Biomarker Analysis of Carbon Rich Shales in the Neoproterozoic Bambuí Group, Brazil

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Acknowledgements

I could not have completed this thesis project if it was not for the help and support of the people around me. I would first like to thank Kristen Miller without whom I could not have completed this project. Her meticulousness and constant guidance and willingness to answer my every question, large or small, made this project a success. I could not have asked for a better advisor. I would also like to thank Dr. Alan J. Kaufman. He has been a fantastic advisor, who always took the time to give me guidance and help with any questions that I had. I would also like to thank Dr. Philip Candela for his advice on how to write my thesis and on the structure of my project. A special word of appreciation goes to Dr. George Cody of the Carnegie Institute for Science for the use of his lab and his GC-MS. Last, but not least, I wish to thank my parents and my younger brother Chris for their love and support, and my friend, Scott, for literally everything.

ABSTRACT

This study focuses on the structural biomarker analysis of carbonatious shales of the Lagoa do Jacaré (Alligator Lake) Formation within the Neoproterozoic Bambuí Group in Brazil. Biomarkers were extracted from 15 samples from one continuous exploratory core (PSB-14-MG) and separated into saturated compounds, aromatic compounds, and polar compounds. The saturated and aromatic compounds were analyzed using a Gas Chromatograph-Mass Spectrometer (GC-MS) at the Carnegie Institute for Science in Washington, D.C. These biomarkers have been structurally identified to determine if they are syngenic to the host rock and biologic in origin. Biomarker abundances and ratios were used to determine thermal maturity, as well as paleoenvironmental conditions. The analysis of biomarkers from the Lagoa do Jacaré Formation provides insight about the organisms that were present during an interglacial interval of the Neoproterozoic, and of the environment in which these organisms lived.

INTRODUCTION

This project analyzes the biomarkers of the Lagoa do Jacaré Formation of the Bambuí Group in order to determine the syngeneity of the biomarkers and the thermal maturity of the organic matter from this formation. Fifteen samples from this Neoproterozoic formation were analyzed using the GC-MS at the Carnegie Institute for Science. The study of biomarkers is an important tool for understanding biological evolution and paleoenvironmental conditions. While biomarkers are not tangible fossil evidence of organisms, they have proven useful for determining phylogenetic relationships between taxa especially when fossil evidence is uncommon (Cartwright et al., 2007). As microorganisms are deposited in the sediment they undergo diagenesis and their biolipids break down into biomarkers. These biomarkers are then extracted from the source rock in a lab, fractionated, and finally analyzed to determine whether the biomarkers are syngenic or non-syngenic, and specific ratios will be analyzed to determine thermal maturity. The biomarker compounds have also been analyzed to determine their biologic origin.

1. BACKGROUND

1.1 BIOMARKERS

Biomarkers are fossilized lipids which have survived degradation (Eigenbrode, 2007). Lipids are fat-soluble, naturally occurring compounds that include: fats, waxes, sterols, and oils. Lipids form the cell membrane, which is the barrier between the cell and the environment. Hopanes and steranes are saturated biomarker compounds that are derived from lipids known to give structural rigidity to the cell wall, and can record a fingerprint specific to an organism (Eigenbrode, 2007). Lipids also differ between organisms because of taxonomy; the genetics of an organism affect its lipid structure (Eigenbrode, 2007). The three domains of life: Archaea, Bacteria, and Eukarya each have distinctions among their lipids, which set them apart from those of other kingdoms. Eukarya for example are the only domain to produce biolipids which rearrange into sterane compounds, therefore the presence of syngenic steranes generally indicates the presence of eukaryotes (Eigenbrode, 2007; Brocks and Summons, 2005).

As lipids undergo diagenesis, they tend to rearrange into more thermodynamically stable forms by cleaving their functional side chains and reducing the number of multiple

bonds through hydration reactions, and thus become more saturated gaining more hydrogen atoms per carbon atom (Summons et al., 2007). The structure of the diagenetic product depends on the structure of the original lipid and the type of degradation, or diagenetic conditions (Figure 1); (Eigenbrode, 2007; Summons et al., 2007). Common fluids vary in their reduction potential so that diagenetic conditions may be oxic, anoxic, or sulfidic (free H_2S). For example, two common biomarkers, phytane and pristane, are formed from phytol (a side chain of chlorophyll a), but their relative abundances depend on diagenetic or depositional conditions (Figure 1). Phytol alters into pristane under oxidizing conditions, but into phytane under reducing conditions. Thus the ratio of pristane and phytane can convey the redox conditions during deposition or diagenesis (Waldbauer et al., 2008; Fischer, 2008; Brody, 2005).

Biomarkers occur in sedimentary rocks and are incorporated into the sediment as kerogen or bitumen (Waldbauer et al., 2008). Kerogen is macromolecular organic matter that is insoluble in organic solvents. Bitumen is the soluble organic matter that can be extracted with solvents such as dichloromethane from the rock. Therefore, biomarkers are from the bitumen fraction. Biomarkers may either be syngenic or non-syngenic. A biomarker is syngenic if it formed from indigenous organic matter during lithification. Non-syngenic biomarkers are introduced to the host rock after lithification (Figure 2). Biomarkers can be introduced into the host rock by the flow through of liquid hydrocarbons from an adjacent formation (Rasmussen et al., 2008). Determining syngeneity is important when trying to identify the organisms present when the host rock was deposited.

Biomarkers are sensitive to contamination in the lab and in the field (Waldbauer et al., 2008; Rasmussen et al., 2008). In the field the core may be contaminated by the drill fluid which is used with the drill which takes the core. Contamination in the lab can come from lab equipment that has not been properly cleaned, or from contact with surfaces which cannot be easily cleaned. Biomarker contaminants can also come from skin oils which are deposited by fingers when handling materials. Anthropogenic contaminants are easily transferred to biomarker samples can be difficult to avoid. These contaminants are considered during extraction and fractionation processes by handling all biomarker samples with care, and by using blank samples for each ten biomarker samples to monitor contamination. A blank sample is silica gel which undergoes crushing, extraction, and fractionation at the same time as the biomarker samples. The blank sample contains no biomarkers, so any contaminants which enter the samples while before GC-MS analysis will appear in the blank sample. Any contaminants found in the blank sample are considered while analyzing results from the GC-MS.

1.2 Biomarker Compounds

Biomarkers are fractionated into three types of compounds: saturate, aromatic, and polar. Saturated compounds are those with only single bonds; these contain the maximum number of hydrogen atoms. Aromatic compounds are unsaturated ring structures. Polar compounds are organic compounds with an asymmetric distribution of charge meaning they possess a dipole moment. These compounds will be separated into their own fraction to prevent them from co-eluting with the aromatic fraction during column chromatography but will not be analyzed. Saturate, aromatic, and polar compounds are analyzed separately to help achieve the highest sensitivity during GC-MS measurements.

1.2.1 Saturated Compounds

Saturated compounds include normal alkanes (*n*-alkanes), acyclic isoprenoids, hopanes, steranes, and diasteranes. *N*-alkanes are symmetric, saturated hydrocarbon chains. These compounds are derived from various precursor molecules (Brody, 2005). *N*-alkanes may show a weak preference for either odd or even numbered alkanes which could change with thermal maturity (Eigenbrode, 2007; Brody, 2005; Summons, 2007). Acyclic isoprenoids are asymmetric compounds with methyl groups which have attached to their hydrocarbon chains (Peters et al., 2005). Pristane and phytane are examples of acyclic isoprenoids, and can be indicative of bacteria activity (Brody, 2005; Peters et al., 2005). Hopanes and steranes are indicative of prokaryotes and eukaryotes respectively because they are the biomarkers which are derived from the lipids responsible for the rigidity of the cell wall in their respective domains (Brody, 2005, Peters et al., 2005). The two types of steranes include regular steranes and diasteranes. Diasteranes are steranes which have rearranged, and they usually do so over time and with thermal maturity. Diasteranes are usually indicative of algae or higher plants and form in clay-rich environments (Peters et al., 2005).

1.2.2 Aromatic Compounds

Aromatic biomarker compounds are those with one or more ring structures with delocalized electrons (Peters et al., 2005). These compounds provide additional measures of thermal maturity. Perhaps the most interesting aromatic compound analyzed in this study are the aryl isoprenoids, which provide evidence for green sulfur bacteria that commonly occur in stratified water columns. It has been suggested that with age and maturation, aromatic compounds will lose multiple bonds, methyl groups and possibly their ring structures (Brody, 2005). For these reasons aromatic compounds are useful indicators of maturity.

2. Geologic Setting

This study focuses on the Neoproterozoic Lagoa do Jacaré Formation, a member of the Bambuí Group in central Brazil (Figure 3). The base of the Bambuí Group has been dated by Pb-Pb carbonate techniques to be approximately 740 Ma, although direct radiometric constraints are lacking for the Lagoa do Jacaré Formation (Alkmim et al., 2006; Babinski et al., 2007). The Neoproterozoic interval records an increase in diversity of life, which may suggest increasingly oxic oceanic and atmospheric conditions (Figure 4) (Canfield et al., 2008).

During the Neoproterozoic, the continent of Rodinia began to break up while major periods of glaciation, including potential "snowball Earths," occurred (Zhu et al., 2007; Kaufman, 2007). It is estimated the main metamorphic event which affected the Bambuí Group was the Brasiliano Orogeny, which is situated to the west and occurred circa 600 Ma, representing the final closure of the ocean separating the Amazon and São Francisco-Congo Cratons (Pimentel et al., 2001; Iyer et al., 1995). The São Francisco Basin, which developed on top of the São Francisco Craton, contains the Bambuí Group (Cruz-Vieira et al., 2007).

It has been determined by Iyer et al. (1995) that the Lagoa do Jacaré Formation is of lower greenschist metamorphic grade. The Sao Francisco Basin is a flat lying basin with the Brasiliano Orogeny to the west and the Araçuaí Orogeny to the east (Santos et al., 2000). The Lagoa do Jacaré Formation experience minor shearing during these events. It has been determined that the level of metamorphism within the fold belt is greenschist

facies. The Lagoa do Jacaré Formation is less metamorphosed that the fold belt and it is therefore likely that these sediments have experience sub-greenschist temperatures but no studies have directly tested this (Santos et al., 2000). This low metamorphic grade makes this an ideal location for a biomarker study (Guido et al., 2007).

The Bambuí Group is a thick carbonate succession which unconformably overlies the Paranoá Group. In some locations this contact is marked by glacial diamictites of the Jequitaí Formation (Santos et al., 2000). The Bambuí Group spans over 300,000 km^2 in central Brazil (Santos et al., 2000; Iyer et al., 1995). The lower section of the Bambuí Group comprises marine strata overlain by strata indicative of shallow water and alluvial deposition (Babinski et al., 2007). Based on this stratigraphic evidence the Bambuí Group was most likely deposited in a shallow marine environment on an epicontinental shelf during three different regressive megacycles (Iyer et al., 1995; Pimentel et al., 2001; Santos et al., 2000). Approximately 250 m above the glacial deposits is the Lagoa do Jacaré Formation, which primarily consists of siltstones, marls, and black organic rich oolitic limestones (Figure 5) (Cruz-Vieira et al., 2007; Kaufman, Pers. Comm.).

