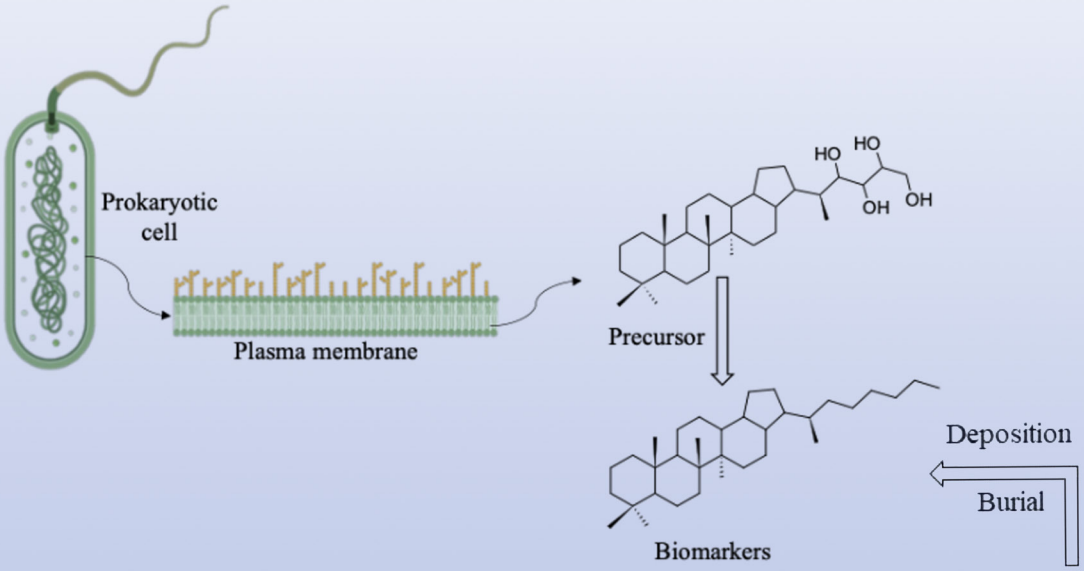
Acidic molecular fossils:
origin, isolation and applications



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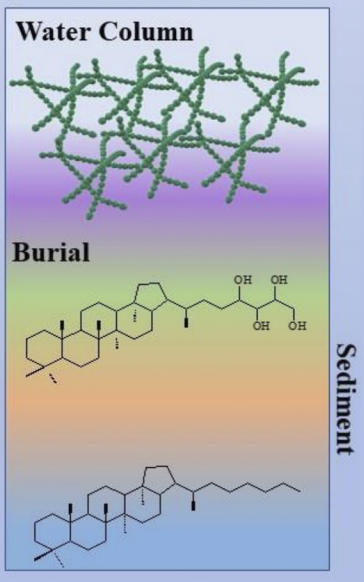

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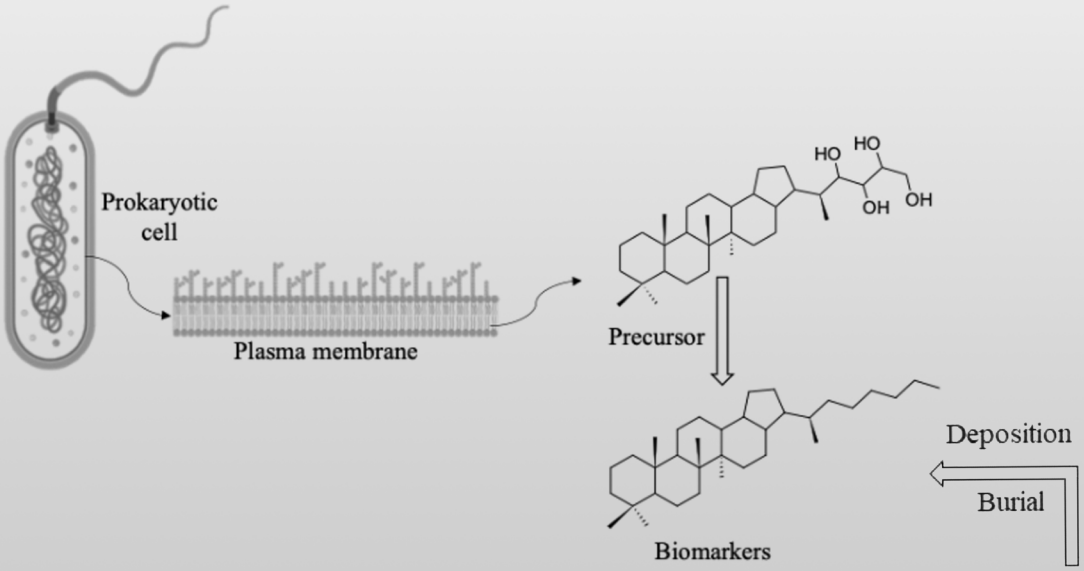

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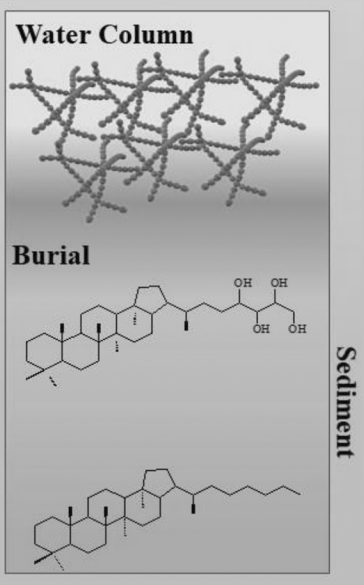

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PREFACE

The researcher team of the Organic Geochemistry Laboratory (Nature Sciences Center, Federal University of Piaui, UFPI, Brazil) has developed an challenging work revising a wide range of topics in Organic Geochemistry with emphasis on acidic molecular fossils, including their origin, isolation, and main applications. A large number of papers cover this theme and huge work was employed to systematize this vast knowledge. This effort is worthwhile for making possible a larger number of students to acquire a deeper understanding of organic geochemistry and become higher-skilled professionals.

Among the main topics discussed in this book are; an introduction to biomarker isolation and analysis techniques, discussions about biological precursors of neutral molecular fossils, involving primary producers, the related processes of transformation of bio- to geolipids with the description in details of the chemical paths of such transformations. The discussion was completed with the introduction to the use of diagnostic compounds as a sophisticated tool to study conditions in the environment where organically enriched sediments were deposited and preserved. Detection and extraction methods of neutral and acidic biomarkers have also been considered in the discussion, including alternative procedures.

The main geochemical characteristics of the representative petroleum group from Brazilian basins are also commented on from specialized literature. Especially, the variations in the relative proportions of diagnostic biomarkers, bulk parameters, and gas chromatographic signatures. Certainly, this valuable information will make more accessible the understanding about how petroleum can be classified based on the inherited composition from sources, history of thermal evolution, and preservation through geological time.

This book is supplemented with a case history using geochemical information of the Codó Formation, Parnaiba Basin. This formation was deposited during the interval Late Aptian-Early Albian (Cretaceous) when most of the sediments in basins alongshore Brazil deposited varying sediments under transitional conditions of the environment and climate. Due to such conditions, highly complex signatures of biomarkers are found with compositions of organic matter from organisms thriving in lacustrine, shallow marine or fluvial-deltaic conditions. The overall procedure emphasizing carboxylic acids involving isolation, separation, and analyses has shown a promising tool for the geochemical characterization of potential source rocks and related oils.

This work represents an intense effort of the Geochemistry Group at (UFPI) and the contribution of Petrobras through the sponsorship of research projects. For this successful partnership, both must be acknowledged.

Dr. Eugênio Vaz dos Santos Neto

Petroleum geologist – Universidade Federal Fluminense

SUMMARY

INTRODUCTION

Organic geochemistry studies the distribution, composition, and fate of organic matter in the geosphere at both bulk and molecular levels, combining aspects of geology, chemistry, and biology. One of the vital roles of organic geochemistry includes the examination of the evolution of organic compounds (hydrocarbons and their derivatives) from the moment of their formation, a study of the subsequent change in their composition and distribution. It has been applied in understanding the phenomena associated with the origin and biogeological transformation undergone by petroleum.

The study of the geographic distribution worldwide of oil, the understanding of its genesis, and the phenomena that control its accumulations are essential in assessing the commercial quality and help provide information for exploratory decision-making. Such a study has been supported by so-called molecular fossils or molecular geochemical indicators, including any distinct organic molecule or molecular fragment recovered from rocks, sediments, fossils, or oils. Such compounds have often been called **biomarkers**, because of their intrinsic relationship to molecules generated by living organisms in ancient environments.

Some of the biomarkers include **sesquiterpanes** and **diterpanes**, which can indicate whether a particular fossil came from a plant/insect or conifer resins, respectively; **biphytanes**, which point to **archaea**; hopanes, which suggest bacteria; **2-methylhopanes and steranes**, which have been associated to cyanobacteria and eukaryotic organisms, respectively. These organic molecules have also been used to provide evidence for oxidation events and the emergence of eukaryotic life on earth. In the oil industry, they are most often used to study the depositional paleoenvironment of organic matter, the sediment maturation stages, the oil migration occurrence, and for oil-oil and oil-rock correlation.

Most of petroleum geochemical research is focused on the presence or distribution of some biomarkers in the saturated hydrocarbon fraction, which can be explained by a more accessible analysis using Gas Chromatography-Mass Spectrometry (GC-MS). However, less attention is given to other fractions containing compounds besides hydrocarbons, oxygen, nitrogen, and sulfur heteroatoms which, at first, provide complementary information about organic matter OM deposition, and at last, geological history associated to such deposition.

Among the main heterocomponents present in oils, carboxylic acids have been the subject of much discussion regarding their origin and economic importance. They have proven helpful in thermal evolution studies, biodegradation-related studies, and in establishing diagenetic pathways in characterizing recent sediments and oils. Although their concentration is low in crude oils, their emulsifying and corrosive characteristics make them especially important as they cause corrosive effects in distillation towers, directly impacting the costs associated with their processing and refining.

A great part of this book has to deal with factual data, research experience of our organic geochemistry group that has been working with oil samples and sedimentary rocks from Brazilian marginal basins, maintaining a close relationship and partnership with PETROBRAS Research and Development Center (CENPES), to whom we are fondly thankful for the partnership, for the incentive to our group and the unconditional support given to the Organic Geochemistry Network.

Our research group, which also works with Phytochemistry, has, among others, the following primary goals:

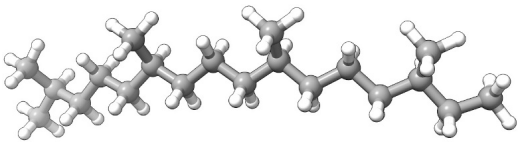
- To evaluate the distribution, identify and quantify neutral and acidic hydrocarbons in oil and source rock samples;
- Assess the distribution, origin, and influence of hopanoic acids in oil samples of different origin and biodegradation;
- Synthesize, identify and quantify biomarkers.

This book focuses on carboxylic acids (biomarkers) present in oil and sedimentary rock samples, but we begin with the neutral molecular fossils routinely applied in the petroleum industry. Then, we wrote a good review about extraction methods of acidic biomarkers, with advantages and disadvantages. In the end, we present a case study, a simple, fast, and inexpensive extraction and analysis method applied to sedimentary oil and rock samples, as a significant contribution of this work.

CHAPTER 1

NEUTRAL MOLECULAR FOSSILS

Molecular fossils are organic molecules derived from once-living organisms. They fall within the category of biomarkers, which are substances or characteristics that indicate a modern or past biological presence, state, or process. Molecular fossils can be a valuable source of information about past organismic physiology and an effective tool for the reconstruction of paleoenvironmental conditions. This unit deals with the origin, definition, and applications of neutral biomarkers, often used in the characterization of oil samples by the oil industry, showing their main classes, with structural examples and chromatographic profiles.



1 | NEUTRAL MOLECULAR FOSSILS (BIOMARKERS): APPLICATIONS

Petroleum is a complex mixture of organic compounds of varying structures, ranging from gases to polymers. Among these compounds are chemical fossils, molecular fossils, or, as they are more commonly called, biomarkers, which can be found in oils, sediments, and rocks. These molecular structures can be correlated to natural products (precursors) found in eukaryotic and prokaryotic organisms, which are suggested to be derived as a result of transformations undergone through biological and thermochemical processes (PETERS; WALTERS; MOLDOWAN, 2005a; SCHWARZBAUER; JOVANČIĆEVIĆ, 2016; TISSOT; WELTE, 1984).

These molecular structures, present in living organisms (plants, plankton, fungi, and bacteria), when incorporated into sediments, their functional groups and bonds undergo modifications, preserving their basic carbon skeleton (**Figure 1)**.

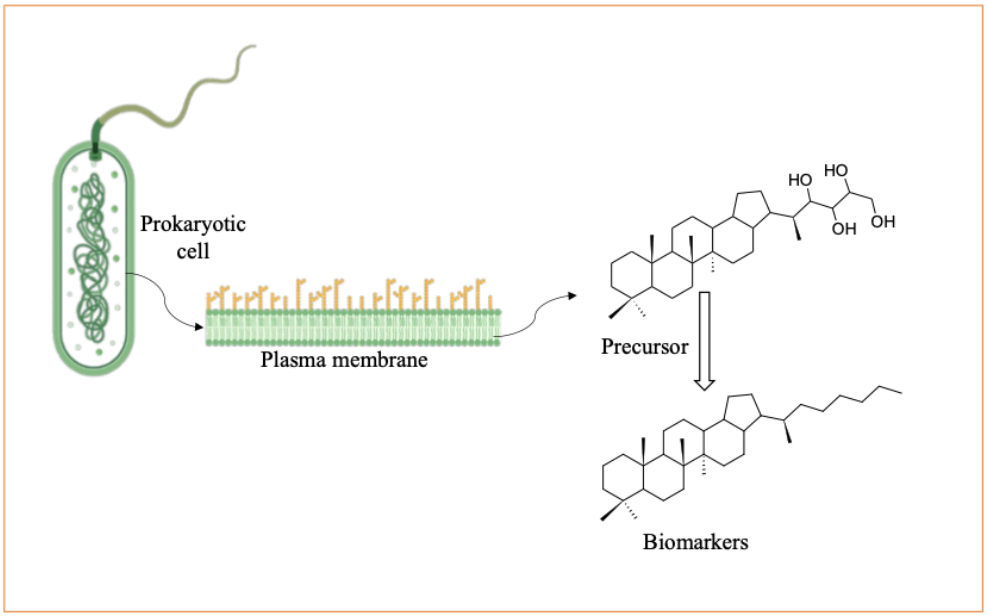


Figure 1. Formation stage of biomarkers, triterpane biomarkers (hopanes). Under appropriate conditions, these compounds are transformed into their hydrocarbon equivalents and can be preserved in sediments and oils for billions of years without compromising the basic information that gives them diagnostic utility.

They have also proven useful in identifying contaminants of the marine environment by petroleum products, indicating their origin whether industrial, urban, or natural. The biomarkers more commonly used in environmental and geochemical studies are terpanes and steranes. Other components (fatty acids, alcohols, photosynthetic pigments, polycyclic terpenoids etc., **Figure 1**) have high preservation potential. Under adequate conditions, such

compounds are transformed into their hydrocarbon equivalents and can be preserved in sediments and oils for millions of years, without compromising the information which confers their diagnostic utility (BRIGGS; SUMMONS, 2014; PETERS; WALTERS; MOLDOWAN, 2005a; SUMMONS; LINCOLN, 2012). An example is the cyclic terpenoid steroids and hopanoids: their hydrocarbon backbone – steranes and hopanes – are biomarkers well known to organic matter (OM) from eukaryotes and bacterial sources (**Figure 1**).

Once preserved, the sediments or oil components are reflexes of those originally found in organism which contributed to OM at deposition time. In this sense, the biomarkers provide a record, with several degrees of specificity, of paleobiodiversity, paleoenvironmental conditions, water chemical composition, redox conditions, as well as sediments thermal history (SUMMONS; LINCOLN, 2012). They also found a great utility on fossil fuel exploration, allowing the characterization and distinction of petroleum source rocks deposited at different environments and the process which affects preservation and quality of sedimentary OM (MELLO et al., 1988a; PETERS; WALTERS; MOLDOWAN, 2005a).

1.1 Organic Geochemistry and Molecular Fossils

Organic geochemistry is a branch of science concerned with the study, occurrence, composition, origin, and fate of organic matter in oils (petroleum), rocks, and sediments. Historically, it developed from the application of methods from sedimentary geology and organic chemistry. Practitioners in this field may be interested in the occurrence and distribution of organic matter, if geologists, or interested in the composition, if organic chemists. However, the common interest is to evaluate a better understanding of the origin and significance of organic matter present in geological materials (KVENVOLDEN, 1967) even-carbon-numbered normal fatty acids are much more abundant than those with odd carbon numbers ; in some sediments, however, concentrations of even- and odd-carbon-numbered normal fatty acids are about equal. Normal fatty acids have been postulated as pos-sible precursors for normal paraffin hydrocarbons in petroleum because of 1.

Interest in the various forms of organic matter associated with rocks dates back to early recorded history, at least 3800 B.C., when asphalt was used to generate light and heat. Much later, in the late 18th century, the use of these materials as sealants, lubricants, and even medicinal uses was reported. Despite this early interest, the composition of these various organic materials was unknown, there was no interest in this kind of information, and the necessary analytical tools were not yet available (KVENVOLDEN, 2008).

In the mid-nineteenth century, geology and organic chemistry - two disciplines that, until then, had originated and developed independently - realized that detailed information about the organic materials present in sediments and rocks was scientifically interesting and of practical importance, strongly influenced by developments in petroleum geochemistry (KVENVOLDEN, 2002, 2008). The stage was set for the emergence of organic geochemistry.

Organic geochemistry as a scientific discipline began in the 1930s, with the first model study of the geochemistry of organic molecules by Alfred Treibs (1936). Treibs identified porphyrinic pigments in shales, oils, and coals in his study and was able to show that the free and metal complexed porphyrins identified in these geological materials were degradation products of chlorophyll and heme. This discovery establishes a clear link between the biochemical compounds present in living organisms and the compounds found in organic matter of geochemical origin, and for this reason, Treibs is considered the "father of organic geochemistry" (KVENVOLDEN, 2002, 2006).

Figure 2 shows the structures of chlorophyll a, essential to most photosynthetic organisms; of Heme B, present in the structures of hemoglobin and myoglobins, the red blood pigments, and directly responsible for oxygen transport in the bloodstream; as well as free porphyrins and those complexed to metals such as vanadium (in the form of vanadyl cation, VO^{2+}) or nickel.

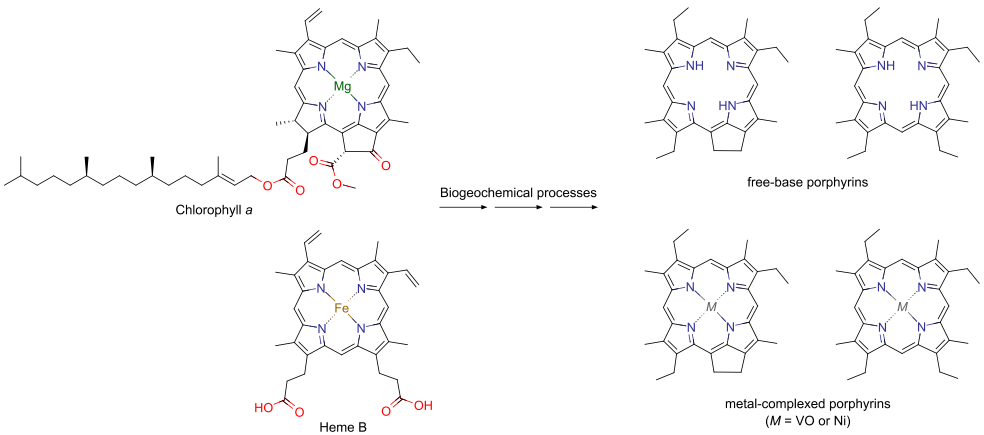


Figure 2. Chlorophyll and biochemical processes. In 1936 Alfred Teibs recognized that vanadyl porphyrin was a molecular fossil of chlorophyll. Teibs discovery helped support a biologic origin for petroleum. Adapted from (KNOLL et al., 2007).

1.2 Organic matter: diagenesis, catagenesis and metagenesis

During diagenesis, organic matter is buried and transformed by various chemical and biological processes until it combines with degradation-resistant macromolecules and forms kerogen. These processes occur at low depths such that temperatures are around 50 °C and pressures are not high (BROCKS; SUMMONS, 2014), **Figure 3**. Kerogen is defined as solid sediment in particle form, insoluble in solvents, but it is associated with organic compounds in the liquid phase, it has MO fractions that can be extracted with organic solvents, and is defined as bitumen.

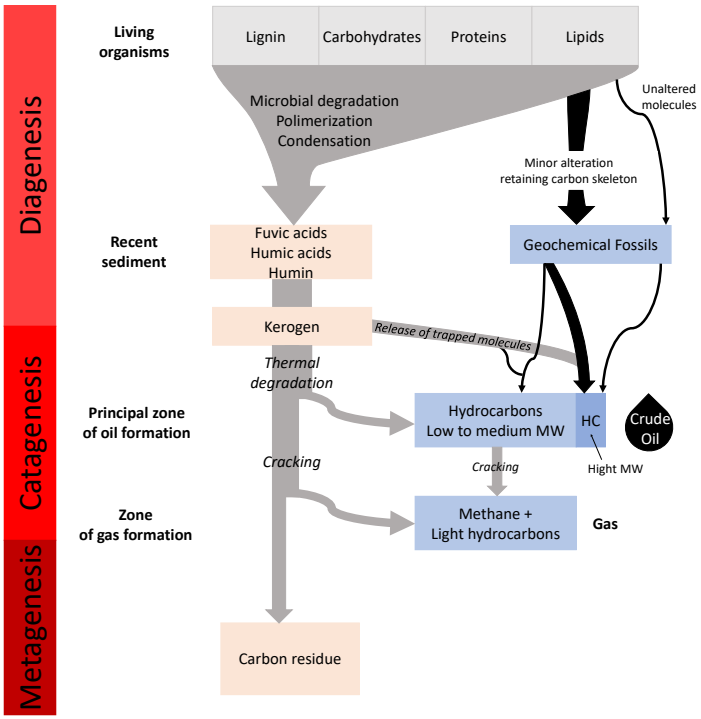


Figure 3. Simplified diagram of oil and gas formation showing the diagenesis, catagenesis, and metagenesis phases. Adapted from Tissot and Welte (1984)

As the depth increases, and consequently the temperature and pressure, the first changes start to appear, such as structural changes of the molecules and bond breaking in the polar compounds, as well as oxidation, reduction, sulfurization, desulfurization, and rearrangements reactions. These changes generate a mixture of partially or fully de-functionalized compounds that may have different isomers and stereochemical but retain the original skeleton of the compounds that gave rise to them (**Figure 4**). These products are known as molecular fossils, chemical fossils, or biomarkers (PETERS; WALTERS; MOLDOWAN, 2005a; TISSOT; WELTE, 1984).

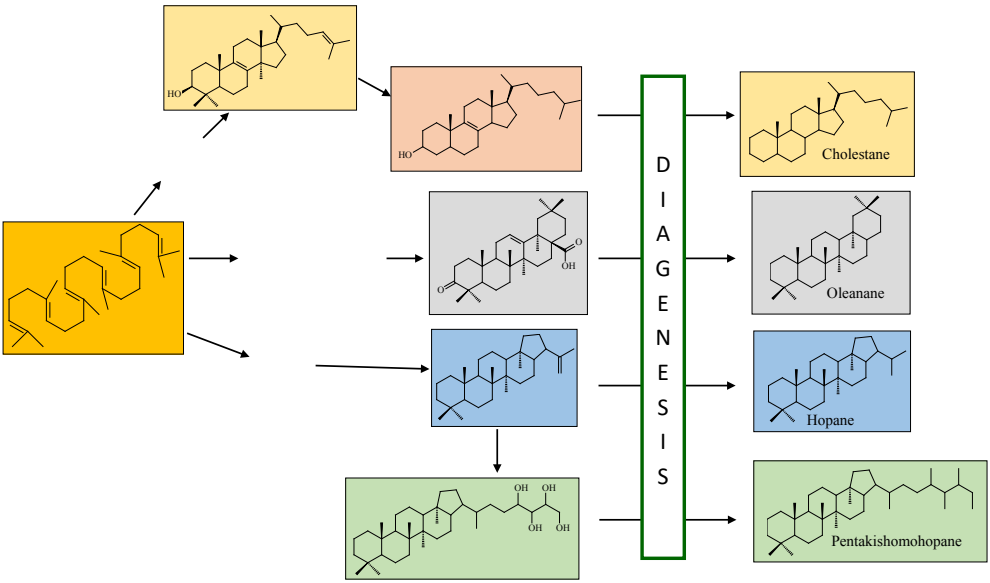


Figure 4. Biosynthesis of some biomarker precursors (biological compounds from a common origin, squalene - C_{30} isoprenoid) and their transformations after diagenesis. These biomarkers can be found in consolidated or unconsolidated sediments and oils. Adapted from HSU et al. (2003). Fossil fuels, petroleum (crude oil), coal, and natural gas result from biological activity and contain chemical fossils.

With successive advancements of burial over time (millions of years - Ma), the temperature increase causes kerogen to crack into lighter compounds that considerably increase the volume of bitumen, which then begins to be expelled as oil. The temperature is between 50 °C and 150 °C. This phase is called catagenesis, the main stage in oil generation and is often referred to as the "window of oil generation" (BROCKS; SUMMONS, 2014; PETERS; WALTERS; MOLDOWAN, 2005a; TISSOT; WELTE, 1984).

Metagenesis is considered the final stage, which reaches high temperatures (>200 °C), where most of the residual bitumen is flushed out or cracked into gas and is then considered the senile stage. kerogen becomes progressively hydrogen-poor, forming a carbon-rich aromatic polycondensate (BROCKS; SUMMONS, 2014; TISSOT; WELTE, 1984).

1.3 Primary Producers and Biomarkers

Molecular fossils help to understand the evolution of primary producers in the oceans. Microfossils and molecular fossils help establish that Earth's oceans have undergone two significant changes in the composition of primary producers (KNOLL et al., 2007):

- Initially, cyanobacteria, along with other photosynthetic bacteria, were the primary producers during the Proterozoic eon (**Figure 5**). The first shift occurred during the early Paleozoic era when eukaryotic green algae joined the cyano-

bacteria in being the primary producers;

- The second shift would occur during the Mesozoic era when diatoms would join dinoflagellates and coccolithophores in the Jurassic. Diatoms, dinoflagellates, and coccolithophores would assume their dominant role as the basis of many modern marine ecosystems in the Cretaceous era.

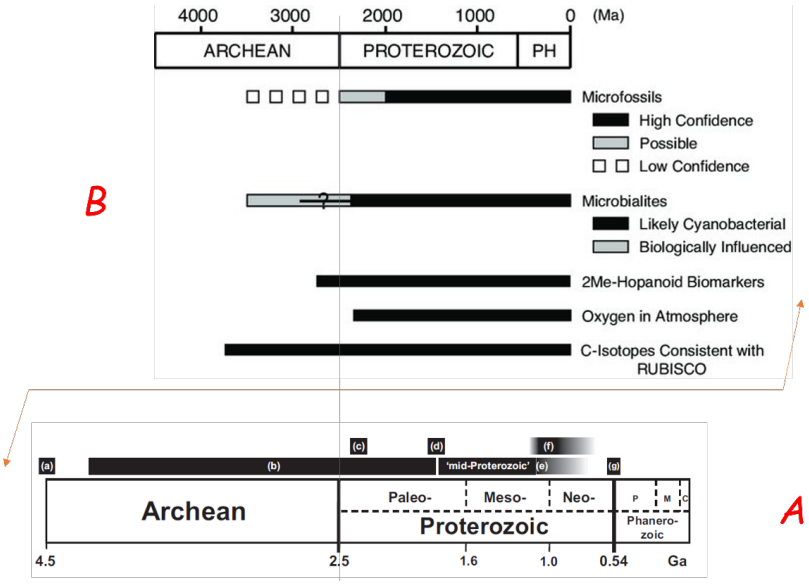


Figure 5. **"A":** Geological time chart beginning with (a) the formation of Earth ~4.6 billion years ago (Ga); (b) anoxic, non-sulfidic oceans; (c) onset of oxygenation of the atmosphere; (d) disappearance of banded iron formations as an indicator of changing ocean chemical banded iron formations as an indicator of changing ocean chemistry; (e) the informally defined "mid-Proterozoic" interval with possible widespread, anoxic and sulfidic marine conditions; (f) major radiation of eukaryotic algae; (g) first appearance and major radiation of multicellular organisms and animals. P = Paleozoic, M = Mesozoic, C = Cenozoic. For "A", caption and image modified from BROCKS and PEARSON (2005). **"B":** Summary of the stratigraphic distribution of fossils and other geobiological features interpreted as evidence for cyanobacteria in Archean and Proterozoic oceans. For "B", caption and image modified from (KNOLL, 2008).

Thus, life was probably present throughout the Archean, but it must have been limited to simple single-celled non-nucleated organisms, called prokaryotes, because there are no fossils of eukaryotes as old as during the Archean. Microfossils, stromatolites, sedimentary carbon, and sulfur isotopes indicate that microorganisms inhabited Earth during the Archean and should be limited to simple single-celled non-nucleated organisms, called prokaryotes (ALLEON; SUMMONS, 2019; BROCKS et al., 2003a, 2003b).

Some studies have linked the presence of biomarkers to specific biological precursors or the depositional paleoenvironment. For example, the establishment of significant petroleum rocks accompanies the increase in diatoms over the last million years (PETERS;

WALTERS; MOLDOWAN, 2005b, 2005a). Bacteria and high relative concentrations of alkyhopanes indicate that cyanobacteria were important primary producers (BROCKS et al., 2003b, 2003a). The often spatial coincidence of silica and fossil fuels, together with the worldwide survey of biomarkers (e.g., 24-norcholestane or C_{28}-C_{29} steranes) in sediments and source rocks, indicate a crucial role of diatoms in the formation of petroleum reserves (BENOISTON et al., 2017; PETERS; WALTERS; MOLDOWAN, 2005a). Furthermore, several oil basins overlap with regions where diatoms thrive, such as coastal oceanic environments and the Arctic Ocean. Although previous assessments suggest that petroleum rocks are relatively low in abundance in the Southern Ocean, this region may also hold significant resources (BENOISTON et al., 2017).

