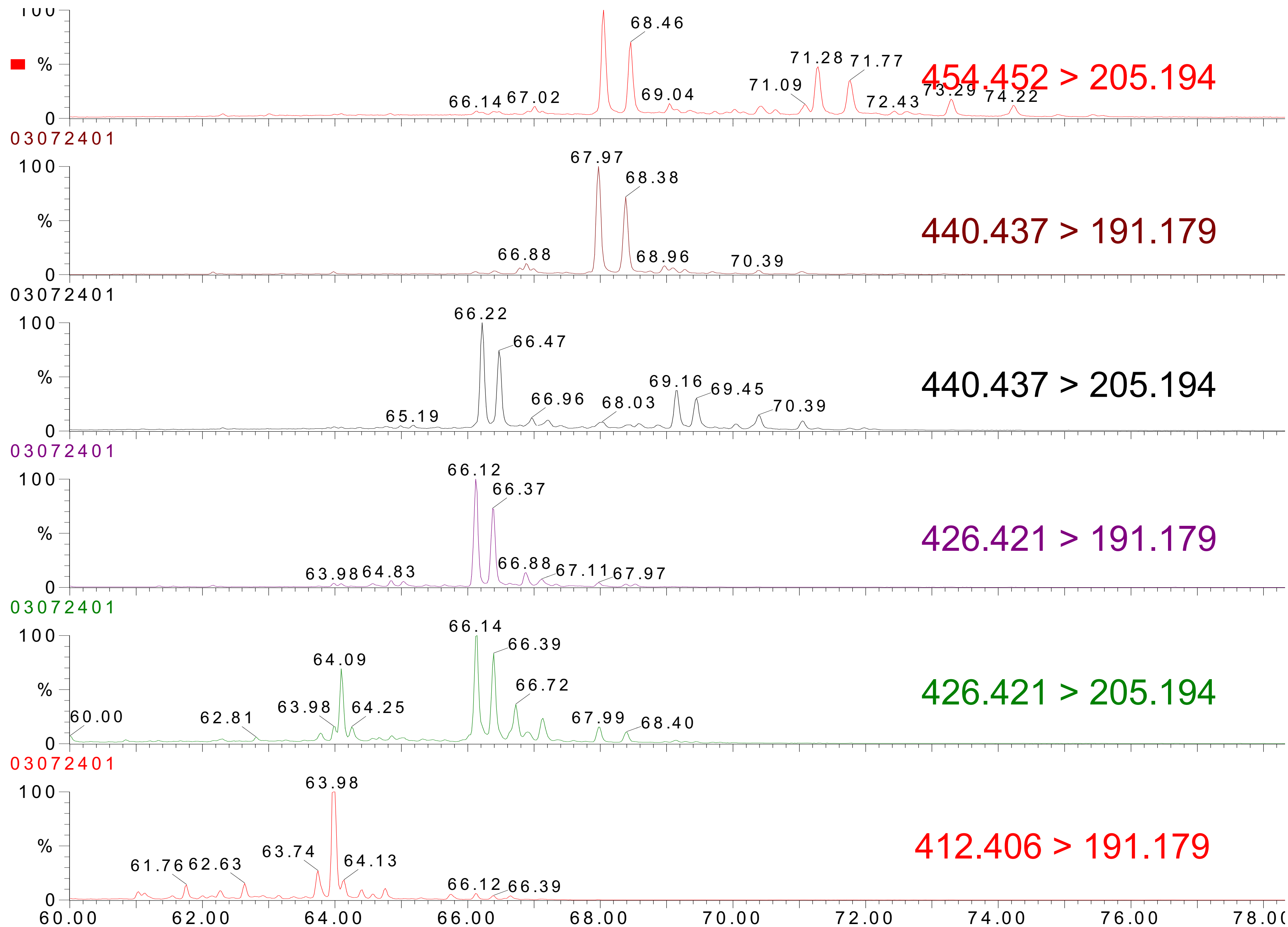
Geological Occurrence of Methyl Hopanoids

- Fossil methyl hopanes reported in sedimentary rocks and oils eg SEIFERT and MOLDOWAN, 1978; ALEXANDER et al., 1984; MCEVOY and GIGER, 1986; SUMMONS and POWELL, 1987
- *Tentatively* but erroneously thought to be 3-methyl hopanes based on the reports of 3-methylhopanoids in methylotrophs
- Complex mixtures precluded any spectroscopic analysis other than GC-MS
- Synthetic approaches already developed by Rohmer but not applied to geo hopanoids

Geological Occurrence of Methyl Hopanoids

Mass spectrum of 17α-hopane





GC with MS-MS -> isomers of each individual homologue to be seen in separate chromatograms

Geological Occurrence of Methyl Hopanoids

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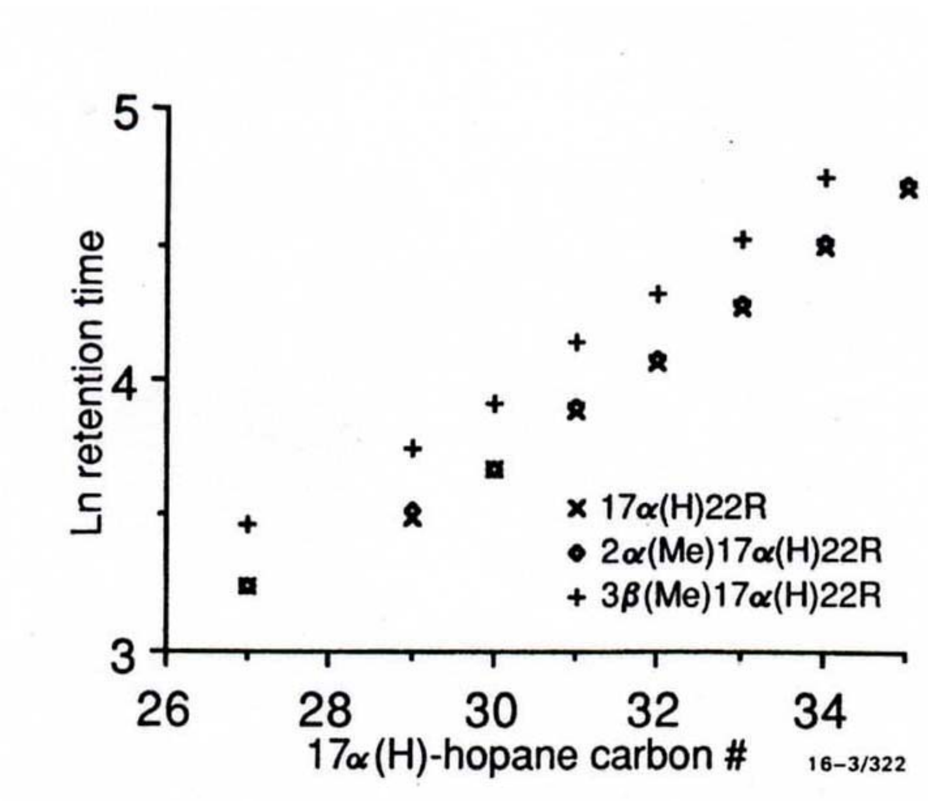
Comparison of 2α-methyl hopane and 3β-methyl hopane with fossil hydrocarbons