3. HYPOTHESES

Based on preliminary observations as well as historical context, I hypothesize that shales in the Lagoa do Jacaré Formation are thermally immature. Petrologic evidence suggests the sediments experienced a sub-greenschist grade metamorphism, indicating temperatures ranging from approximately 250°C to 300°C (Iyer et al., 1995; Brocks et al.; 2005). Secondly, I hypothesize that the biomarkers within the Lagoa do Jacaré Formation are syngenic. The lack of veining and the low permeability of these fine grained sediments suggest that diagenetic fluids did not flow through these rocks since they were deposited. I will also be determining the organisms responsible for the deposition of the biomarkers in the Lagoa do Jacaré Formation. Because this formation is Neoproterozoic and these sediments formed in a quiet shallow marine setting, I expect the biologic community to have consisted of green sulfur bacteria, cyanobacteria, and aerobic eukaryotes which are likely representative of algal contributions (Olcott et al., 2005).

4. METHODS

4.1 SAMPLES

Thirty organic rich black shale samples from the Lagoa do Jacaré Formation were collected from one continuous core (PSB-14-MG). The samples were collected by Nick Geboy in 2005. The core was separated into individual samples based on depth (Table 1). Each depth was chosen based on which parts of the core were most organic rich, and had not shown any secondary mineralization, or veining. The samples collected from the core range in depth from 12.6 m to 145.55 m with an average spacing of approximately 4.7 m.

4.2 MATERIALS

Gas chromatograph grade organic solvents (dichloromethane, hexane, and methanol) were used in sample extraction, fractionation, and cleaning of all laboratory equipment. Biomarkers of extremely low abundance (ppb) are very susceptible to contamination, and will become contaminated easily. For this reason every surface or tool the biomarker samples touch, even the core samples, must be handled with the utmost care.

All of the glassware was cleaned by sonicating three times with heat, once with a combination of Alconex solution and de-ionized (DI) water, then with DI water only, and lastly with Milli-Q water; the glassware it must be sonicated to remove contaminants, which may be on the glassware prior to combusting. All glassware and metal, including aluminum foil were then combusted in the furnace at 450°C for at least six hours in order to remove any remaining organic residues; a process called ashing.

All glassware and lab equipment were cleaned three times with dichloromethane immediately before use. This affects the methods used in handling the samples because the materials must be handled carefully to prevent contaminating sensitive surfaces especially with anthropogenic contaminants which could be deposited by touching any glass, metal, or hardware.

Silica gel and activated silica gel were also used. To activate silica gel it is sonicated for 20 minutes with dichloromethane and methanol, decanted, then rinsed with dichloromethane and methanol and decanted twice. This is done in a safe fume-hood environment. The silica gel is then left to dry in the fume hood. Once the silica gel has dried it is baked at 200°C for several hours and removed to be stored in a drying oven until use.

4.3 Sample Preparation

Approximately 100 g of each sample were taken from the core. The outer 0.5 cm was removed, and then the residual sample was broken in 0.5 cm^3 pieces. These pieces were cleaned with Milli-Q water and each piece dipped in dichloromethane. The cleaned chips were then crushed into a fine powder using a mill and puck shatter box. All of my samples were previously crushed by Kristen Miller. All materials used in the processing and crushing of the samples were cleaned with quartz sand and then rinsed with dichloromethane three times to prevent contamination. The samples were then extracted using an Accelerated Solvent Evaporator (ASE) at the Virginia Institute of Marine Science (VIMS).

4.4 Extraction and Fractionation

The samples were extracted at VIMS and the extracts were concentrated by Kristen Miller. I then placed 20 μL of each extract onto activated silica gel in an ashed aluminum cup and left the samples to dry over night. The extract was then poured into a pipette packed with 0.6 g of activated silica gel. Using liquid column chromatography the samples were fractionated into saturated, aromatic, and polar compounds using 1.5 mL of hexane, 3 mL hexane:dichloromethane (4:1 ratio), and dichloromethane:methanol (7:3 ratio) respectively.

4.5 Gas Chromatograph-Mass Spectrometer (GC-MS) Analysis

The saturated and aromatic compound fractions were analyzed using a GC-MS. The GC-MS is a gas chromatograph coupled to a mass spectrometer based on their affinity for the stationary phase of the column. The gas chromatograph separates compounds using a gas with a high diffusivity such as hydrogen or helium (Hites, 1997). The mass spectrometer then separates compounds based on their *m/z* ratio and analyzes the compounds for their abundances and identifies what compounds they are as ions (Hites, 1997). The GC-MS model used at the Carnegie Institute for Science is the 6890 N Network Gas Chromatograph and the 5973 Network Mass Spectrometer manufactured by Agilent

Technologies. The software for obtaining and analyzing the data is ChemStation MS Data Analysis manufactured by Hewlett-Packard.

During twelve different analytical sessions at the Carnegie Institute for Science in Washington, D.C., the aromatic and saturate fractions were analyzed. The fractions were first concentrated with nitrogen (N_2) gas while replacing the hexane solvent with dichloromethane. Once the hexane has been completely replaced by dichloromethane these samples are then concentrated to 80μL under the stream of nitrogen. Internal standards were added to each fraction. Internal standards IS-2, 0.1 μg 3-methylheneicosane (1000 μg/mL) and IS-3, 1.0 μg d_4C_{29}ααα(20R)-Ethylcholestane (0.01 μg/mL), were added to the saturate fractions. While IS-2 showed up in abundance, IS-3 did not appear on the chromatographs even after increasing the amount injected. The internal standard IS-1, 1.0 μg p-Terphenyl-d_{14} (0.3 μg/mL) was added to the aromatic fraction. The fractions were then placed into the individual wells of the GC-MS. For each fraction run the GC-MS oven begins at a temperature of 60°C for two minutes and increases at a rate of 10°C per minute until it reaches a temperature of 140°C. At this point the temperature increases at a rate of 3°C per minute until it reaches a final temperature of 300°C. This final temperature is held for 24 minutes. The total run time for each analysis is 87 minutes.

The saturate fractions were analyzed by Total Ion Count (TIC) and by Selected Ion Monitoring (SIM). The aromatic fractions were analyzed only by SIM. A TIC analyzes all of the ions present within the fraction and measures their abundances. A SIM run analyzes ions with specific *m/z* ratios. These *m/z* ratios are selected based on the compounds of interest within the fraction being analyzed. A SIM run increases the sensitivity of the probe in the MS because it is monitoring specific ions. Not more than eight ions were chosen per run because the sensitivity of the machine decreases as the number of ions being monitored increases. The ions which were run for each fraction are listed in Table 1.

Each ion is representative of a certain type of geolipid, for example, a run including ions *m/z* 191.2, *m/z* 205.4, and *m/z* 412.0 test the presence hopanes. Certain ions can be selected and to monitor for types of compounds which may provide evidence for the existence of certain organisms. For example, 24-Isopropylcholestane (24-IPC) is a sterane which is indicative of demosponges. In order to test for the presence of 24-IPC the *m/z* 217 is monitored because *m/z* 217 tests the presence of steranes, but *m/z* 414 must also be monitored. *M/z* 414 is the parent ion for 24-IPC. The peaks from each ion are then compared and where these peaks overlap is where it is most likely to find evidence for 24-IPC.

4.6 Peak Integration and Error Analysis

To determine any ratios which are used to measure thermal maturity, syngeneity, or conditions of deposition, as well as error, the peaks on each of the chromatograms produced by the GC-MS are integrated to determine the area beneath the peaks (Figure 6). Each peak is integrated three times and the average area is determined. This average peak area is the area used to compute biomarker ratios. The standard deviation of each average peak area is the error. The percent error for each measurement is calculated by dividing the standard deviation by the average and multiplying by 100. The error on average for each peak area is approximately 5%.

The error of the GC-MS used at the Carnegie Institute for Science was also determined by running the external standard ALK1 five consecutive times during the same day. The peaks obtained from these runs were manually integrated each three times and the

standard deviation was taken each peak. These standard deviations were averaged to find the average error of the GC-MS, which is 12%.

5. Results and Discussion

During the Neoproterozoic life was largely microbial (Eigenbrode, 2007) and mainly consisted of cyanobacteria, green sulfur bacteria, aerobic eukaryotes, as well as other microbial life (Olcott et al., 2005). Because some biomarkers are indicative of individual organisms, the presence of these biomarkers has been specifically noted. It has been suggested that 2α-Methylhopane is indicative of cyanobacteria (Summons et al., 1999), aryl isoprenoids are indicative of green sulfur bacteria (Brody, 2005; Brocks et al., 2003), and 24-Isopropylcholestane (24-IPC) which is likely indicative of demosponges. This is important because carbon evidence of sponges has been reported in the Neoproterozoic Huqf Formation (~547-540 Ma) in Oman (Love et al., 2009).

5.1 The Saturated Fraction

The saturated fractions for samples 15.70 and 38.00 did not contain biomarkers. The 13 samples which from the Lagoa do Jacaré Formation did contain biomarkers contained *n*-alkanes and acyclic isoprenoids. Eight of the samples from the Lagoa do Jacaré contained hopanes. Nine samples contained steranes, but the sterane abundances were low in six of the samples (Table 2).

5.1.1 *N*-alkanes

The samples from the Lagoa do Jacaré Formation which produced higher abundances of *n*-alkanes showed a unimodal distribution of *n*-alkanes, however most of the samples from the Lagoa do Jacaré Formation did not produce high enough abundances to effectively determine whether the *n*-alkane distribution unimodal or bimodal. The abundances of the *n*-alkanes was calculated better determine the type of distribution (Table 3). These abundances were also compared to determine whether the *n*-alkane preference was for odd or even *n*-alkanes. To determine whether the preference was for odd or even *n*-alkanes the sum of the abundances of odd *n*-alkanes was divided by the sum of the abundances of even *n*-alkanes. *N*-alkanes with maturity lose their preference for either odd or even *n*-alkanes (Peters et al., 2005). Three of the values (Table 4) calculated for this ratio were close to or greater than 1, however the overall preference for all of the samples was even *n*-alkanes.

N-alkanes are an integral part of determining the source of the organic matter. If the *n*-alkane distribution is unimodal, this indicates a single source of organic matter; however a bimodal distribution of *n*-alkanes indicates a secondary source of organic matter. Of the samples which did produce *n*-alkanes, the preference is for even *n*-alkanes. The preference for odd *n*-alkanes comes from terrigenous input. Contamination from higher plants will show an odd *n*-alkane preference. An even *n*-alkane preference usually implies aqueous input. While the predominant preference was for even *n*-alkanes, it is important to note that this could be an unreliable statistic because *n*-alkanes, which are normally the most abundant compound, were not abundant in these samples.