Biomarkers of bacterial origin are detected from 1.73 Ga in several mid-Proterozoic settings, while the oldest distinct eukaryotic biomarkers (e.g., C_{27} cholestane) are detected from 800 million years onwards (BROCKS et al., 2017). The difficulty in linking older microfossils to a biological precursor becomes more evident when looking at the more recent work by BOBROVSKIY *et al.*, (2020), who suggest that 24-isopropylcholestane (C_{30}) is not diagnostic for demosponges (Cryogenian, >635 million years ago), as proposed in earlier work, and likely formed in Neoproterozoic sediments through geological methylation of sterols (C_{29}) from chlorophyte algae.

Thus, science needs to move forward and better settle these speculations about the lack of evidence linking any biomarker (which is commonly found in the Ediacaran) to older microfossils found in the middle Proterozoic.

Source- and age-related biomarkers can directly correlate crude oil samples with other oils or with thermally mature source rock extracts. When potential source rock samples are not available, source- and age-related biomarkers in oil can be used for indirect correlation (PETERS; WALTERS; MOLDOWAN, 2017). However, strictly speaking, biomarkers alone are not markers for taxonomic groups or environmental conditions; however, their specific isotopic analysis is valuable for understanding the modern and ancient geological record (BROCKS; PEARSON, 2005).

More recently, SUMMONS; WELANDER; GOLD (2021) have discussed how advances in molecular biology have helped elucidate the origins of biomarkers and allowed more robust interpretations of fossil lipids and how the rock record is a vital calibration point for molecular clocks.

1.4 Biomarkers: details about their origin and applications

The distribution and relative abundance of these biomarkers make it possible to identify and evaluate the source rocks and correlate the oils among themselves and with the respective rocks, aiming to guide exploration to specific areas and depths that are more favorable to the existence of commercial oil and gas accumulations (KILLOPS; KILLOPS,

2005; MILANI et al., 2000).

Such compounds may also provide information about: anoxic conditions in the water column (SUMMONS; POWELL, 1986), hypersalinity in evaporitic environments (GRICE et al., 1998), the emergence of groups of organisms (LOVE et al., 2009), perturbations and reorganization in geochemical cycles (LOGAN et al., 1995), photosynthetic organisms during global glacial events (OLCOTT et al., 2005), and catastrophic losses in biodiversity (GRICE et al., 2005).

In order to distinguish a biomarker from other organic molecules, three essential characteristics must be taken into consideration (PETERS; WALTERS; MOLDOWAN, 2005b):

I. Its chemical structure must show information that indicates its biological origin (coming from living organisms);

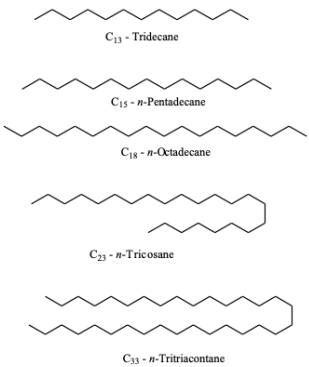
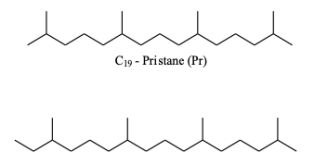
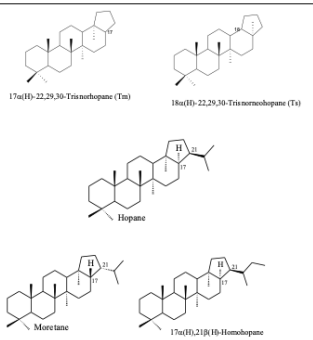
II. Present in high concentrations in living organisms with speciation;

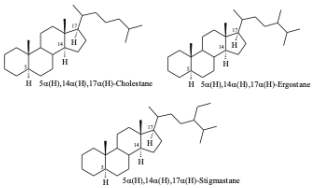
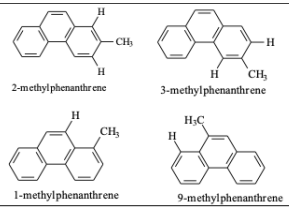
III. The main structural feature of the compound (skeleton) must be chemically stable when subjected to sedimentation and burial of organic matter.

Some bio-compounds such as carbohydrates, proteins, and nucleic acids usually do not survive long after the organism's death since such compounds are an accessible food source for bacteria and fungi. Other components (fatty acids, alcohols, photosynthetic pigments - chlorophylls and carotenoids - polycyclic terpenoids, etc.) have high preservation potential. Most effective biomarkers have a well-defined number of sources, are resistant to geochemical changes, and are easily analyzed in samples of sedimentary origin. The most valuable biomarkers for environmental and geological studies are lipids, such as steranes and hopanes.

The following is a brief overview of the main characteristics of the principal families of compounds used in oil, rock, or sediment sample analysis and petroleum system studies. **Table 1** shows the main parameters of saturated and aromatic hydrocarbons, commonly used in interpretations of origin, depositional environment, and degree of maturation. **Figure 6** shows the main parameters used and the types of information obtained from their interpretations.

Table 1. briefly shows the main parameters calculated from saturated and aromatic biomarkers, which can be used in interpretations of origin, depositional environment, and degree of maturation.

Compound/ Biomarker	Structure	Parameter, *m/z*	Interpretation
***n*-alkanes**		PCI- Preferred Carbon Index, TIC; *m/z* 71, 85 or 99 CPI = [$(C_{25} - C_{33})_{odd}$/ $(C_{24} - C_{32})_{even}$ + $(C_{25} - C_{33})_{odd}$/ $(C_{26} - C_{34})_{even}$]/2.	**Maturity index:** The values for immature kerogen will be > 1 (> 3), since biologically odd-numbered n-alkanes are preferred. In the course of diagenesis and catagenesis, even n-alkanes are produced through conversion processes so that the value approaches 1 with increasing maturity.
		The terrestrial over aquatic ratio of n-alkanes (TAR) TIC; *m/z* 71, 85 or 99	**Origin indicators:** TAR>1; indicates terrestrial OM TAR<1; indicates aquatic OM
		% C_{13}-C_{18} TIC; *m/z* 71, 85 or 99	**Origin indicators:** Indicative of OM from phytoplankton and zooplankton
		% C_{19}-C_{24} TIC; *m/z* 71, 85 or 99	**Origin indicators:** Indicative of bacterial organic matter
		% C_{25}-C_{33} TIC; *m/z* 71, 85 or 99	**Origin indicators:** Indicative of organic matter from higher vascular plants
		n-alkanes (C_{15}, C_{17}, C_{19}) TIC; *m/z* 71, 85 or 99	**Origin indicators:** Predominance of C_{15}, C_{17}, C_{19} n-alkanes is indicative of organic matter from lacustrine and/or marine environments.
		n-alkanes (C_{27}, C_{29}, C_{31}) TIC; *m/z* 71, 85 or 99	**Origin indicators:** Predominance of n-alkanes C_{27}, C_{29}, C_{31} is indicative of OM from terrestrial environments (higher plants)
***Isoprenoids* (branched alkanes)**		Pristane/Phytane TIC; *m/z* 71, 85 or 99	**Depositional paleoenvironment:** Pr/Ph > 3 indicate oxic depositional environment; Pr/Ph > 1 indicate suboxic conditions; Pr/Ph < 1 for anoxic environments.
		Pr/(Pr+Ph) TIC; *m/z* 71, 85 or 99	**Depositional paleoenvironment:** Pr/(Pr+Ph) > 1 indicates suboxidic conditions; Pr/(Pr+Ph) < 1 for anoxic environments.
		Pr/*n*-C_{17} e Ph/*n*-C_{18} TIC; *m/z* 71, 85 or 99	***Degree* of thermal *maturity*:** Pr/*n*-C17 and F/n-C18 > 1 indicate immature MO Pr/*n*-C17 and F/n- C18 < 1 indicate mature OM High values for Pr/*n*-C17 indicate OM of terrestrial origin High values for Ph/*n*-C18 indicate OM of marine origin
***Terpanes Pentacyclics* (Hopane)**		Ts/Tm *m/z* 191	***Degree* of thermal *maturity*:** The ratio increases in proportion to maturity
		Ts/(Tm+Ts) *m/z* 191	***Degree* of thermal *maturity*:** Low values indicate a suboxide environment, probably originating from carbonate rocks, while higher values are related to anoxic environments where deposition occurred under hypersaline conditions. (*m/z* 191)
		22S/(22S+22R) *m/z* 191	***Degree* of thermal *maturity*:** Biologically produced hopane has the 22R configuration, it is gradually converted to the 22S configuration. Equilibrium values between 0.57-0.62
		Moretane/Hopane *m/z* 191	***Degree* of thermal *maturity*:** The value decreases with increasing thermal maturity, and can range from 0.15 to a minimum of 0.05 in mature extracts. In

			immature bitumen its value is approximately 0.8.
		Hopane/Sterane *m/z* 191 and 217	**origin indicators:** High sterane concentrations and low values of the Hopane/Esterane ratio (less than or equal to 4) indicate deposition of marine OM with higher contribution of planktonic organisms and/or algae. Low sterane concentrations and high values for the Hopane/Esterane ratio (greater than 7) indicate deposition of terrestrial OM. C_{30}, it's associated bacteria, a few eukaryotic species (for example, some cryptogams, mosses, lichens, filamentous fungi, and protists)
Steranes	 5α(H),14α(H),17α(H)-Cholestane 5α(H),14α(H),17α(H)-Ergostane 5α(H),14α(H),17α(H)-Stigmastane	C_{29} ββ/(ββ +αα) *m/z* 217	***Degree* of thermal *maturity:*** The ratio increases with progressive increase in maturity. Equilibrium values between 0.67 and 0.71.
		C_{29} ααα 20S/(20S+20R) *m/z* 217	***Degree* of thermal *maturity:*** The 20R configuration sterane, with increasing maturity is converted into the 20S and 20R mixture. Equilibrium values are between 0.52-0.55.
		Regular sterane ratio C_{29}-C_{28}-C_{27} *m/z* 217	**Origin indicators**: The predominance of sterane C_{27} indicates a contribution from marine plankton. Sterane C_{28}, when in higher proportion, indicates a higher contribution from lacustrine algae. A predominance of sterane C_{29} indicates a terrestrial contribution. Cholestane in water sources is almost exclusively derived from various Eukaryotes. Ergostane and stigmastane are derived exclusively from Eukaryotes.
Methyl Phenanthrenes	 2-methylphenanthrene 3-methylphenanthrene 1-methylphenanthrene 9-methylphenanthrene	Distribution of methyl-phenanthrenes (MP) *m/z* 192	**Origin indicators:** The distribution of methyl-phenanthrenes (MP) depends on the origin of the organic matter. Samples of marine origin show a higher abundance of 9-MP (α-isomer) and low concentration of 3- and 2-MP (β-isomer), while the abundance of 1-MP (α-isomer) and 2-MP (β-isomer) may be associated with the input of organic matter of terrestrial origin.

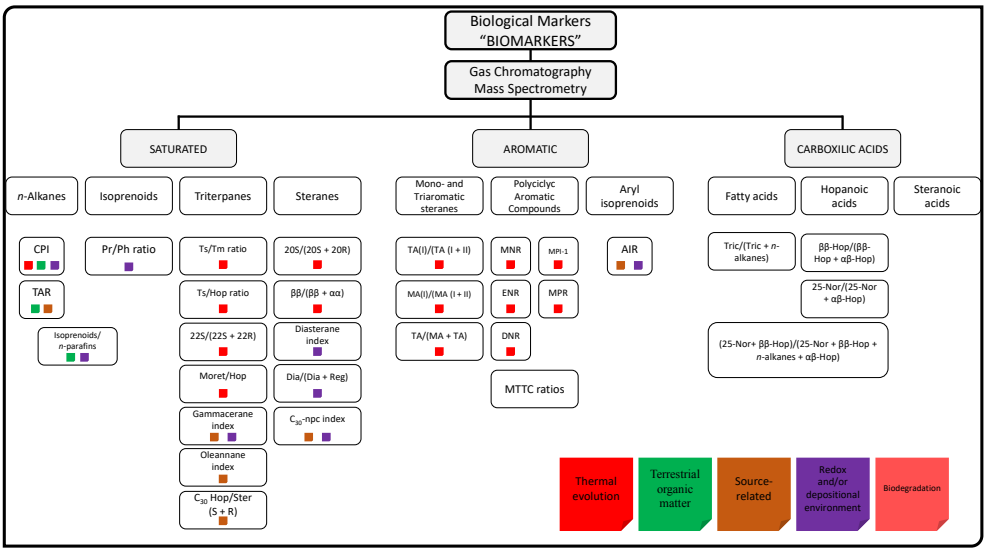


Figure 6. most common general parameters used for the evaluation of thermal evolution, organic matter contribution, source, depositional paleoenvironment and biodegradation in oil samples and sedimentary rock extract.

1.4.1 n-alkanes

n-Alkanes are classified according to their molecular weight into four groups: gaseous alkanes, low molecular weight alkanes (C_8–C_{16}), medium molecular weight alkanes (C_{17}–C_{28}), and high molecular weight alkanes (>C_{28}). Linear hydrocarbons (*n*-alkanes) present in geological samples are derived from long-chain fatty acids by decarboxylation. They are generally the most abundant compounds in the saturated fraction of rock extracts and crude oils, present in terrestrial and marine organisms. They have a high sensitivity to microbial and/or geochemical processes (PETERS; WALTERS; MOLDOWAN, 2005a).

Oil and sediment samples contributed by terrestrial plants predominantly contain long-chain *n*-alkanes in the C_{27}-C_{33} range. However, certain lake algae, such as *Botryococcus braunii*, can produce *n*-alkanes in the C_{29}-C_{31} range (GELPI et al., 1970). However, their contribution is easily identified since they give rise to specific biomarkers such as macrocyclic alkanes (AUDINO et al., 2001) and the irregular isoprenoid C_{34} botryococcane (MOLDOWAN; SEIFERT, 1980). The absence of the latter biomarker does not necessarily exclude the contribution of these algae to the *n*-alkane profile (DERENNE et al., 1988). Oil and sediment samples contributed by terrestrial plants predominantly contain long-chain *n*-alkanes in the C_{27}-C_{33} range. However, certain lake algae, such as *Botryococcus braunii*, can produce *n*-alkanes in the C_{29}-C_{31} range (GELPI et al., 1970). However, their contribution is easily identified since they give rise to specific biomarkers such as macrocyclic alkanes (AUDINO et al., 2001) and the irregular isoprenoid C_{34} botryococcane (MOLDOWAN; SEIFERT, 1980). The absence of the latter biomarker does not necessarily exclude the

contribution of these algae to the *n*-alkane profile (DERENNE et al., 1988).

The *n*-alkanes are easily recognized in GC-MS by their characteristic fragments *m/z* 57, *m/z* 71, and *m/z* 85 (**Figure 7**) in higher relative intensity. The distribution and abundance of these compounds are sensitive to the maturation and biodegradation process, so they should not be the only source of geochemical information in oil samples and rock extracts (PETERS; WALTERS; MOLDOWAN, 2005a).

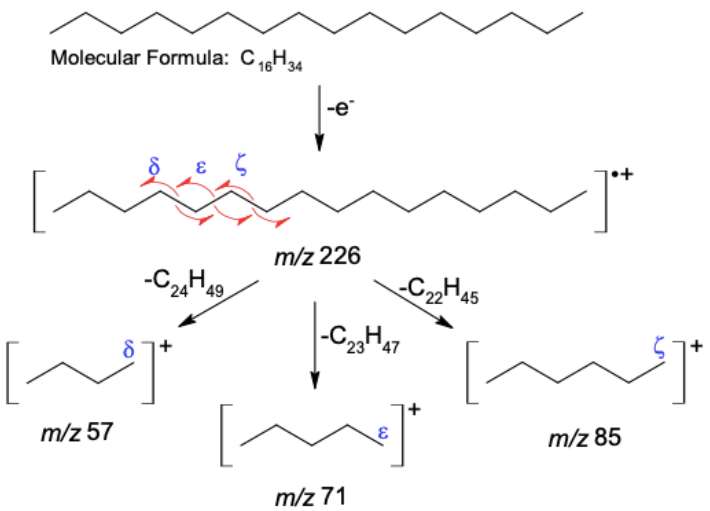


Figure 7. The general structure of *n*-alkanes, with fragmentation pattern. When fragmented, the linear hydrocarbons produce the *m/z* 57, 71, and 85 ions, obtained through sequential fragmentations with a difference of 14 Da, characteristic of CH_2 units. The fragmentation mechanism of these compounds is accomplished through homolytic breaks in the δ, ε, and ξ bonds counting from the terminal methyl.

Aquatic plants generate *n*-alkanes from C_{21}-C_{25}, whereas algae and bacteria mainly produce short-chain hydrocarbons. In addition to this information, the distribution of *n*-alkanes provides preliminary clues regarding the degree of thermal evolution and biodegradation processes (BUSH; MCINERNEY, 2013; LIU; LIU, 2016). **Figure 8** shows the influence of biodegradation on the distribution of saturated hydrocarbons in crude oils.

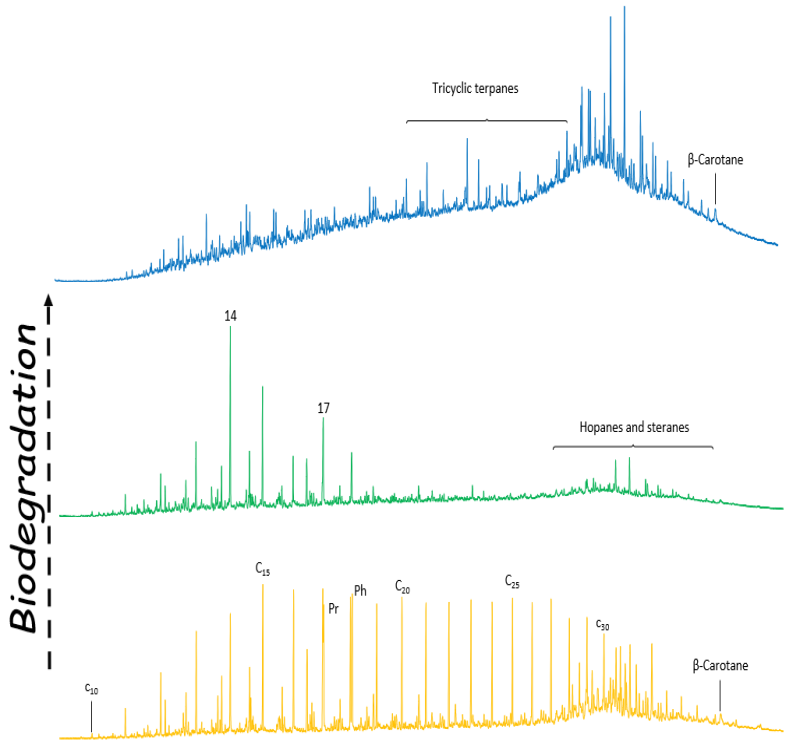


Figure 8. TIC of the saturated hydrocarbon fraction of oil samples from different biodegradation levels, Campos Basin. The biodegradation decreases in the following order: *n*-alkanes > cyclic and acyclic isoprenoids > regular steranes > terpanes. An "unresolved complex mixture" is related to naphthenic compounds produced through biodegradation processes. Petroleum accumulations in the Campos Basin occur from Neocomian to Miocene reservoirs, and it has characteristics of saline lacustrine source rock (DE LIMA et al., 2010).

1.4.2 Acyclic Isoprenoids

Isoprenoids are branched hydrocarbons derived from C_5 isoprene (2-methyl-1,3-diene), one of the main building blocks of carbon chains in nature. Among the isoprenoids, the most prominent in organic geochemistry are pristane (**Pr** - 2,6,10,14-tetramethylpentadecane) and phytane (**Ph** - 2,6,10,14-tetramethylhexadecane). These compounds are derived from the phytyl side chain of chlorophyll, present in phototropic organisms (**Figure 9**), but can also be derived from tocopherols found in most algae cyanobacteria, and higher plants (KILLOPS; KILLOPS, 2005).

Under anoxic conditions, the phytyl side chain is cleaved, producing phytol, which is reduced to dihydrophytol, followed by phytane (**Figure 9**, right). Under oxic conditions, phytol is oxidized to phytenic acid, which by decarboxylation generates pristene and, by reduction, pristane (**Figure 9**, left). Thus, the pristane/phytane ratio may indicate fluctuations

in the oxidation process during the early stages of chlorophyll decomposition, with high pristane/phytane ratios indicating an oxidizing terrestrial environment and low values may be indicative of reducing marine environment organic matter.

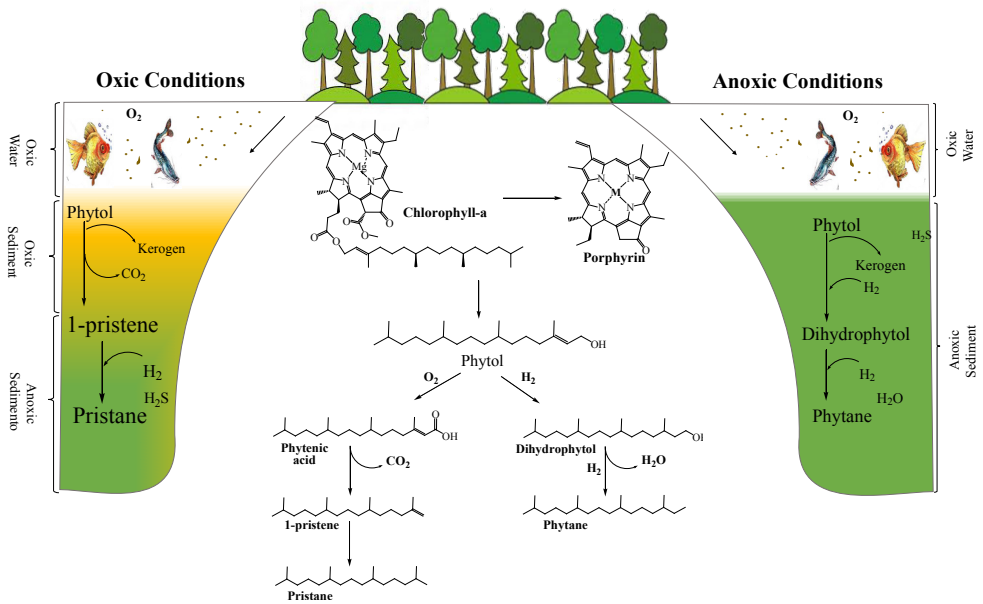


Figure 9. Origin of the acyclic isoprenoids Pristane and Phytane from the side chain of Chlorophyll a. Source: Modified from material available at the XV Latin American Congress on Organic Geochemistry (ALAGO 2018) in the Introduction to Petroleum Geochemistry course. Phytane is formed when phytol, a constituent of chlorophyll, loses its hydroxyl group. When phytol loses a carbon atom, it gives rise pristane. Other sources of phytane and pristane have also been proposed in addition to phytol.

However, pristane/phytane ratios are not restricted simply to anoxic/oxic conditions of sedimentation, reflecting more the chemistry of the environments (salinity and alkalinity of the water) as suggested by (MELLO et al., 1988a). Thus, the more saline environment contributes to the higher concentration of phytane precursors and a low salinity environment is related to the higher concentration of pristane.

The Pristane/Fitane (Pr/Ph) ratio is an early indicator of the redox conditions of the depositional environment, rock type, and OM input. Values of Pr/Ph > 3.0 indicate the contribution of terrestrial OM deposited under oxic conditions, and values of Pr/Ph < 0.8 represent anoxic conditions quite common in carbonate or hypersaline environments. Values between 0.8 and 3.0 should be interpreted with caution. The ratios give essential information about the abundances of pristane and phytane relative to the C_{17} and C_{18} *n*-alkanes, respectively. Pr/*n*-C_{17} and Fi/*n*-C_{18}

ratios above 1.0 (>1), indicate immature oil or extract, whereas values less than 1.0 (<1), indicate mature oil or extract (ASIF; FAZEELAT; GRICE, 2011; PETERS; WALTERS; MOLDOWAN, 2005a)Pakistan is carried out in this study. Their relative thermal maturities, environment of deposition, source of organic matter (OM. Isoprenoids can be analyzed by TIC or *m/z* 183, *m/z* 113 or *m/z* 111.

1.4.3 Pentacyclic biomarkers

The most common functionalized forms of hopanoids found in some aerobic bacteria are the amphipathic bacteriohopanepolyols (BHP), in which the side chain is a part derived from sugar. This side chain can have, in addition to hydroxyls, amino groups (NH_2), additional sugar, and others. Studies suggest that these compounds perform functions in the plasma membranes of prokaryotic cells (BROCKS; SUMMONS, 2014). Despite being derived from aerobic bacteria, oxygen is unnecessary in their biosynthesis, which consists of the cyclization of squalene. Bacteriohopanepolyols undergo three major transformations until they reach their corresponding geochemical fossils: defunctionalization, side-chain reduction, and epimerization at specific chiral centers (SCHWARZBAUER; JOVANČIĆEVIĆ, 2016).

Defunctionalization and shortening of the side chain are related to two distinct processes. One of these processes is the oxidation of hydroxyl groups to carboxyl groups, followed by decarboxylation. Defunctionalization and shortening of the side chain are related when (1) reduction of the hydroxyl groups occurs or (2) oxidation of the hydroxyl groups to carboxyl groups followed by decarboxylation.

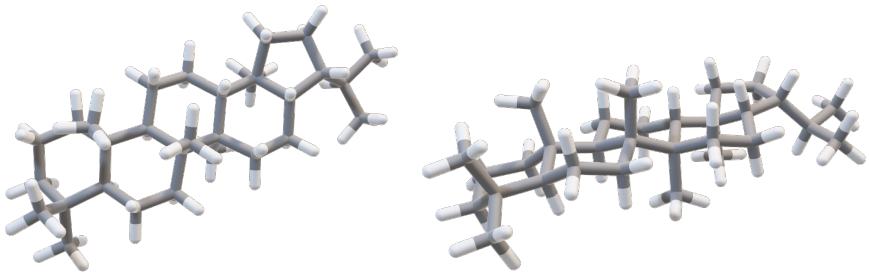


Figure 10. Two representations of the structure of C_{30} hopane ($C_{30}H_{52,}$ 412 Dalton). Hopanoids have a fixed stereochemistry and differ in the orientation of Carbon-17 and Carbon-21 (α or β) and Carbon-22 (R or S). Hopanoids are formed by the squalane cyclization, a pentacyclic triterpanoid with a hopane skeleton.

With a naphthenic structure consisting of four six-membered rings and a five-membered ring as the base skeleton, pentacyclic terpanes with thirty carbon atoms (C_{30}) are called “hopanes” (**Figure 10** and **Figure 11**), while those pentacyclic terpanes with less than thirty carbon atoms are then called “norhopanes” (in this case, nor = loss of CH_3) (**Figure**

11, left). The smallest ever found is tetrakisnorhopane (loss of 4 methyls) (SUBROTO; ALEXANDER; KAGI, 1991). Pentacyclic terpanes above C_{30} are called “homohopanes” (**Figure 11**, right); these homologs can have C_{40} carbon atoms (PETERS; WALTERS; MOLDOWAN, 2005a; RULLKÖTTER; PHILIP, 1981).

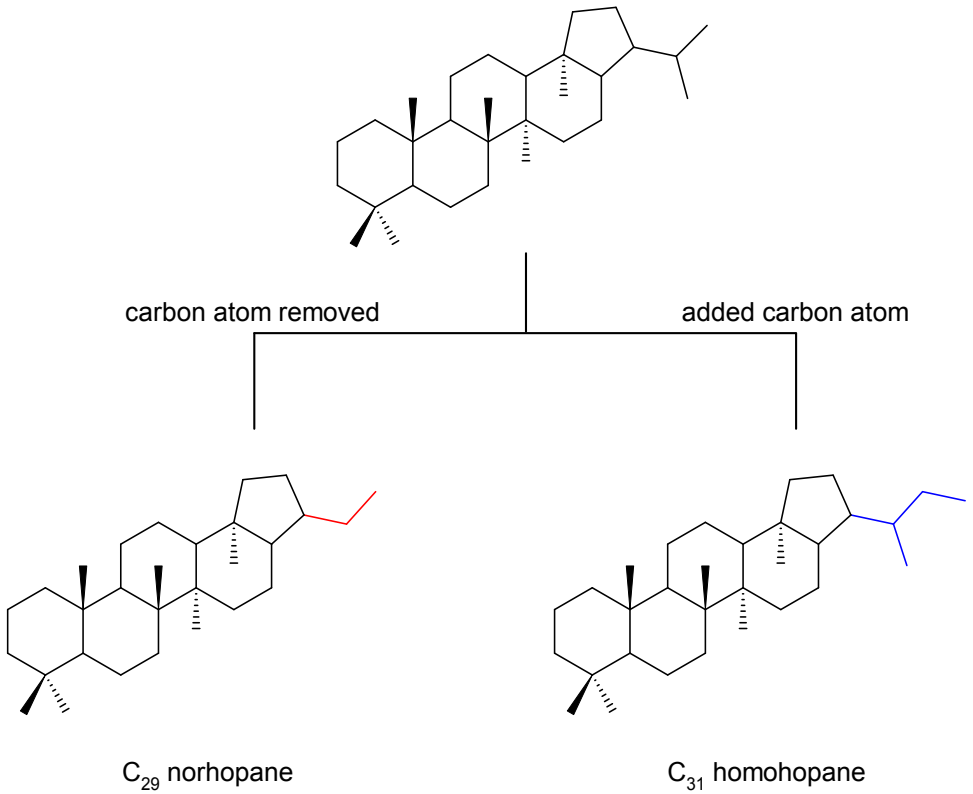


Figure 11. Schematic diagram showing structures of hopanes with carbon atom removed (C_{29} norhopane) and with carbon atom added (C_{31} homohopane). Adapted from Schwarzbauer and Jovančićević (2016). The formation of 25-norhopanes by microbial removal of the methyl groups on C-10 during petroleum biodegradation is well documented in the literature.