Sample was an immature Ordovician sediment with two series of methyl hopanes

Geological Occurrence of Methyl Hopanoids

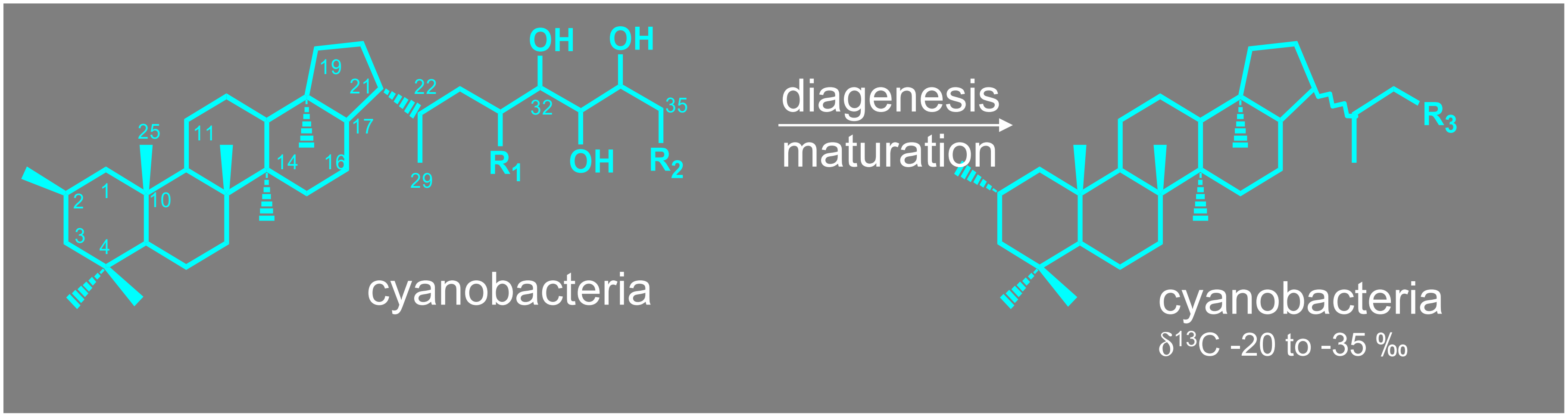


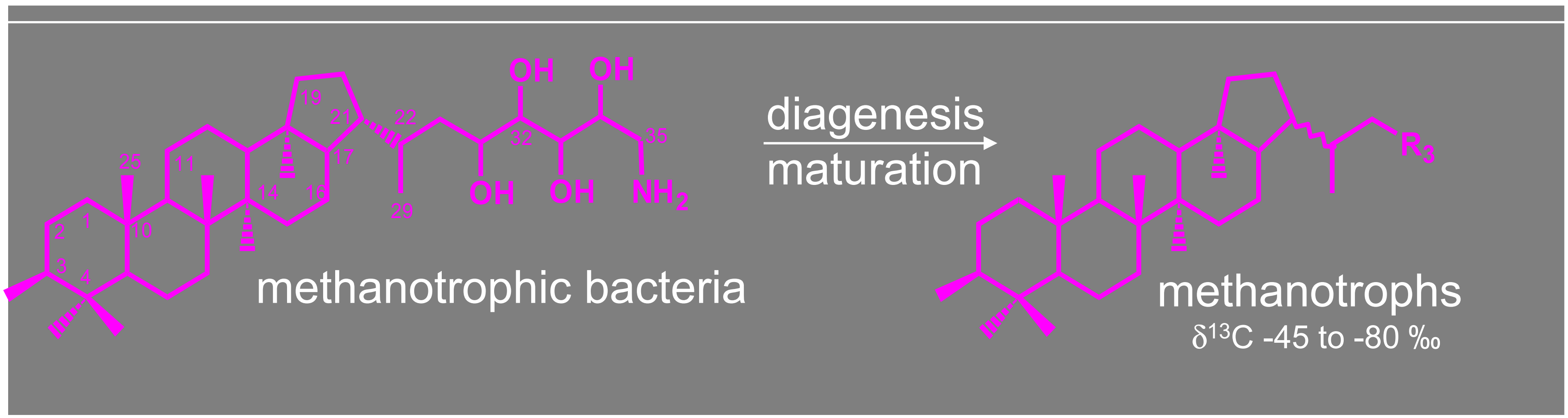
Relative retention times used to assign identity to C_{27}-C_{35} series'

Sample s an immature Ordovician kukersite

Sedimentary methyl hopanes derived from methyl analogues of BHP

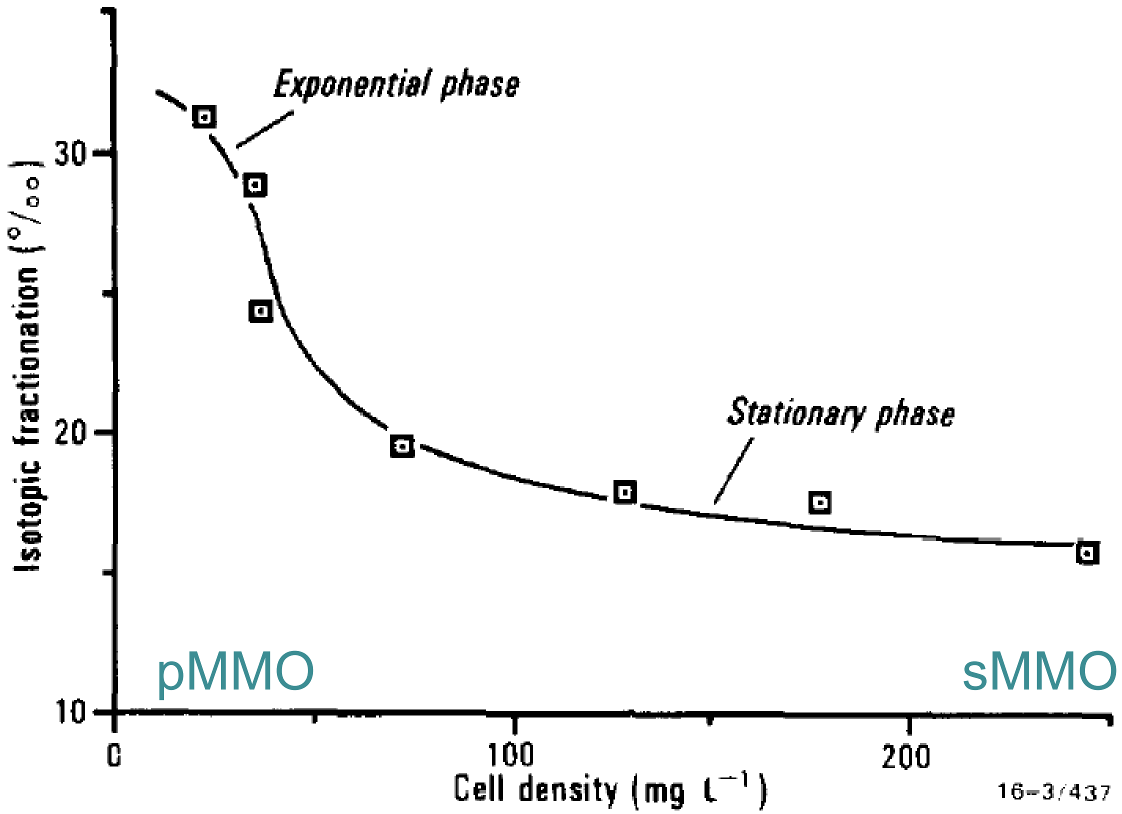
O_2-Diagnostic Bacteriohopanes ??





Jahnke et al., 1992, 1993; 1999; Summons & Jahnke, 1988, 1990; Summons et al., 1994, 1999

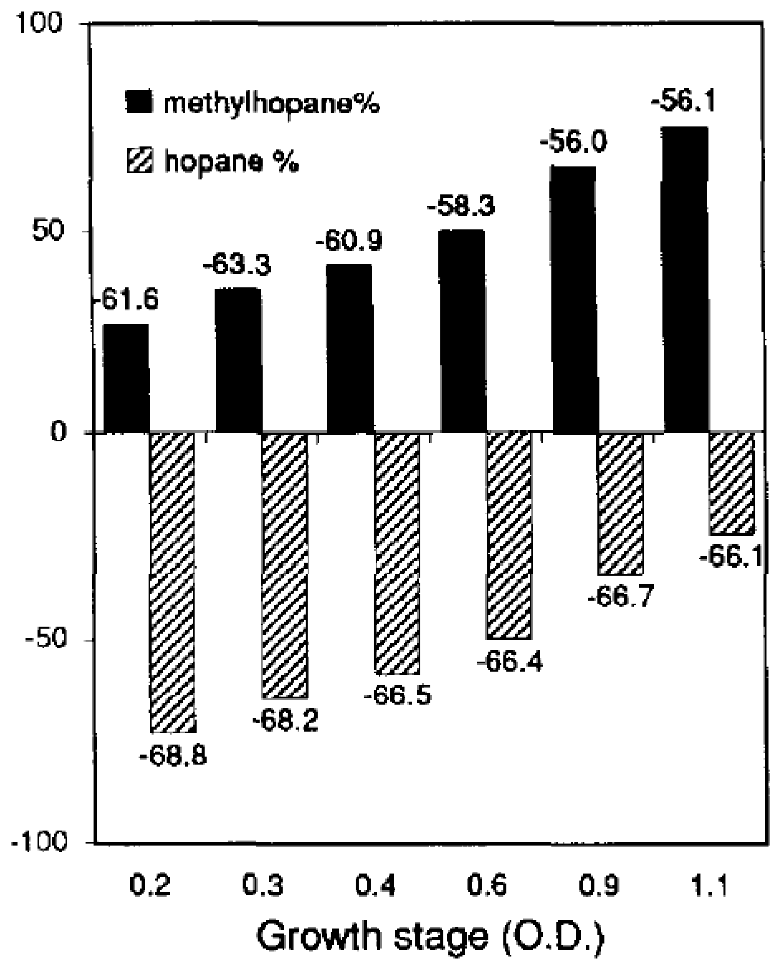
Isotopic Signature of 3-Methyl Hopanoids