The low abundance or absence of *n*-alkanes could have been caused by dry extracts. When I received the samples in January, some of them (noted in Table 2) had completely evaporated and needed to be rehydrated with dichloromethane. *N*-alkanes, especially

lighter alkanes, tend to be lost if the extract dries completely because they are highly volatile.

5.1.2 PRISTANE AND PHYTANE

Pristane (Pr) and phytane (Ph) are the most important acyclic isoprenoids in the context of this study. The Pr/Ph ratio value for the Lagoa do Jacaré Formation ranges from 0.33-0.64 (Table 5). These values are less than 1.0, indicating that phytane is more abundant than pristane throughout the formation. This suggests that the conditions of deposition were reducing (Huang and Pearson, 1999). Figure 9 shows the Pr/Ph ratio values for each sample plotted against increasing sample depth. The overall trend shows increasingly reducing conditions of deposition with time which implies an increasingly anoxic marine environment with time.

The lipid phytol upon diagenesis rearranges and will alter into pristane under oxidizing conditions and phytane under reducing conditions (Figure 1). Under oxidizing conditions there will be a higher abundance of pristane, but under reducing conditions a higher abundance of phytane will be present (Eigenbrode, 2007, Cao et al., 2009). Pristane and phytane are two isoprenoids which are homologues for the n-alkanes for C_{17} and C_{18} respectively (Eigenbrode, 2007). In a more reducing environment, phytane will be relatively abundant and the numerical value of the Pr/Ph ratio will be smaller (<1.0). In an oxidizing environment, pristane will be more abundant though which will be reflected by a larger numerical ratio value (>1.0)

5.1.3 HOPANES

Hopanes were not detected in all samples. Only relative hopane abundances could be calculated because IS-3 was not visible on the full scan or SIM analyses. The detectable hopanes ranged from C_{27} to C_{31} in all samples and depending on the sample the range increased to a maximum C_{27} to C_{35} hopane spectrum. In all of the hopane-bearing samples $C_{29}17\alpha,21\beta$(H)-30-norhopane and $C_{30}17\alpha,21\beta$(H)-hopane were most abundant. Many of the samples contained detectable amounts of C_{31} hopanes, and two samples contained detectable amounts of C_{34} and C_{35} hopanes.

Hopane ratios may also be used to calculate the relative thermal maturity of the organic matter. Hopane molecules are chiral molecules, meaning two molecules have the same chemical formula, but are asymmetric. Chiral molecules can be sinistral (left-handed) or dextral (right-handed). The chirality of all biolipids tends to be sinistral (S). As biolipids undergo diagenesis and become biomarkers their chirality changes, and the biomarkers become dextral (R). This process happens with time but is expedited with stressful conditions causing maturation of the organic matter. For example, the ratio of C_{30} hopanes is determined by C_{30} $(\beta\alpha)/(\beta\alpha+\alpha\beta)$. The $C_{30}17\beta,21\alpha$(H)-hopane (Moretane) is the sinistral hopane molecule and rearranges into the dextral $C_{30}17\alpha,21\beta$(H)-hopane molecule. The ratio of $(\beta\alpha)/(\beta\alpha+\alpha\beta)$ molecules conveys the relative abundance of sinistral molecules compared to the overall abundance of C_{30} hopane molecules, thus this ratio is used as a relative indicator of maturity. If a higher abundance of sinistral molecules remain relative to the total C_{30} hopane abundance, then the thermal maturity is low.

The values calculated for the hopane ratios, including Ts/(Ts+Tm), are all contained within a relatively narrow range and have been classified as immature in comparable studies (Table 5). Brocks et al. (2003) extracted biomarkers from the Mt. McRae Shale in Australia which were also considered sub-greenschist to lower greenschist facies. The majority of the ratios calculated for the Mt. McRae Shale have values contained within the

same range as the Lagoa do Jacaré Formation. It can therefore be inferred that the biomarkers from the Lagoa do Jacaré Formation are thermally immature. These values are slightly higher than I expected, however they still support my hypothesis that the biomarkers of Lagoa do Jacaré Formation are thermally immature.

5.1.4 Steranes

Steranes were detected in approximately 9 of the biomarker samples for the Lagoa do Jacaré Formation. Only relative sterane abundance ratios could be calculated for sterane compounds, as opposed to sterane abundaces because IS-3 was not visible on either the full scan or SIM analyses. The most relatively abundant steranes are 5α,14β,17β(H)-cholestane, and 13β,17α(H)-diastigmastane. When these steranes were present their abundance was low which lead to small or hard to identify peaks in the data. This was especially the case for C_{27} steranes.

5.1.5 24-Isopropylcholestane

To test for 24-IPC, *m/z* 414 was run separately using SIM on the biomarker samples with the highest abundances of steranes. The abundances of ions with *m/z* 414 were either below the limits of detection by the GC-MS, or were not present all together. One sample, 65.17, did produce data for *m/z* 414, however the signal from the chromatographs on this *m/z* was too low to be adequately compared with the signal from *m/z* 217. It is therefore undeterminable whether 24-IPC is present within the Lagoa do Jacaré Formation. There are 15 samples from the Lagoa do Jacaré Formation which have not yet been extracted. When these samples are analyzed in the future, they should be analyzed for 24-IPC because samples with a more recent extraction may produce a better signal on *m/z* 414.

5.2 The Aromatic Fraction: Aryl Isoprenoids and Dibenzothiophene

The aromatic fraction was analyzed by SIM run only. The aromatic fractions were tested for aryl isoprenoids (*m/z* 133) and dibenzothiophenes (*m/z* 184). Aryl isoprenoids are indicative of green sulfur bacteria and thus a stratified water column (Figure 8) (Brody, 2005, Summons and Powell, 1987). Aryl isoprenoids were detected in 12 samples (Table 1). While it is currently not clear which organism is responsible for the production of dibenzothiophene, it is has been proposed that abundant dibenzothiophenes indicate a carbonate-evaporite source environments (Peters et al., 2005). Dibenzothiophene can also be used as a thermal maturity parameter (Brody, 2005). Damsté et al. (1989) determined that the abundant presence of dibenzothiophene meant a low thermal maturity.

Dibenzothiophene has been detected in the aromatic fractions for the Lagoa do Jacaré Formation and is relatively abundant. Dibenzothiophenes are often analyzed in ratios with phenanthrene to determine relative thermal maturity; however the presence of phenanthrene was not tested. It would be beneficial to test for phenanthrene in aromatic fractions from the Lagoa do Jacaré Formation in the future. While the presence of dibenzothiophene could be indicative of low thermal maturity, it needs to be compared with phenanthrene before this can be an absolute parameter.

5.3 Thermal Maturity Ratios

The thermal maturity of the Lagoa do Jacaré Formation was determined by calculating multiple hopane and sterane ratios based on the samples collected and classifying the amount of thermal maturity based on parameters set by Peters et al. (2005) as well as by comparing the values with values from other sub-greenschist facies shales.

One study in particular by Brocks et al. (2003) analyzed the sub-greenschist Mt. McRae Shale. The organic matter from this formation produced similar ratio values to those in this study; however the syngeneity of the biomarkers within the Mt. McRae Shale has recently come into question. The ratios used to determine the thermal maturity are listed in Table 6 and Table 7. These ratios provide evidence from different molecules about the thermal maturity of the organic matter of the rock.

5.3.1 Ts/(Ts+Tm)

Ts/(Ts+Tm) is one of the ratios used to determine thermal maturity. 22, 29, 30-Trisnorhopane (Tm) and 22, 29, 30-Trisnorneohopane (Ts) are C_{27} hopanes. Ts is the diagenetic rearrangement product of Tm (Brocks et al., 2003). Comparing the abundance of Ts to the total abundance (Ts+Tm) is a way of determining thermal maturity because as sediments and organic matter mature, Tm will rearrange into Ts. If Ts is much less abundant than Tm then it can be inferred that there has been little thermal maturation because Tm has not rearranged. If Ts however is much more abundant then it can be inferred that the organic matter in the formation is more mature because Tm has rearranged into Ts. The range for the Ts/(Ts+Tm) ratio value for the Lagoa do Jacaré Formation is 0.45-0.60 for the seven samples which produced hopane data. This value indicates an immature to early thermal maturity (Peters et al., 2005).

5.3.2 $C_{30} - C_{35}$ Hopane Isomerization Ratios

C_{30} and C_{31} hopane ratios were calculated for all fractions containing hopanes (Table 6). The C_{30} hopane ratio for the Lagoa do Jacaré Formation has a range of 0.03-0.14. The C_{31} hopane ratio has a range of 0.54-0.62. These values reflect a low thermal maturity based on comparable data from other studies (Brocks et al., 2003; Li et al., 2003).

Hopanes are also thought to rearrange over time so thermal maturity can also be measured by comparing abundances of heavier hopanes to lighter hopanes (Brody, 2005). For this reason it is useful to calculate ratios between heavier and lighter hopanes to determine relative thermal maturity. In this study, ratios were calculated for C_{27}/C_{29}, C_{29}/C_{30}, and C_{30}/C_{31}. These ratio values were calculated range from 0.32-0.62, 0.89-1.2, and 1.7-2.4 respectively.

5.3.3 Sterane Isomerization Ratios

Sterane isomerization ratios were also used to calculate relative thermal maturity (Table 7). Chirality with biomarkers changes over time in sterane biomarkers as well. Therefore comparing ratios such as $C_{27}S/(S+R)$ gives a relative thermal maturity for C_{27} steranes which aids in determining in overall thermal maturity. Each type of sterane ratio produced approximately the same range and average value. The overall range for these sterane values is 0.35-0.77. These values are also similar to those found by Brocks et al. (2003) in the Mt McRae Shale. It should also be noted that these samples contained low sterane abundances, which made calculating accurate thermal maturity ratios difficult.

5.4 Isotopic Data

Isotopic data has been determined for the Lagoa do Jacaré Formation using samples from PSB-14-MG. $\delta^{13}C_{TOC}$ and $\delta^{34}S$ have been analyzed on core PSB-14-MG and the %TOC and %S has been analyzed (Figure 7). The $\delta^{13}C_{TOC}$ data values do not show any large anomalies. The range of the values (-29‰ to -24‰) is 5‰ which is typical of $\delta^{13}C_{TOC}$ isotope values. This narrow range does not reflect a change in the source of the

organic matter or a change in the chemistry of the sea water. The %TOC values for the Lagoa do Jacaré Formation also possesses a narrow range from 0.56% to 1.01% and has an average value of 0.83% total organic carbon which is not anomalous and close to the current average value of %TOC in shales today which is 0.5%.