Hopanes substituted at C-21 (E-ring) have asymmetric centers at this position and at all junctions at C-5, C-8, C-9, C-10, C-13, C-14, C-17, and C-18. Homohopanes, on the other hand, have an additional asymmetric center at C-22. Under the chromatographic conditions commonly employed, homohopanes show two signals in the chromatogram, one corresponding to the “S” configuration and another signal corresponding to the “R” configuration, eluting in this order. The ratio between these homohopane homologous series is widely used for the evaluation of thermal evolution through the S/(S+R) ratio for which values 0.6 suggest 60 % equilibrium concerning the S configuration, which is the most stable (PETERS; WALTERS; MOLDOWAN, 2005b; SCHWARZBAUER; JOVANČIĆEVIĆ, 2016).

Another significant isomerization with diagenesis and catagenesis occurs in positions C-17 and C-21. These positions have 17β(H) and 21β(H) positions in biological precursors. Due to the instability of this configuration, with increasing temperature and pressure, changes to βα configurations (called moretanes) and then to αβ occur (**Figure 12**), allowing the use of the relationship between these isomers as a parameter of the thermal evolution of OM. Isomers with the βββ configuration have been reported in oils with recent OM overlay (WENGER; ISAKSEN, 2002), and in sedimentary rocks, their presence represents OM immaturity.

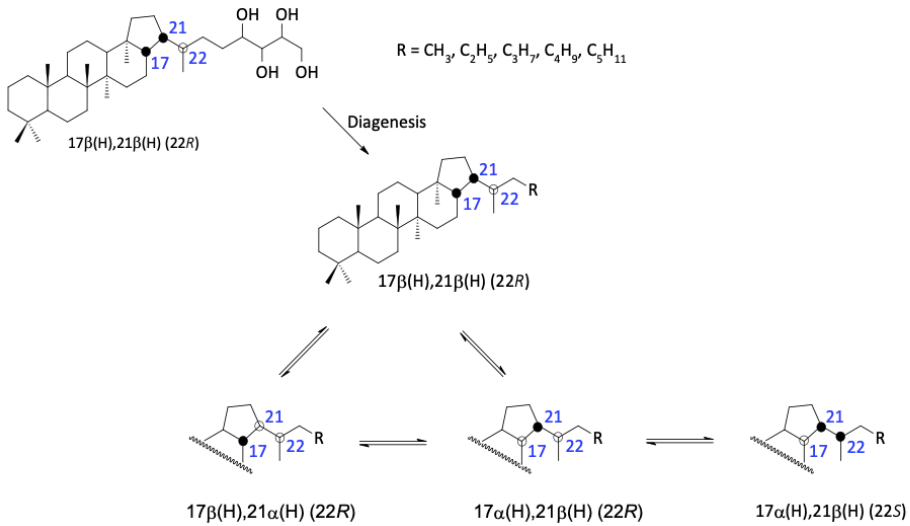


Figure 12. Origin of some hopanes of different configurations at positions C-17, C-21, and C-22 from **bacteriohopanetetrol**. Bacteriohopanepolyols are more complex forms of molecules present in bacteria. Many different structures occur from simple hopanoids, to long-chain polyfunctional compounds and extend from living organisms to "fossil molecules" at least 2.7 billion years ago in geological sediments.

Rearranged hopanes (neohopanes) comprise a compound subclass with an identical five-membered skeleton as hopanes, with the difference in the position of the methyl in C-18, which is rearranged to the C-17 position. These compounds also include norhopanes and hopenes with variations in the double bond position and are also used to evaluate thermal evolution (SINNINGHE DAMSTÉ; SCHOUTEN; VOLKMAN, 2014).

Hopanes (and neohopanes) show in the mass spectra the molecular ion ($M^{+\cdot}$), the ion corresponding to the loss of methyl (M-15), the ion corresponding to the loss of the side chain at C-21, the fragment ion consisting of rings A and B with their respective methyls, and the *m/z* 369 ion of the side chain at C-21 in addition to the ion containing the DE rings resulting from the C ring break at C-8 - C-14 and C-12 - C-13. They are monitored by the

m/z 191, the base peak, as in tricyclic terpanes. Homohopanes have the same *m/z* 191 fragments as the base peak (BP); however, the breaking of the C ring generates a fragment containing the DE rings + side chain, which results in the ions; *m/z* 191 + 14n (*m/z* 205, *m/z* 219, *m/z* 233, *m/z* 247, *m/z* 261, etc.), **Figure 13**.

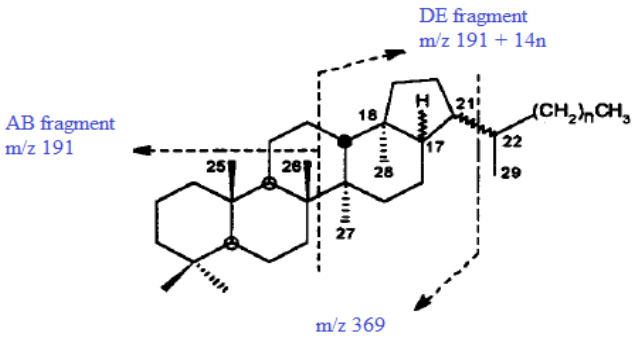


Figure 13. Main normal sterane fragments.

1.4.4 Steranes

Steranes present in crude oils and sedimentary rocks are derived from the sterols of ancient organisms. Their precise structure and stereochemistry can give important clues about the nature of the original biological materials and the geochemical processes they underwent (SUMMONS; WELANDER; GOLD, 2021). Predominant in OM from sedimentary rocks and oils are steranes (molecular fossil) of C-27, C-28 (methyl in C-24), and C-29 (Ethyl in C-24) carbon atoms, which are cholestanes, ergostanes, and stigmastanes, respectively (SCHWARZBAUER; JOVANČIĆEVIĆ, 2016), **Figure 14**.

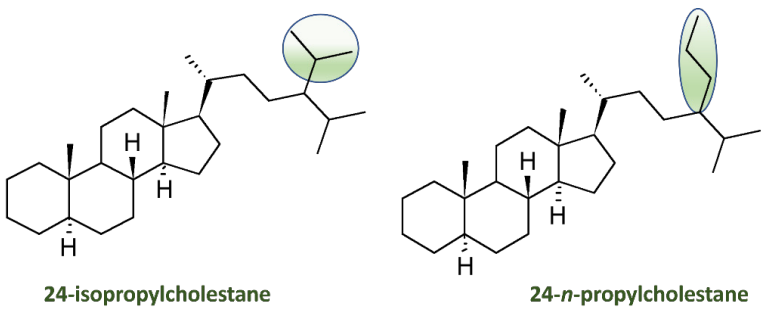


Figure 14. Typical structures of C_{30} steranes are markers for input into sedimentary lipids from marine organisms.

According to MCCAFFREY et al. (1994), petroleums and bitumens from Early Proterozoic (≈1800 Ma) to Miocene (≈15 Ma) in marine strata contain 24-isopropylcholestanes. The abundance of these compounds, relative to 24-*n*-propylcholestanes, varies with source rock age.

The so-called regular steranes are usually the majority compounds present in sedimentary rocks, generated initially from the de-functionalization (loss of functional groups) of sterols and further by displacement, double bonds loss, and epimerizations at specific chiral centers, **Figure 15**.

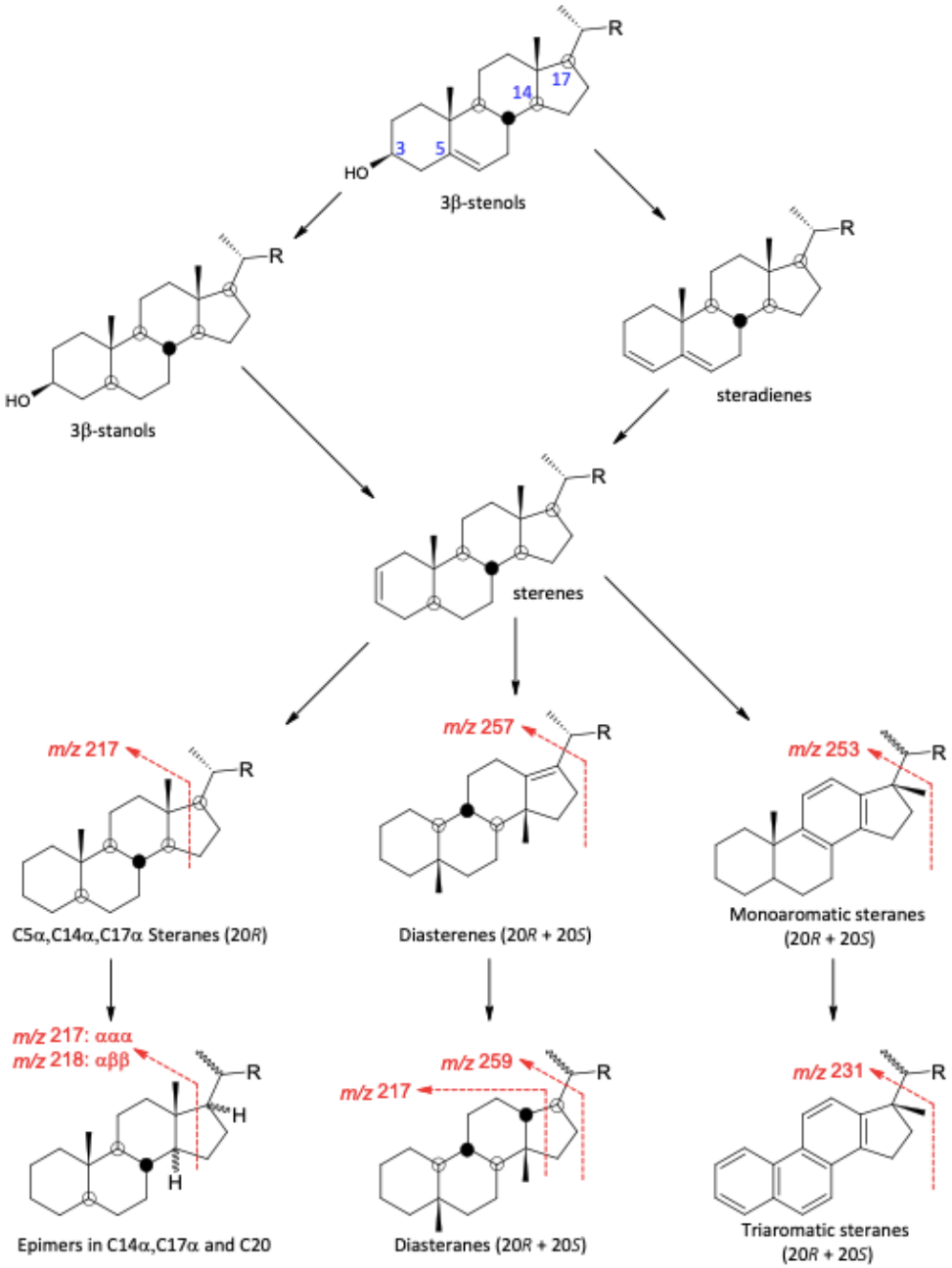


Figure 15. General scheme of the conversion of sterols into steranes, diasterenes, diasteranes, monoaromatic and triaromatic. Adapted from Schwarzbauer and Jovančićević (2016). Variations in the stereochemistry of given hydrogen (S, R, ααα, αβββ, βββββ) are dependent on the origin, degree of maturity, or level of degradation of a given oil sample. The steranes in petroleum originate from sterols in the cell membranes of eukaryotes, whereas prokaryotes use hopanoids rather than steroids in their cell membranes and these precursors account for the hopanes in petroleum. Rearranged steranes (diasteranes) are relatively more abundant in classic sediments than carbonates (VAN KAAM-PETERS et al., 1998).

The lithology can influence the transformation of compounds, as is the case for the so-called diasterenes (rearranged steranes), which are products of the acid catalytic effect of clay minerals. The concentration of diasterenes depends on the proportion of clay minerals present in the organic matter of the rock (VAN KAAM-PETERS et al., 1998)the mineral compositions of three sample sets of sedimentary rocks displaying a wide range of diasterane/sterane ratios were analysed quantitatively. Diasterane/sterane ratios do not to correlate with clay content but depend on the amount of clay relative to the amount of organic matter (clay/TOC ratios. The double bond is reduced with thermal evolution, leading to saturated products, such as diasteranes, which also undergo the epimerization process. Alternatively, during diagenesis, sterols can generate stable products through aromatization of the rings, generating first the mono- (*m/z* 253) and from these the triaromatic (*m/z* 231) steranes.

The stereochemical changes at positions C-5, C-14, C-17 (α or β), and in C-20 (R or S) are evaluated for steranes. These configurations are commonly abbreviated by ααα R, ααα S, αββ R, αββ S, etc. Isomerization at carbons C-14, C-17 and C-20, which occur in both defunctionalization and aromatization, are transformations that occur at the beginning of catagenesis. Therefore, both the concentrations and the parameters that relate to the different isomers are used in organic geochemistry to measure the thermal evolution of OM and the sedimentary rock type.

Steranes are monitored by the *m/z* 217 (base peak, BP), the fragment corresponding to the loss of methyl (M-15), and also the molecular ion peak ($M^{+\cdot}$), as well as other ions (e.g., *m/z* 149 or *m/z* 151) that can indicate stereochemistry at specific positions in the hydrocarbon chain (**Figure 15**).

The change in configuration from α to β in C-17 leads to a change in the base peak intensity from *m/z* 217 to *m/z* 218. In addition, the *m/z* 259 fragment is more intense. The diasterenes are identified through the BP *m/z* 257, due to the doublet between C-17 and C-21. In addition, to the molecular ion ($M^{+\cdot}$) and the loss of methyl (M-15), the diasteranes have the same PB as the regular steranes (*m/z* 217); the difference lies in the intensity of the fragment *m/z* 259, which is more intense, also the *m/z* 189 fragment is observed.

Regular steranes are monitored by the *m/z* 217 (BP) that corresponds to the stereochemistry of the C/D ring junction. Furthermore, the fragment corresponding to the loss of methyl (M-15), molecular ion ($M^{+\cdot}$) helps in the identification of such components. The change in configuration of C-17 from α to β leads to a shift in the base peak to *m/z* 218. The *m/z* 149 and *m/z* 151 ions indicate the stereochemistry of the hydrogen in the A/B fragment; if the configuration is **α,** the peak generated preferentially will be *m/z* 149, and if it is β, the peak generated preferentially will be *m/z* 151. The diasteranes have the same BP as the regular steranes (*m/z* 217); the difference lies in the fragment *m/z* 259, which is more intense. Additionally, the *m/z* 189 fragment is also observed. Diasterenes are

identified through the PB *m/z* 257 due to the doublet between C-17 and C-21, the molecular ion ($M^{+\cdot}$), and the loss of methyl (M-15), **Figure 16**.

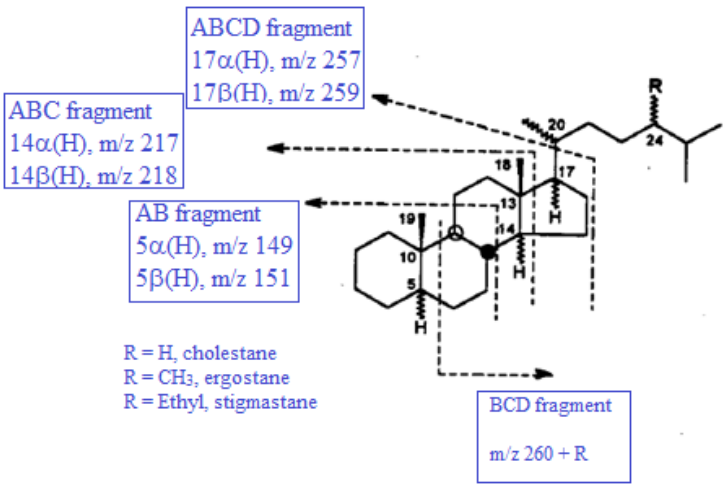


Figure 16. Main normal sterane fragments

In mono- and triaromatic steranes, the breaking of the side chain favors the formation of fragments composed of the rings due to the high stability. Thus, monoaromatic steranes are monitored by *m/z* 253 and triaromatic ones by the fragment *m/z* 231. The configurations at C-20 (R and S) are differentiated only by elution order in the chromatogram, the S isomer eluting first than the R isomer. The application of sterols as biomarkers of source type is based on the concentrations found in organisms. C_{27} sterols could act as indicators of some animals, marine zooplankton and some types of plants, (C_{28}) as indicators of algae and fungi and (C_{29}) as indicators of higher plants and phytoplankton (MACKENZIE et al., 1968).

Aromatic steranes are another group of biomarker compounds that are highly resistant to biodegradation and can be used for oil/oil correlation and for tracing oil sources. **Figure 17** shows the mass chromatograms of monoaromatic (MA, *m/z* 253) and triaromatic (TA, *m/z* 231, *m/z* 245) steranes in aromatic hydrocarbon fractions of representative crude oils.

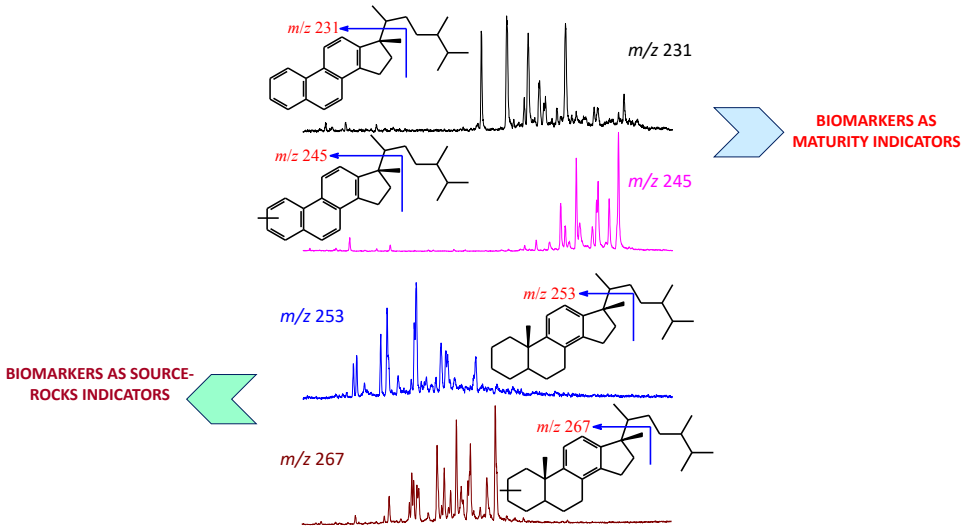


Figure 17. Typical distribution of aromatic steranes in oils from the Sergipe-Alagoas Basin, Brazil (unpublished data). Monoaromatic steroids in crude oils give information on source-rock characteristics. Triaromatic steroids can originate from aromatization and loss of a methyl group from monoaromatic steroids. Triaromatic steroid ratios should be more sensitive to thermal maturation than those for monoaromatic steroids or steranes because the triaromatic steroids appear to be maturation products from aromatization of monoaromatic steroids (PETERS; WALTERS; MOLDOWAN, 2005a).

1.4.5 Aromatic alkylphenanthrene hydrocarbons

Alkylphenanthrenes are a class of aromatic hydrocarbons that provide information about the maturity of oils and rocks in organic geochemistry. The distribution of alkylphenanthrenes, depends on the origin of the organic matter (marine or lacustrine). Alkylphenanthrenes are used as maturity parameters because of their high resistance to transformations under (reasonably high) temperature and pressure conditions. The most abundant isomers are 2-, 3-, 9- and 1- methylphenanthrenes **Figure 18** and are monitored through *m/z* 191 or 192 ions. Although they are not considered biomarkers, due to the changes suffered in their original basic structures containing their precursor molecules and for their low concentrations in living organisms, it is still possible to use alkylphenanthrene hydrocarbons in the determination of various geochemical parameters (SOUZA, 2012).

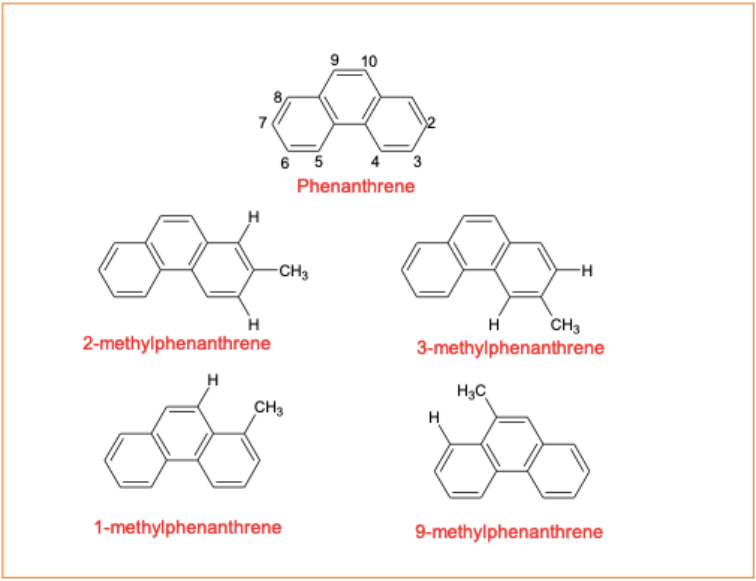


Figure 18. Methylphenanthrenes (MF) series and the representation of steric interactions. Phenanthrene and methyl-phenanthrenes are major aromatic pollutants originating particularly from fuel oil. Phenanthrene usually degrades faster than methyl-phenanthrenes under geological and environmental conditions. Generally this fraction is said to be more resistant to biodegradation than the fraction of saturated hydrocarbons.

The methyl groups of the 9- and 1- methylphenanthrenes exhibit steric tension with hydrogen atoms on carbon atoms 8 and 10 (**Figure 18**), whereas in 2- and 3-methylphenanthrenes, these interactions are minimized. With increasing temperature, the methyl groups become more mobile, and consequently, with increasing thermal maturity, the 9- and 1-MP are converted into the 2- and 3-MP, which are the most stable isomers. It is the basis of the methylphenanthrenes index (DE LIMA, 2005; HECKMANN et al., 2011; SHEPPARD; POLISSAR; SAVAGE, 2015).

1.4.6 Dibenzothiophenes and alkyldibenzothiophenes

One of the primary forms of organic sulfur in crude oil is thiophene-class compounds where sulfur is bound to polycyclic aromatic hydrocarbons, including alkylated benzothiophenes (BTs), dibenzothiophene (DBT) and its C_1C_3 alkyl derivatives (DBTs), Meijun Li et al., 20214. Dibenzothiophenes and alkyldibenzothiophenes (DBTs) are compounds in which the sulfur atom is incorporated into aromatic hydrocarbons. They have a wide distribution in ancient sediments and oils. Their distribution patterns, relative and absolute concentrations have been widely used to correlate oil-rock and oil-oil to assess thermal maturity, depositional environment, and thermochemical processes in petroleum reservoirs (LI et al., 2013; SIVAN; DATTA; SINGH, 2008; ZHANG et al., 2015)the relationship between

DMDBTs and dimethylbiphenyls (DMBPs.

The generation of DBTs is poorly understood, and there may be a variety of precursors for this type of structure. The most common origin of these compounds is inorganic sulfur reduced in functionalized lipids producing organosulfur compounds, preserved in rock extracts and crude oil (ZHANG et al., 2015) NW China were quantitatively analyzed by gas chromatography-mass spectrometry to elucidate the influence of thermal maturation, multiple charges and mixing, and thermochemical sulfate reduction (TSR. Another widespread form of occurrence of dibenzothiophenes would be the catalytic reaction of biphenyls and sulfur (**Figure 19** and **Figure 20**) adsorbed on carbonate surfaces (ASIF; GRICE; FAZEELAT, 2009; XIA; ZHANG, 2002).

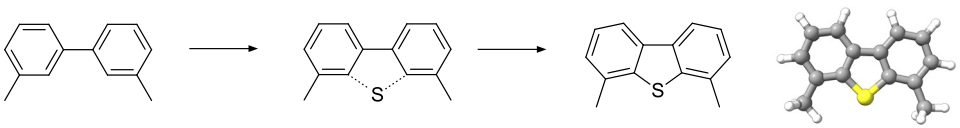


Figure 19. (**A**) Proposed reaction pathway scheme for 4,6-DMDBT (ASIF; ALEXANDER; FAZEELAT; PIERCE, 2009). (B) A 3D model of 4,6-DMDBT.

Some works correlate the distribution of these compounds with thermal evolution, using the methyl-, ethyl- and dimethyl dibenzothiophene isomers, showing results comparable to those obtained by the reflectance of vitrinite (HECKMANN et al., 2011).

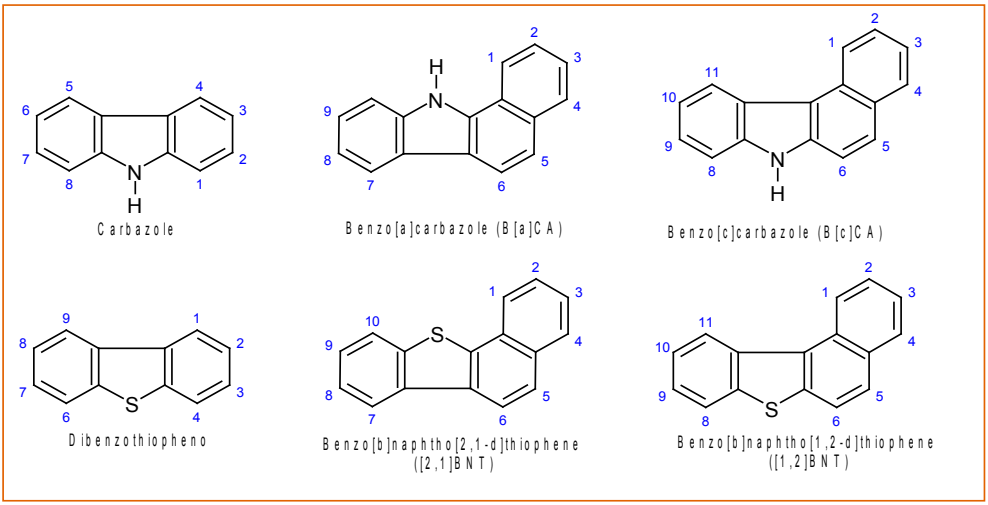


Figure 20. Structures of benzo[b]naphtho[2,1-d] thiophene ([2,1]BNT) and benzo[a] carbazole (B[a]CA), benzo[b]naphtho[1,2-d] thiophene ([1,2]BNT) and Benzo[c]carbazole (B[c]CA). Adapted from LI et al. (2014).

1.4.7 Aromatic carotenoids and aryl isoprenoids

Three other classes of compounds of great relevance in organic geochemistry, although they cannot be classified as biomarkers because they are present in more than one type of organism, are the pigments called aromatic carotenoids, their saturated derivatives (perhydro), and the aryl isoprenoids that can be related to specific types of bacteria. These compounds can vary in the substitution pattern of the aromatic rings. SCHAEFLE et al. (1977) were the first to report aromatic carotenoids and their perhydro derivatives with different substitution patterns on the aromatic rings in rock samples: isorenieratane, renieratane, and renierapurpurane (**Figure 21**).

Isorenieratane has two aromatic rings linked via the isoprenoid tail chain. These rings have three methyls at C-2, C-3, and C-6 relative to the isoprenoid chain. In the low-fragmentation mass spectrum, it is possible to see the presence of the molecular ion *m/z* 546 and the base peak is *m/z* 133 with *m/z* 134 at almost the same intensity, coming from β-type fragmentation and McLafferty rearrangement, respectively (HARTGERS et al., 1994; HUANG et al., 2007; KOOPMANS; DE LEEUW; SINNINGHE DAMSTÉ, 1997; REQUEJO et al., 1992; SCHRÖDER-ADAMS et al., 2001). 3,4-trimethyl substitution pattern characteristic of diaromatic carotenoids found in the Chlorobiaceae family of photosynthetic sulfur bacteria. Two C40perhydrodiaromatic carotenoids which are structurally related to the presumed precursors of the aryl isoprenoids have also been identified in oils and source rock bitumens of the Duvernay (Late Devonian). On the other hand, paleoenvironmental interpretations and the link to possible organisms, more specifically, were only made later by SUMMONS AND POWELL (1987) in Silurian oils from the Michigan Basin (Canada). The authors identified aryl isoprenoids from C_{13} to C_{31} of different substitutions. With the aid of synthetic standards and ^{13}C isotope ratio data from a previous study (SUMMONS; POWELL, 1986), they proposed the isoprenoids with substitutions 2,3,6- are derived from isorenieratene, although they did not identify this compound or its perhydro derivative, isorenieratene, in that study.

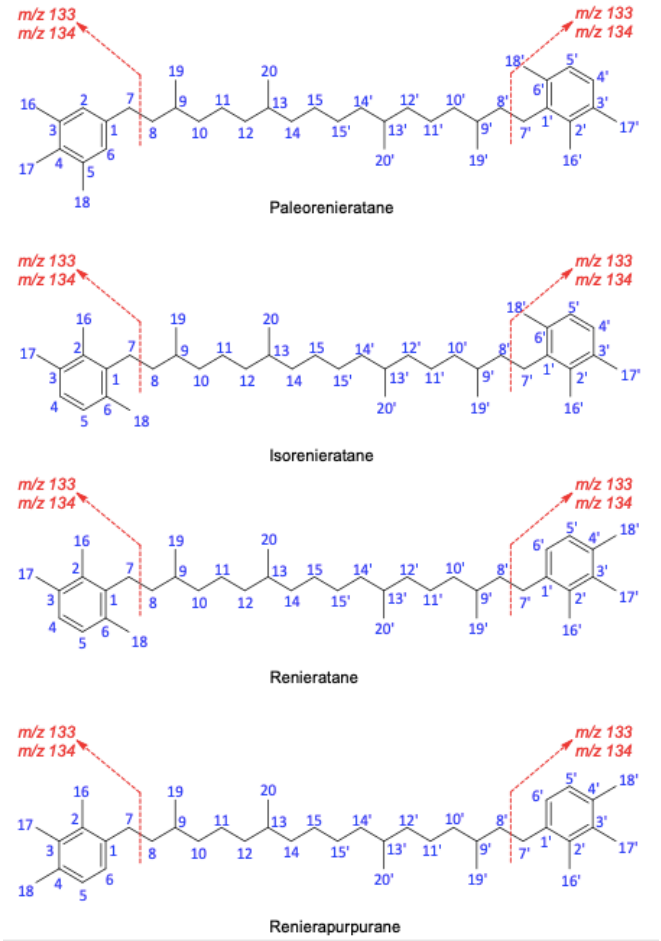


Figure 21. Structures of aromatic carotenoids with different patterns of substitution. From top to bottom: paleorenieratane, isorenieratane, renieratane, and renierapurpurane. The diagnostic fragment varies between *m/z* 133/134. Carotenoid occurrences in modern environments serve as analogs for interpreting fossil hydrocarbons in an ancient setting.