C-isotopic fractionation in biomass of *M. capsulatus*:
varies as a function of substrate, growth stage, MMO type

Summons et al., GCA, 1994

Isotopic Signature of 3-Methylhopanoids



Biomass-triterpenoids ε ~ 36‰

Distribution and C-isotopic fractionation in hopanoids of *M. capsulatus* as function of growth stage

Summons et al., GCA, 1994

Geological Occurrence of 3-Methylhopanoids

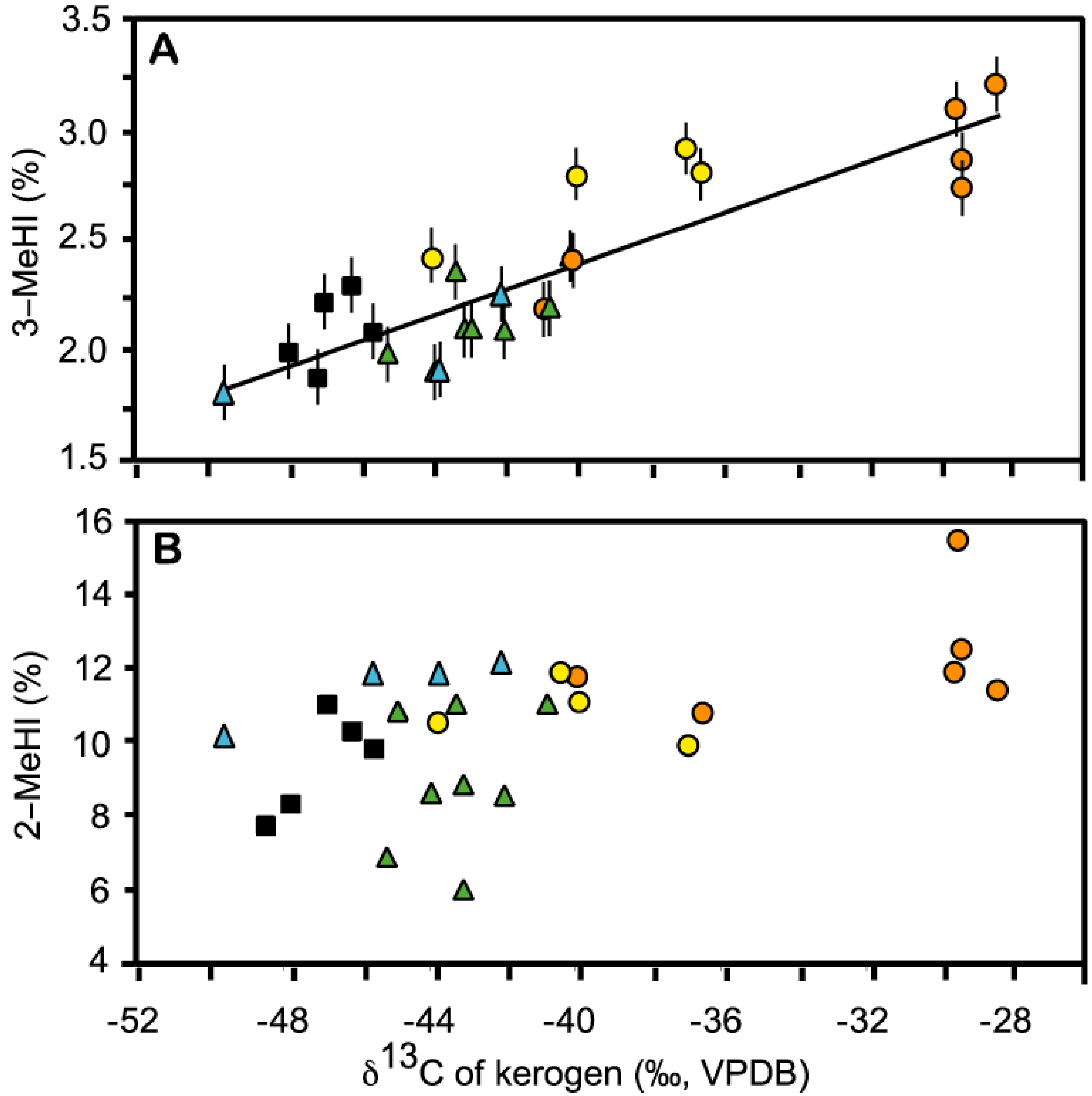
Advances in Organic Geochemistry 1991
Org. Geochem. Vol. 19, Nos 1-3, pp. 265-276, 1992
Printed in Great Britain

An isotopic biogeochemical study of the Green River Oil shale

JAMES W. COLLISTER, ROGER E. SUMMONS, ERIC LICHTFOUSE, and J. M. HAYES
Biogeochemical Laboratories, Department of Geological Sciences and Chemistry Geology Building, Indiana University, Bloomington, IN 47405, U.S.A. and Bureau of Mineral Resources, G.P.O Box 378, Canberra, A.C.T 2601, Australia

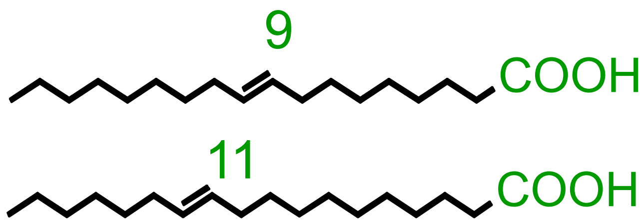
This image has been removed due to copyright restrictions. Please see Figure 7 in the above article.