The $\delta^{34}S$ and %S data however show an interesting trend. The $\delta^{34}S$ shows a positive trend with decreasing depth. $\delta^{34}S$ values at greater depths (approximately 90 m) are relatively low and within a range of 0‰-10‰. The $\delta^{34}S$ values show a clear trend and increase going up strata to values of almost 30‰. This most likely indicates that the abundance of sulfate (SO_4^{2-}) within the reservoir was low initially and was enhanced by bacterial sulfate reduction. The presence of aryl isoprenoids in the Lagoa do Jacaré Formation indicates the presence of green sulfur bacteria which reside in a stratified water column (Figure 8). This indicates an anoxic ocean beneath the oxic photic zone, the boundary of which is marked by the green sulfur bacteria which require sunlight for photosynthesis. This is consistent with the data provided by the $\delta^{34}S$ data indicating an increasingly anoxic ocean with time.

5.5 SYNGENEITY

The syngeneity of the biomarkers in the Lagoa do Jacaré Formation can be determined by comparing the thermal maturity indicated by the ratios calculated with the known thermal maturity of the sediments which was previously determined petrologically. The ratios used to determine thermal maturity show a low thermal maturity of the organic matter in the Lagoa do Jacaré Formation. These values are within the expected range for shales which have been classified as sub- to lower greenschist facies. These values are consistent with the petrologically determined thermal maturity by Iyer et al. (1995), and are evidence supporting that these biomarkers are syngenic.

Syngeneity can also be determined by comparing the abundance of odd *n*-alkanes to even *n*-alkanes, because an odd *n*-alkane preference suggests input from higher plants. The *n*-alkanes within the Lagoa do Jacaré Formation indicate an overall preference for even *n*-alkanes, which suggests these biomarkers are not contaminants. Overall the evidence for syngeneity outweighs the evidence for contamination.

6. CONCLUSIONS

These biomarkers are biologic in origin. The presence of hopanes and steranes indicates that prokaryotes and eukaryotes were present at the time of deposition. Analysis of the aromatic fraction of these biomarker samples indicates the presence of green sulfur bacteria. Green sulfur bacteria are evidence for a stratified water column. In this case green sulfur bacteria would have existed at the boundary between the oxic waters of the photic zone within the ocean above, and the anoxic waters of the ocean beneath (Figure 8).

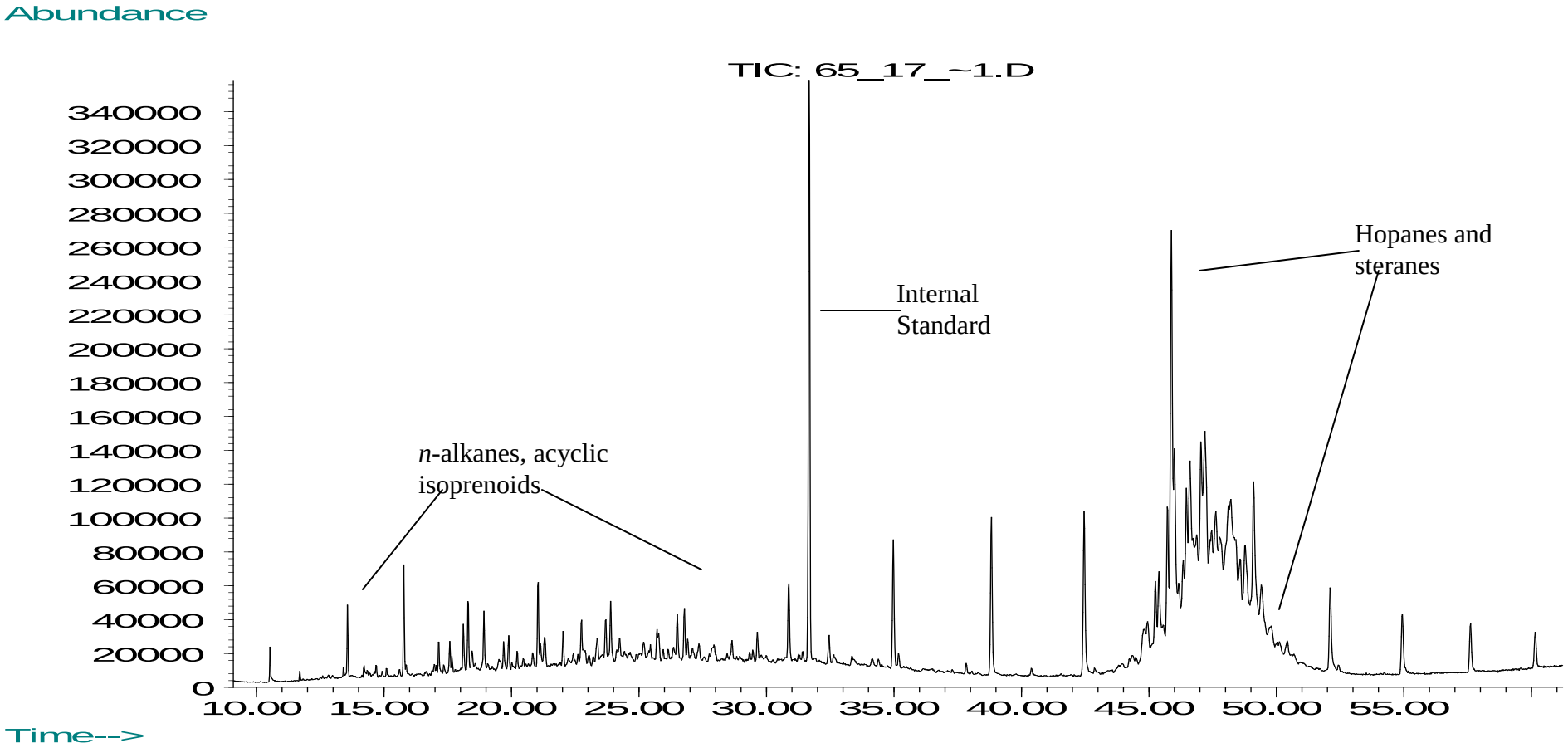
Paleoenvironmental conditions were also determined using biomarker ratios from the Lagoa do Jacaré Formation. Relative abundances of pristane and phytane, which are acyclic isoprenoids, were compared to determine whether the depositional conditions were oxidizing or reducing. The values of this ratio indicated an increasingly reducing environment. This is consistent with the $\delta^{34}S$ isotope for the Lagoa do Jacaré Formation which indicates an increase in the amount of sulfate within the reservoir and thus increasingly reducing conditions.

Based on the analysis of the biomarkers extracted from the Neoproterozoic Lagoa do Jacaré Formation in Brazil, it can be inferred that the organic matter within this

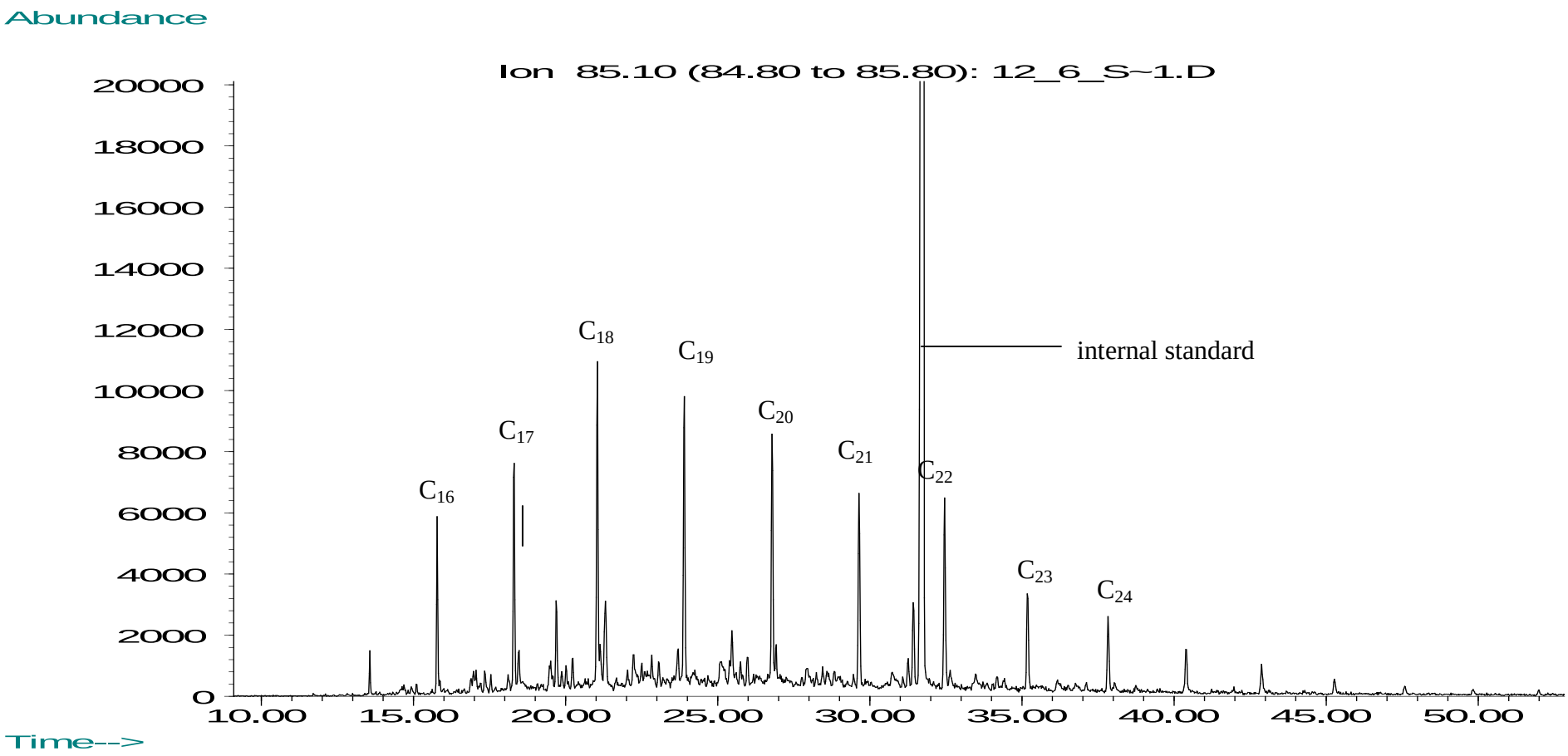
formation is syngenic. The unimodal distribution of the n-alkanes indicates a single source of organic matter; therefore secondary organic matter has not flowed through the Lagoa do Jacaré Formation. The Lagoa do Jacaré Formation also shows a preference for even n-alkanes which indicates that organic matter is from aqueous input as opposed to terrigenous plants. The thermal maturity ratios calculated using relative hopane and sterane abundances also provide evidence for syngeneity.