SUMMONS AND POWELL (1986 and 1987) confirmed the structural proposals of the aryl isoprenoids found by OSTROUKHOV et al. (1982) but refuted the single origin from β-carotane, indicating a more robust origin from bacteria belonging to the Chlorobiaceae family as major contributors. However, the study does not exclude the possibility of different sources of these compounds, which highlights the need for isotopic data, which are also advocated as necessary by KOOPMANS; SCHOUTEN; KOHNEN; SINNINGHE DAMSTÉ (1996).

Bacteria of the family Chlorobiaceae are anaerobic and use aromatic carotenoids as secondary pigments for photosynthesis with hydrogen sulfide (H_2S) instead of water as an electron donor, in the presence of light and low oxygen concentrations, thus creating

special environmental conditions called Photic Zone Euxinic (PZE) (PETERS; WALTERS; MOLDOWAN, 2005a). **Figure 23** gives a general illustration of euxinic environments with the presence of sulfur bacteria, the main source for specific aromatic carotenoids (e.g., isorenieratane) in sedimentary environments and are molecular indicators of photic zone euxinia (PZE). Other important occurrences of carotenoids are in sponge species; since these organisms cannot biosynthesize such compounds, their presence is due to the symbiotic relationship (LU et al., 2015).

These environmental conditions of euxinic photic zones do not develop easily and may be linked to water column stratification, resulting from two distinct factors: hyper salinity or temperature gradients. The first is usually related to a specific biomarker, the non-hopanoid gammacerane (**Figure 22**), derived from species of protozoa and possibly some other types of organisms. In the absence or low intensity of gammacerane, water column stratification can be justified by temperature gradients due to mixing cold and warm waters (PETERS; WALTERS; MOLDOWAN, 2005a; SINNINGHE DAMSTÉ et al., 1995a, 1995b).

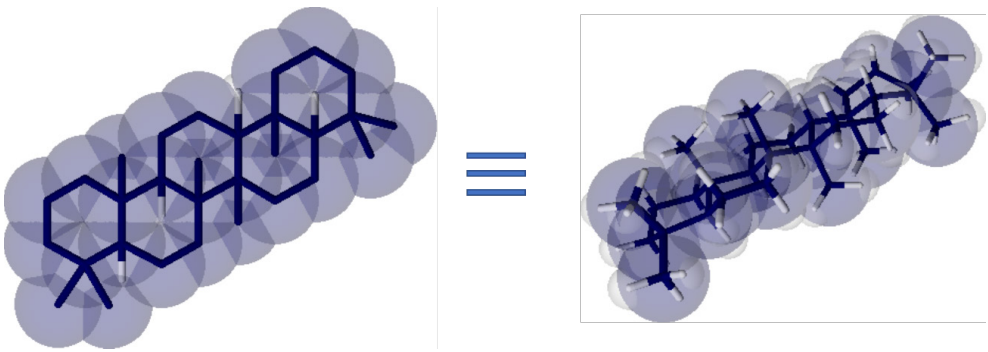


Figure 22. Gammacerane is a pentacyclic triterpene compound with the formula $C_{30}H_{52}$ and five six-membered rings. Among its precursors is tetrahymanol - TH (gammaceran-3β-ol). After millions of years of diagenesis, the TH becomes gammacerane and can be used as biomarkers in petroleum to study the origin of oil. Structures built in the program ACD/ChemSketch (Freeware version).

The first hint in the literature of paleorenieratane (**Figure 21**) seems to have been made by Requejo et al. (1992)3,4-trimethyl substitution pattern characteristic of diaromatic carotenoids found in the Chlorobiaceae family of photosynthetic sulfur bacteria. Two C40perhydrodiaromatic carotenoids which are structurally related to the presumed precursors of the aryl isoprenoids have also been identified in oils and source rock bitumens of the Duvernay (Late Devonian when reporting the mass spectrum of a compound termed “Carotenoid A” with a base peak *m/z* 134 but eluting before isorenieratane. Structural elucidation was only accomplished by (HARTGERS et al., 1993) with 1H NMR data, which assigned the substitution patterns 2,3,6 and 3,4,5 to paleorenieratane. The research study

in which this compound is identified, mandatorily, isorenieratane is also present, both in oils and sedimentary rocks. A study with isotopic ratios of δ 13C performed by Hartgers et al. (1994) 2,3,4- and 1,2,3,5-tetramethylbenzene, which, generated via β-cleavage, indicate the presence of diaromatic carotenoids in the macromolecular aggregates. This was substantiated by desulphurization of sulphur-rich aggregates of the polar fraction, which released (partly), in which paleorenieratane and isorenieratane have very similar δ 13C discrimination ranges, suggests biological sources of close families common to these two compounds, perhaps extinct species of the Chlorobiaceae family (ARMSTRONG et al., 2009; BROCKS; SCHAEFFER, 2008; HARTGERS et al., 1994; HUANG et al., 2007; LU et al., 2015; SCHRÖDER - ADAMS et al., 2001; SINNINGHE DAMSTÉ et al., 1995b; SOUSA JÚNIOR et al., 2013) northeastern Brazil, were analyzed using full scan gas chromatography-quadrupole mass spectrometry (GC-qMS). Thus, paleorenieratane is another compound associated with the photic zone euxinia

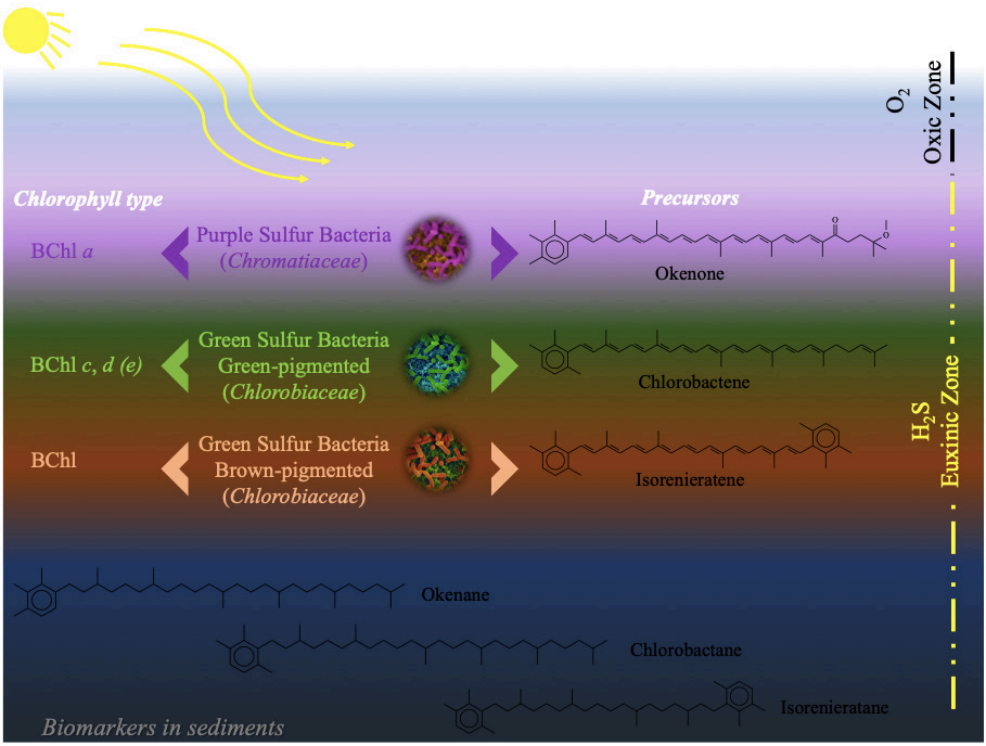


Figure 23. Simplified representation of Photic Zone Euxinia type environments illustrating sulfur bacteria of the Chromatiaceae and Chlorobiaceae families, their respective chlorophylls, precursors, and derivatives. Meromictic lakes with shallow chemoclines provide examples of the type of environments where PSB and GSB flourish today and where we might expect to find okenane and chlorobactane in the fossil record. Adapted image from Sousa Júnior et al. (2013).

Although Xu; Georgee and Hou (2018) reported its presence in the Cenozoic, this compound has often been associated with the Paleozoic age (ADEROJU; BEND, 2018; CONNOCK; NGUYEN; PHILIP, 2018; HEFTER; NAAFS; ZHANG, 2017; MARYNOWSKI; FILIPIAK, 2007; MELENDEZ et al., 2013; SPAAK et al., 2018; TULIPANI et al., 2015).

Renieratane (substitution patterns 2,3,6 and 2,3,4) and Renierapurpurane (substitution pattern 2,3,4-) (**Figure 21**) are likely to have another type of bacterium, Purple Sulphur Bacteria (PSB) (from the family Chromatiaceae), as their likely source, which also requires the same euxinic conditions for growth. However, cyanobacteria should not be dismissed as a source of these compounds (BROCKS AND SCHAEFFER, 2008). These authors further propose the carotenoid okenane (monoaromatic C_{40} with pattern 2,3,4-) as a biomarker for PSB.

In Brazil, isorenieratane and its derivatives were first reported by HEIMHOFER et al., (2008). Later, SOUSA JÚNIOR et al. (2013) performed the identification of C_{40} carotenoids and derivatives in oil samples from the Sergipe-Alagoas Basin (**Figure 24**).

The aryl isoprenoids, have been reported as diagenetic and catagenetic derivatives of carotenoids via OM maturation (HARTGERS et al., 1994; REQUEJO et al., 1992). However, short-chain ($< C_{17}$) aryl isoprenoids can originate via aerobic degradation (SCHWARK; FRIMMEL, 2004; SINNINGHE DAMSTÉ; KOOPMANS, 1997; SPAAK et al., 2018).

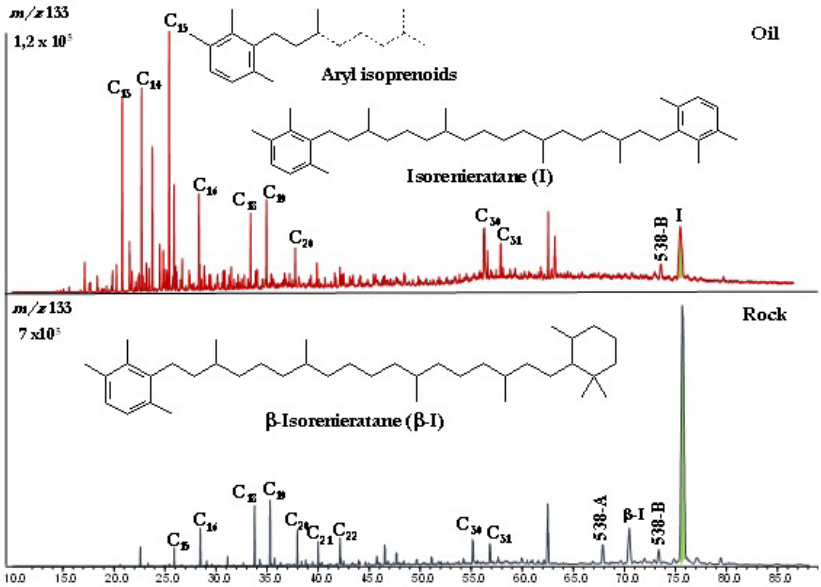


Figure 24. Mass chromatogram of *m/z* 133 showing homologous series of aryl isoprenoids (numbers, representing the number of carbon atoms in individual homologues) and diaryl isoprenoids, (Sergipe-Alagoas Basin, oil) and rock (São Luiz Basin, rock) - The signals represented by 538-A and 538-B are isomers, of molar mass 538 Daltons and base peak at *m/z* 134. The aromatic carotenoids isorenieratane and β-isorenieratane are the degradation products of isorenieratene and β-isorenieretene, which are the major carotenoids of brown pigmented strains of green sulfur bacteria (Chlorobi).

1.5 General characteristics of oils and sediments of Brazilian sedimentary basins

Brazil has about 60% of its territory occupied by sedimentary basins, **Figure 25.** They are divided into three different types: Large sedimentary basins - examples: Amazon basin, Parnaíba basin, Paraná basin, and Central basin. The sedimentary basins of Brazil date back to the Paleozoic, Mesozoic, and Cenozoic (CAINELLI; MOHRIAK, 1999; KOIKE et al., 1992; LOPES et al., 1999; MILANI et al., 2000, 2007) giant oil fields of Campos Basin (offshore Brazil). Most of the oil reserves are in offshore fields, which have led Petrobras to drill to ever-increasing depths.

Figure 25 Location map of Brazilian sedimentary basins. Image adapted from Souza-Lima; Silva, (2018)equatorial and interior basins from Northeastern Brazil contain a diverse sedimentary record representative of the events that culminated in the rupture of the Gondwana megacontinent and the genesis of the South Atlantic Ocean. Those events that occurred between the neo-Aptian and neo-Albian (Mid-Cretaceous.

A large part of the oil reserves discovered in Brazilian territory consists of heavy oil of low API grade (<20º) high viscosity, and high total acidity (MOHRIAK; SZATMARI; ANJOS, 2012). The Campos and Santos basins are Brazil's most important offshore oil reserves and can be the largest offshore oil exploration fields in the next decades, mainly due to the oil located in the pre-salt layer. According to the Brazilian Statistical Yearbook 2020 (ANP - AGÊNCIA NACIONAL DO PETRÓLEO, 2021), the national oil production grew 5.7% and reached 2.9 million barrels/ day (B/D). This increase was led by the pre-salt oil supply, which reached an average of 2 million B/D in the year, about 69.4% of the country's production. Natural gas production rose by 4.3%, corresponding to the 11th consecutive year of increase, and reached 128 million cubic meters

per day. In the pre-salt layer, natural gas production represents 65.7% of national production (ANP - AGÊNCIA NACIONAL DO PETRÓLEO, 2021).

An advantage in examining the geochemical and biological characteristics of petroleum rocks is the availability of samples from different environments whose geological and paleontological characteristics are well described. The Brazilian marginal basins provide an ideal investigation opportunity since they contain several sediments deposited in different deposits and within a single geographic domain.

The assessment and differentiation of the depositional palaeoenvironments of organic-rich sedimentary rocks using geochemical and biological marker parameters have significantly increased in the last years. Concerning biomarker studies, although, more recently, many works on biomarkers in Brazilian sedimentary basins have been published, the work conducted by Mello et al., (1987) is noteworthy because it performs a multidisciplinary study (geochemical, geological, paleontological, and statistical) to assess the depositional environments of source rocks and oils in the main Brazilian marginal basins.

Mello *et al*., (1987) analyzed many oil and rock samples recovered from reservoirs and sedimentary successions from the lower Neocomian to Oligocene. **Table 2** shows bulk and molecular data for Brazilian oils and sediment, adapted from Mello et al. (1987), that supports, expands, and assists in investigations that use or may use molecular parameters to characterize samples of oils and rocks from different sources.

Table 2. Bulk and molecular data for Brazilian oils and sediments. Adapted from Mello et al., (1987).

Environment of deposition	Lacustrine freshwater	Lacustrine saline	Marine evaporite	Marine carbonate	Marine deltaic	Marine calc. lith.	Marine silic. lith.
^{0}API (oils)	30-39	24-32	20-30	25-30	42-44	-	-
% saturates (oils)	60-73	45-65	30-59	20-60	60-70	-	-
% sulphur (oils)	<0.1	0.2-0.4	0.3-1.5	0.4-0.7	0.3-0.4	-	-
V/Ni (oils)	<0.05	0.3-0.4	0.2-0.3	0.4-0.5	0.8-1.0	-	-
% R_0 (rocks)	0.4-0.7	0.4-0.8	0.5-0.7	0.4-0.6	0.5-0.6	0.4-0.6	0.5-0.7
% saturates (rocks)	40-60	25-55	25-40	20-45	27-30	22-34	25-44
% sulphur (rocks)	0.2-0.3	0.1-0.5	0.3-2.5	0.2-0.6	0.6-0.7	0.4-0.5	0.3-0.7
% $CaCO_3$	< 7	2-30	5-25	15-65	50-70	15-48	6-20
Δ ^{13}C (PDB‰)	< -28	-23: -27	-25: -27	-26: -28	-24: -26	-26: -28	-26: -27
n-alkane max.	~C_{23}	~C_{19}	~C_{18}|	C_{20}-C_{22}	C_{20}-C_{22}	~C_{20}	~C_{17}
Odd/Even	≥1	≥1	≤1	≤1	≤1	<1	>1
Pr/Ph	>1.3	>1.1	<1.0	<1	<1	<1	>1
1. *i*-C_{25} + *i*-C_{30}[a]	<170	70-700	300-1500	100-500	150-300	10-100	40-180
2. β-carotane (ppm)	ND	10-200	100-400	20-60	5-10	10-30	ND
3. C_{21} + C_{22} steranes (ppm)	Tr	10-30	10-60	10-60	30-50	10-30	25-35
4. C_{27} steranes (ppm)	10-50	50-150	500-4000	50-300	50-350	30-200	20-400
5. C_{27}/C_{29} steranes	1.5-4.0	1.5-2.5	1.0-2.2	1.1-1.5	1.3-1.8	0.8-1.2	1.5-2.5
6. Diasterane index	20-40	10-50	6-20	20-30	30-60	10-30	30-80
7. C_{30} steranes (MS-MS)	ND	ND	Low	High	High	High	High
8. 4-Me-sterane index	30-50	30-150	30-80	30-80	<10	20-40	10-20
9. Hopane/Steranes	5-15	5-15	0.4-2.0	0.9-3.0	0.5-3.0	0.3-0.9	1.5-3.0
10. Tricyclic index	30-100	100-200	10-60	60-200	60-180	50-100	70-100
11. C_{34}/C_{35} αβ hopanes	>1	>1	<1	≤1	<1	≤1	>1
12. Bisnorhopane index	0	3-15	15-40	10-30	0	20-1000	1-5
13. 18α(H)-oleanane index	0	0	0	0	20-40	0	0
14. Ts/Tm	>1	<1	≤1	<1	>1>1	<1	>1
15. C_{30} αβ hopane (ppm)	200-500	200-1600	300-2000	80-300	100-250	10-70	50-800
16. Gammacerane index	20-40	20-70	70-120	10-20	0-5	0-25	1-5
Ni-porphyrins (ppm)	Tr	0-2800	0-1900	0-400	Tr	0-1700	0-800
V = 0-porphyrins (ppm)	Tr	0-150	0-600	0-3000	Tr	0-4000	0-130
Ni/Ni+V = 0 porphyrins[b]	-	0.9-1.0	0.6-0.9	-0.1-0.3	-	0.3-0.9	0.8-1.0
% amorphous	55-65	85-90	45-60	50-60	60-70	60-70	85-95
% herbaceous	25-35	5-10	15-25	10-15	10-15	5-10	5-10
% woody + coaly	5-10	5-10	10-25	20-30	15-25	20-25	0-5

[a]For measurement see Appendix 2. [b]Ratios range given Only When V=0 porphyrins > trace quantities. Tr- trace. ND- not detected. **1. i-C_{25} + i-C_{30}:** Sum of 2,6,10,14,18 + and/or 2,6,10,15,19-pentamethyleicosane (i-C25) and squalane (i-C30) peak areas in RIC trace and normalised to added sterane standard. **2. β-carotane (ppm):** Peak area β-carotane in RIC trace and normalised to added sterane standard. **3. C_{21} + C_{22} steranes (ppm):** Sum of peak areas 13β(H),17α(H)-diapregnane (C_{21})+ 5α(H),14β(H),17α(H)-pregnane (C_{21}) + 5α(H),14β(H),17β(H) + 5α(H),14α(H),17α(H)-pregnane (C_{21}) + 5α,(H),14β(H),17β(H) + 5α(H),14α(H),17α(H)-homopregnane (C_{22}) in *m/z* 217 chromatogram and normalised to added sterane standard (*m/z* 221 chromatogram). **4. C_{27} steranes (ppm):** Sum of peak areas for *20R* and *20S* 5α,14α,17α,(H)- cholestane in *m/z* 217 chromatogram and normalised to added sterane standard (*m/z* 221 chromatogram). **5. C_{27}/C_{29} steranes:** Peak area of *20R* 5α,14α,17α,(H)-cholestane over peak area of *20R* 5α,14α,17α,(H)-ethyicolestane in *m/z* 217 chromatogram. **6. Diasterane index:** Sum of peak areas of C_{27} *20R* and *20S* 13β,17α-diasteranes in *m/z* 217 chromatogram over sum of peak areas of C_{27} *20R* and *20S* 5α,14α,17α-cholestane × 100. Low, <30; medium, 30–100; high, >100. **7. C_{30} steranes (MS-MS):** Monitored using linked scan techniques (cf. Moldowan; Seifert; Gallegos, 1985). **8. 4-Me-sterane index:** Sum of peak areas of all C_{30} 4-methylsteranes in *m/z* 231 chromatogram and *m/z* 414 chromatogram over sum of peak areas of C_{27} *20R* and *20S* 5α,14α,17α(H)-cholestane × 100. Low, <60; medium, 60–80; high, >80. **9. Hopane/Steranes:** Peak area of C_{30} 17α,21β(H)-hopane in *m/z* 191 chromatogram over sum of peak areas of C_{27} *20R* and 20*S* 5α,14α,17α,(H)-cholestane in *m/z* 217 chromatogram. Low <4, medium 4-7, high >7. **10. Tricyclic index:** Sum of peak areas of C_{19}-C_{29} (excluding C_{22} and C_{27}) tricyclic terpanes in *m/z* 191 chromatogram over peak area of C_{30} 17α,21β(H)-hopane × 100. Low <50, medium 50-100, high >100. **11. C_{34}/C_{35} αβ hopanes**: Peak areas of C_{34} *22R* and *22S* 17α,21β(H)-hopanes in *m/z* 191 chromatogram over peak areas of C_{35}, counterparts. Low < 1, high >1. **12. Bisnorhopane index**: Peak area of C_{28} 28,30-bisnorhopane in RIC chromatogram over peak area of C_{30} 17α,21β(H)-hopanes in *m/z* 191 chromatogram. Low <10, medium 10-50, high >50ppm. **13. 18α(H)-oleanane index:** Peak area of 18α(H)-oleanane in *m/z* 191 chromatogram over peak area of C_{30} 17α,21β(H)-hopanes in *m/z* 191 chromatogram. **14. Ts/Tm:** Peak area of 18α(H)-trisnomeohopane (Ts) over peak area of 17α,(H)-trisnorhopane (Tm) in *m/z* 191 chromatogram. **15. C_{30} αβ hopane (ppm):** Peak area of 17α,21β(H)-hopanes measured in RIC and normalized. **16. Gammacerane index:** Peak area of gammacerane in *m/z* 191 chromatogram over peak area of 17α,21β(H)-hopanes × 100. Low < 50, medium 50-60, high > 60

1.5.1 Biomarker isolation and analysis techniques

Concerning the separation and analysis of biomarkers, classical chromatographic processes usually are used on silica gel or silica modified with bases, acids, or silver nitrate, depending on the class of composites of interest. Similar chromatographic processes are followed for the analytical procedure of biomarkers in rock samples; extraction of the soluble fraction of organic matter is performed.

The samples are treated, crushed, and submitted to extraction in Soxhlet or ultrasonic systems, when necessary derivatized, and analyzed by classical and modern chromatographic techniques, including LC-MS-MS, GC-MS-MS and HS-GC-MS, and high-resolution GC-MS, IR, atmospheric pressure gas chromatography–mass spectrometry (APGC–MS), RAMAN, 1H, and 13C NMR. GRICE AND EISERBECK (2014) address some analytical techniques employed in the biomarkers analysis, using, for example, 2D chromatographic techniques and other state-of-the-art mass spectrometry and various pyrolysis techniques (GRICE; EISERBECK, 2014).

The GC-MS technique was one of the significant advances for developing biomarker geochemistry, initially for petroleum exploration research and then for organic geochemistry in general. Current instrumentation and derivatization methods have expanded the biomarker concept to other disciplines and applications, such as chemical ionization (CI) MS, GC-high-resolution MS, field-ionization and desorption MS, tandem mass spectrometry (MS-MS), pyrolysis GC-MS, and high-pressure liquid chromatography (HPLC)-MS, all with associated online computers and processors, these techniques are being applied in

biomarker fingerprinting.

GC-MS is frequently used for determining hydrocarbons in crude oils and petroleum products because of its specificity, selectivity, and sensitivity. GC-MS can provide excellent separation and accurate quantification of volatile and semi-volatile organic compounds, and polar derivatives to methyl or silylated esters in petroleum. **Figure 26** shows a general scheme of a GC-MS system.

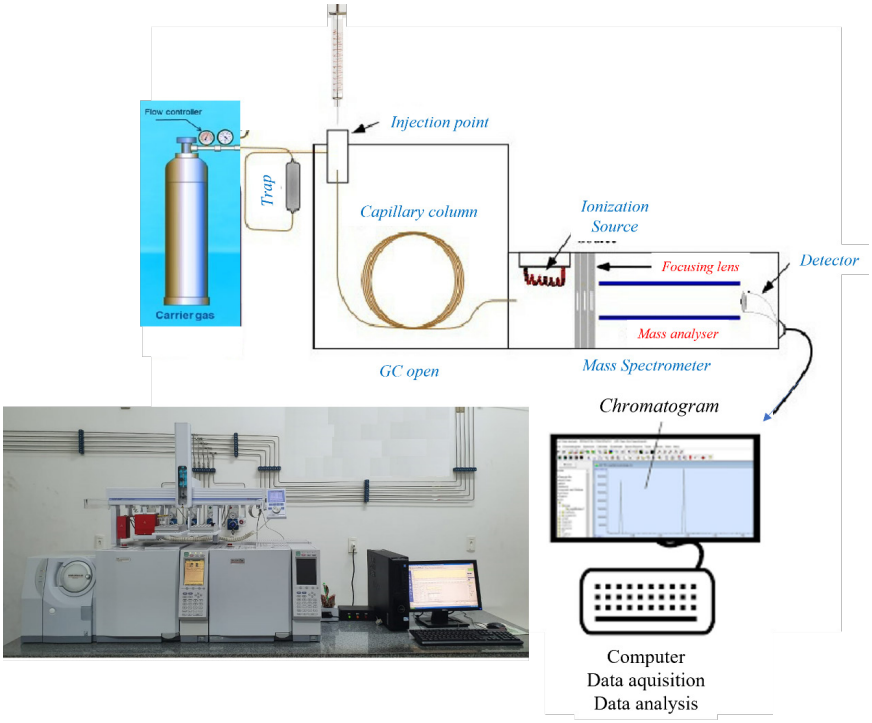
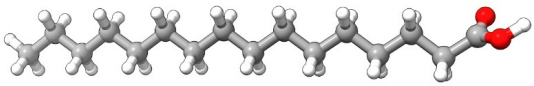


Figure 26. Schematic plot of the main components of GC–MS instruments. GC-MS can be used to separate complex mixtures, quantify analytes, identify unknown peaks and determine trace contamination levels. Despite the evolution of analysis techniques, It continues being “blue-eyed girl” of the industry in general.

CHAPTER 2

REVIEW ON EXTRACTION METHODS FOR ACIDIC BIOMARKERS IN OILS AND SEDIMENTARY ROCKS

Acidic biomarkers can provide relevant information parallel to neutral biomarkers, providing additional clues about reservoir oils. They have been used as indicators of migration, maturation, and biodegradation of oils. Like neutral biomarkers, they can be distributed into different classes of compounds according to their carbon skeletons. This unit discusses the importance of acidic biomarkers, their genesis, and main extraction methods, with advantages and disadvantages.



1 | NAPHTHENIC ACIDS X MOLECULAR FOSSIL (ACIDIC BIOMARKERS)

Naphthenic acids are mixtures of naturally occurring cycloaliphatic carboxylic acids recovered from petroleum distillates. The acids are present in varying amounts in crude oil and are typically recovered by caustic extraction of petroleum distillates with boiling ranges of 200–370°C (BRIENT; WESSNER; DOYLE, 2008).

Naphthenic acids in crude oils are of concern in the petroleum industry due to their corrosivity to refinery units. It is desirable to determine the ring type and carbon number distributions because the corrosivity of naphthenic acids is dependent on the sizes and structures. The characterization of naphthenic acids is also of interest to geochemical studies, particularly migration and biodegradation and refinery wastewater treatment for environmental compliance (HSU et al., 2000).

The term “naphthenic” acid has been used to refer to all of the carboxylic acids present in petroleum, whether cyclic, acyclic, or aromatic compounds and carboxylic acids containing heteroatoms such as N and S (HEADLEY; PERU; BARROW, 2009; QIAN et al., 2001; YANG et al., 2019)or is a negative, even integer. Commercial naphthenic acids are sold in various grades of purity. Although commercial naphthenic acids often contain primary cycloaliphatic acids, some studies have shown they also contain straight chain, branched aliphatic and aromatic acids. It can be represented by a general formula $C_nH_{2n-z}O_2$, where n indicates the carbon number and z specifies a homologous series. Z is equal to 0 for saturated, acyclic acids and increases to 2 in monocyclic naphthenic acids, 4 in bicyclic naphthenic acids, 6 in tricyclic acids, and 8 in tetracyclic acids (**Figure 27**) (YANG et al., 2019).