Hamersley Basin biomarker & isotopic records

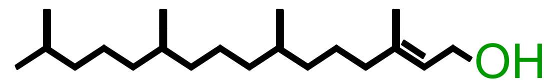


Eigenbrode et al, PNAS 2008

Cyanobacterial Lipids

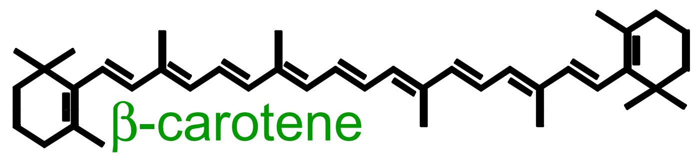


C $_{16\text{-}18}$ fatty acid and PUFA

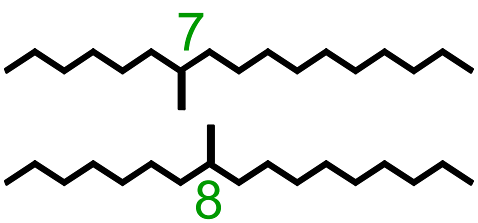


phytol

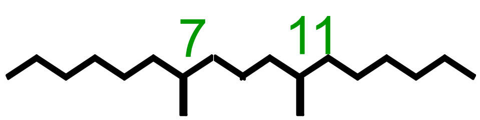
n-heptadecane



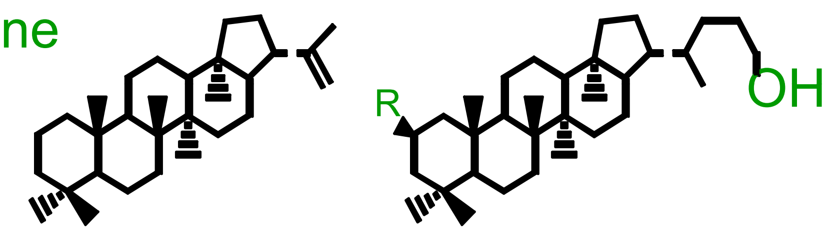
β-carotene



7- & 8-
methylheptadecane



7,11-dimethylheptadecane

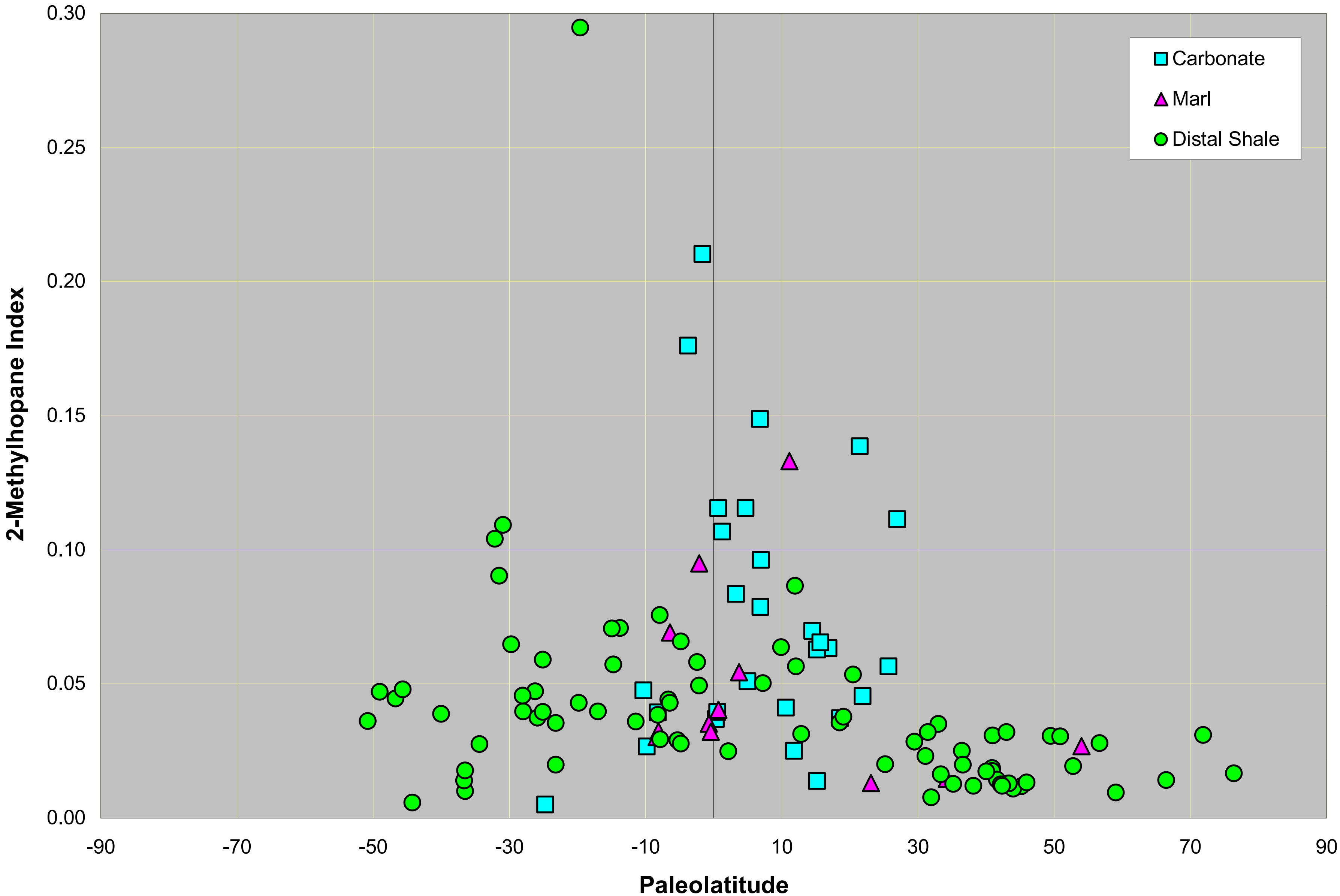


hopanoids & 2-methylhopanols

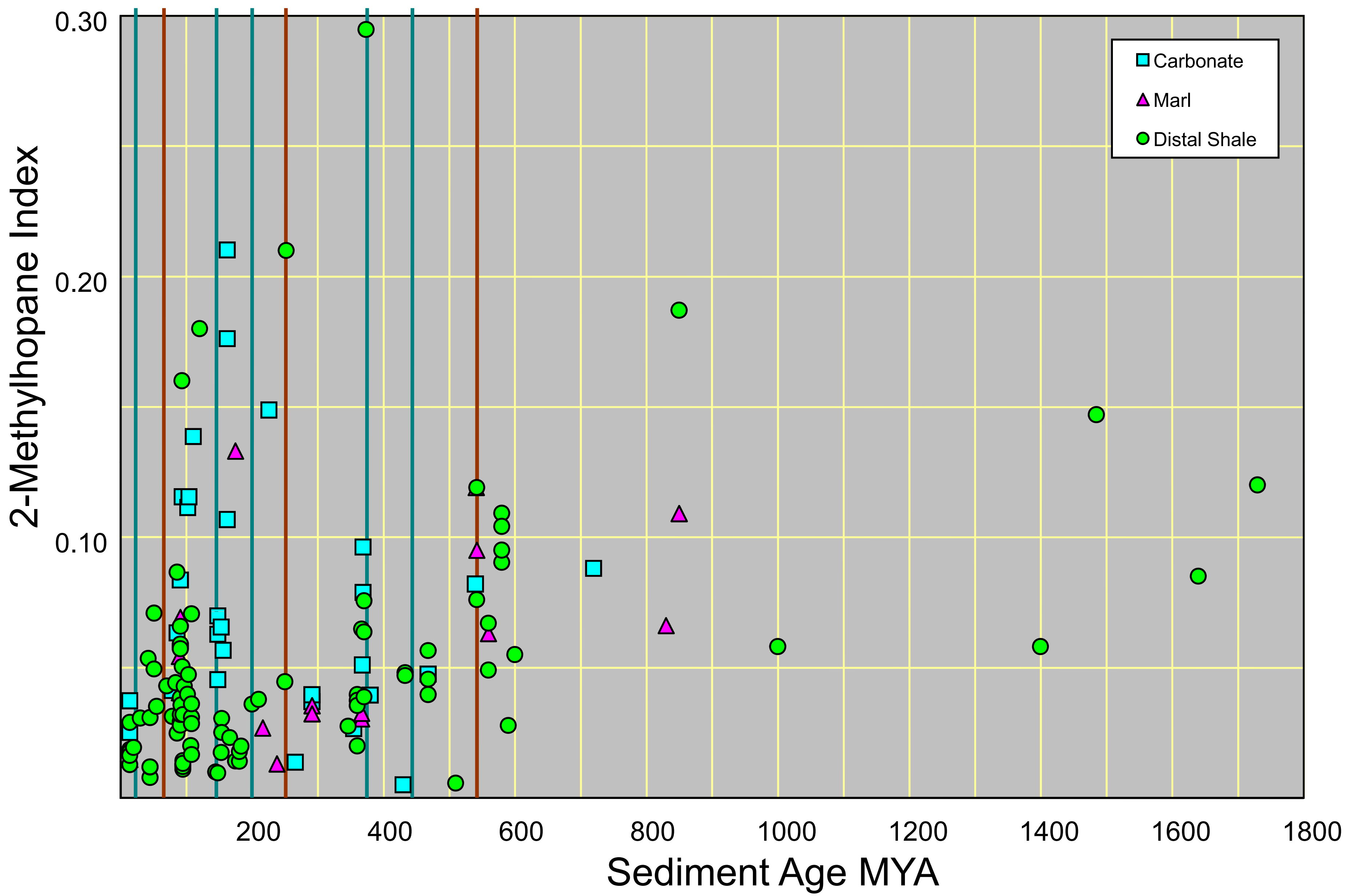
Are 2-methylhopanes biomarkers for cyanobacteria and O_2-photosynthesis?