The hopane and sterane ratios calculated indicate a low thermal maturity. The presence of dibenzothiophene is also an indicator of low thermal maturity. Dibenzothiophene was relatively abundant in the biomarker samples of the Lagoa do Jacaré Formation. This is consistent with the sub-greenschist thermal maturity determined by Iyer et al. (1995). Therefore it can be concluded that the relative thermal maturity of the organic matter of the Lagoa do Jacaré Formation is low. Overall the data produced by the biomarkers from the Lagoa do Jacaré Formation support the hypotheses that the organic matter within this formation is thermally immature and the biomarkers are syngenic and were not deposited by the flow through of a secondary organic source.

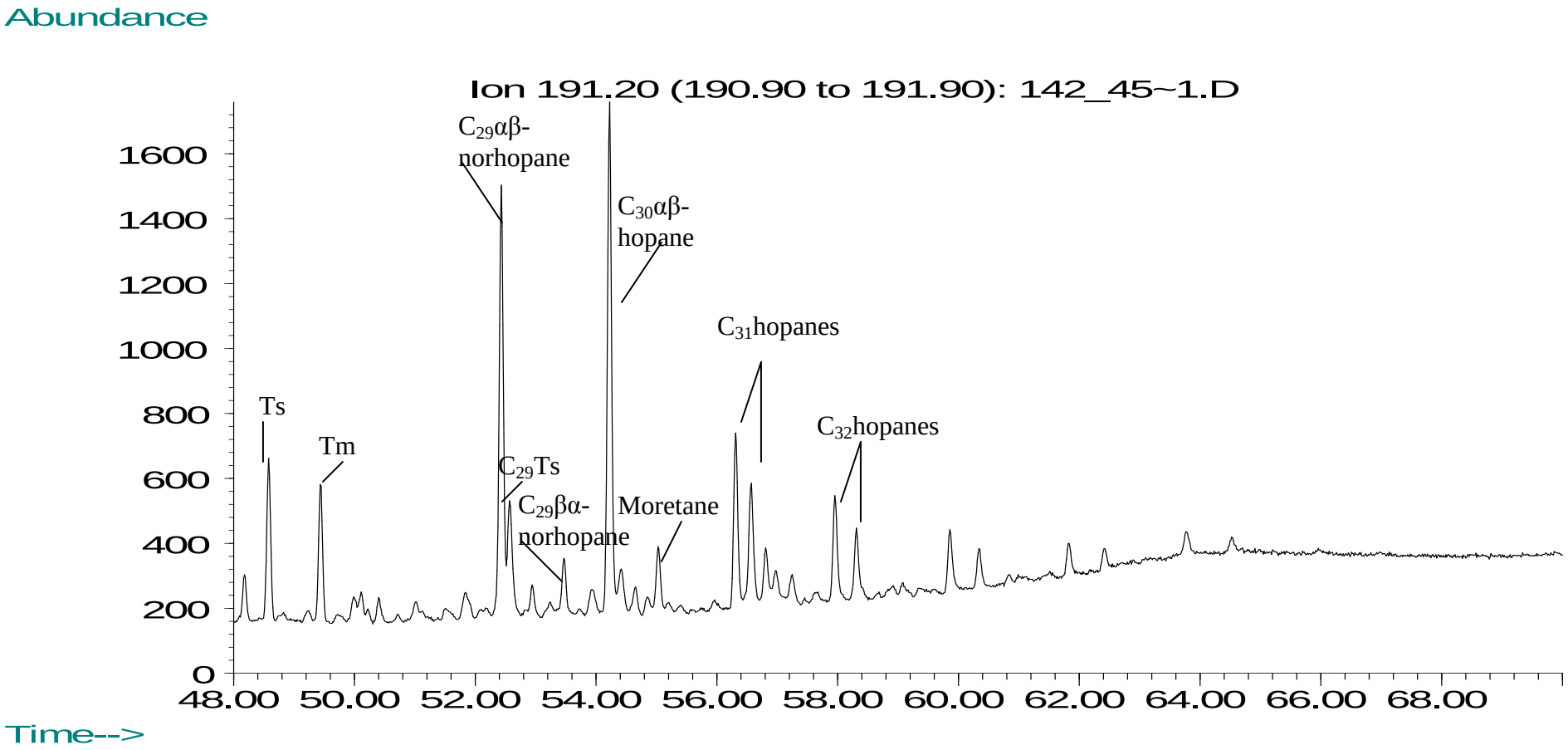
Chromatogram 1. Representative Full Saturate Fraction Scan



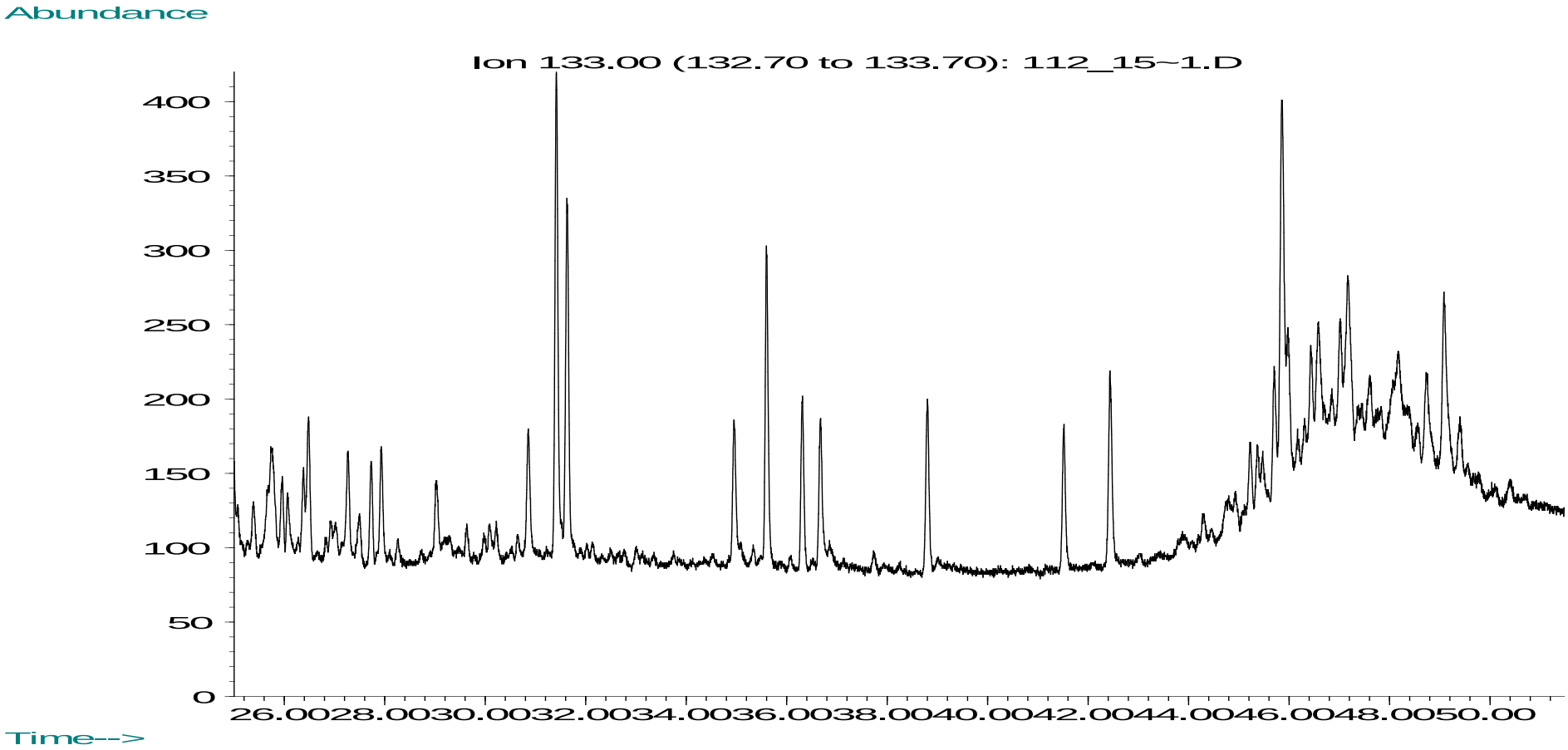
Chromatogram 2. Unimodal *n*-Alkane Distribution *m/z* 85.1 SIM.