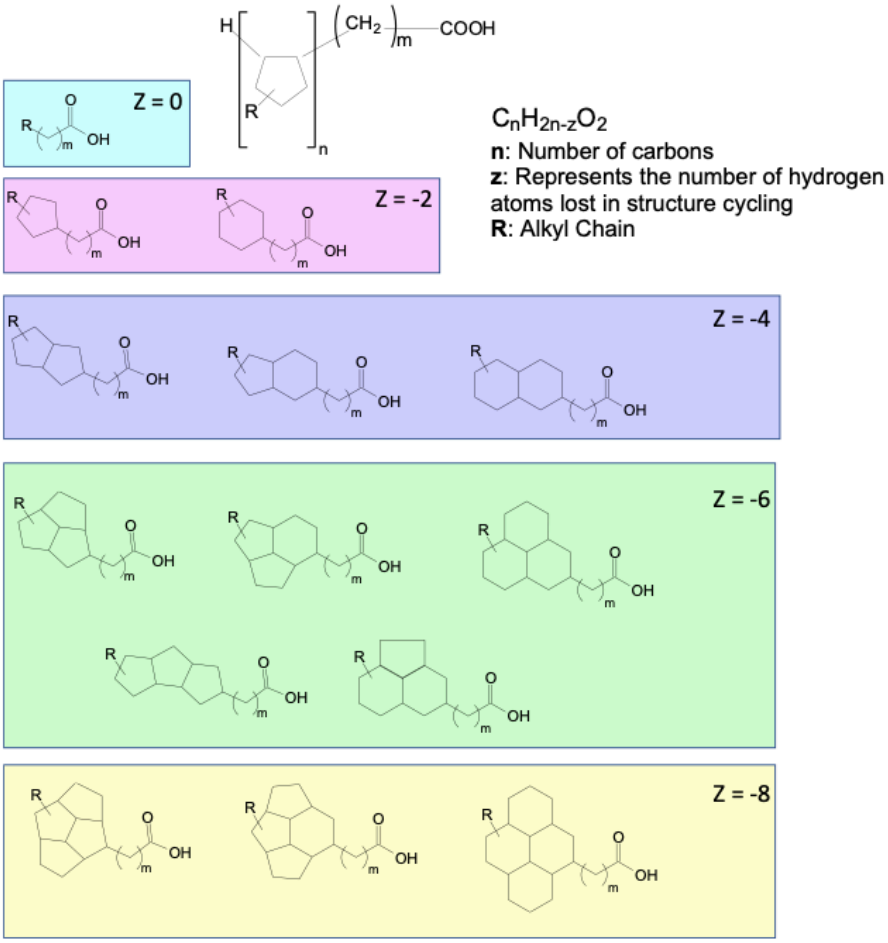


Figure 27. The general structure of some naphthenic acids. Structures of the same Z belong to the same series.

The structural elucidation of these compounds has been occurring more recently with the use of high (TOF-MS) or ultra-high resolution (Orbitrap-MS) mass spectrometers (HEADLEY; PERU; BARROW, 2009; QIAN et al., 2001; TOMCZYK et al., 2001) and liquid chromatography coupled to these spectrometers has shown promise for naphthenic acid analysis (BATAINEH et al., 2006; DAMASCENO et al., 2014) in water and soil samples from areas associated with oil refineries (GREWER et al., 2010; HAN et al., 2016; HEADLEY; PERU; BARROW, 2016; HINDLE et al., 2013; HUGHES et al., 2017; SHANG et al., 2013; WANG; KASPERSKI, 2010; YANG et al., 2019) or in crude oils (HEADLEY; PERU; BARROW, 2016; QIAN et al., 2001; RUDZINSKI et al., 2002; YANG et al., 2019).

GC-Ms technique is most commonly used to analyze these compounds. However, the major obstacle to these analyses is the identification of higher molecular weight compounds,

which have higher boiling points, and the need for derivatization for their proper identification. These limitations do not apply to LC-MS, and this technique can provide complementary information to that which is obtained by traditional GC-MS methods. **Figure 28** shows the distribution of Naphthenic acid in a commercial sample as a silylated derivative. In this sample, there are mainly cycloaliphatic carboxylic acids.

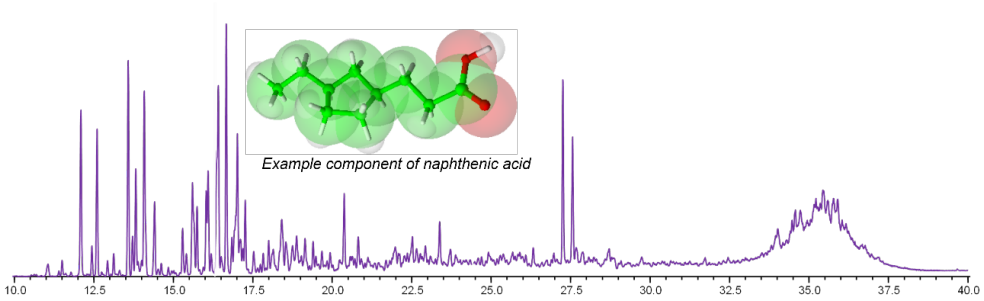


Figure 28. ICT (GC-MS) of a commercial sample of naphthenic acids with a silylated derivative (Naphthenic acid, technical, Aldrich, Lot # BCCB6544). On the silica gel column, these naphthenic acids do not elute with hexane when using a silica/sample (1:70) but elute gradually as the polarity is increased with ethyl acetate. They are easily entrained, even on base-modified silica, with ethyl ether.

In the following, we will present some basic information about higher molar mass carboxylic acids whose structures are associated with neutral biomarkers often analyzed in oil samples and sedimentary rock extracts, such as sesquiterpanoic, diterpanoic, steranoic and therpanoic acids, and their isolation processes, according to literature data. In the following chapter, we present a broad review of the carboxylic acid extraction processes, showing the advantages and disadvantages of the respective methods

1.1 Acidic molecular fossils (biomarkers) detected in petroleum and sedimentary rocks

The carboxylic acids found in petroleum are usually called “naphthenic” acids. Although the term corresponds to saturated cyclic compounds, it is common to apply it to all classes of carboxylic acids present in oils and rock extracts. Due to their very heterogeneous composition, they are very difficult to characterize individually (VAZ DE CAMPOS et al., 2006).

Besides the geological interest, the study of the composition of the acid fraction is also of economic interest. Although the amount of acids in crude oil is generally low, their emulsifying and corrosive characteristics make them especially important because they cause corrosive effects in distillation towers, directly impacting the costs associated with their processing and refining (VAZ DE CAMPOS et al., 2006).

Acid biomarkers can provide relevant information parallel to those obtained by neutral

biomarkers, providing additional clues about reservoir oils. Although there are fewer studies compared to saturated or aromatic fractions, many research groups have been interested in petroleum polar fractions, mainly the acid fraction (AF). Many works are reporting carboxylic acids identification from sedimentary rock samples (BARAKAT; RULLKÖTTER, 1995; EL-SHAFEIY et al., 2014; HUANG et al., 1999; LI et al., 2015; NISHIMURA et al., 2006; SAITO; SUZUKI, 2007; THIEL; HOPPERT, 2018; VENKATESAN; KAPLAN, 1987). For example, Farrimond et al. (FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002) reported fatty and hopanoic acid composition from thirty rock samples spanning Triassic to Cretaceous.

In Brazil, some studies were performed using oil samples from many basins across the east coast (DE LIMA et al., 2010; LOPES et al., 1997, 1999; NASCIMENTO et al., 1999; RODRIGUES et al., 2000). Besides the extreme complexity of component mixtures, the acidic fraction is composed mainly of carboxylic acids, which have been analyzed using an approach based on their conversion to their equivalent hydrocarbons (R–COOH→R–CH_3), making the GC-MS analysis easier. However, the complexity of the acid fraction (AF), the laborious separation techniques, purification, and derivatization needed have delayed the research toward the understanding of the distribution of these compounds and, consequently, studies highlighting the organic geochemistry relevance of Acid fraction (AF) are limited.

In this sense, methodologies that separate the acid components fast, quantitative, and using few steps are desirable. Between the methodologies used to recovery AF the most used are liquid-liquid (LLE) and solid-phase (SPE) extractions (GRUBER et al., 2012).

The first articles to use LLE on carboxylic acid isolations were the studies of Seifert and coworkers (SEIFERT; GALLEGOS; TEETER, 1972; SEIFERT; HOWELLS, 1969; SEIFERT; TEETER, 1969, 1970). However, the method used on those papers demands considerable time to prepare the samples, besides a large solvent volume. Because of these drawbacks, the SPE, which use less solvent volume and demands lesser analyst time, have been used by industries and many other fields, including petrochemical, which applied these methodologies in the analyses of AF from oils and rocks as reported by several authors (JONES et al., 2001; LAMORDE; PARNELL; BOWDEN, 2015; SESSIONS et al., 2002; ZHU et al., 2017)

1.2 Carboxylic acids and oil biodegradation processes

Frequently, microbial degradation in oil reservoirs has been associated with increased acidity of the oils (BARTH et al., 2004; BEHAR; ALBRECHT, 1984).Experiments of oil biodegradation can be performed in the laboratory to produce significant amounts of carboxylic acids. The work done by WATSON et al. (2002) demonstrated that medium molecular weight acids (C_{10} - C_{20}) were rapidly produced, which coincided with the removal of *n*-alkanes from the samples studied. Other examples include the research perfomed

by (MEREDITH; KELLAND; JONES, 2000), who investigated the influence of carboxylic acids on the acidity of crude oils. The comparison between acidity values and analysis of the acid fraction of 33 different oil samples indicated that biodegradation is one of the main processes responsible for the high concentrations of carboxylic acids in petroleum. HUGHEY et al. (2008) demonstrated the potential of ESI FT-ICR MS as a semi-quantitative tool to monitor the production of naphthenic acids during crude oil biotransformation in the environment. DE LIMA et al. (2010) evaluated three typical oil samples of similar maturity, derived from saline lacustrine source rock and accumulated at different depths in stacked reservoirs in the Campos Basin, Brazil. These authors observed systematic variations in the relative abundance of acidic biomarkers with the extent of biodegradation. **Figure 29** shows, as an example, the presence and variation in the distribution of C_{32} ββR hopanoic acids as biodegradation increases (A < B < C).

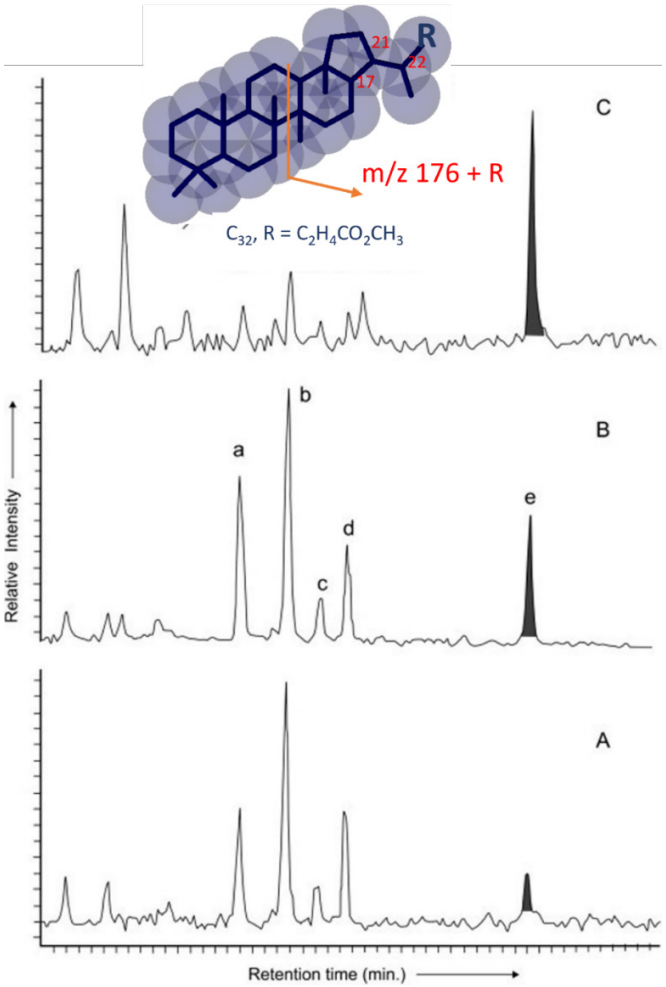


Figure 29. RICs (*m/z* 263) for C_{32} homologues of hopanic methyl esters, showing progressive increase in C_{32} ββR biological isomer in oils A–C: (a) 17α(H), 21β(H) 22 S; (b) 17α(H), 21β(H) 22

R; (c) 17β(H), 21α(H) 22 S; (d) 17β(H), 21α(H) 22 R and (e) 17β(H), 21β(H) 22R. Ion analysis the *m/z* 235, *m/z* 249 and *m/z* 263 BERs allow the identification of the series of hopanoics acids with configuration 17α(H), 21β(H), 22(R or S); 17β(H), 21α(H), 22(R or S) and 17β(H), 21β(H), 22(R or S). The presence of the less stable 17β(H), 21β(H) isomer in oils of different origins and levels of biodegradation has been associated with the incorporation of immature organic matter during migration.

Although polar compounds are often a significant fraction of the oils, biomarkers obtained from saturated and aromatic fractions have been prioritized in geochemical studies, providing essential indications of oil origin and maturation. The projects developed by NASCIMENTO et al. (1999) and (GALIMBERTI; GHISELLI; CHIARAMONTE, 2000) propose using polar compounds as biomarkers. These compounds can be a complementary source of relevant information because their properties give more specific indications since the processes involved recreate oil/water and oil/rock interactions and data on secondary migration, origin, and preservation.

Acid biomarkers, like neutral biomarkers, can be distributed into different classes of compounds according to their carbon skeletons. In the following subsections, the classes most commonly observed in studies of acidic fractions will be presented.

1.2.1 Fatty acids

Fatty acids are major components in most organisms, and after suffering deposition and burial, they persist for a long time on the geological scale. Perhaps due to their endurance, among the polar compounds, fatty acids are frequently reported constituents in the analysis of petroleum and sedimentary rock samples. **Figure 30** shows a typical profile of linear fatty acids and isoprenoids.

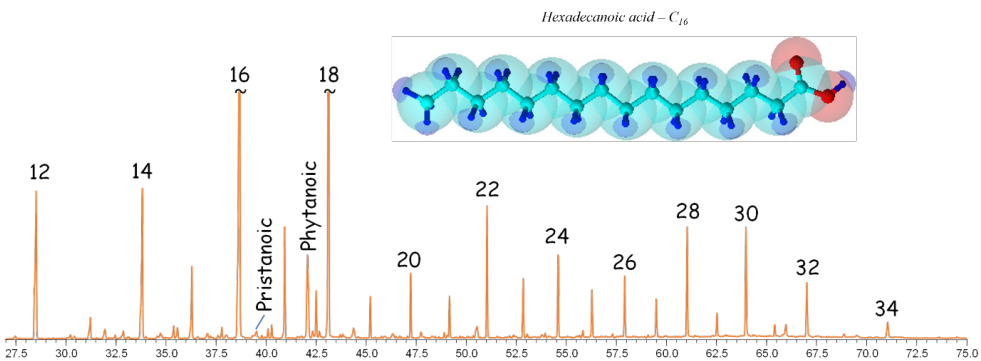


Figure 30. Representative mass chromatogram (GC-MS) of n-alkanoics acids as a silylated derivative, obtained from Potiguar Basin oil samples with 10% KOH-modified silica phase. In oils, if the distribution of n-alkanoic acids shows similarity to the distribution of n-alkanes, it can be suggested that both series have, at least in part, a diagenetic relationship by decarboxylation of the acids.

There are reports of the presence of C_{14}, C_{16}, C_{18}, C_{20}, and C_{22} fatty acids in sedimentary rocks and C_{14}, C_{16}, $C_{18,}$ and C_{20} fatty acids in petroleum, both reported since the Precambrian (KVENVOLDEN, 1967) and apparently without the presence of fatty acids with an odd number of carbons. Although even-numbered carbon compounds are the most commonly reported in most ancient samples (Precambrian samples), odd-numbered carbon compounds of low molecular mass (C_1 to C_9) have also been reported (KVENVOLDEN, 1967). The constituents with an odd number of carbon atoms, both lower and higher molecular mass, become more significant as more recent geological samples are analyzed, suggesting that acids with an odd number of carbon atoms are formed during diagenesis.

In nature, fatty acids with an even number of carbon atoms between C_4 and C_{26} predominate; they are maintained in recent sediments and, consequently, reflect the original composition in organisms. However, since fatty acids are also susceptible to diagenetic changes, this pattern does not persist in ancient sedimentary rocks (Schwarzbauer and Jovančićević, 2016).

It is now believed that fatty acids are, at least in part, the precursors of geochemically formed n-alkanes (COOPER; BRAY, 1963). This hypothesis was experimentally demonstrated by Welte and Ebhardt (WELTE; EBHARDT, 1968), who studied sediments from the Persian Gulf. According to these authors, the relative abundances of n-acids and n-paraffins are almost identical when comparing the n-acid and the respective n-paraffin with one carbon atom less. The data obtained suggest that the overall process responsible for converting n-acid to n-paraffin is decarboxylation (**Figure 31**).

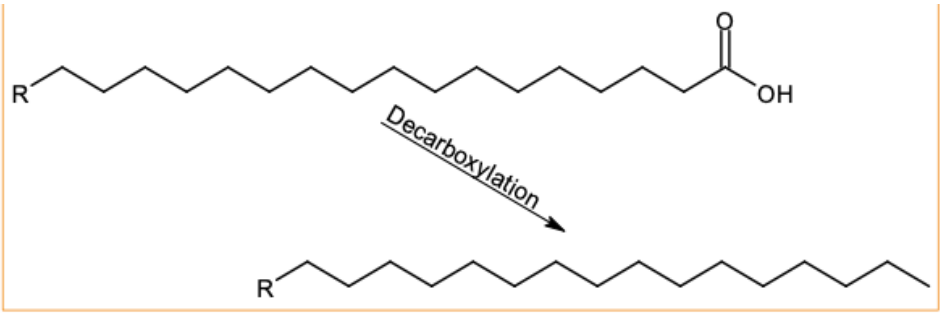


Figure 31. Decarboxylation process in fatty acids.

The change in chain size predominance caused by decarboxylation can be monitored by the CPI parameter (Carbon Preference Index). The most abundant fatty acids in nature have C_{16} or C_{18} (**Figure 30**) carbon atoms in their constitution and are also the most abundant in oil and sediments. The CPI parameter for fatty acids

can thus be calculated from the simplified expression (C_{16} + C_{18})/2×C_{17} (COOPER; BRAY, 1963). The interpretation of this parameter is similar to that used for the CPI parameter calculated for *n*-paraffins: recent sediments have high values (>1.0) and indicate low thermal evolution, older sediments, the value decreases to closer to 1.0, and in oil samples, the value is around 1.0 indicating more significant maturation (SCHWARZBAUER; JOVANČIĆEVIĆ, 2016).

1.2.2 Acyclic isoprenoid acids

Pristanic and phytanic acids (**Figure 32**) are the most studied compounds within the class of acyclic isoprenoid acids. They are present in living organisms, and, like fatty acids, their hydrocarbon skeletons are pretty resistant and can be preserved in sediments and oils. The chlorophyll side chain (the phytol) is considered the main source; however, both acids have other possible sources, such as halophilic bacteria (KATES; YENGOYAN; SASTRY, 1965).

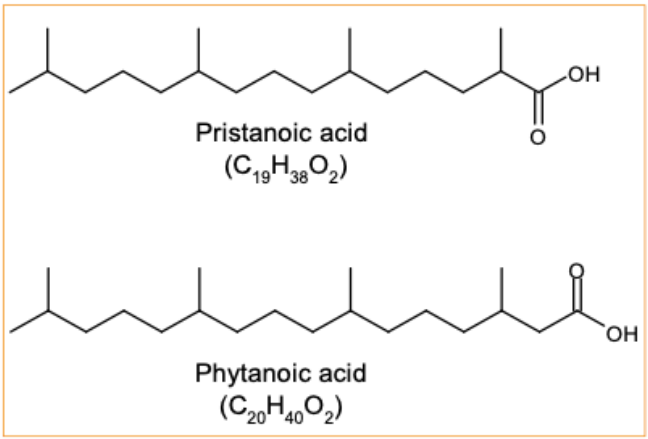


Figure 32. Structure of Pristanic (C_{19}) and Phytanic (C_{20}) acids. Phytanic acid is a methyl-branched fatty acid present in the human diet.Due to its structure, degradation by beta-oxidation is impossible. Instead, phytanic acid is oxidized by alpha-oxidation, yielding pristanic acid.

1.2.3 Bicyclic terpanoic acids

Bicyclic terpanoic acids are not very common, but they have been reported in some works that studied both rock and oil samples (NASCIMENTO et al., 1999). The composition of the bicyclic terpanoic acids can vary from C_{15} to C_{22} and be different from that present in the neutral fraction (DE LIMA, 2005; LOPES, 1995; NASCIMENTO et al., 1999). The **Figure 33** shows a typical profile of bicyclic acids present in oils from Campos Basin - Brazil

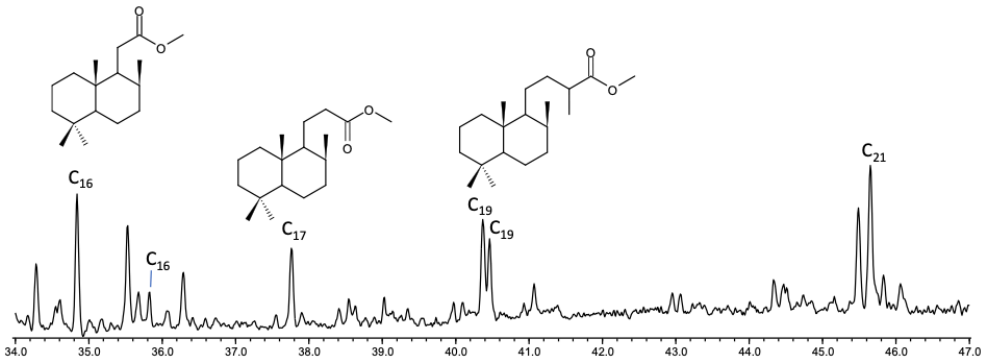


Figure 33. Distribution of bicyclic acids (*m/z* 123, GC-MS) in an oil sample from the Campos Basin, obtained by SPE (silica modified with KOH, Organic Geochemistry Laboratory of the Federal University of Piaui - Brazil).

Several reports show the occurrence of bicyclic sesquiterpanes in crude oils, but the significance of most of these compounds remains unknown due to lack of precise structure assignments (PETERS; WALTERS; MOLDOWAN, 2005a). Bicyclic biomarkers in biodegraded oils from the Campos Basin have been attributed to preferential removal of *n*-alkanoic and isoprenoic acids, thus selectively concentrating the labdanic acids (NASCIMENTO et al., 1999) that have been related to higher plant diterpenoids.

1.2.4 Tricyclic and pentacyclic terpanoic acids

As with the saturated constituents, tricyclic and pentacyclic terpane acids are the most commonly reported constituents among the terpenoids present in the acidic fraction of petroleum and sediments (KOIKE et al., 1992). The tricyclic acids (**Figure 34 A**) occur as a pseudo-homologous series in the C_{20} to C_{26} range (CYR; STRAUSZ, 1984). Due to the existing stereocenter at the C-22 position, the signals from the C_{24} acid occur as isomers pairs. A chromatographic profile of tricyclic acids is shown in **Figure 35**. These acids are present in higher abundance in biodegraded samples.

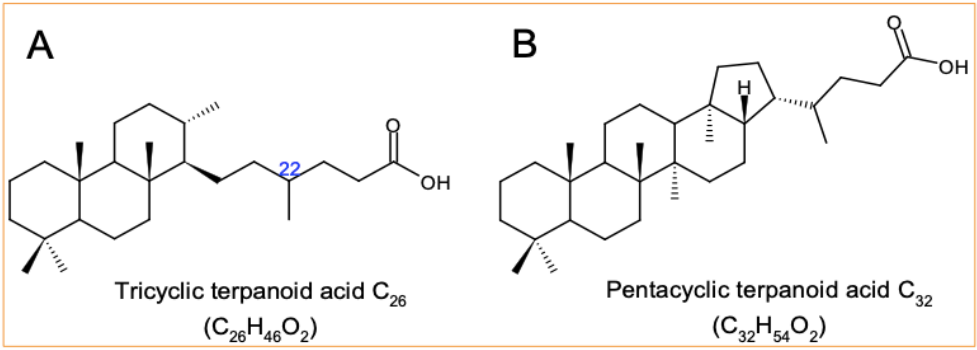


Figure 34. Examples of tricyclic (A) and pentacyclic (B) acids, whose precursors are found in the organic matter of sedimentary rock samples and crude oil.

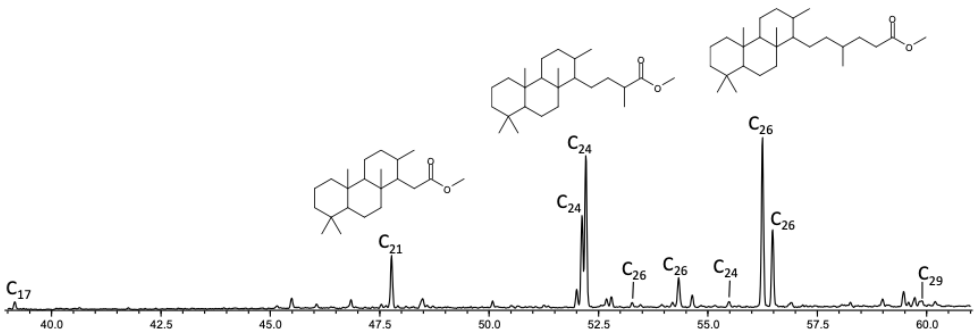


Figure 35. Example of GC-MS profile showing tricyclic acids as methyl esters in a sample of biodegraded oil (Campos Basin - Brazil) obtained through extraction with Lewis base modified silica phase.

Pentacyclic terpanoic acids have been reported in rock and oil samples of various geological ages (FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002; RODRIGUES et al., 2000). This class of compounds is believed to give rise to the pentacyclic terpanes (hopanes, moretanes, etc.) present in oils and sediments from simple decarboxylation (LOPES, 1995). **Figure 36** shows an example of hopanoic acids present in a sample of crude oil from the Campos Basin

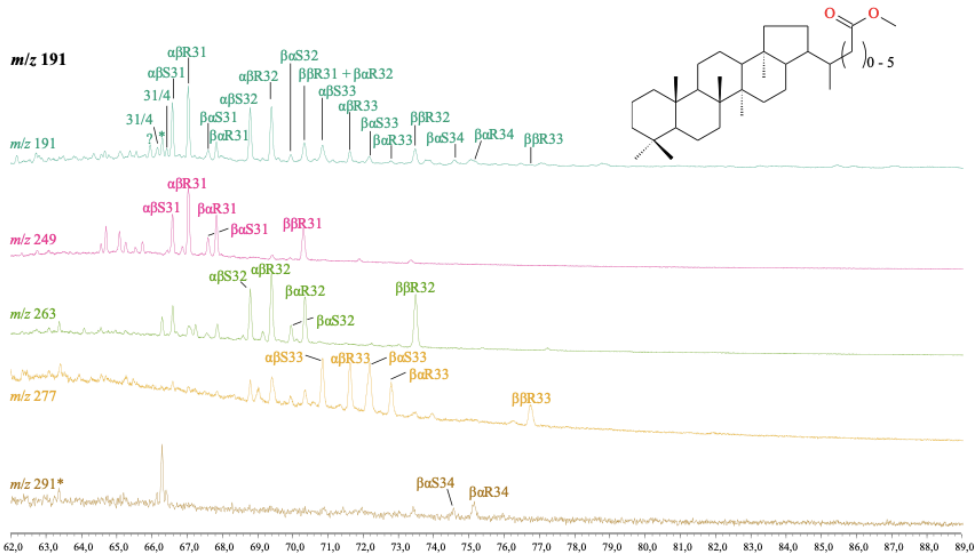


Figure 36. Distribution of hopanoic acids in an oil sample from Campos Basin - Brazil, isolated by SPE (silica modified with KOH) and analyzed as methyl esters (BF_3/CH_3OH). Organic Geochemistry Laboratory of the Federal University of Piauí.

Usually, hopanoic acids in the C_{30} to C_{35} range are reported. However, the smallest hopanoic acid reported in the literature has the carboxyl group directly attached to C-21; this acid was obtained after the mild oxidation of kerogen from the Green River Formation (Eocene) (BARAKAT; YEN, 1990). In general, the predominant stereochemistry in recent samples is biological (βββ), which isomerizes to αβ with maturation, which is the predominant stereochemical form in older oils and rocks (LOPES, 1995). In these acids, the carboxyl group is usually attached to the terminal carbon of the side chain.

1.2.5 Steranoic acids

Steranoic carboxylic acids are another class that may be present in sedimentary rock and oil samples, but unlike *n*-alkanoic and terpanoic acids, the reports in the literature are much less frequent. This class can present itself as a series that has the carboxyl group attached to the terminal carbon of the side chain (e.g. De Lima et al. (2010)) (**Figure 37 A**). Another group is the series of alkyl-esteranoics that have the carboxyl linked to an alkyl group attached to C-3 (e.g. Lopes et al. (1999)) (**Figure 37 B**). The complete series, which includes cholestane-type skeletons, ergostanes, and stigmastanes, has already been detected by GC-MS in samples from the Potiguar Basin (LOPES et al., 1997, 1999). **Figure 38** shows a complete series of 3-carboxyesterane acids (cholestane, ergostane, and stigmastane) and 3-carboxymethylesterane acids (cholestane, ergostane, and stigmastane) is observed. Dinosterane acids are present only in the 3-carboxymethyl form. These

compounds are present in the 5α(H) and 5β(H) configurations.

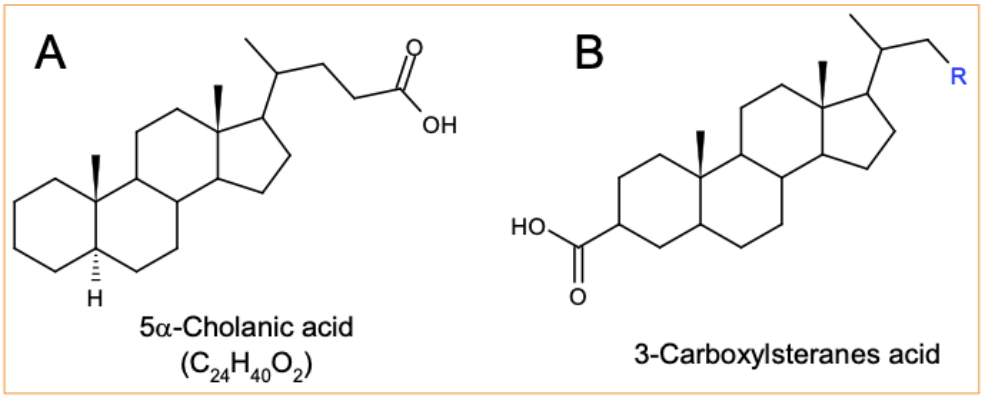


Figure 37. Structure of 5-cholanic acids (Structure A) and the 3-carboxyesteranoic acid series (Structure B).