- Specificity of 2-MeBHP for Cb? ~30% of Cb surveyed (Talbot et al, OG 2008)
  - *Methylobacterium*, N-fixing soil bacteria (Rhizobiales)
  - *Rhodopseudomonas palustris* (Rashby et al., 2007)
  - Commonly present in heterocystous cyanobacteria
  - Relatively abundant in a Baltic Sea strain of *Nodularia*
- What are the environmental occurrences?
  - Very abundant in hot spring mats; low levels in marine hypersaline Cb mats
- What are the biological functions of BHP & methylated BHP?
  - Membrane-associated in *P luridum* and Mc Bath; pCO_2 control in *Phormidium*?
- Geological occurrences? Secular in shales; Always at OAEs
- What is the biosynthetic pathway?
  - No unsaturated precursors; Radical SAM? See Paula's talk
- Are biosynthetic pathways conserved over geological time? Does a biomarker found today mean the same thing as one 500Myr old?

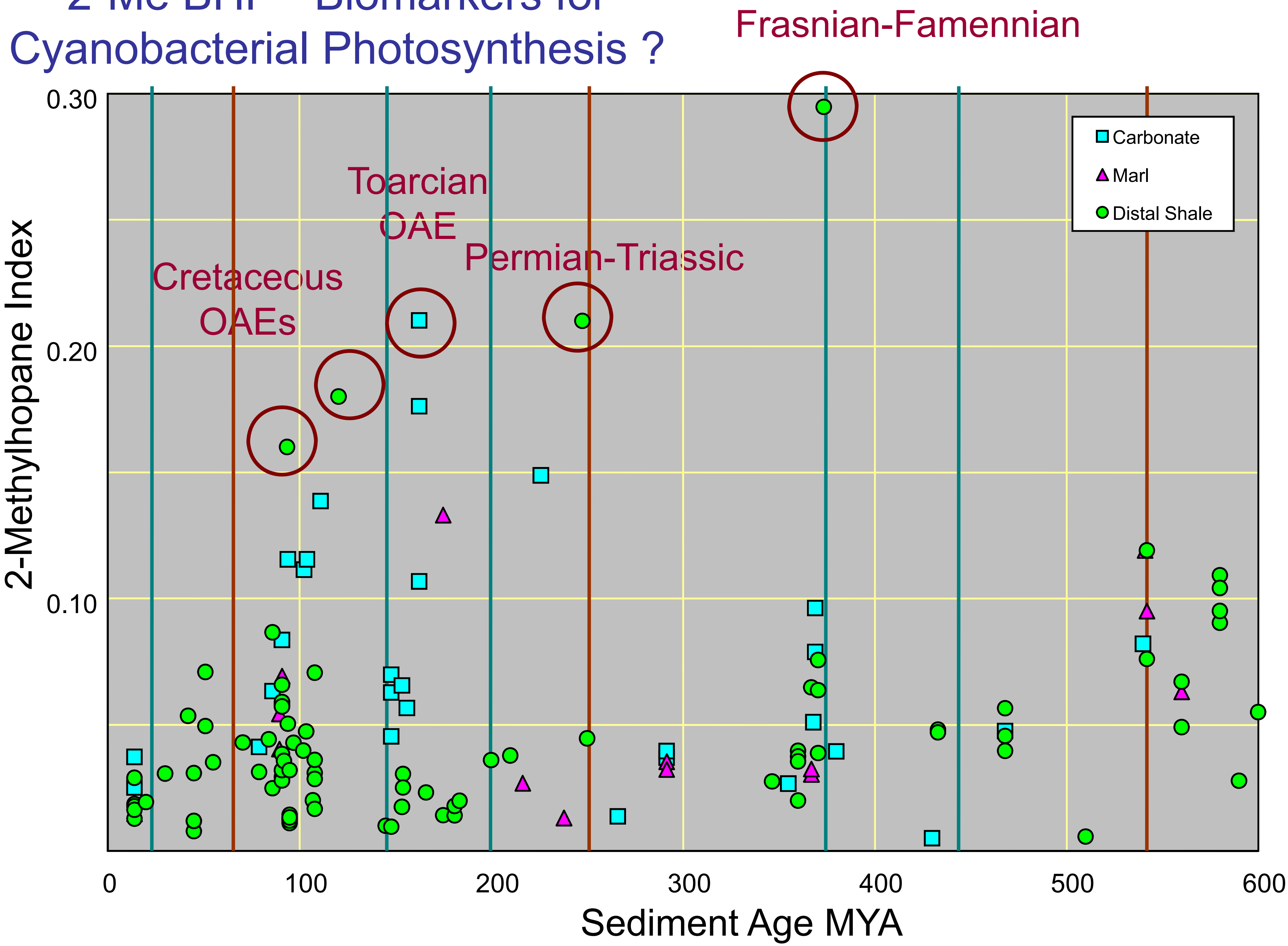
2-MeHopanes vs Lithology & Paleogeography



2-Me BHP - Biomarkers for Cyanobacterial Photosynthesis ?

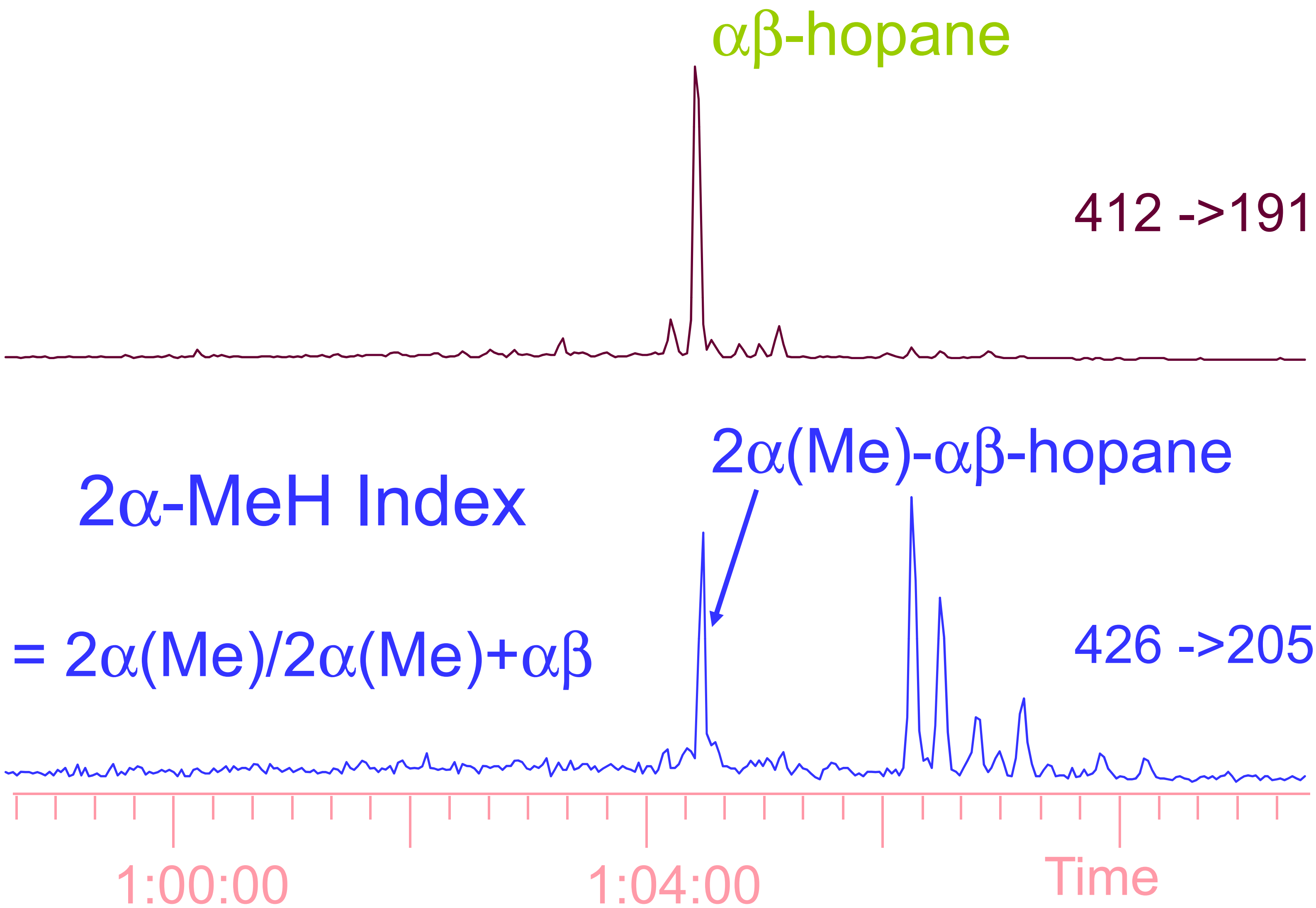


2-Me BHP - Biomarkers for Cyanobacterial Photosynthesis ?