Chromatogram 3. Hopanes *m/z*191.2 SIM

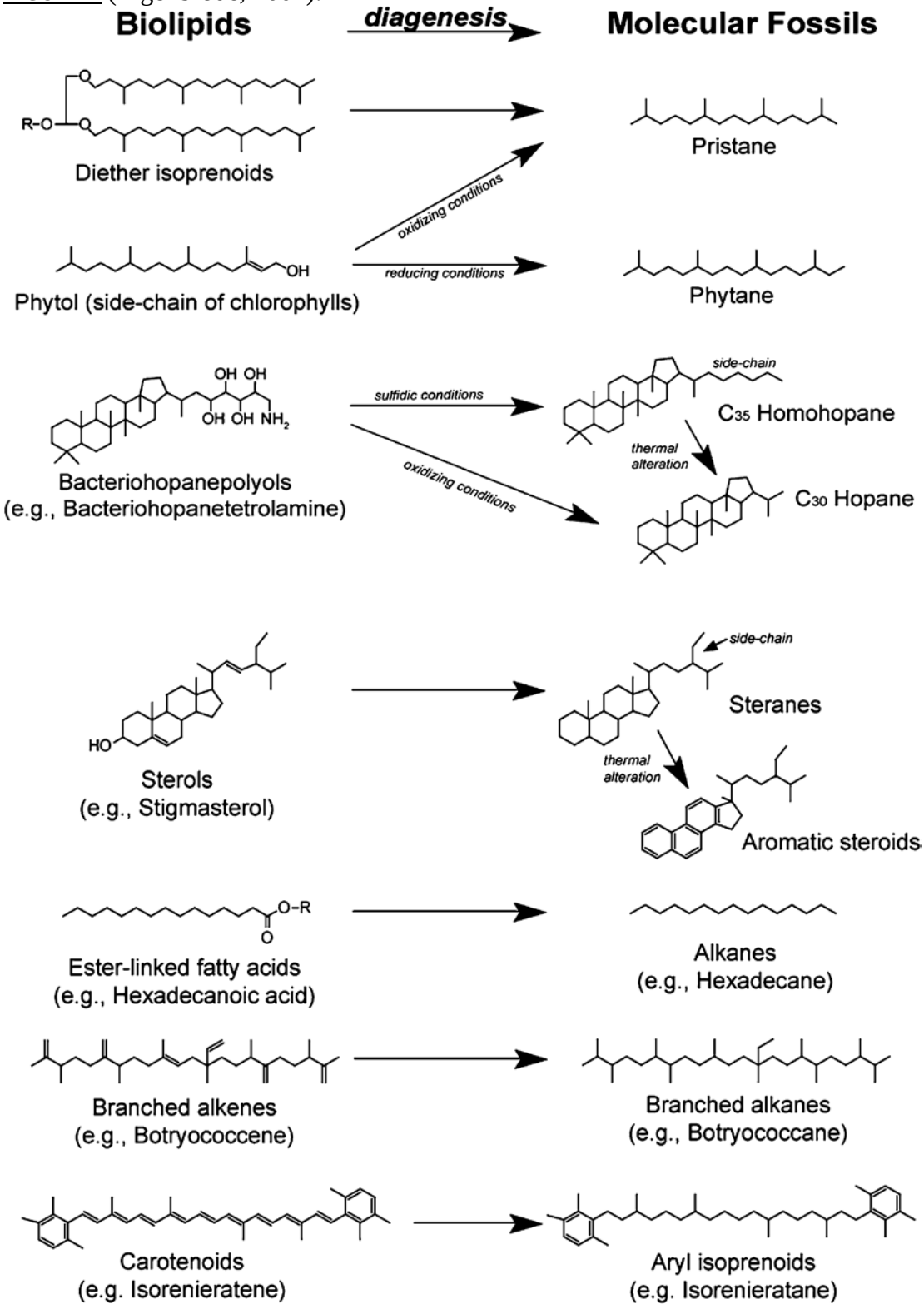


Chromatogram 4. Aryl Isoprenoids indicating green sulfur bacteria



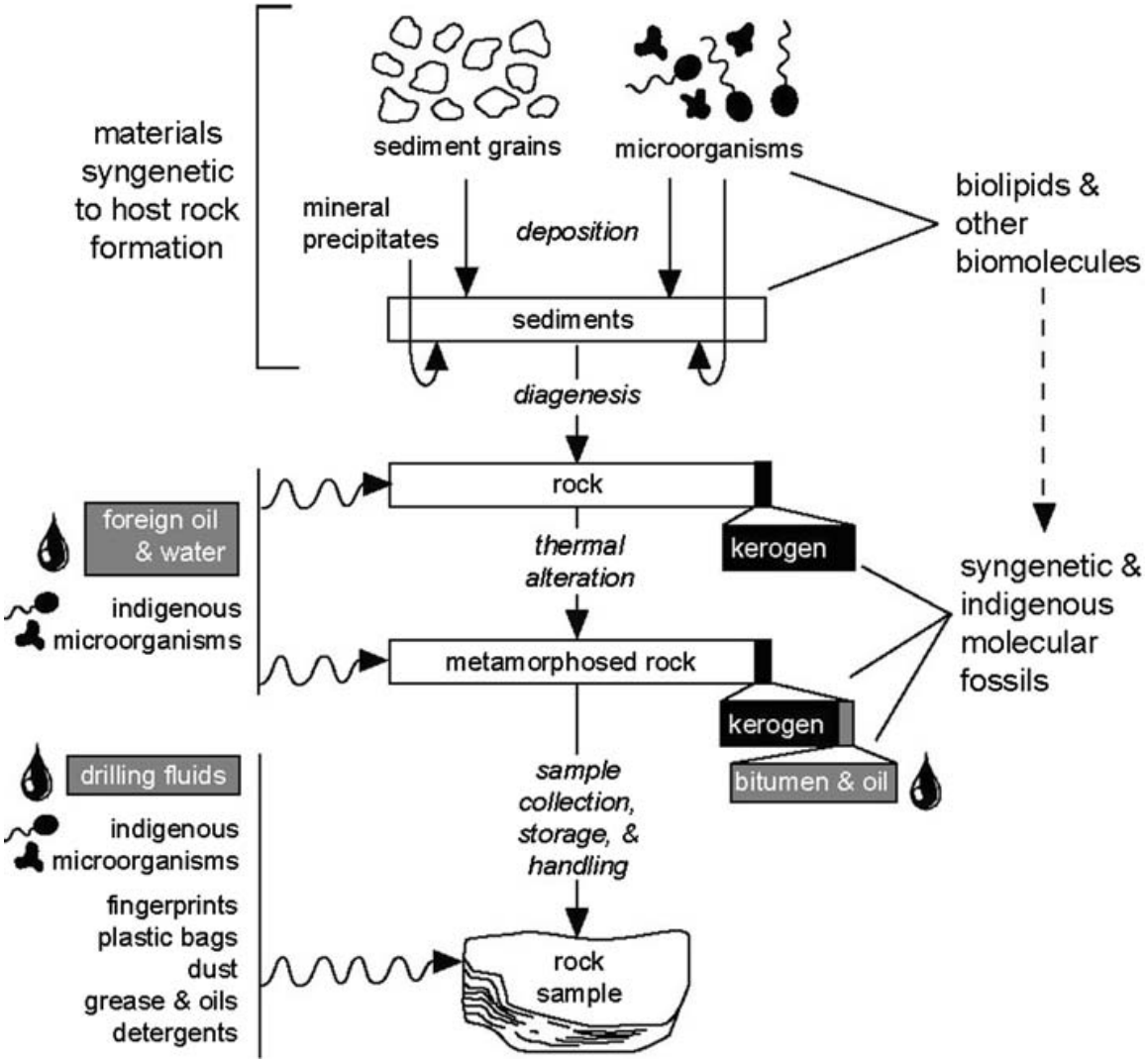
FIGURES

FIGURE 1 (Eigenbrode, 2007):



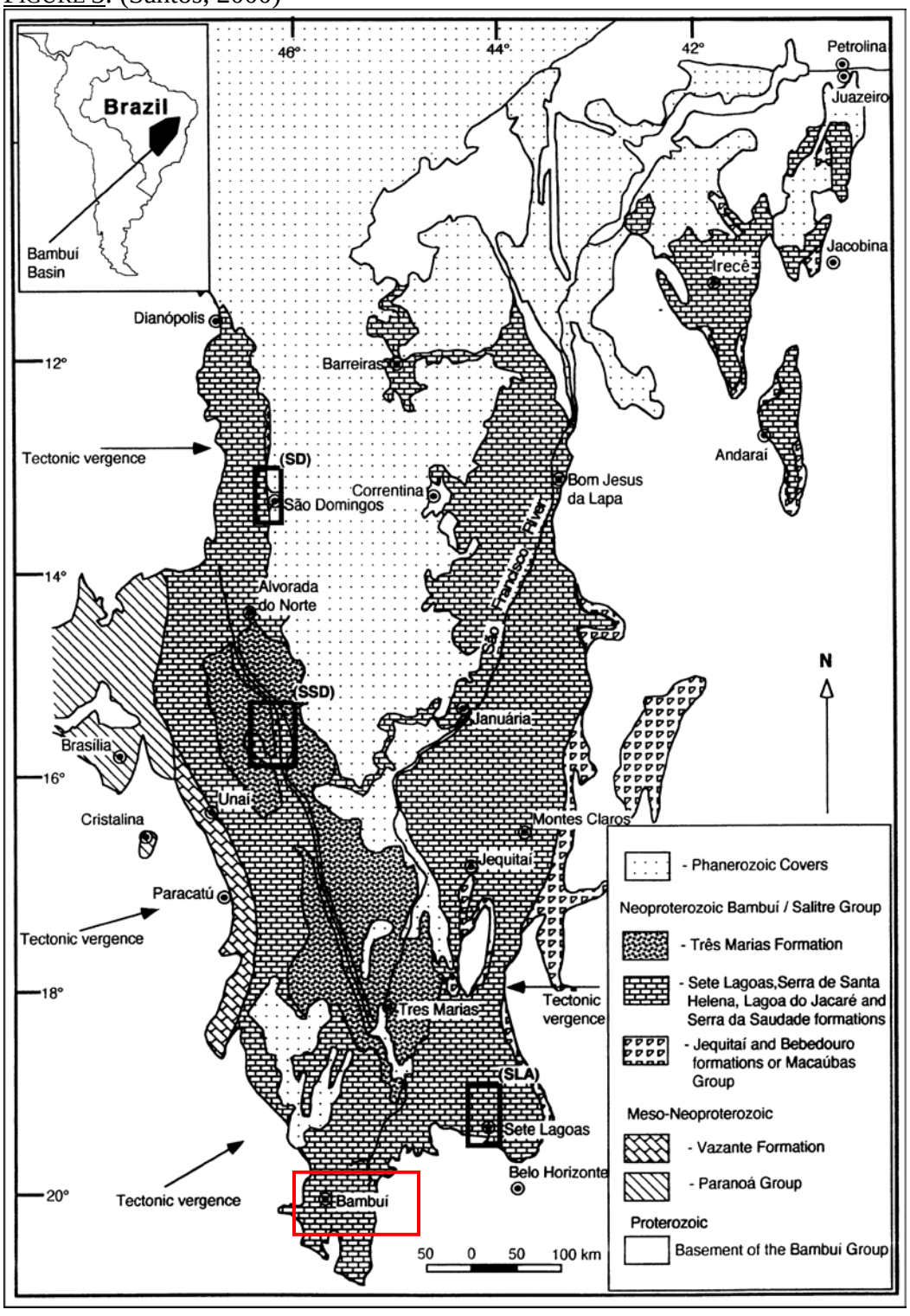
Shown here are common biomarkers which are formed from the degradation of common lipids. This figure also shows how the biomarkers may differ based on thermal alteration or under different diagenetic conditions.

FIGURE 2 (Eigenbrode, 2007)



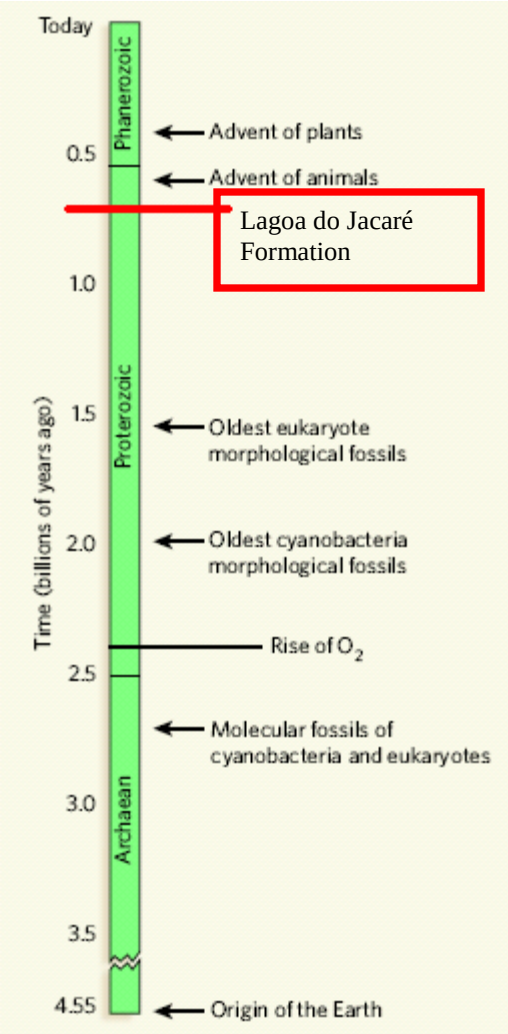
This flow chart shows the process in which microorganisms become biomarkers in rock samples. It also gives a sense of how non-syngenic biomarkers flow into the source rock and how they may become contaminated.