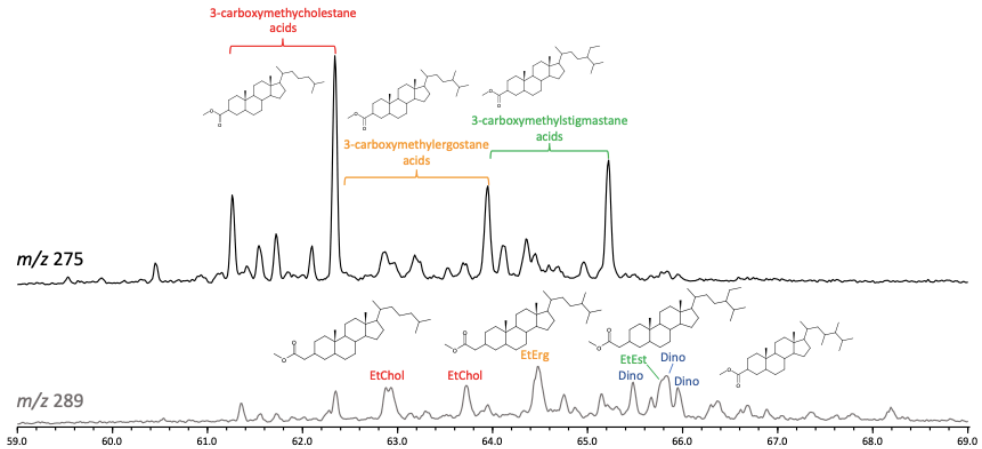


Figure 38. Typical GC-MS Profiles of 3-carboxysteranoic acids. Carboxilic acids more recently extracted in Lewis-based modified silica phase in our laboratory. We previous report a series of alkylsterane hydrocarbons and carboxyalkylseranoic acids in different Brazilian basins (Lopes et al., 1997 and 1999; De lima et al., 2010). These compounds belongs to the cholestane (C-24: H), ergostane (C-24: Me) and stigmastane (C-24: Et) families.

1.3 Carboxylic acids extracting methods from petroleum and sedimentary rocks

Methodologies capable of separating these components quickly and quantitatively with as few steps as possible are desirable. Among the methodologies employed in the recovery of acids, the most widely used is liquid-liquid extraction (LLE) and solid-phase extraction (SPE) (GRUBER et al., 2012).

Because of the nature of acidic compounds in oils and rock extracts, their removal in oils is possible using liquid-liquid extraction (LLE) (**Figure 39**). Alkaline solutions with sodium (NaOH) and potassium hydroxide (KOH) are most often employed for separation. For a long time, this methodology has been used in the literature. For example, the works performed by Seifert and Teeter (1969, 1970); Seifert and Glenn (1969)"abstract":"This report is part of a general study of the naturally occurring anionic surfactants in a Midway Sunset (California; Seifert, Gallegos and Teeter (1972) can be cited in which the authors extract terpenoid acids, saturated polynuclear acids, mono- and polynuclear aromatic acids, naphthenic acids, and heterocyclic acids, and C_{22} and C_{24} phenols and steroids, using isopentane and 1% NaOH in 70% ethanol. Despite being a widely used method, the effectiveness of this methodology is impaired by the extensive formation of emulsions and coextraction of acidic impurities such as phenols and carbazoles in addition to the time required for sample processing and the use of large amounts of solvent (CLEMENTE; FEDORAK, 2005; GRUBER et al., 2012; VAZ DE CAMPOS, 2005).

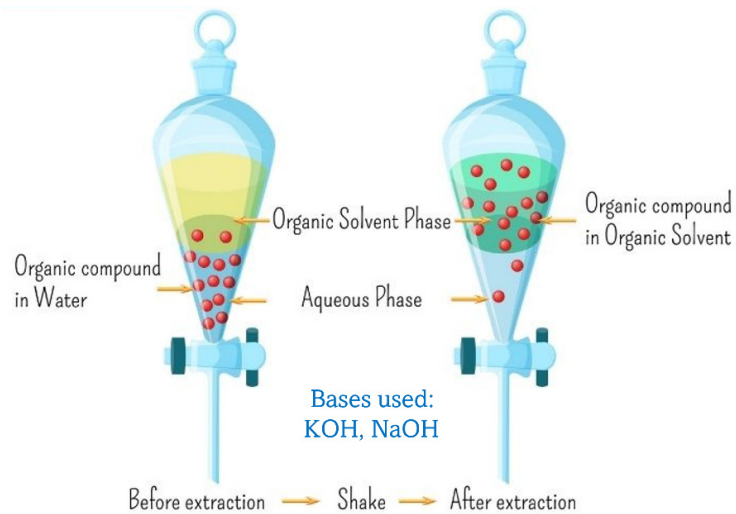


Figure 39. Liquid-liquid extraction system for extraction of carboxylic acids in oils or rock extracts. This technique is a widely used step in sample preparation. However, it has drawbacks when carried out with a separating funnel: multiplication of extraction steps, large volumes of organic solvents, emulsion formation, and traces of eluent in the raffinate requiring additional sample processing before the evaporation stage. Image adapted from https://www.chromtech.com, on November 18th, 2021.

Recently many papers in the literature have been replacing liquid-liquid extraction with ionic liquids (ILs) (**Figure 38**), which are a class of solvents that are generally non-volatile, non-flammable, and thermally stable. ILs have been widely used in purification, electrochemical, extraction, and catalysis processes. This method has been applied to

extract naphthenic acids in crude oils (ANDERSON et al., 2013, 2015; MANDAL; ISKANDAR, 2015; NASIR SHAH et al., 2015; SHI; SHEN; WANG, 2008). In the work of Shi et al. (SHI; SHEN; WANG, 2008), 67% efficiency of naphthenic acids extraction was observed using imidazole derivatives associated with different organic solvents. Anderson et al., (2013), on the other hand, uses tributylmethylammonium, tetrabutylphosphonium, serinate, threonate, cystinate, prolinate, valinate, lysinate, taurinate and bis(trifluoromethanesulfonyl)imide in crude oil to remove naphthenic acids. Anderson et al., (2015) also report extraction efficiencies of 71.1 to 94.8 % using ionic liquids derived from tributylnethylammonium and triethylmethylammonium. Mandal and Iskandar, (2015) obtained a 25% removal efficiency of naphthenic acids using methylimidazolium, 1-hexyl-3-methylimidazolium, and 1-ethyl-3-methylimidazolium, which are derived from imidazole. Nasir Shah et al., (2015) used Tetramethylammonium, Tetrabutylammonium Methyltributylammonium, choline, and tetrabutylphosphonium to recover 95% of the naphthenic acids sold commercially.

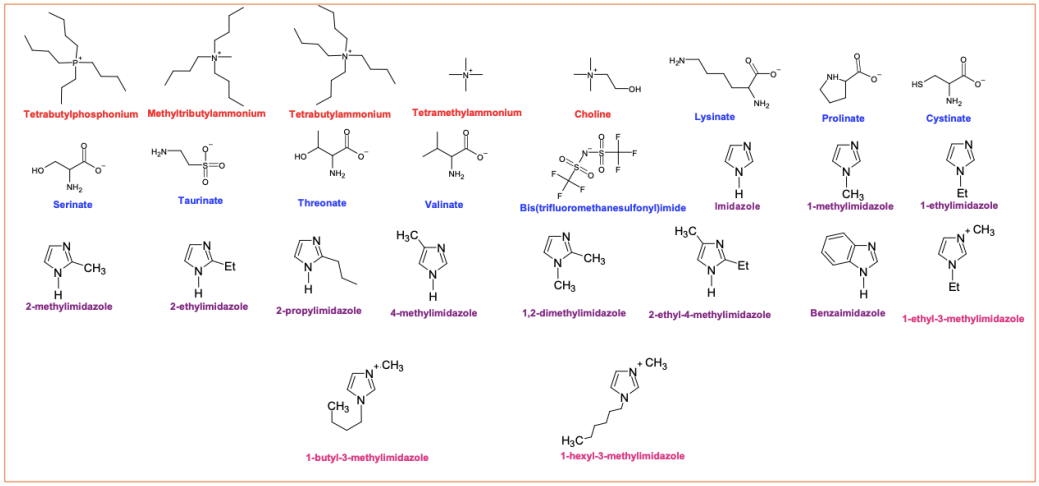


Figure 40. Structure of the main ionic liquids used for extraction of carboxylic acids in petroleum.

A classic method widely used in petrochemical industries is open column chromatography (OCC) and high-efficiency chromatography (HPLC), using silica (SiO_2) as the stationary phase. Isolation by OCC and HPLC provides fractions of saturated compounds, aromatics, resins, and asphaltenes, with an elution gradient. This analysis is called SARA, where the first letter refers to saturates, and the next three letters refer to aromatic hydrocarbon, resins (polar), and asphaltenes (GRUBER et al., 2012).

The use of the normal phase is limited for obtaining acids because the silane groups can promote irreversible adsorption of polar compounds. As an alternative for obtaining acids, many studies started to employ modifications on silica, using bases such as KOH or aminopropyl and cyanopropyl groups (BARAKAT; RULLKÖTTER, 1995; BORGUND;

ERSTAD; BARTH, 2007a; MCCARTHY; DUTHIE, 1962; SCHMITTER; ARPINO; GUIOCHON, 1978).

Barakat and Rullkötter (BARAKAT; RULLKÖTTER, 1995) also used COL with KOH-impregnated silica to isolate acids in five sediment samples from the Niirdlinger Ries. The authors used 1% formic acid in CH_2Cl_2/CH_3OH (99:1) as eluent for acid fraction, obtaining a yield ranging from 10% to 57%, detecting fatty, steranoic and hopanoic acids as methyl derivatives. Some authors have adapted the use of potassium hydroxide modified silica to other glassware such as Soxhlet and pressure-equalizing funnels (or solvent recycling column) (**Figure 41**). An example of the application of this procedure is the work carried out by Pan and Philp (PAN; PHILP, 2006), in which the authors use KOH-modified silica in the Soxhlet-type system for the isolation of carboxylic acids in the oil and rock samples from different basins and geological periods. As eluent, dichloromethane was used for elution of neutral hydrocarbons and ethyl ether/formic acid 20% to extract the acids.

Among the constituents, 17,21-seco-hopanoic, 25-nor-hopanoic, and 28-nor-hopanoic acids have been identified. However, the process is time-consuming and uses high quantities of stationary phase and sample, besides HPLC purification steps for the methylated carboxylic acids. The studies performed by Lima et al. (DE LIMA et al., 2010), Lopes et al. (LOPES et al., 1999), Nascimento et al. (NASCIMENTO et al., 1999), Rodrigues et al. (RODRIGUES; MARSAIOLI, 2000), use the modification on silica with potassium hydroxide in a funnel with pressure equalizer, the same system used by Ramljak et al. (RAMLJAK et al., 1977) and Schmitter et al. (Schmitter et al. (SCHMITTER; ARPINO; GUIOCHON, 1978).

Koike et al. (KOIKE et al., 1992) isolated carboxylic acids from the Marlim and Albacora wells of the Campos Basin, using open column chromatography with KOH-impregnated silica, obtaining a yield of 3.5% and 1.8%. To analyze the acidic fraction by GC-MS, the authors used diazomethane for esterification and reduction to hydrocarbon, detecting fatty acids, tricyclic acids, and hopanoic acids as hydrocarbon derivatives.

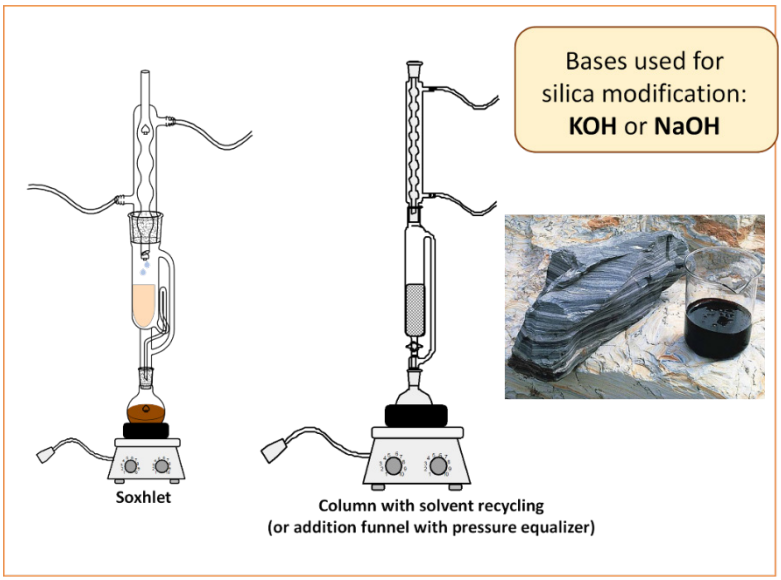


Figure 41. Soxhlet and column with solvent recycling (or addition funnel with solvent recycling). Systems used to extract carboxylic acids in oils and rock extracts with base-modified silica. Oil shale (right image), is generally described as a fine-grained sedimentary rock containing organic matter that, upon destructive distillation, yields significant amounts of oil and combustible gas.

The studies conducted by Mccarthy and Duthie (MCCARTHY; DUTHIE, 1962) and Keeney (KEENEY, 1954) were some of the first studies to apply a Lewis base to modify a stationary phase in order to extract carboxylic acids. In these works, the authors use the KOH base to modify silicic acid for the isolation of fatty acids in soybean oil and butter. Other authors have adapted the methodology for use in the isolation of carboxylic acids in petroleum and rocks, for example, the works of Jaffé et al. (JAFFE; ALBRECHT; OUDIN, 1988; JAFFÉ; ALBRECHT; OUDIN, 1988), Jaffé and Gallardo (JAFFÉ; GALLARDO, 1993), Farrimond et al. (FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002).

Some works also report the use of high-efficiency chromatography (HPLC) for the isolation of acids. In this methodology, columns with stationary phases with different bonded organic groups are used, silane groups, such as NH_2 and CN. Borgund et al. (BORGUND; ERSTAD; BARTH, 2007b, 2007a), Green (GREEN, 1986), Green et al. (GREEN et al., 1985), use HPLC equipped with specific columns to obtain an acid fraction. Green et al. (GREEN et al., 1985), developed a method for HPLC isolation of acids, using a normal phase column specific for acids, to perform the isolation in the shortest possible time. The authors identified only tricyclic acids of molecular weights 320, 334, and 376.

More recently, solid-phase extraction (SPE) has been gaining attention for the isolation of carboxylic acids in sediments and oils. The advantages of using this method are numerous for the petrochemical industry, such as uniformity of the stationary phase, low

solvent consumption, and speed in the separation process.

The SPE method (**Figure 42**) consists of solid particles (stationary phase) contained in a cartridge-type device. Cartridges with a strong anion exchanger, SAX (Strong Anion Exchanger), e.g., SPE filled with quaternary amine, are generally used for obtaining acid fractions in sediments and oils. Many studies in the literature use SPE for the isolation of carboxylic acids in sediments and oils, such as Jones et al. (JONES et al., 2012, 2001), Lamorde et al. (LAMORDE; PARNELL; BOWDEN, 2015), Sessions et al. (SESSIONS et al., 2002), Zhu et al. (ZHU et al., 2017).

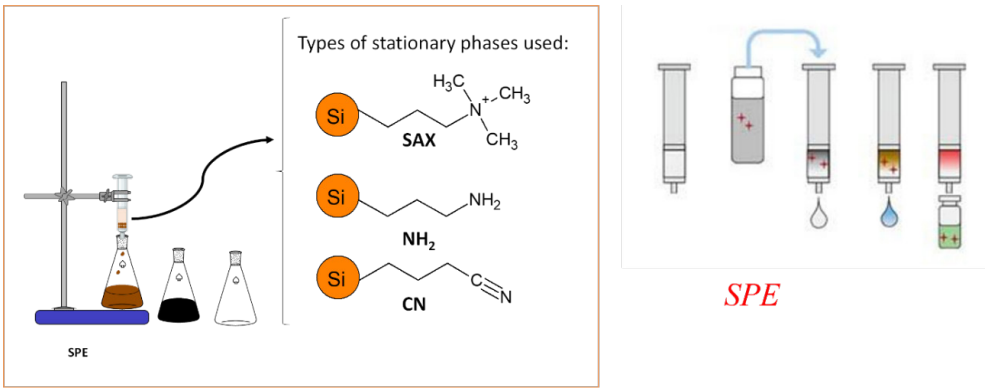


Figure 42. Solid-phase extraction (SPE) system and the stationary phases used to isolate carboxylic acids in petroleum and rock extractor. SPE uses a solid phase material (there are many to choose from) that retains the interfering substances, while solvents elute the sample, which is collected and analyzed.

Jones and co-workers (JONES et al., 2001) used quaternary amine SPE to isolate carboxylic acids in oil samples. To extract the acids, the eluents used were hexane, to extract the apolar hydrocarbons, and ethyl ether/formic acid 2%. 1-adamantane carboxylic acid and 5β(H)-cholanic acid were used in the procedure to evaluate recovery. The authors detected fatty, naphthenic, and hopanoic acids. **Table 3** summarizes the papers reporting various methods for the isolation of acidic compounds, the economic viability of their application in routine crude oil analysis was considered. The research was performed in Science Direct, Scopus, and Wiley databases and the citations of the papers found.

Table 3. Some studies reporting the different methods of extraction of carboxylic acids in oils and sediments

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Soy and butter oils	Classic open silicic acid/KOH column (selected particles only)	F1 - Et_2O F2 - Et_2O/form. acid 2%	Methylation (N.I.).	*n*-Alkanoic acids	(MCCARTHY; DUTHIE, 1962)	Stationary phase of high economic cost. Slow chromatographic process because of the smaller particle diameter. No profiling, so a further particle selection step is required before use. Recovery: 96-99.6%.
Oil from the Midway-Sunset Field 31E, Canada.	Isopentane NaOH 1% in ethanol 70%.	N.A.	Analyzed as methyl esters (CH_2N_2).	Alkyl-phenanthrenes Carboxylic acids	(SEIFERT; HOWELLS, 1969)	Multi-step extraction. No profiles. Yield of the acid fraction: 2.5% (w/w). No recovery.
	Isopentane NaOH 1% in ethanol 70%, followed by preparative plate.	N.A.	Multi-step derivatization: analyzed as methyl esters (BF_3/etherate) and then reduced to hydrocarbons ($LiAlH_4$).	Terpenoid acids Monoaromatic acids Polyaromatic acids Naphthenic acids	(SEIFERT; TEETER, 1969)	Multi-step extraction. It presents only direct infusion profiles. Yield of the acid fraction: 2.5% (w/w). Recovery as methyl esters: 5% (w/w).
	Isopentane NaOH 1% in ethanol 70%, followed by preparative plate. Reduction to hydrocarbons.	N.A.	Multi-step derivatization: analyzed as methyl esters (BF_3/etherate) and then reduced to hydrocarbons (LiAlH4).	Polycyclic and heterocyclic aromatic acids	(SEIFERT; TEETER, 1970)	Multi-step extraction. No profiles. Yield of the acid fraction: 2.5% (w/w). Low recovery (40%).
	Isopentane NaOH 1% in ethanol 70%, followed by preparative plate. Reduction to hydrocarbons.	N.A.	Multi-step derivatization: analyzed as methyl esters (BF_3/etherate) and then reduced to hydrocarbons ($LiAlH_4$ e $LiAlD_4$).	Steranoic acids C_{22} and C_{24}	(SEIFERT; GALLEGOS; TEETER, 1972)	Multi-step extraction. Yield of the acid fraction: 2,5% (w/w). 40% recovery of all petroleum acids.
Oil from the Midway-Sunset Field 31E, Canada.	Isopentane NaOH 1% in ethanol 70%, followed by preparative plate. Reduction to hydrocarbons. Combination of silica gel and gel permeation chromatography: Waters gel.	N.A.	Multi-step derivatization: analyzed as methyl esters (BF_3/etherate) and then reduced to hydrocarbons ($LiAlH_4$).	Polycyclic naphtenic acids mono-, di-, and polynuclear aromatic acids mono- and dibenzothiophene acids Carbazolic acids Phenolic acids	(SEIFERT et al., 1969)	multi-step extraction. Yield of the acid fraction: 2,5% (w/w). 40% recovery of all petroleum acids.
Asphalt, supplied by the INA Petroleum refinery Rijeka, Yugoslavia.	Solvent-recycled column with silica/KOH 9.1% phase	F1 – $CHCl_3$ F2 - $CHCl_3$/form. acid 20%	Analyzed as methyl esters (CH_2N_2).	N.I.	(RAMLJAK et al., 1977)	Multi-step extraction, using a lot of material and time-consuming processing. Presents IR profile only. Yield of acid fraction: 0.36-4.9% (w/w). No recovery.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Petroleum from Nigeria and Germany.	Solvent recycling column with the silica/KOH phase.	F1 - Et_2O F2 - Et_2O/form. acid 20%.	Analyzed as methyl esters (BF3/MeOH).	*n*-Alkanoic and Hopanoic Acids	(SCHMITTER; ARPINO; GUIOCHON, 1978)	Multi-step extraction, using lots of samples and solvents. Time-consuming processing. Profiles show probable separation inefficiency Yield of acid fraction: 0.3-5% (w/w). No recovery data.
Oil from the fields of Anguille (Gabon), Bati-Raman (Turkey), Boscan (Venezuela), Grenade (Aquitaine, France).	Reflux followed by liquid-liquid extraction with KOH and diethyl ether.	N.A.	Multi-step derivatization: analyzed as methyl esters (CH_2N_2) and then reduced to hydrocarbons ($LiAlH_4$).	*n*-Alkanoic acids, tricyclic acids, and hopanoic acids	(BEHAR; ALBRECHT, 1984)	Multi-step extraction, using a lot of samples, shows only profiles of acids reduced to hydrocarbons. Saponification yield: 0.28-2.87% (w/w).
Sediments of the Priest Pot, English Lake District	Liquid-liquid extraction with aqueous KOH and hexane-ethyl ether.	N.A.	It was analyzed as methyl esters (BF3/MeOH). BSTFA for alcohols and acids with hydroxyl group.	Sterols	(ROBINSON et al., 1984)	Multi-step extraction. No profiles. The acid fraction yields obtained range from 6.4 to 127 μg/L and 10.4 to 44 μg/g.
Sediments from Bransfield Strait, Antarctic Peninsula.	Liquid-liquid extraction with 0.5 M KOH in MeOH, H_2O 1:1)	N.A.	Analyzed as methyl esters (BF_3/MeOH).	*n*-Alkanoic acids Sterols Hopanoic acids	(VENKATESAN; KAPLAN, 1987)	Very long sample processing time and no acid profiles. Recovery of *n*-alkanoic acids: 40-85%.
Standard lipid mixture	Aminopropyl column	obtaining multiple fractions with an elution gradient	Analyzed as methyl esters (BF_3/MeOH).	Only standards used.	(KALUZNY et al., 1985)	Many fractions. No chromatographic profiles. Good recovery: 98.4-101.4%.
Oils were obtained from the Tulare, Beta, Santa Maria fields and wastewater from a refinery (California)	Liquid-liquid extraction with pentane. NaOH 1% in 70% ethanol.	N.A.	N.I.	Paraffins Naphthenic Acids Polycyclic Acids	(DZIDIC et al., 1988)	Multi-step extraction. Presents chemical ionization profiles. Yield of the acid fraction: 0.7-3.5% (w/w).
Oils and coals from the Mahakam field Delta, Indonesia, and oil from Nigeria.	Classic open column with silicic acid/KOH	F1 - Et_2O F2 - Et_2O/formic acid 2%	Analyzed as methyl esters (CH_2N_2).	*n*-Alkanoic acids Hopanoic acids.	(JAFFE; ALBRECHT; OUDIN, 1988)	Slow chromatographic process because of the smaller particle diameter. Time-consuming preparation of the stationary phase. Good chromatographic profiles of hopanoic acids.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Oils and coals from the Handil fields, Mahakam Delta, Calimantan, Indonesia.	Classic open column with silicic acid/KOH.	F1 - Et_2O F2 - Et_2O/form. acid 2%	Analyzed as methyl esters (CH_2N_2).	*n*-Alkanoic acids Hopanoic acids.	(JAFFÉ; ALBRECHT; OUDIN, 1988)	Slow chromatographic process because of the smaller particle diameter. Time-consuming preparation of the stationary phase. Good chromatographic profiles of hopanoic acids.
Oils, Marlim and Albacora fields, Campos Basin, Brazil	Solvent recycling column using KOH-modified stationary phase.	F1 - Et_2O F2 - Et_2O/form. acid 20%	Analyzed as methyl esters (CH_2N_2)	**As hydrocarbon derivatives:** *n*-Alkanoic acids Tricyclic acids Hopanoic acids	(KOIKE et al., 1992)	methodology with high loading of stationary phase and sample. Presents just profiles of acids reduced to hydrocarbons. acid fraction: 1.8-3.5%. No recovery.
Petroleum, La Luna Formation, Maracaibo Basin, Venezuela.	Solvent recycling column with 9.1% silica/KOH phase	F1 - $CHCl_3$ F2 - $CHCl_3$/form. acid 20%	Analyzed as methyl esters (CH_2N_2)	*n*-Alkanoic acids Hopanoic acids.	(JAFFÉ; GALLARDO, 1993)	Stationary phase of high economic cost. A further particle selection step is required before use. Slow chromatographic process because of the smaller particle diameter. No profiling, no yield and recovery.
Sediments (core) of the Nördlinger Ries **Crater, Germany**	Classic open column with silica/KOH	Free fatty acids: extracted from silica/KOH column with 1% formic acid in DCM/MeOH (99:1) Fatty acids in the polar fraction: eluted with MeOH. Saponified and extracted with DCM; fractionated again on silica/KOH column. Fatty acids from kerogen: Extracted with DCM/MeOH (1:1). Saponified and extracted with DCM and fractionated again on silica/KOH column.	Analyzed as methyl esters (CH_2N_2).	*n*-Alkanoic acids Steranoic acids Hopanoic acids.	(BARAKAT; RULLKÖTTER, 1995)	Multi-stage extraction. Use of high quantities of materials. Does not present detailed profiles of detected acids. Does not present yields and recovery
Sediments (core) from Sacred Lake, Mount Kenya, Kenya.	Solid-phase extraction with Aminopropyl Bond Elute®.	F1 - DCM/iPOH F2 - DCM/HOAc 2%	Analyzed as trimethylsilyl esters (BSTFA)	*n*-Alkanoic acids Linear alcohols	(HUANG et al., 1999)	Fast process, but only obtains *n*-alkanoic acids. Does not show yields for the acid fraction.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Petroleum from the Fazenda-Belém field, Açu **Formation, Potiguar Basin.**	Solvent recycling column using KOH-modified stationary phase.	Extraction with Et_2O, followed by 20% Et_2O/formic acid	**Two types of derivatization:** as methyl esters (CH_2N_2) and as hydrocarbon derivatives ($LiAlH_4$ e $LiAlD_4$)	**As methyl esters:** *n*-Alkanoic acids **As hydrocarbon derivatives:** Tricyclic and hopanoic acids. Homologous series of 3-alkyl and 3-carboxyalkyl-5α(H) steranes 3-alkyl and 3-carboxyalkyl-5β(H) ssterane series 4-Carboxyalkyl sterane series	(LOPES et al., 1999)	Methodology with high loading of stationary phase and sample. Many processing steps and time-consuming. Excellent profiles, but the procedure is impractical for large numbers of samples. Multi-step derivatization for analysis as hydrocarbons.
Oils from the Albacora Field, and the Lagoa Feia Formation, Campos Basin, Brazil	Solvent recycling column using KOH-modified stationary phase.	F1 – DCM F2 - Et_2O/form. acid 20%	**Mult-step derivatization:** as methyl esters (CH_2N_2) and then reduced to hydrocarbons ($LiAlH_4$ and $LiAlD_4$).	*n*-Alkanoic acids Carboxylic acids Acyclic acids Bicyclic acids Tricyclic acids Hopanoic acids Norhopanoic acids	(NASCIMENTO et al., 1999)	methodology with high use of stationary phase and solvent. Processing time greater than 8 h. Shows only profiles of acids reduced to hydrocarbons.
Oils from the Muribeca-Carmopolis Formation, Sergipe-Alagoas Basin, Brazil.	Solvent recycling column using KOH-modified stationary phase	F1 - DCM F2 - Et_2O/form. acid 20%	multi-step derivatization: as methyl esters (CH_2N_2) and then reduced to hydrocarbons ($LiAlH_4$ and $LiAlD_4$).	*n*-Alkanoic acids isoprenoid acids Hopanoic acids Alkanoic Steroid	(RODRIGUES et al., 2000)	methodology with high use of stationary phase and solvent. Processing time longer than 8 h. Yield of acid fraction: 1.5-3.0%. No recovery data.
Oil from fields in the UK, Italy and California	New SPE in quaternary mine - strong anion exchanger	F1 - Hex F2 - DCM F3 - Et_2O/form. acid 2%	It is analyzed as methyl esters (BF_3/MeOH), purified on a second silica SPE column.	*n*-Alkanoic acids Hopanoic acids	(MEREDITH; KELLAND; JONES, 2000)	Methodology with 90% extraction efficiency of 5β(H)-cholanic acid. The profiles presented are only of *n*-alkanoic acids and hopanoic acids from C_{30} to C_{32}. There is no yield of the respective fractions, and no information on the identity of the stationary phase.
Oil provided by Enterprise Oil plc (U.K.). The origin of the oil is not informed.	Quaternary amine SPE ion exchange column (SAX), named as new phase, but not specified	F1 - Hex F2 - DCM F3 - Et_2O/form. acid 2%	Analyzed as methyl esters (BF_3/MeOH).	*n*-Alkanoic acids Naphthenic acids Hopanoic acids	(JONES et al., 2001)	Time-consuming extraction process, with 90% recovery of 5β(H)-cholanic acid.
Bacterial culture of *M. capsulatus*	Aminopropyl SPE ion-exchange column (SAX)	F1 - DCM/Ace (9:1) F2 - DCM/form. acid (50:1)	Analyzed as methyl esters (MeOH/ MeCOCl).	*n*-Alkanoic acids Esterols Hopanols	(SESSIONS et al., 2002)	Fast extraction process, but no acidic biomarker data.
Light Arabian Oil (supplied by AEA Technology PCL), Seawater and marine sediment collected in the UK	Liquid-liquid extraction with KOH and purification of the acids on a quaternary amine ion-exchange column (SPE - SAX)	Et_2O/form. acid 2%	Analyzed as methyl esters (BF_3/MeOH).	*n*-Alkanoic acids Hopanoic acids (C_{29}-C_{32}). C_{27} and C_{28} are uncertain.	(WATSON et al., 2002)	The rapid extraction process, but the phase has a very high cost. A range of hopanoic acids from C27 to C32 were detected in work.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Sedimentary rocks from Italy, United Kingdom, Tunisia, Isle of Skye and Switzerland	Classic open column with silicic acid/KOH	F1 - Et_2O F2 - Et_2O/form. acid 2% F3 - Et_2O	Analyzed as methyl esters (BF_3/MeOH).	Hopanoic acids	(FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002)	Time-consuming process but with acid values ranging from 0.4 to 66.6 ppm.
Heavy oil from the Marlim field Campos Basin	Liquid-liquid extraction with NaOH	Hex	Analyzed as silylated derivatives (*t*-BDMS)	Naphthenic acids	(VAZ DE CAMPOS et al., 2006)	multi-step process, only naphthenic acids were identified. The yield obtained is 0.98±0.01%.
Oil and rock from Russia, California, USA, and Seychelles	Soxhlet extraction using KOH-modified silica	F1 - DCM F2 - Et_2O/form. acid 20%	Analyzed as methyl esters (BF_3/MeOH).	17,21-seco-hopanoic acids 25-nor-hopanoic acids 28-nor-hopanoic acids.	(PAN; PHILP, 2006)	Time-consuming process and uses high amounts of stationary phase and HPLC purification steps.
Recent sediments from Toyama Bay (Japan Sea) and from Lake Baikal (Rússia)	Reflux with 1N KOH/MeOH (30 mL)/ Dichloromethane (15 mL)	N.A.	Analyzed as silylated derivatives (*t*-BDMS)	Alcohol Sterols *n*-Alkanoic acids Hopanoic acids	(NISHIMURA et al., 2006)such as alkanols, sterols, diols, keto-ols and hydroxy fatty acids (FAs	Methodology with a long extraction time. No profiles for carboxylic acids. To improve the fractionation efficiency for hydroxylated compounds, the authors derivatized the fresh sediment extracts with t-BDMS
Recent sediments collected in the Nankai Trough	Saponification with KOH/MeOH	Saponification with KOH/MeOH	**Two types of derivatization:** as methyl esters (BF_3/MeOH) and as trimethylsilyl esters (BSTFA) and acetylated (Acetic anhydride/Pyridine)	**Methyl esters:** Hopanoic acids **Trimethylsilyl: esters:** Hopanols	(SAITO; SUZUKI, 2007)	A process with very long extraction time shows hopanoic acid profiles and reports the presence of pentaquis-homo-hopano-32,33,34,35-tetrol in the samples.
N.I.	Four types of SPE, filled with aminopropyl and using cartridges of different materials: 2 high-density polyethylene cartridges; 1 Teflon-like fluorinated polymer cartridge; 1 glass cartridge, prepared by hand	F1 - DCM/iPOH (2:1) F2 - Et_2O/HOAc 4%	Analyzed as methyl esters (MeOH/ MeCOCl)	*n*-Alkanoic acids	(RUSSELL; WERNE, 2007)	Fast process, but the authors only emphasize the presence of fatty acids.
Safaniya (Middle East), Ardjuna (Java), Cold Lake and Pelican Lake (Canada) and a West African oil and Potiguar basin (Brazil)	Liquid-liquid extraction with KOH	cyHex DCM	Multi-step derivatization: as methyl esters (CH_2N_2) and as hydrocarbon derivatives ($LiAlH_4$ e $LiAlD_4$)	*n*-Alkanoic acids Naphthenic acids Hopanoic acids	(FAFET et al., 2008)	Multi-step extraction. The authors present only the fatty acid profiles