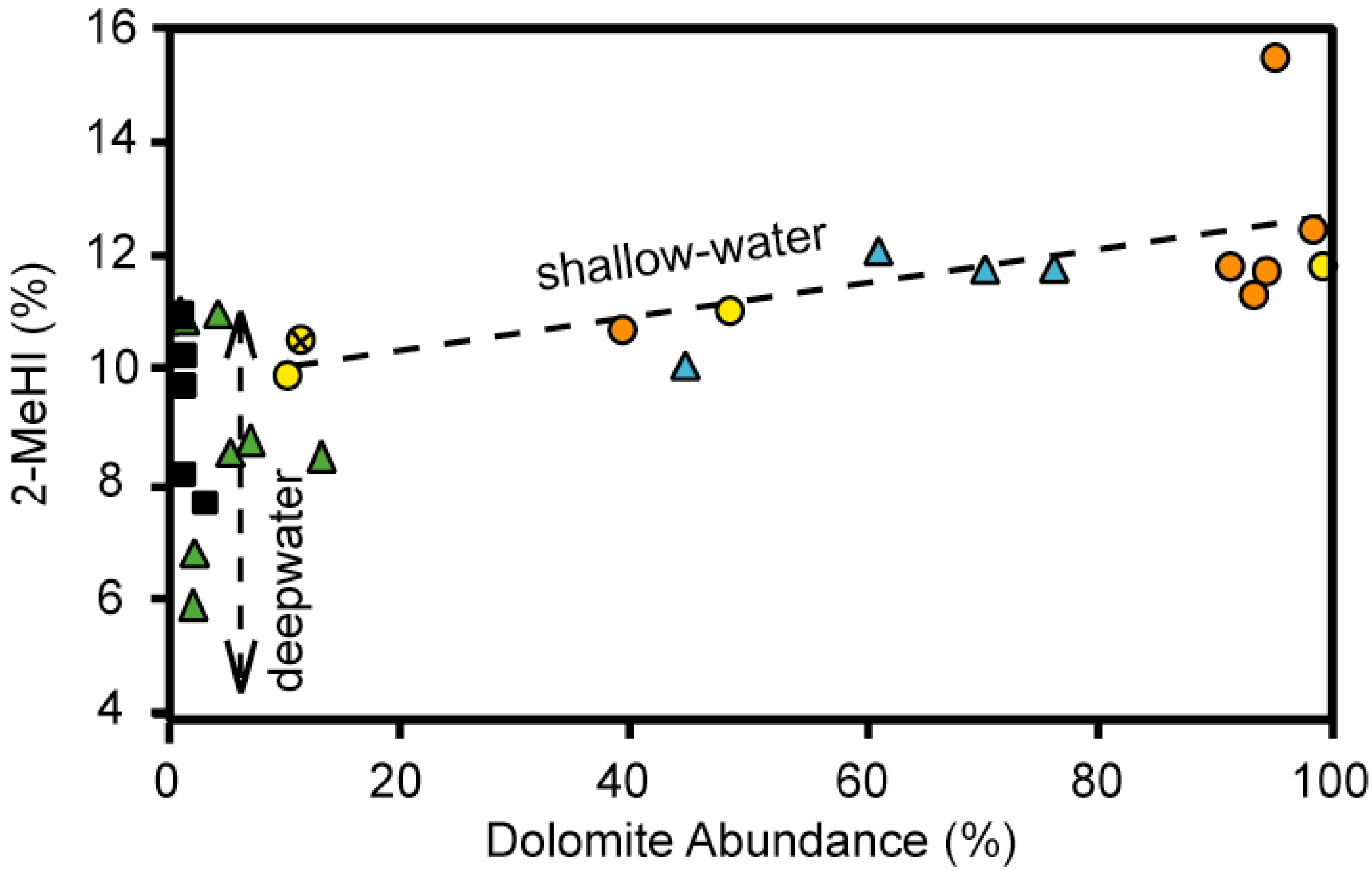


2-Methylhopane Index for Sediments/Oils

Barney Creek Fm #1698



2α-Methylhopane index 2.72-2.56 Ga samples from the Hamersley Province



Eigenbrode et al, PNAS 2008

Meishan Drilling Project 2004

c.120 samples Late Permian-Mid Triassic for bulk geochemistry: $^{87}Sr/^{86}Sr$, TOC, $^{13}\delta_{carb}$, $^{13}\delta_{org}$, $^{15}\delta_{org}$ and detailed lipid biomarkers

Meishan-1 core
drilled near GSSP
Jan 2004

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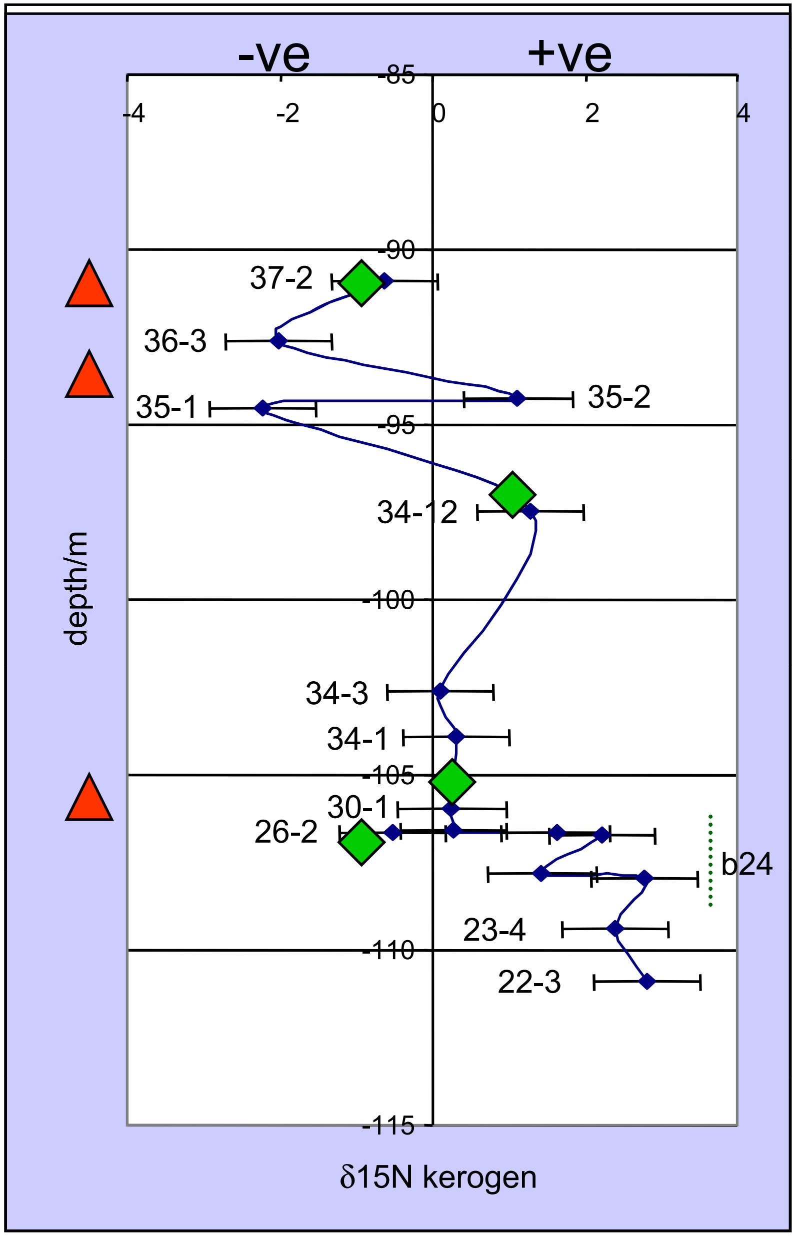
Multiple radiometric ages constrain pace; Ash in bed 25 = 251.4 ± 0.3 Ma, Bowring et al, 1998; Revised 252.6 ± 0.2 Ma Mundil, 2004; & 252.25 ± 0.06 Crowley, Bowring 2008