FIGURE 3: (Santos, 2000)



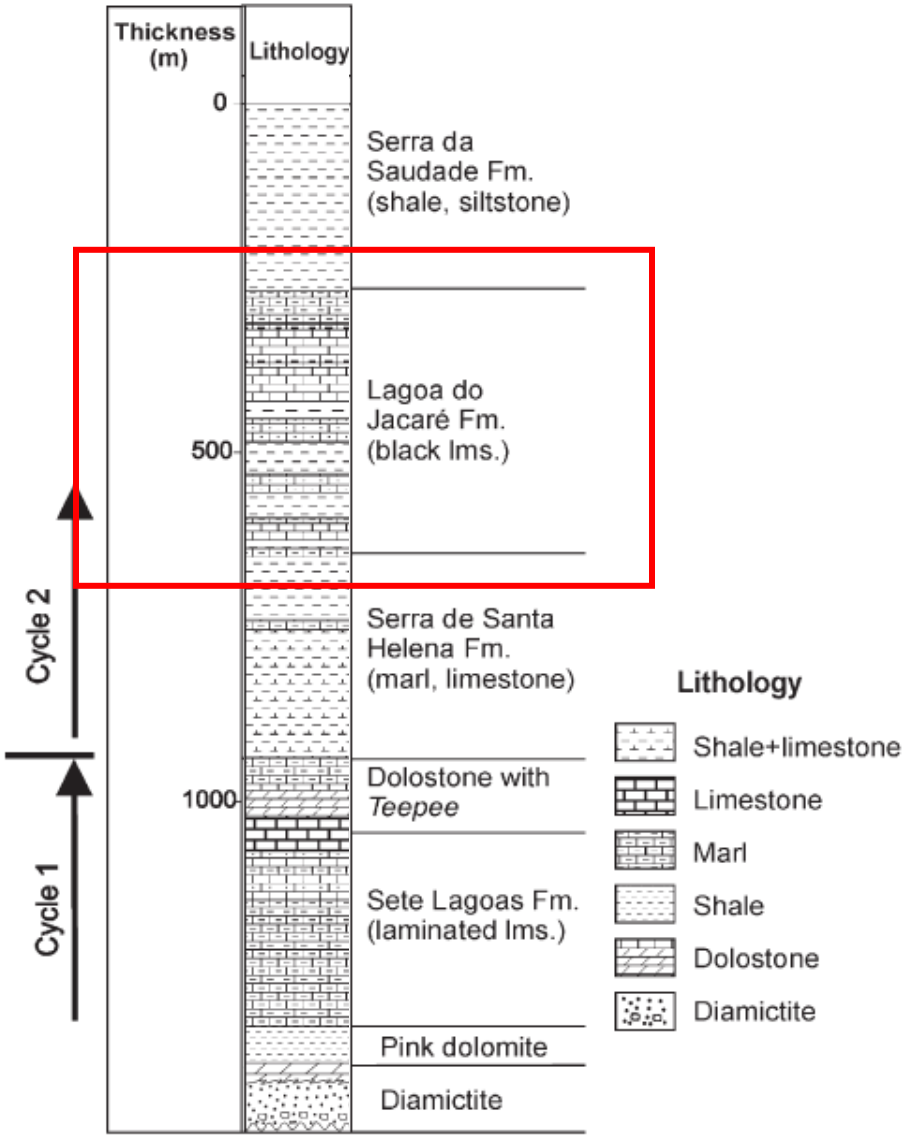
Noted by the red box is the geographic location of the Bambuí Group, from which my samples were collected.

FIGURE 4: (Fischer, 2008)



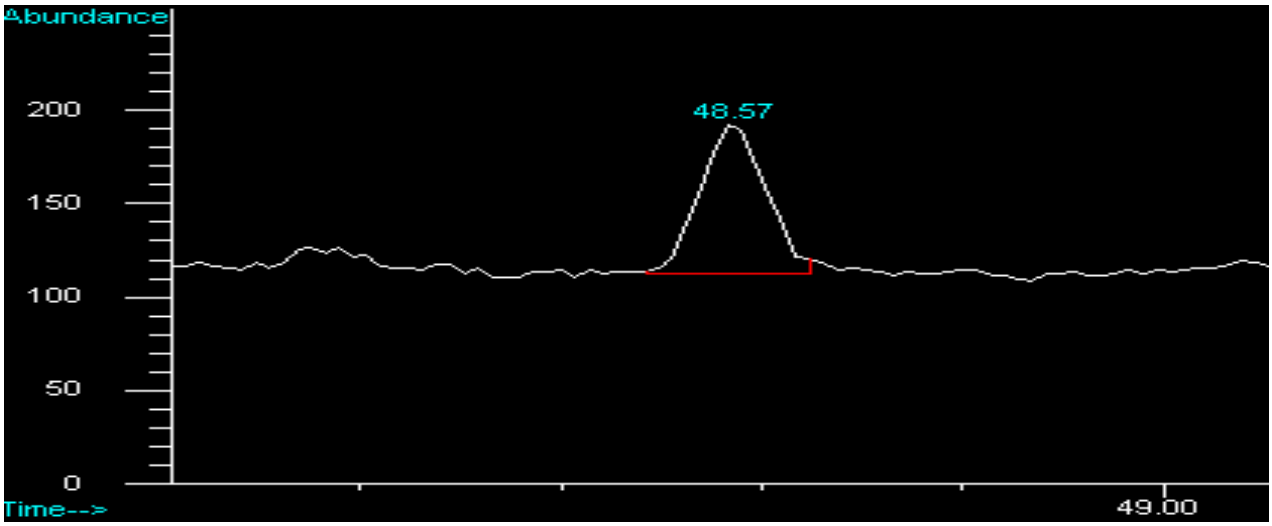
This figure shows the age of my biomarker samples on the geologic time scale as well as where they lie relative to early signs of life.

FIGURE 5: (Misi et al.., 2007)



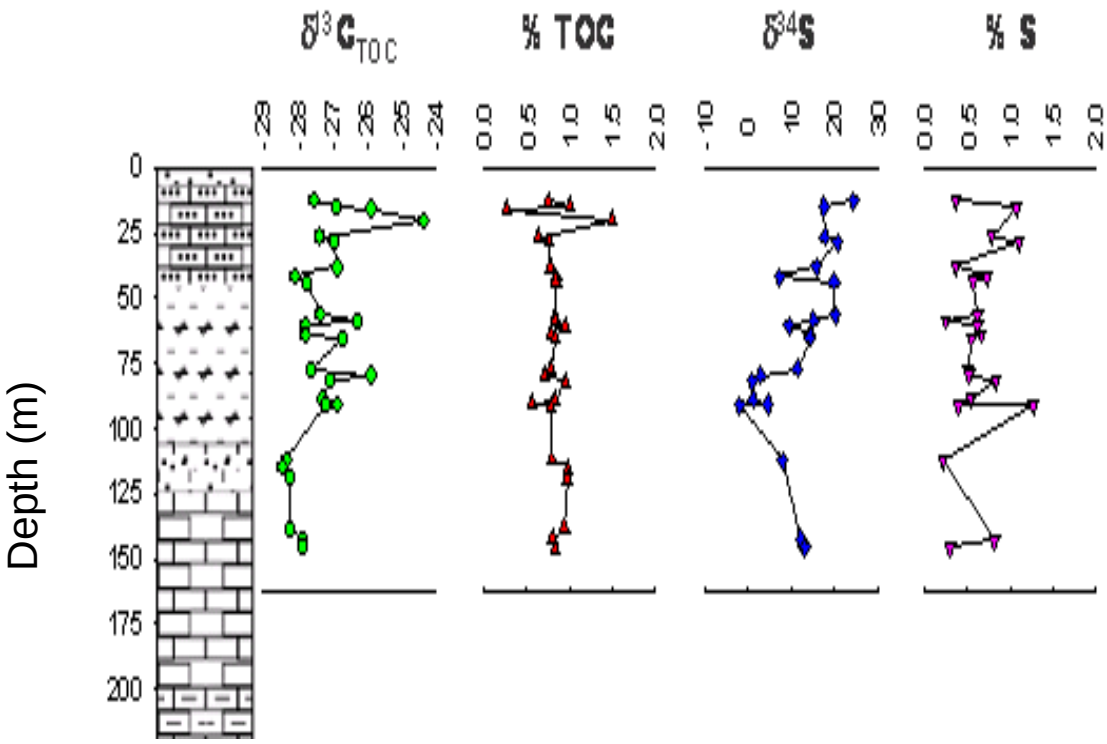
This figure shows the stratigraphic location of the Lagoa do Jacaré Formation within the Bambuí Group. This stratigraphic column depicts the types of lithologies which are found within this formation.

FIGURE 6: Peak Integration



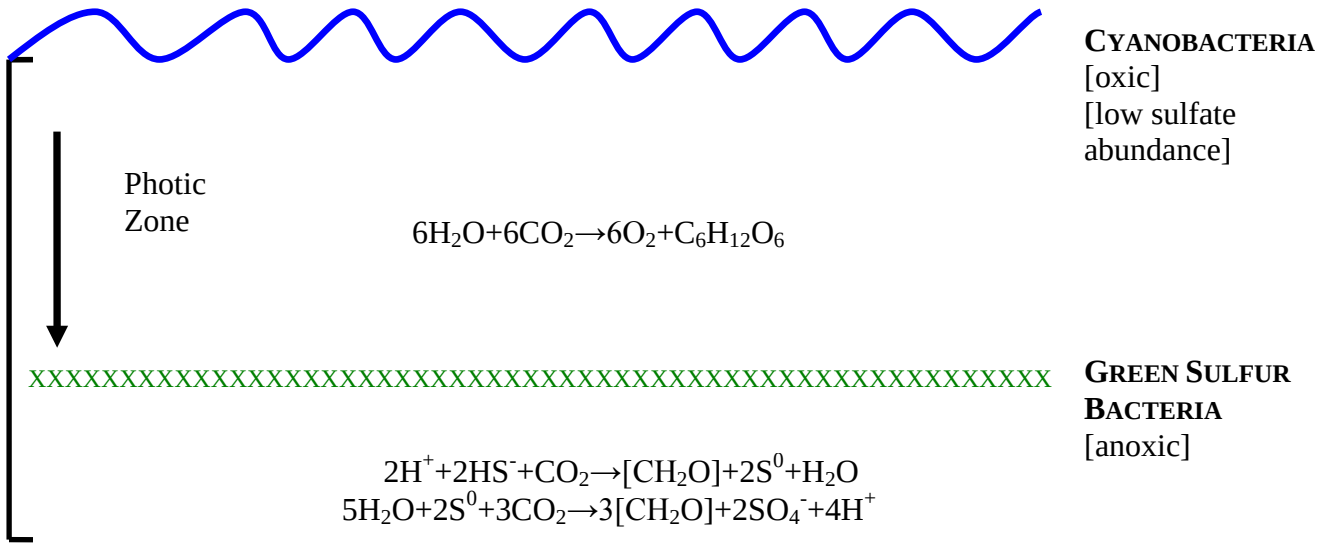
Each peak is manually integrated three times and the average peak area is taken and used in determining ratios for thermal maturity.