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Crude oil from Beijiang, China	Liquid-liquid extraction with ionic liquids	2-methylimidazole in EtOH 20% (w/w)	N.I.	N.I.	(SHI; SHEN; WANG, 2008)	Multi-step extraction No chromatographic profiles and does not mention identified compounds.
Oil collected in the Pampo field, Campos Basin	Solvent recycling column using KOH-modified stationary phase	F1 - Et_2O F2 - Et_2O/form. acid 20%	**Multi-step derivatization:** as methyl esters (CH_2N_2) and then reduced to hydrocarbons ($LiAlH_4$ e $LiAlD_4$)	Tricyclic acids Hopanoic acids Steranoic acids	(DE LIMA et al., 2010)	A very long extraction procedure using a high mass of stationary phase. The yield of the isolated acid fraction is not shown.
Recent sediments from Lake El Junco, Galapagos Islands.	Aminopropyl SPE ion exchange column (SAX)	F1 - DCM/iPOH (3:1) F2 - Et_2O/HOAc 4%	Analyzed as trimethylsilyl esters (BSTFA)	Alcohol Diols Triols Cetones Acetols Ceto-acids Hydroxy-acids Esterols	(ZHANG; METZGER; SACHS, 2011)	The SPE method provides a fast extraction process, but the type of phase used usually has economic elevator. In addition, the authors only present chromatographic profiles of structures with up to two hydroxyls. The system applied to recent sediments.
Padrões de ácidos Naftênicos sintetizados e amostras OSPW (areias petrolíferas)	Silver ion-exchange column SPE	F1 - Hex F2 - Hex/Et_2O 5% F3 - Hex/Et_2O 10% F4 - Et_2O 100%	Analyzed as methyl esters (BF_3/MeOH)	Aromatic naphthenic acids	(JONES et al., 2012)	The extraction procedure is quick, but the type of SPE cartridge used has a high economic value. The authors present GC × GC chromatographic profiles and mass spectra for aromatic naphthenic acids, but few signs are attributed. Another observation is about the recovery of materials applied in the SPE; the authors confirm the recovery, but in different fractions, causing solvent waste.
Crude oil supplied by the Petronas company from the Chad field in Central Africa	Liquid-liquid extraction with ionic liquids	Tributylmethylammonium hydroxide Tetrabutylphosphonium hydroxide	N.I.	Naphthenic acids	(ANDERSON et al., 2013)	Multi-step procedure. Distribution profiles of naphthenic acids are not shown. The authors do not comment on extraction yield. The infrared profile is unique to the authors.
Rocks were collected on the Abu Tartur plateau (Maghrabi-Liffiya sector) in the Western Desert of Egypt.	Ion Exchange Column (SPE) Supelco DSC-NH	F1 - Hex F2 - Hex: DCM (3:1) F3 - DCM/Ace (9:1) F4 - DCM/form. acid 2%	Analyzed as methyl esters (BF_3/MeOH).	*n*-Alkanoic acids Hopanoic acids	(EL-SHAFEIY et al., 2014)	The SPE method provides a fast extraction process, but the type of phase used usually has high economic value. Profiles to show the distribution of hopanoic acids are not present.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Oil from the Niger Delta Basin (Nigeria).	Quaternary Amine SPE Ion Exchange Column (SAX)	F1 - Hex/MeOH F2 - Et_2O/form. acid 2%	Analyzed as methyl esters (BF_3/MeOH).	Hopanoic acids norhopanoic acids	(LAMORDE; PARNELL; BOWDEN, 2015)	The SPE method provides a fast extraction process, but the phase used usually has high economic value. Profiles for identified acidic biomarker classes are presented. Data on yields of the acid fraction and recovery are not presented to evaluate the extraction efficiency.
Commercial mixture of naphthenic acids	Liquid-liquid extraction with ionic liquids	tetramethylammonium hydroxide tetrabutylammonium hydroxide Methyltributylammonium hydroxide choline hydroxide tetrabutylphosphonium hydroxide	N.I.	Naphthenic acids	(NASIR SHAH et al., 2015)	The procedure consists of multi-steps; profiles with the distribution of naphthenic acids are not present. The authors do not comment on extraction yield.
Peat collected in Dajiuhu city in China.	Liquid-liquid extraction with KOH	N.I.	**Multi-step derivatization:** analyzed as methyl esters (BF_3/MeOH) and as trimethylsilyl esters (BSTFA).	**Methyl esters:** Hopanoic acids **Trimethylsil:** Hopanols **Underivatized:** Hopanones	(HUANG et al., 2015)	The procedure consists of multi-steps. Chromatographic profiles of the distribution of hopanoic acids, hopanols, and hopanones are shown.
Doba crude oil supplied by the Petronas company from the Chad field in Central Africa.	Liquid-liquid extraction with ionic liquids	Triethylmethylammonium methylcarbonate Triethylmethylammonium hydrogencarbonate Tributylmethylammonium methylcarbonate tributylmethylammonium hydrogencarbonate	N.I.	Monocyclic and tetracyclic naphthenic acids (C_{12} a C_{40})	(ANDERSON et al., 2015)	The authors test different proportions between the ionic liquid and the samples, obtaining an efficiency between 71% and 95%.
Crude oil collected in Beijiang city in China.	Liquid-liquid extraction with ionic liquids	Imidazolium chloride	N.I.	Naphthenic acids	(MANDAL; ISKANDAR, 2015)	The multi-step process, a material used at a very high cost, has only an infrared profile.

Sample Type	Extraction Method	Eluents/Solvent[a]	Derivatizing Reagent	Identified classes	References	Remarks
Synthetic patterns of acids with smaller molecular structures and petroleum	Amino functionalized silica micro-SPE	TFA/EtOAc (1/99, v/v).	Analyzed as silylated derivatives (MTBSTFA)	Low Molecular Mass Standards	(ZHU et al., 2017)	Fast microscale process, using adsorbent in a *Pasteur* pipette, but the authors do not present acid biomarker profiles.
Acidic oil degradation products spilled into waters at the port of Svanemøllehavnen in Denmark	q-p (NVI-EGDMA): *imidazole-based polymer.* Oasis MAX (Waters): *Divinyl-benzene and vinyl-pyrrolidone copolymer* Strata XA 9 (Phenomenex): *non-polar, fully aromatic poly(styrene-divinylbenzene) polymer.*	MeOH/form. acid (1-10%)	N.I.	Low molecular weight mono- and dicarboxylic acids	(SCHEMETH; NIELSEN; CHRISTENSEN, 2018)	The authors use several high-cost adsorbents for extracting naphthenic acids to analyze in LC-MS and LC-DAD.
Rocks in an open clay pit near Lias d, Amaltheenton Formation	Saponified using 6% KOH in methanol	Hex	Analyzed as methyl esters (TMCS/MeOH)	*n*-Alkanoic acids Hopanoic acids	(THIEL; HOPPERT, 2018)	The authors do not present individual profiles for each class of acidic compounds identified in the rock samples, only the total ion chromatogram.
Crude oil and Refined Products from the US, Canada, Alaska, and the Gulf of Mexico	Silica gel column	F1 - Hex F2 - DCM/Hex (1:1) F3 - DCM/Ace (98:2) F4 - DCM / MeOH/form acid (50:50:0,1)	N.I.	Naphthenic acids	(YANG et al., 2019)	The fractionation procedure was carried out in a sample, the analyses of the fractions obtained were carried out in LC-MS. As *surrogate* standards, the authors used deuterated *n*-alkanoic acids. However, chromatographic profiles and mass spectra of isolated naphthenic acids are not shown.

[a] Fn (n = 1, 2, 3, ...) is the fraction number in the methodology.

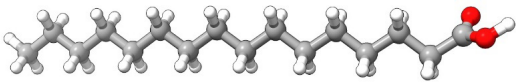
N.A.: not applicable.

N.I.: not informed.

CHAPTER 3

A CASE STUDY: AN ALTERNATIVE METHOD FOR THE EXTRACTION AND ANALYSIS OF ACIDIC MOLECULAR FOSSILS FROM CRUDE OIL

Three oil samples of different origins and biodegradation were analyzed concerning their molecular composition, neutral and acid. We apply a new methodology for the separation and analysis of neutral hydrocarbons, without the use of chlorinated solvents or modified silica, with quantitative separation of saturated and aromatic hydrocarbons. Furthermore, we present a simple methodology for isolating acidic biomarkers, using silica modified with potassium hydroxide in SPE, using a few milligrams of crude oil.



1 | A CASE STUDY: AN ALTERNATIVE METHOD FOR THE EXTRACTION AND ANALYSIS OF ACIDIC MOLECULAR FOSSILS FROM CRUDE OIL

1.1 Introdution

The major focus of petroleum geochemical research is on the presence or distribution of some biomarkers into saturated hydrocarbons fractions. This is explained by easy analysis using Gas Chromatography-Mass Spectrometry (GC-MS), for example (PETERS; WALTERS; MOLDOWAN, 2005a). However, less attention is given to other fractions containing compounds besides hydrocarbons, such as oxygen, nitrogen, and sulfur heteroatoms which, at first, provide complementary information about OM deposition, and at last, provide information on the geological history associated with such deposition.

Although there are fewer studies compared to saturated or aromatic fractions, many research groups have been interested in petroleum polar fractions, mainly the acid fraction (AF). Many works are reporting carboxylic acids identification from sedimentary rock samples (BARAKAT; RULLKÖTTER, 1995; EL-SHAFEIY et al., 2014; HUANG et al., 1999; LI et al., 2015; NISHIMURA et al., 2006; SAITO; SUZUKI, 2007; THIEL; HOPPERT, 2018; VENKATESAN; KAPLAN, 1987). Farrimond et al. (FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002) report fatty and hopanoic acid composition from thirty rock samples spanning Triassic to Cretaceous.

In the Brazilian context several studies were performed using oil samples from many basins across the east coast (DE LIMA et al., 2010; LOPES et al., 1997, 1999; NASCIMENTO et al., 1999; RODRIGUES et al., 2000). Besides the extreme complexity of component mixtures, the AF is composed mainly of carboxylic acids, which have been analyzed using an approach based on their conversion to their equivalent hydrocarbons ($R–COOH \rightarrow R–CH_3$), making the GC-MS analysis easier. However, the complexity of AF, the laborious techniques of separation, purification, and derivatization needed have been delayed the research toward the understanding of distribution of these compounds and, consequently, studies highlighting the organic geochemistry relevance of AF are limited.

In this sense, methodologies that separate the acid components fast, quantitatively, and using a few steps are desirable. Among the methodologies used to recover AF, the most used are liquid-liquid (LLE) and solid-phase (SPE) extractions (GRUBER et al., 2012).

The first articles to use LLE on carboxylic acid isolations were the studies conducted by Seifert and coworkers (SEIFERT; GALLEGOS; TEETER, 1972; SEIFERT; HOWELLS, 1969; SEIFERT; TEETER, 1969, 1970). However, the method used on those papers demands a significant amount of time to prepare the samples, besides a large solvent volume. Because of these drawbacks, the SPE, which use less solvent volume and demands lesser analyst time, have been used by industries and many other fields, including petrochemical applied in the analyses of AF from oils and rocks. as reported by several authors (JONES et al.,

2001; LAMORDE; PARNELL; BOWDEN, 2015; SESSIONS et al., 2002; ZHU et al., 2017)

In this context, the main aim of this unit is to present a method for extraction of acidic biomarkers in oil samples, using a classic stationary phase (in SPE), which is easy and inexpensive to prepare. Furthermore, to perform a detailed study of the molecular composition of aromatic and acid hydrocarbons in the geochemical characterization of three oil samples, of different depositional paleoenvironments, from Brazilian marginal basins. Some figures, images and/or text in this chapter were adapted from a paper published by our research group, and more details can be seen in Sousa et al. (2022)

1.2 Experimental

Three oil samples, identified as A, B, and C, were provided by PETROBRAS Research and Development Center (CENPES), Rio de Janeiro, Brazil, without any information about origin, formation, biodegradation, or maturity (Sousa et al., 2022).

1.2.1 Neutral fractions preparation

For neutral (non-acid) biomarkers analysis, aliquots of 40 mg of each oil sample were fractionated using open chromatographic columns using silica gel (60–230 mesh) using 1:70 (m/m) sample/silica ratio.

The fractions were eluted using *n*-hexane (10 mL, saturated fraction), *n*-hexane/ethyl acetate (92:8 v/v, 30 mL, aromatic fraction), *n*-hexane/ethyl acetate (88:12 v/v, 30 mL, heterocomponentes-containing fraction), and ethyl acetate/methanol (95:5 v/v, 20 mL, polar fraction). All procedure was performed in triplicates.

1.2.2 Acid Fractions Preparation

A stationary phase based on silica and KOH was prepared to extract acid biomarkers from oil samples. 5 g of KOH was added to an Erlenmeyer containing 100 mL of isopropanol and manually mixed at 80 °C using a water bath until dissolution. 45 g of silica gel, previously activated, was gently added and manually homogenized, and manually homogenized by 1 minute more. Then, the silica KOH-modified 10%-weight mixture was vacuum filtered and solid dried on the oven at 100 °C at least two hours before use.

For extractors assembly, 5 g of silica gel KOH-modified were transferred to a glass small (ø 20 mm) column. The SPE extractors were coupled to the vacuum system eluted three times with 10 mL of ethyl ether to pack and clean up the stationary phase. For acid biomarkers analysis, aliquots of 250 mg of each oil sample were spiked with five μg of ibuprofen (internal standard) and 2.5 μg of homopregnanic acid (5β(H)-pregnane-20*S*-carboxylic acid, surrogate standard) and submitted to SPE extraction. For elution, 80 mL of ethyl ether was used to remove saturated and aromatic hydrocarbons, and 60 mL of ethyl

ether/formic acid 95:5 (v/v) was used for the acid fraction (AF) recovery.

Before GC-MS analysis, acid compounds were converted to their trimethylsilyl (TMS) esters. About 4–7 mg of each sample was added to a 5 mL one-neck rounded bottom flask containing a stirrer bar, added 200–350 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 200 μL of pyridine using a syringe. The flask was placed on sand bath at 60 °C for 60 min. Blank runs for SPE KOH-modified extractions were carried out. In this case, only the standards (ibuprofen and homopregnanic acid) were eluted from SPE and derivatized the same way as oil samples. After that, both derivatized AF and blank runs were analyzed using GC-MS

1.2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

For neutral biomarkers GC-MS analysis, only saturated and aromatic fractions were used. Before GC-MS analysis about 1 mg each fraction was spike with 5 μg of 3-methylheneicossane (*anteiso*-docosane, *ai*-C_{22}, ULTRA Scientific) and 500 ng of 5β(H)-cholane (Chiron) (saturated fraction) and 5 μg de anthracene-d10 (Sigma-Aldrich) (aromatic fraction) for quantification purposes, without any correction for possible response differences between classes. The saturate and aromatic fractions were analyzed using GC-MS at a concentration of 1 mg mL^{-1} in *n*-hexane.

All GC-MS analyses were carried out using a GCMS-QP2010SE, AOC-500 (Shimadzu, Japan) instrument. For saturated and aromatic fractions, chromatographic separation was achieved on an SLB-5MS (30 m × 0.25 mm diameter × 0.1 μm film thickness, Sigma-Aldrich) using two different temperature programs. For the saturated fractions, the temperature program was initially held at 60 °C for 1 min, then ramped at 4 °C per minute to 315 °C and held for 15 min for a total run time of 79.75 min including a 5 min for solvent cut time. For aromatic fractions, the temperature program was the same, except the final temperature which was 315 °C held for 25 min (89.75 min total run time). The injection method was split with a ratio 1:10 with helium (99.9999%) flow rate of 1.0 mL min^{-1} as carrier gas. The injector temperature was held at 290 °C, and transfer line temperature was held at 320 °C. For quadrupole was held at 230 °C and operated at 70 eV electron ionization on a full scan (*m/z* 57–600 mass range) and SIM modes.

For acid fractions, the chromatographic separation was achieved on a DB-5MS UI (30 m × 0.25 mm diameter × 0.25 μm film thickness, Agilent Technologies) using the same oven program for aromatic fractions. The injection method was split with a ratio 1:1 with helium (99.9999%) flow rate of 1.0 mL min^{-1} as carrier gas. The injector temperature was held at 290 °C, and the transfer line temperature was held at 320 °C; for quadrupole, it was held at 230 °C and operated at 70 eV electron ionization on a full scan (*m/z* 60–600 mass range) and SIM modes.

Compounds identification was based on mass spectra, relative elution order, and literature data. For parameter, calculations were performed using the signal areas taken from auto integrated and non-smoothed SIM-data chromatograms. For a few cases, signals were integrated by hand.

1.3 Results and discussion

First, the results for compound distributions from a saturated fraction (*n*-alkanes, acyclic isoprenoids, cheilanthanes, hopanes, and steranes) will be discussed, followed by those from an aromatic fraction (naphthalenes, phenanthrenes, methyltrimethyltridecylchromans [MTTCs], benzohopanes, mono- and triaromatics steranes, and aromatic carotenoids). After that distributions of acid fraction compounds are presented.

1.3.1 Neutral fraction

Regarding neutral fraction analysis, elution solvents were changed to avoid chlorinated ones aimed at good separation between saturated and aromatic fractions. A high sample/silica ratio was used, without alumina or silver nitrate use, resulting in good separation between such classes as shown using *n*-alkanes/*n*-alkylbenzenes and aromatics. Saturate hydrocarbons TIC of three studies samples are shown in **Figure 43**.

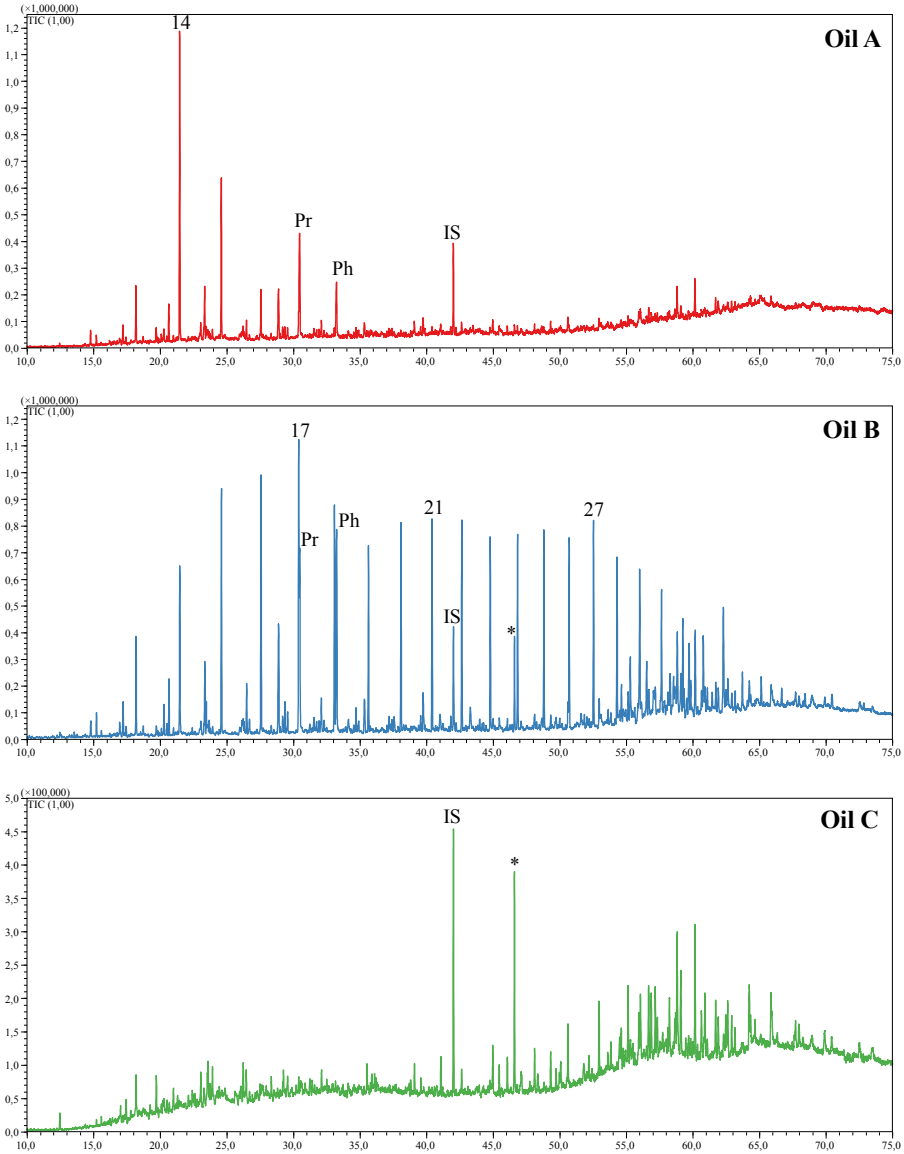


Figure 43. Saturated fraction Total Ion Chromatograms (TIC) for A, B, and C oils, under investigation. IS indicates 3-methylheneicosane. * indicates a contaminant.

1.3.1.1 Biomarkers related to thermal evolution

Pristane (Pr)/*n*-C_{17} and Phytane (Ph)/*n*-C_{18} ratios are used to evaluate thermal evolution for rock and oil samples. For **Oil B**, Pr/*n*-C_{17} < 1 and Ph/*n*-C_{18} > 1, suggesting a more evolved oil. For **Oil A**, both ratios are greater than 1, suggesting a less evolved sample. **Oil C** lacks both *n*-C_{17} and *n*-C_{18}. However, such ratios are affected by biodegradation (PETERS; WALTERS; MOLDOWAN, 2005a) and are only used as initial thermal evolution

indicators. Thus, such information must be supported by other parameters.

Terpanes distributions for **A**, **B**, and **C** oils are presented in **Figure 44**. Steranes distributions for **A**, **B**, and **C** oils, **Figure 43**. Ts/(Ts + Tm) ratio for all samples remains 0.28, indicating a similar thermal evolution for **A**, **B**, and **C** oils. The ratio C_{31} 22*S*/(22*S* + 22*R*) exhibit values at 0.52–0.55 (indicating the samples do not entered on main oil generation phase yet (PETERS; WALTERS; MOLDOWAN, 2005a). A similar conclusion can be obtained from C_{30} *βα*/(*βα* + *αβ*) with values around 0.13–0.15. The C_{29} ααα 20*S*/(20*S* + 20*R*) and C_{29} *αββ*/(*αββ* + *ααα*) sterane ratio exhibit values from 0.33 to 0.41 and 0.16 a 0.34, respectively, indications of a low thermal evolution level for all of the samples also. Despite these values, factors such as lithology, high salinity during sedimentation, and higher sulfur content on source rock can affect these ratios, making an organic matter at low-level thermal evolution exhibits values of that at higher-level thermal evolution (PETERS; WALTERS; MOLDOWAN, 2005a).

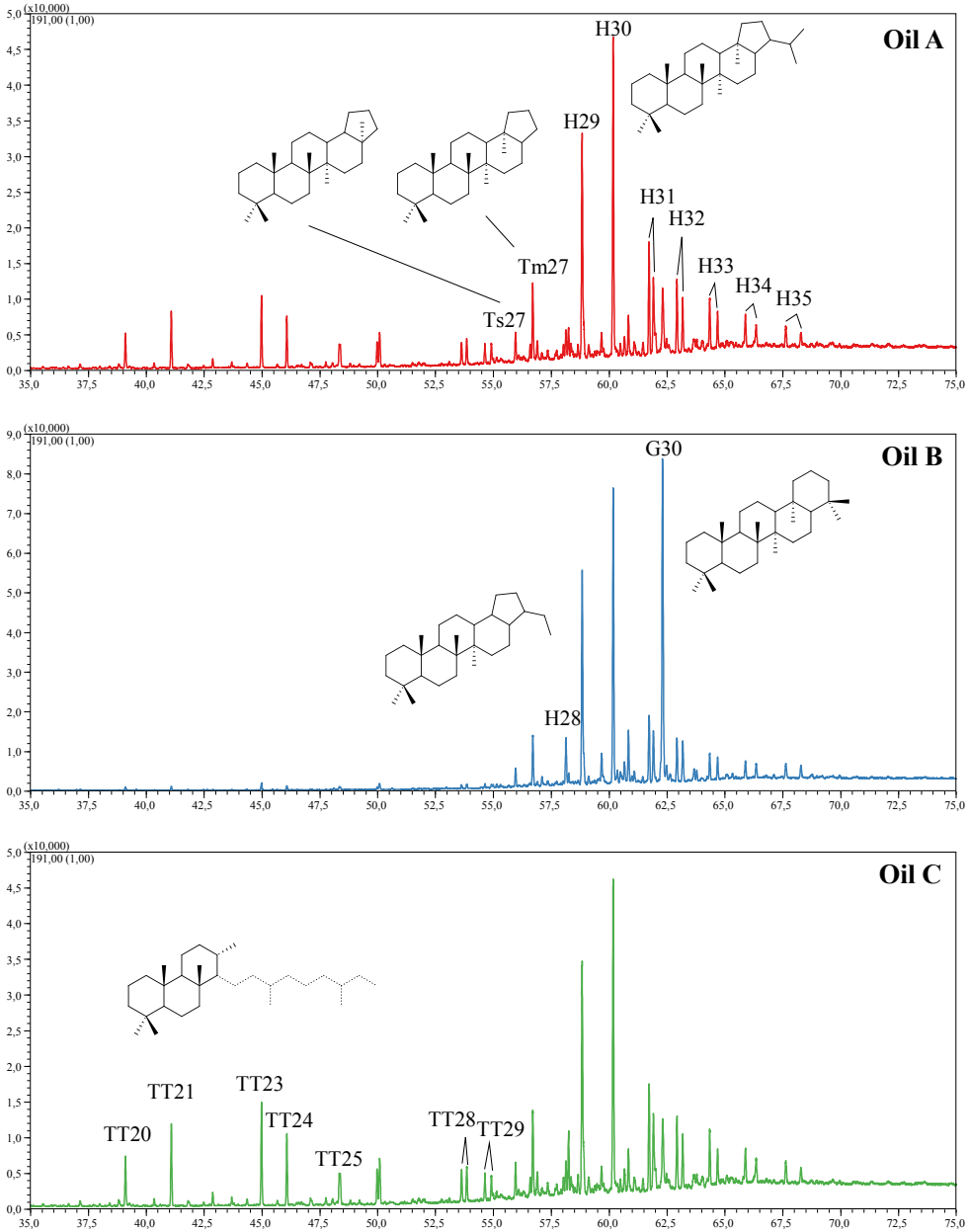


Figure 44. *m/z* 191 Mass chromatogram showing terpanes distribution. TT, H, and G indicates tricyclic terpanes, hopanes, and gammacerane, respectively. Numbers indicates carbons total atoms in each structure.