$\delta^{15}N$ of Meishan Organic Matter



- Positive values (+3 to +2) in late Permian Beds 6 -24
- Trend to zero or negative values of $\delta^{15}N$ in latest Permian reflects depletion of nitrate/nitrite pool driven by euxinic conditions
- Large swings in E. Triassic may reflect waxing and waning of euxinia
- Predominantly cyanobacterial primary N fixation

◆ Peaks in 2-MeHI >15%

▲ Peaks of aryl isoprenoids

EARTHRISE

Certified Pesticide
FREE

SPIRULINA

Blue-Green Algae
Rich in Beta Carotene
Essential Vitamins & Phytonutrients
Biologically Grown in the USA

100 TABLETS
Net Wt. 50 grams

Meishan-1 Isotope Geochemistry

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Meishan-1 Euxinia/Redox

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Meishan-1 Microbial Physiologies

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Hopanoids

The effect of thermal stress on source-rock quality as measured by hopane stereochemistry

WOLFGANG K. SEIFERT and J.MICHAEL MOLDOWAN
Chevron Oil Field Research Company, P.O. Box 1627, Richmond, California 94802, U.S.A.

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In: A.G. Douglas and J.R. Maxwell, Editors, *Advances in Organic Geochemistry*, Pergamon Press, Oxford (1980), pp. 229–237

Meishan-1 Maturity Parameters

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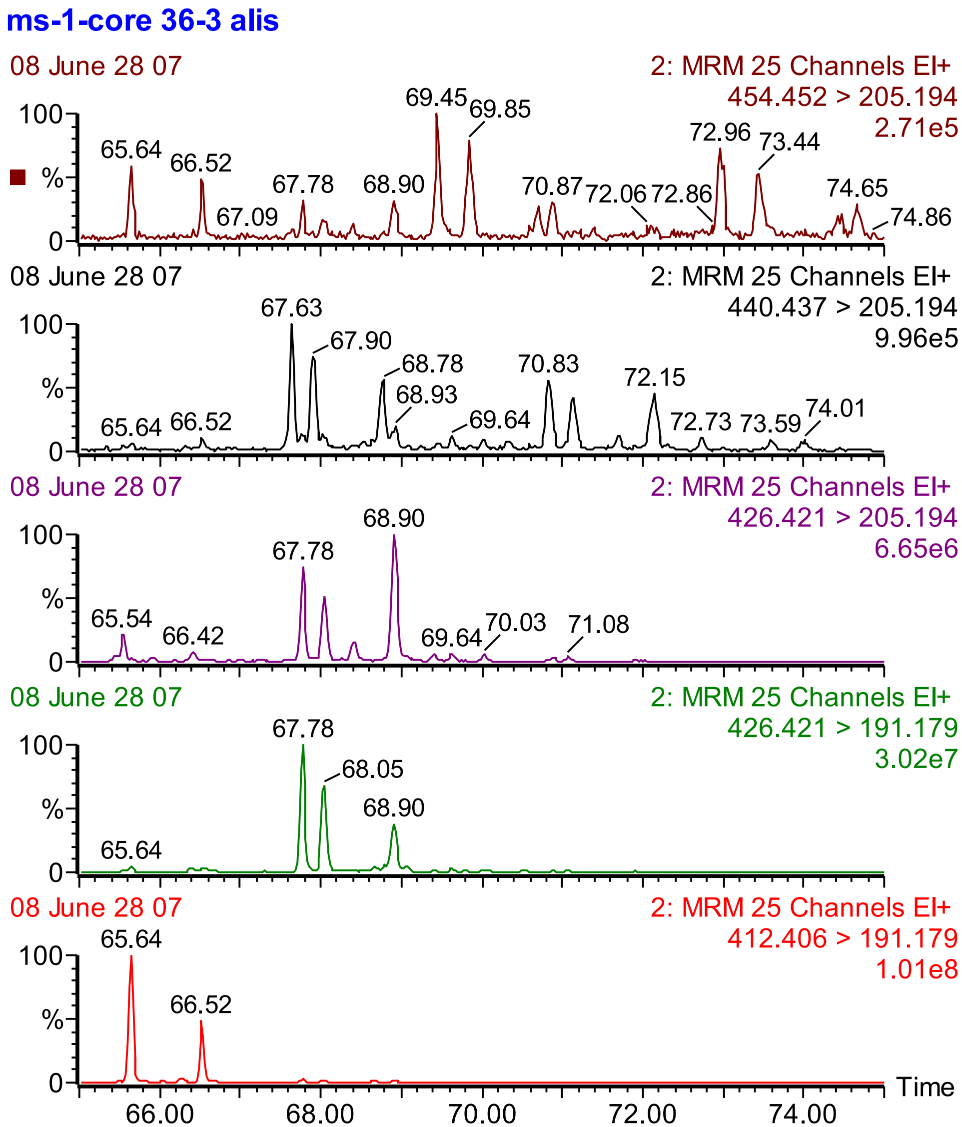
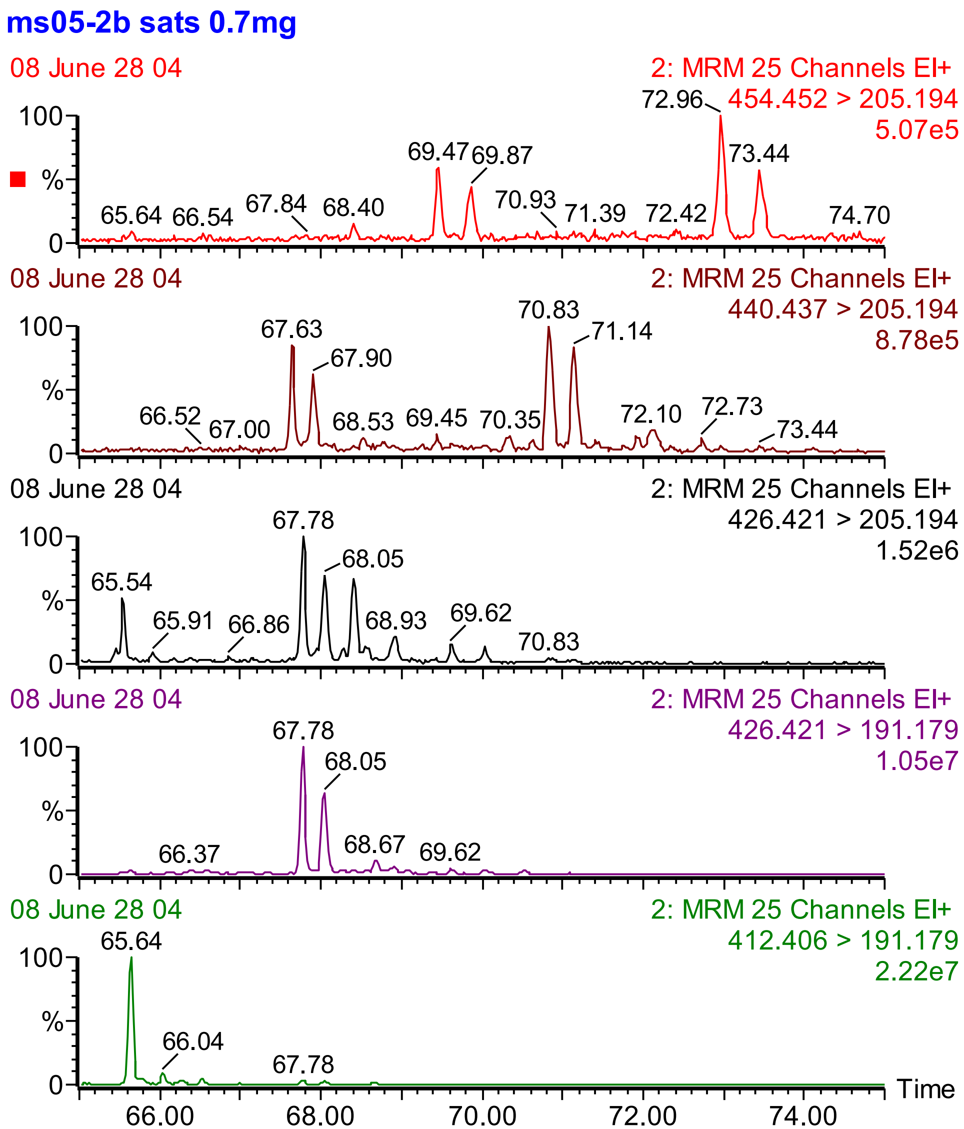
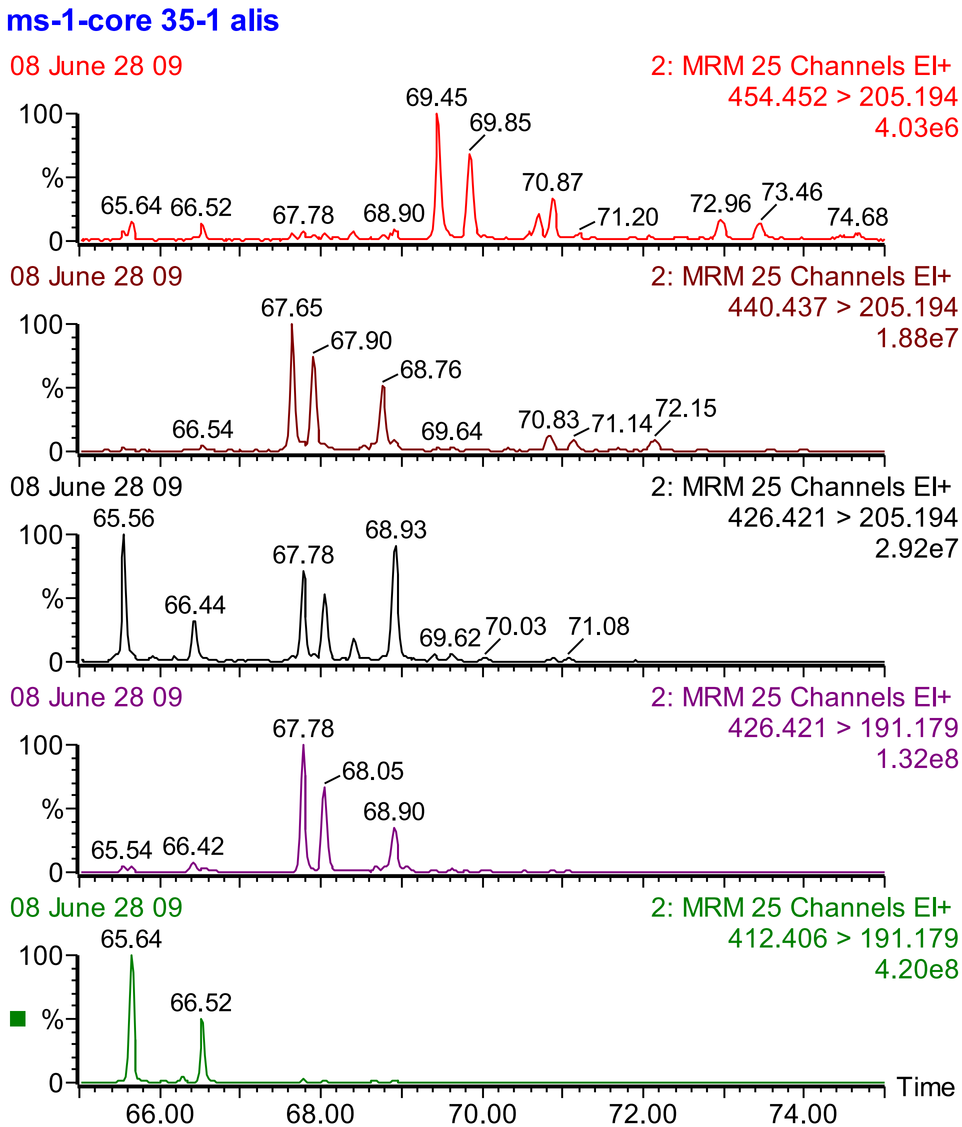
Sample with high moretanes, 2-methylmoretanes and 3-methylmoretanes