FIGURE 7: (K. Miller, pers comm)



This figure shows isotopic data for $^{13}C_{TOC}$ and ^{34}S as well as %S and % TOC plotted against the stratigraphic column for the Lagoa do Jacaré Formation.

FIGURE 8



This schematic diagram represents the position of Green Sulfur Bacteria within a stratified water column. Green Sulfur Bacteria are found at the base of the photic zone, and mark the boundary where the ocean becomes anoxic. This boundary is found at depths of less than 20 m (Brocks et al., 2005).

FIGURE 9: Pr/Ph

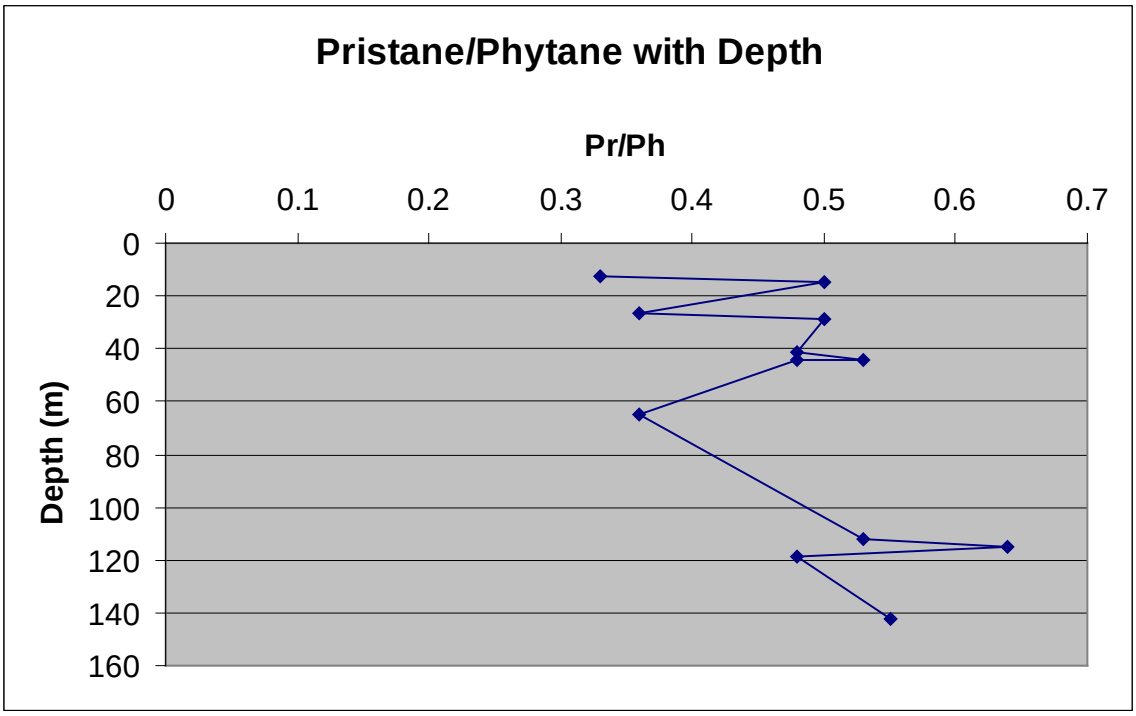


TABLE 1: Ions Scanned During SIM analysis

	Saturate Fraction				Aromatic Fraction	
	n-Alkanes	Acyclic Isoprenoids	Hopanes	Steranes	Aryl Isoprenoids	Dibenzo-thiophenes
m/z	85	183	191, 205, 412	217, 259, 414	133	184

TABLE 2: Index of compounds produced by each sample

		Saturates				Aromatics		
Depth	Extracts dry before being set on SI gel	n-Alkanes	Acyclic Iso-prenoids	Hopanes	Steranes	Aryl Iso-prenoids	Dibenzo-thiophene	Comments
12.60	x	x	x	x	--	--	--	
15.00		x	x	--	x	x	x	
15.70		--	--	--	--	--	--	
26.50		x	x	x	x	x	x	
28.50		x	x	x	x	x	x	
38.00		--	--	--	--	x	x	
41.60		x	x	x	--	x	x	
43.90a		x	x	--	--	x	x	Aryl isoprenoids and dibenzothiophene almost below detection limits.
43.90b		x	x	--	--	--	--	
65.17		x	x	x	x	x	x	Aryl isoprenoids and dibenzothiophene almost below detection limits.
112.15	x	x	x	x	x	x	x	
115.00	x	x	x	x	x	x	x	
119.00	x	x	x	--	--	--	--	
142.45	x	x	x	x	x	x	x	
145.55		x	x	--	--	--	--	n-Alkanes and acyclic isoprenoids were present, but too low to calculate

x- compound is present
-- -compound is not present

TABLE 3: *N*-alkane abundances

Normal Alkane Abundances (ppm)

depth	C_{15}	C_{16}	C_{17}	C_{18}	5	6	7	8	9	10	11	12	13	14
12.60	0.06	1.3	2.9	5.4	4.5	3.6	2.3	2.1	0.64	0.38	0.14	0.07	0.039	0.06
15.00	--	0.0016	0.004	0.0044	0.0041	--	--	--	--	--	--	--	--	--
26.50	0.23	0.2	0.12	0.38	0.15	0.15	--	--	--	--	--	--	--	--
28.50	0.301	0.402	0.55	2.07	0.80	0.82	0.23	0.17	0.070	0.309	0.049	0.047	--	--
41.60	0.13	0.12	0.18	0.07	0.086	0.041	0.022	--	--	--	--	--	--	--
43.90a	--	0.053	0.10	0.26	0.14	0.11	0.022	0.018	0.0056	0.024	--	--	--	--
43.90b	--	0.00063	0.0019	0.0011	0.00099	0.00077	0.00071	0.0012	--	--	--	--	--	--
65.17	0.85	8.8	5.0	6.8	2.4	1.6	0.74	1.06	0.21	0.13	0.090	0.040	--	--
112.15	0.00092	0.0018	0.0013	0.0024	0.00063	0.00068	0.00052	0.00049	0.00041	0.00039	0.00025	0.00017	--	--
115.00	0.012	0.0074	0.0044	0.012	0.0056	0.0059	0.0036	0.00051	0.0019	0.0013	0.0011	--	--	--
119.00	0.067	0.83	0.29	0.67	0.17	0.25	0.14	0.073	0.072	--	--	--	--	--
142.45	0.09	1.2	0.4	1.8	--	--	--	0.32	--	--	--	--	--	--
145.55*	--	--	--	--	--	--	--	--	--	--	--	--	--	--

-- abundance below detection limits or compound was not present

*Sample 145.55 did produce *n*-alkane peaks, however the abundance was too low for analysis.

TABLE 4: n-alkane odd/even ratio

depth	odd/even
12.60	0.91
15.00	1.2
26.50	0.55
28.50	0.73
41.60	0.67
43.90a	0.72
43.90b	0.89
65.17	0.73
112.15	0.77
115.00	1.1
119.00	0.58
142.45	0.37
145.55	--

The area of the odd *n*-alkane peaks was compared with the area of the even *n*-alkane peaks. This gave a ratio of odd to even normal alkanes. A preference for odd *n*-alkanes, values >1, indicates contamination by terrigenous sources. A preference for even *n*-alkanes, values <1, indicates aqueous input.

TABLE 5: Pristane/Phytane with Depth

Depth	Pr/Ph
12.60	0.33
15.00	0.50
15.70	--
26.50	0.36
28.50	0.50
38.00	--
41.60	0.48
43.90a	0.53
43.90b	0.48
65.17	0.36
112.15	0.53
115.00	0.64
119.00	0.48
142.45	0.55
145.55	--

TABLE 6:Hopane Ratios

HOPANES

Depth	C_{27}/ C_{29}	C_{29}/ C_{30}	C_{30}/ C_{31}	Ts/ Ts+Tm	C_{29}Ts/ C_{29}	C30βα/ (βα+αβ)	C31S/ (S+R)
12.60	0.49	1.2	2.2	0.53	0.13	0.10	0.61
15.00	--	--	--	--	--	--	--
15.70	--	--	--	--	--	--	--
26.50	0.52	1.0	1.7	0.48	0.20	0.03	0.61
28.50	0.60	--	--	0.45	0.17	--	0.55
38.00	--	--	--	--	--	--	--
41.60	0.52	--	--	0.51	0.18	--	0.54
43.90a	--	--	--	--	--	--	--
43.90b	--	--	--	--	--	--	--
65.17	0.62	0.93	2.4	0.54	0.22	0.09	0.55
112.15	0.37	0.89	--	0.50	0.22	0.13	--
115.00	0.32	0.97	2.1	0.60	0.18	0.14	0.62
142.45	0.46	1.0	1.8	0.53	0.19	0.10	0.59
145.55	--	--	--	--	--	--	--
Average	0.49	1.0	2.0	0.52	0.19	0.10	0.58
1σ	0.10	0.11	0.29	0.04	0.03	0.04	0.03

TABLE 7: STERANE RATIOS

	STERANES					
Depth	C27 S/(S+R)	C28 S(S+R)	C29 S/(S+R)	C27 ββ/ (αα+ββ)	C28 ββ/ (αα+ββ)	C29 ββ/ (αα+ββ)
12.6	--	--	--	--	--	--
15	0.45	0.36	0.64	0.77	0.64	0.47
15.7	--	--	--	--	--	--
26.5	0.59	0.47	0.52	0.67	0.54	--
28.5	0.58	0.39	0.60	0.50	0.49	0.46
38	--	--	--	--	--	--
41.6	--	--	--	--	--	--
43.90a	--	--	--	--	--	--
43.90b	--	--	--	--	--	--
65.17	0.57	0.40	0.50	0.70	0.61	0.35
112.15	0.57	0.70	--	0.43	0.41	0.40
115	0.41	0.38	0.48	0.68	0.52	--
119	0.57	0.35	0.68	0.77	0.54	--
142.45	0.57	0.69	0.49	0.44	0.41	0.44
145.55	0.45	0.35	0.54	0.67	0.53	--
Average	0.53	0.45	0.56	0.63	0.52	0.42
1σ	0.07	0.14	0.07	0.13	0.08	0.05

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"I pledge on my honor that I have not given or received any unauthorized assistance or plagiarized on this assignment."