Sample with low moretanes, 2-methylmoretanes and 3-methylmoretanes

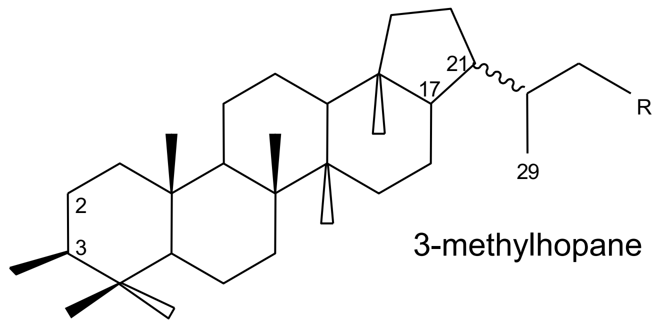


Sample with high moretanes and 2-methylmoretanes



Information from Molecular Fossils

A marker for aerobic methanotrophy



3-methylhopane

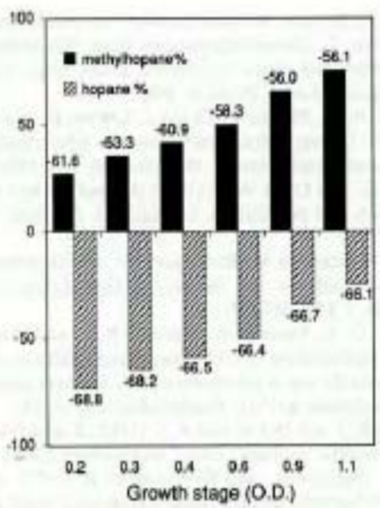


FIG. 6. Changes in the proportions and isotopic compositions of the hopane skeletons of bacteriohopanepolyols during growth of *M. capsulatus*. The data were obtained by CSIA analysis of the hopane-29-ols prepared by cleavage of the polyhydroxy side-chain using periodate oxidation-NaBH$_4$ reduction.

Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers

ROGER E. SUMMONS,[1] LINDA L. JAHNKE,[2] and ZARKO ROKSANDIC[1]

Summons et al., GCA, 2004

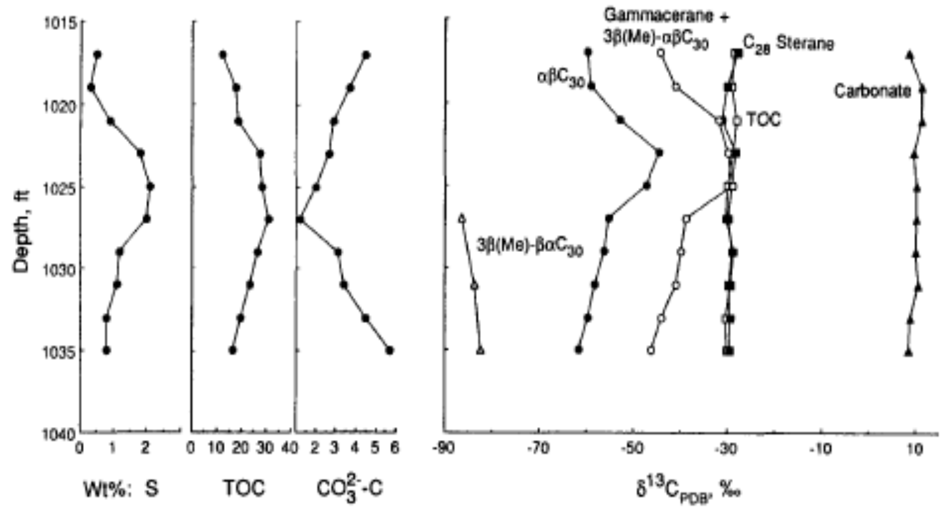


Fig. 7. Stratigraphic variations of abundances and isotopic compositions of selected components in the M cycle.

An isotopic biogeochemical study of the Green River oil shale

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Collister et al., Organic Geochemistry, 1992

Life's History on Earth

Cloud, Holland, Walker Paradigm