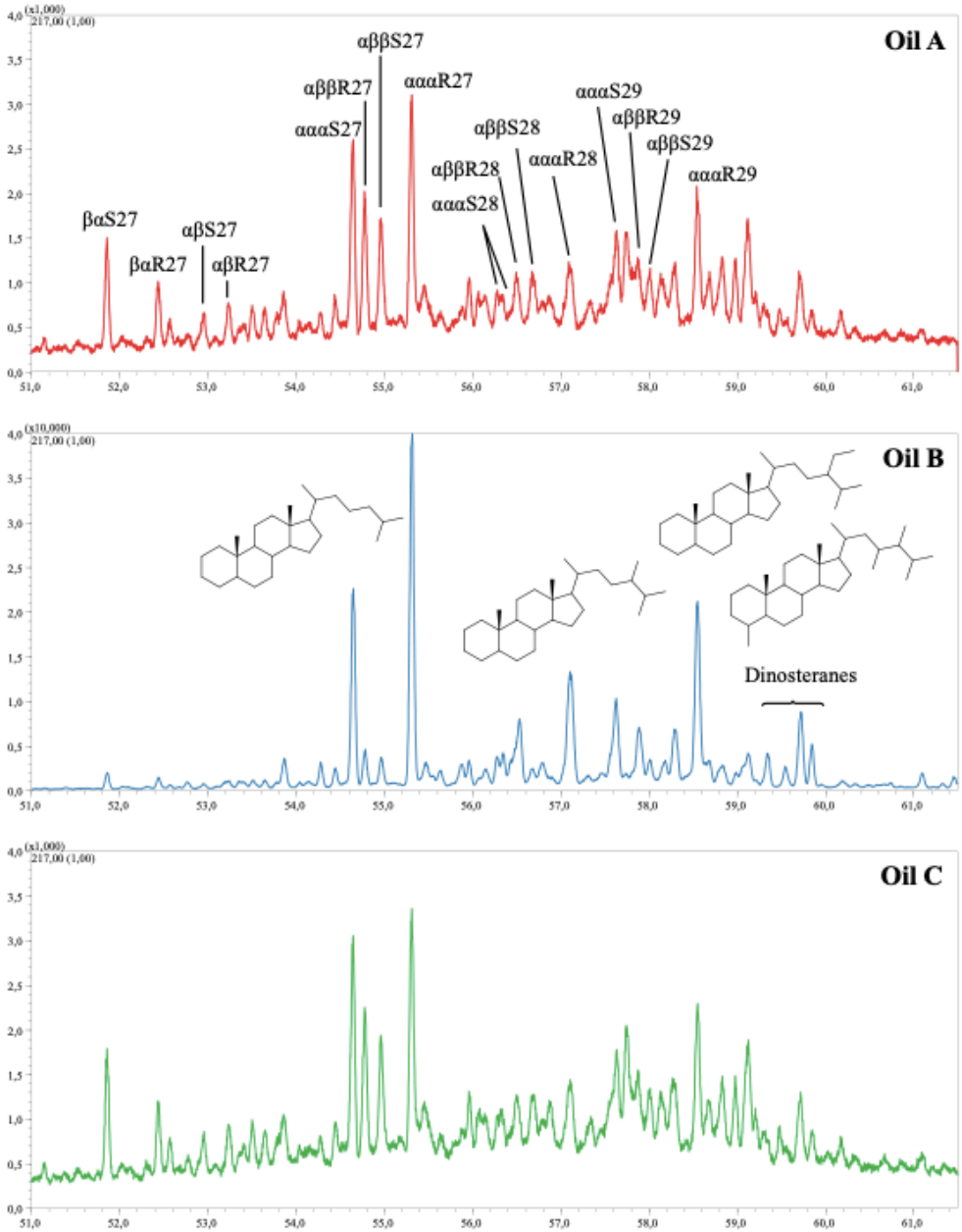


Figure 45. *m/z* 217 Mass chromatogram showing cholestanes, ergostanes, and stigmastanes distribution.

1.3.1.2 Biomarkers related to terrestrial organic matter contribution

n-Alkanes distribution, commonly used as marine or terrestrial organic matter relative contribution initial indicator, are shown in **Figure 43**. Its distribution is significantly different between samples, with respect to OM source and mainly with respect to biodegradation stages. Oil B shows a complete essentially bimodal *n*-alkane distribution, ranging from *n*-C_{12} to at least *n*-C_{40} with maxima at *n*-C_{17} and *n*-C_{21}. The signals *n*-C_{21+} show a light predominance for homologues of odd-numbered carbon atoms, specifically *n*-C_{27}. These characteristics, allied to CPI(1) values of 1.07 and TAR values of 0.50, suggests a marine depositional paleoenvironment with a somewhat contribution of terrestrial OM. However, since terrestrial OM usually has more n-alkanes than aquatic organic matter, the contribution of terrestrial OM affects the total distribution of n-alkanes, and therefore, terrestrial contributions should be supported by other indicators. Due to biodegradation processes, *n*-alkanes from Oil A ranged from *n*-C_{12} to *n*-C_{18} only, and no *n*-alkane was observed on Oil C.

Figure 44 presents *m/z* 191 mass chromatogram showing tricyclic terpanes (TT) distribution for A, B, and C samples. For “Oil B” absence of TT was almost noticed which is commonly observed on marine-sourced OM (PETERS; WALTERS; MOLDOWAN, 2005a). A similar distribution was noticed among A and C samples, which strengthens the idea that these samples differ only in their biodegradation stages. Previous works support that $C_{19,}$ and C_{20} tricyclic terpanes are more abundant in terrigenous oils, with C_{24} tetracyclic terpane (TeT) dominant *(OURISSON; ALBRECHT; ROHMER, 1982; TAO et al., 2015; ZUMBERGE, 1987)paralic/deltaic*. The C_{19}/C_{23} TT, C_{20}/C_{23} TT, C_{21}/C_{23} TT, C_{26}/C_{25} TT, C_{24} TeT/C_{26} TT ratios, and Tricyclic index suggest a marine-source to B sample and a non-marine source to both A and C samples.

1.3.1.3 Source-related biomarkers

The Hopane/Sterane (Hop/St) ratio, calculated from the abundance of C_{30} 17α(H),21β(H) hopane and 5α(H),14α(H),17α(H) (20*S* + 20*R*) cholestanes, is commonly used as a qualitative indicator of relative contributions of organic matter of eukaryotic and bacterial origin (PETERS; WALTERS; MOLDOWAN, 2005a). For sample B, the low value of 1.24 indicates an OM deposited in a marine depositional paleoenvironment, while for samples A and C, the high values of 9.11 and 8.58, respectively, indicate a non-marine (lacustrine fresh water or saline) depositional paleoenvironment.

Diasteranes are formed during diagenesis from sterols transformation, catalyzed by acidic sites in argilominerals (RUBINSTEIN; SIESKIND; ALBRECHT, 1975; SIESKIND; JOLY; ALBRECHT, 1979). Diasteranes/Regular cholestanes ratio helps differentiate oils derived from rocks deposited under carbonate conditions, common in marine paleoenvironments, from clastic rocks, commonly observed on lacustrine paleoenvironments. For sample B,

values of 4.71 suggest a marine paleoenvironment, while values of 38.13 and 40.21 for oils A and C, respectively, pointing to a non-marine paleoenvironment.

4-Methylsteranes and tricyclic indices help differentiate marine oils from non-marine oils, subdividing them into freshwater lacustrine and saline lacustrine (MELLO et al., 1988b; PETERS; WALTERS; MOLDOWAN, 2005a). The 4-methylestanes seem to be associated with carbonate and hypersaline environments (ABOGLILA et al., 2011), and tricyclic terpanes are less abundant in samples of marine origin. The high 4-methylsterane index values for samples A, B, and C suggest a depositional environment of high salinity (lacustrine brackish or marine-evaporitic), whereas tricyclic index values suggest a marine-evaporitic depositional environment for sample B and a lacustrine brackish depositional environment for samples A and C.

Several studies have been observed high concentrations of tetracyclic polyprenoid (TPP) in oils and rocks deposited under lacustrine freshwater/brackish conditions. A characteristic is an excellent tool for recognizing algal OM from these depositional environments, especially with other geochemical indicators (HOLBA et al., 2000; PETERS; WALTERS; MOLDOWAN, 2005a). The values for the TPP (**Figure 45**) ratio implies differences in salinity levels in depositional environments, one of lower salinity (freshwater or brackish) for samples A and C and the other of higher salinity (lagoon/estuary) for sample B, as also indicated by other parameters.

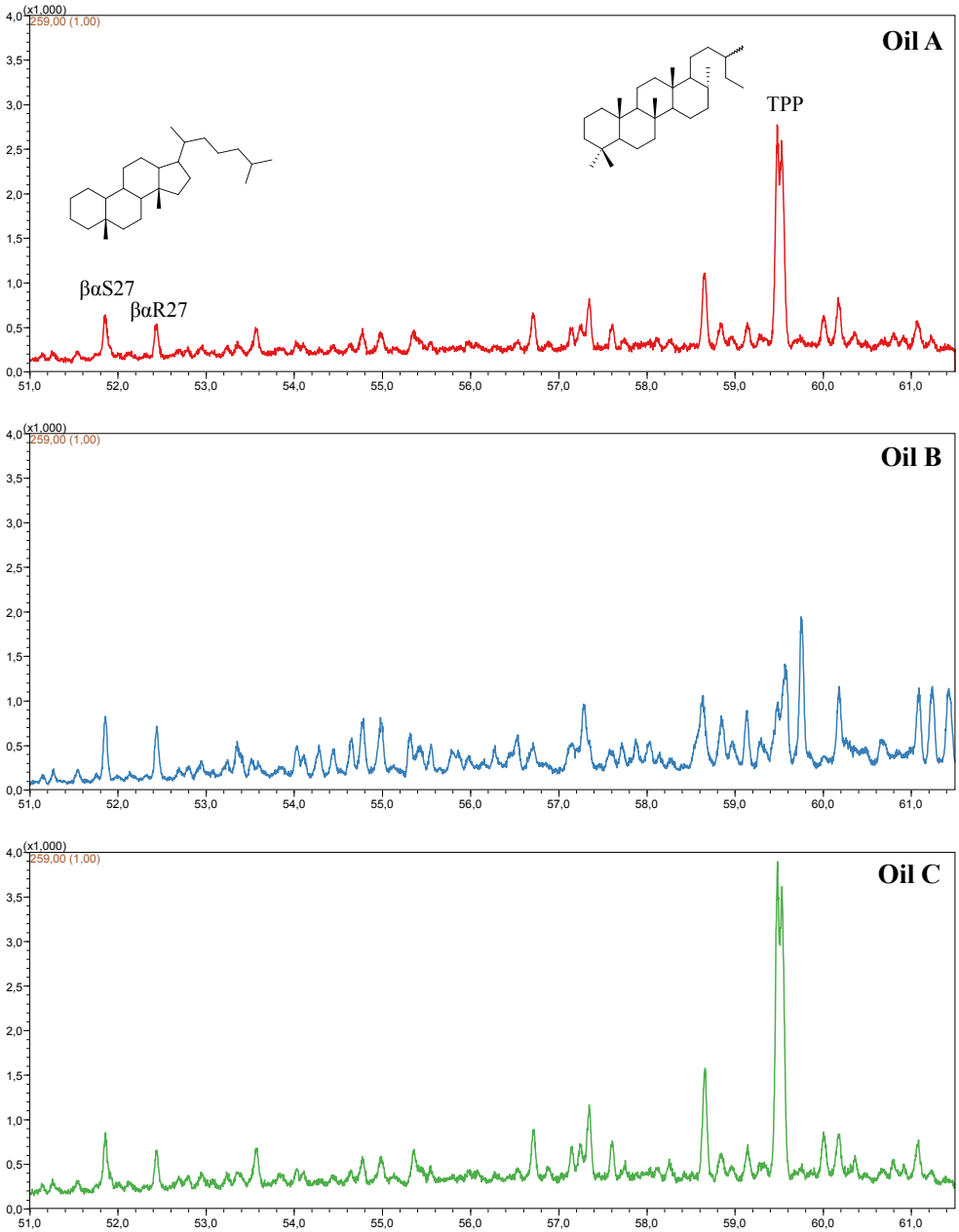


Figure 46. *m/z* 259 Mass chromatogram showing diacholestanes (βα*S* and *R*) and tetracyclic polyprenoid (TPP) isomers, respectively.

1.3.1.3 Redox and depositional environment biomarkers

Pristane (Pr), phytane (Ph), gammacerane, and β-carotane are commonly used to evaluate redox potential, salinity, and water column stratification. High values (>3.0) for the

Pr/Ph ratio indicate a contribution of terrestrial organic matter deposited under more oxic conditions, while low values (<0.8) indicate anoxic conditions, often related to hypersaline or carbonate environments (PETERS; WALTERS; MOLDOWAN, 2005a). The value of 1.73 observed for "A" sample suggests a more oxidizing environment, such as lacustrine environments. The value of 0.72 for B sample is according to expected for a sample deposited in a marine environment. Due to the advanced biodegradation stage, pristane and phytane were not detected in "C" sample.

In "B" sample, the gammacerane is the main signal (**Figure 44**), and the Gam/Hop ratio was 1.17, indicating a stratified water column depositional environment (SINNINGHE DAMSTÉ et al., 1995a), perhaps due to the high salinity of a probably restricted marine environment. For A and C samples, values were 0.29 and 0.31, a non-stratified freshwater lacustrine environment.

β-Carotene is another biomarker commonly associated with salinity in the depositional environment, frequently co-occurring with the gammacerane, both in oil samples (SOUSA JÚNIOR et al., 2013) and in sediments (Sousa et al., 2019). Although widely distributed, its presence, in high abundance, is associated with both highly anoxic lacustrine and restricted marine depositional environments (PETERS; WALTERS; MOLDOWAN, 2005a). More recent studies report high amounts of β-carotane associated with evaporated, reducing, shallow water and carbonate-rich environments (DING et al., 2020). This indicator was detected in the three oil samples, with more intensity in oil B (~756 ppm) and less intensity in oils A (~330 ppm) and C (~360 ppm), indicating marine-evaporitic depositional environment for Oil B and lacustrine saline/brackish conditions for A and C (MELLO et al., 1988b).

1.3.1.4 Biodegradation-related biomarkers

Hopanes and 25-norhopanes distributions for three oils samples are shown in **Figure 47**. For Oils A and C, 25-norhopane distribution ranging from 27 to 32 carbon atoms was observed, while in Oil B, only isomers of 27 and 28 carbon atoms were detected.

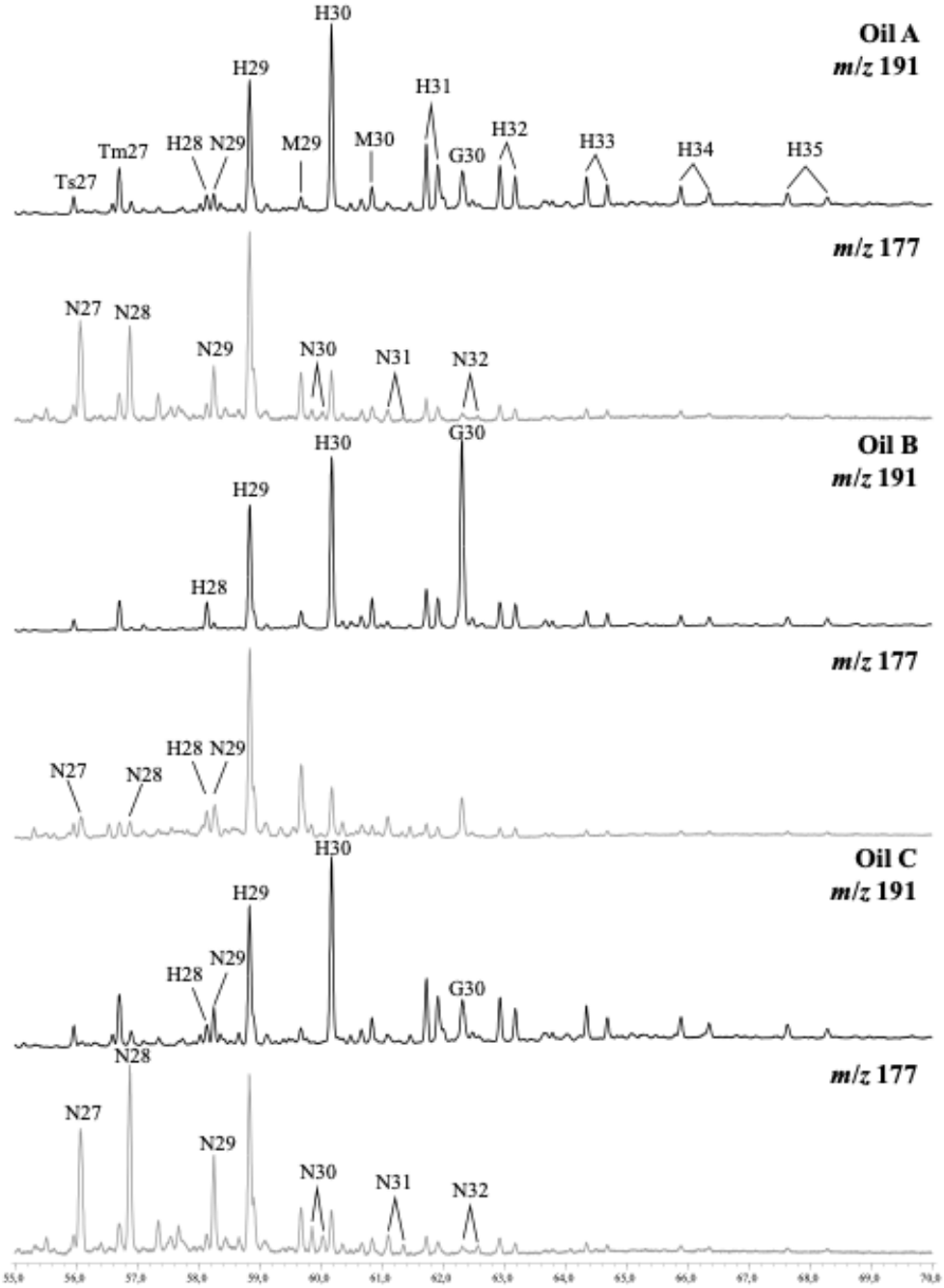


Figure 47. *m/z* 191 and 177 Mass chromatograms showing hopanes and 25-norhopanes distributions. H, M, N, and G indicate hopanes, moretanes, 25-norhopanes, and gammacerane. Numbers indicate total number of carbon atoms in each structure.

From **Figure 43** it is possible to observe (i) distribution of *n*-alkanes of C_{12} to C_{40}, with no apparent changes in the distribution of *n*-alkylcyclohexanes (not shown) in sample B; (ii) an almost complete absence of *n*-alkanes in sample A, with some change in the distribution of *n*-alkylcyclohexanes and (iii) a complete absence of any acyclic *n*-alkanes and isoprenoids (Pr and Ph) in sample C, suggesting biodegradation scale values of Peters and Moldowan (PM) of PM3 for sample A, PM0 for sample B and at least PM5 for sample C. The values of the 25-norhopane C_{29}/ C_{30} Hopane ratio, which increase with biodegradation, are in accordance with this observation.

The occurrence of 25-norhopanes in oil is commonly associated with biodegradation processes (PETERS; WALTERS; MOLDOWAN, 2005a). The mechanism for the formation of these compounds is not fully understood yet, and several factors seem to influence the formation of these compounds (BENNETT et al., 2006). It is believed that formations occur through oxidation with consequent decarboxylation of C-25 from the hopane backbone due to reworking of OM by microorganisms (MOLDOWAN; MCCAFFREY, 1995; SEIFERT; MICHAEL MOLDOWAN, 1979). However, there are several reports of the occurrence of these compounds in samples with no evidence of biodegradation (BLANC; CONNAN, 1992), suggesting that (i) 25-norhopanes may have a direct biological precursor (PETERS; WALTERS; MOLDOWAN, 2005a), or (ii) are formed during diagenesis (PETERS; WALTERS; MOLDOWAN, 2005a) or yet (iii) the occurrence of oil mixture of different biodegradation levels during reservoir filling (LÓPEZ; LO MÓNACO; VOLKMAN, 2015; VOLKMAN et al., 1983).

There are reports of 25-norhopanes presence in source rocks (BLANC; CONNAN, 1992). Nevertheless, the C_{28} and C_{29} demethylated hopanes are less concentrated than the C_{29} hopane. Their distributions are almost always represented by the 25-norhopane C_{29}, with a few members of the series instead of a complete pseudo-homologous series of $C_{29–35}$, as commonly observed in degraded oils. In the present study, it is worth noting that even though the C sample is one with the highest level of biodegradation, the expected series of 25-norhopanes was not observed (**Figure 47**), indicating that biodegradation did not reach advanced stages to the point of degrading biomarkers. This same conclusion can be reached from the similarity between the distribution of steranes and terpanes between samples A and C (**Figure 47**).

In the three studied samples, 25,28,29,30-tetranorhopanes (N27), and 28,29,30-trisnorhopaness (N28) is observed (**Figure 47**). Their abundance increases according to the C > A >> B biodegradation sequence. The presence of these two compounds in a sample with a low thermal evolution degree and no evidence of biodegradation, such as sample B, does not support the hypothesis that their presence is as a result of biodegradation. On the contrary, it reinforces the hypothesis of a direct biological precursor or formation during diagenesis. However, the hypothesis that the detection of these compounds is a result of oil mixture during reservoir filling cannot be ruled out.

1.3.1.5 Aromatic carotenoids and aryl isoprenoids

Aromatic carotenoid derivatives are observed in aromatic hydrocarbon fractions also. **Figure 48** presents the *m/z* 134 mass chromatogram showing the distribution of these compounds. Isorenieratane is present in all samples. This compound is related to sulfur bacteria from *Chlorobiaceae* family, the brown-pigmented Green Sulphur Bacteria (GSB), which live in anoxic environments characterized by low sunlight, low O_2 concentrations, and high dissolved oxygen and H_2S. Thus, isorenieratane allows the assessment of redox conditions of the depositional environment. Euxinic environments are particularly important for oil generation as they contribute to preserving organic matter (SUMMONS; POWELL, 1986, 1987).

In addition to isorenieratane, several diagenetic and catagenetic aryl isoprenoids derivatives of isorenieratane have also been detected. The series ranged from C_{13} to C_{25} (C_{23} absent), with C_{30} and C_{31} are observed in all samples (**Figure 48**). These compounds were identified based on molecular ion ($M^{+\cdot}$) consistent with the general formula C_nH_{2n-6} and base peak *m/z* 134. Only aryl isoprenoids with a 2,3,6- substitution pattern are present, which agrees with the exclusive presence of isorenieratane. Thus, together with isorenieratane, there is another evidence of GSB contribution (SUMMONS; POWELL, 1987).

The Aryl Isoprenoids Ratio (AIR) parameter (SCHWARK; FRIMMEL, 2004) is calculated using the ratio of short-chain (C_{13-17}) to long-chain (C_{18-22}) aryl isoprenoids, allowing access to variability degree and photic zone euxinic (PZE) persistence. Low values (≤2.0) for the AIR parameter should reflect the persistence of the photic zone, and higher values (>2.0) should reflect less intense and episodic photic zone (ADEROJU; BEND, 2018; SCHWARK; FRIMMEL, 2004).

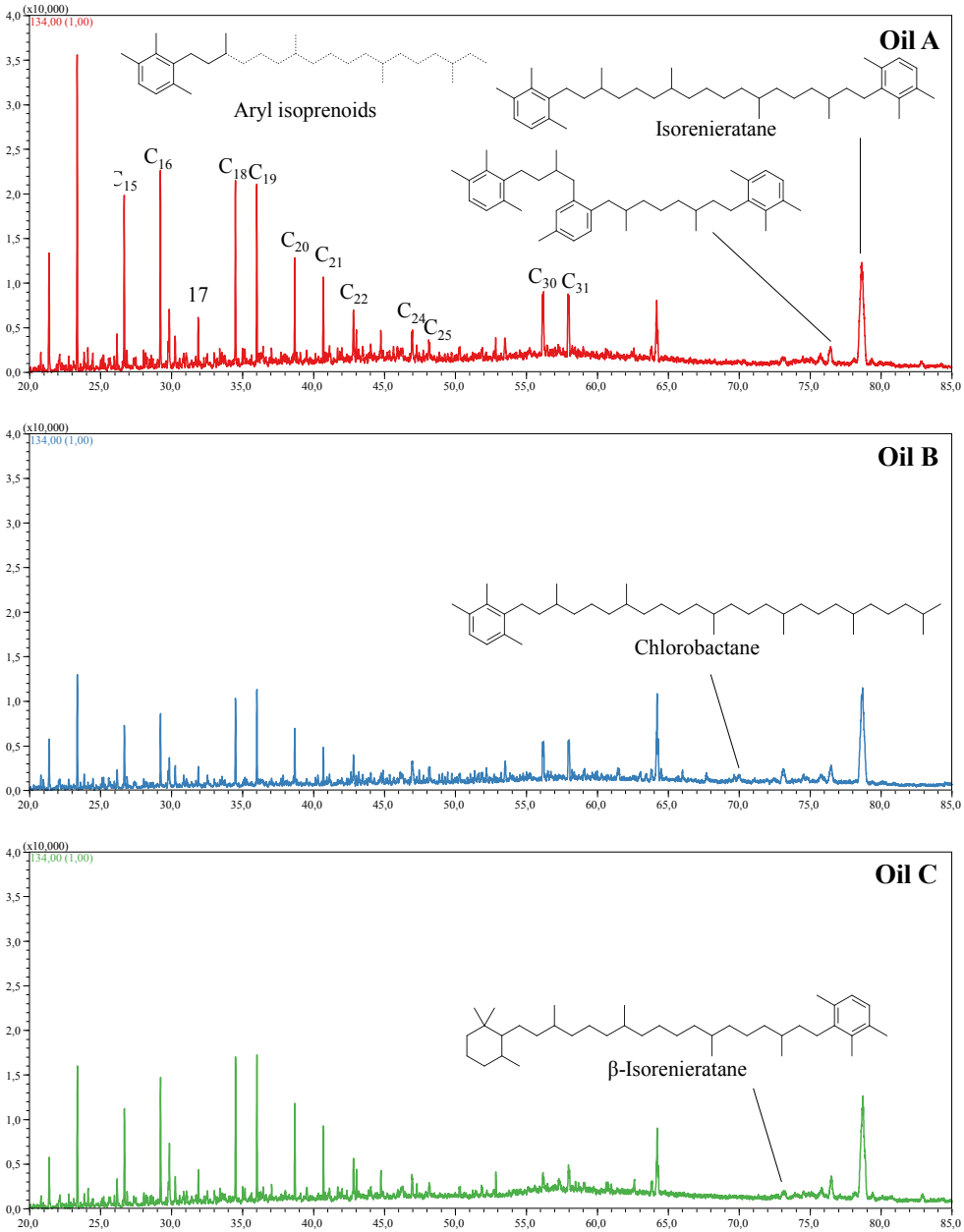


Figure 48. *m/z* 134 Mass chromatograms showing aromatic carotenoids and aryl isoprenoids distributions. Cn (n = 13–31) indicates aryl isoprenoids. 544 indicates molecular ion for an unidentified aromatic carotenoid. * indicates an unidentified compound.

A and C samples show AIR values of 3.01 and 1.86, respectively, which would place them, perhaps, in episodic PZE. However, these samples have light and heavy biodegradation levels, respectively, which can alter the AIR values. The less biodegraded B

sample shows a value of 1.74, indicating a persistent photic zone.

A low amount of chlorobactane was detected in the B sample. This compound is another diagenetic aromatic carotenoid produced exclusively by green-pigmented GSB, which dominates at shallow oxic/anoxic boundaries and requires higher light intensities than the brown-pigmented bacteria (VILA; ABELLA, 1994). The presence of chlorobactane in B sample reinforces the contribution of such bacteria to sediment. It is evidence of a very shallow euxinic layer (depths of 15 m or less) in the marine depositional environment (KUYPERS et al., 2002), and the occurrence may have occurred during the deposition of MO.

1.4 Acid fraction

As seen earlier, the term “naphthenic” acid has been used to refer to all the carboxylic acids present in petroleum. Besides the geological interest, the study of the composition of the acid fraction is also of economic interest. Although the amount of acids in crude oil is generally low, their emulsifying and corrosive characteristics make them especially important because they cause corrosive effects in distillation towers, directly impacting the costs associated with their processing and refining.

The acidic fraction of crude oil contain biomarkers that can yield important information about their geological history, although these compounds are not routinely analyzed in oil exploration. However, the concentration and distribution of hopanoic acids have been used as maturation, biodegradation, and migration indicators (Meredith et al., 2000).

Several methods and stationary phases are used in the literature for carboxylic acid extraction, many of which are more related to the extraction of low molar mass cyclic naphthenic acids. A few others are applied to acidic biomarkers (hopanoic and steranoic); however, economically unfeasible due to low yields, long extraction times, high amounts of oil samples, or the use of chlorinated solvents.

In this context, besides samples characterization by saturated and aromatic biomarkers, we present a simple methodology for isolating acidic biomarkers, using silica modified with potassium hydroxide in SPE, using a few milligrams of crude oil.

1.4.1 Extraction and analysis of carboxylic acids using KOH-modified silica chromatography minicolumns (SPE-type cartridge).

To evaluate the efficiency of the extraction method using mini SPE cartridge columns, samples A, B, and C were submitted to extraction in cartridges packed with silica modified with KOH. The parameters analyzed were chromatographic profile analysis, extraction yield, solvent volume, stationary phase amount, and a load of sample mass (Sousa et al., 2022).

1.4.1.1 n-Alkanoic acids

In all samples a predominance of hexadecanoic (palmitic, *n*-$C_{16:0}$) and octadecanoic (stearic, *n*-$C_{18:0}$) acids was observed (**Figure 49**). Such predominance has been observed in previous studies on the acidic fractions of samples from the Brazilian Campos (KOIKE et al., 1992; NASCIMENTO et al., 1999), Potiguar (LOPES et al., 1999), and Sergipe-Alagoas (RODRIGUES et al., 2000) basins, as well as others around the world (JAFFE; ALBRECHT; OUDIN, 1988; JAFFÉ; ALBRECHT; OUDIN, 1988).

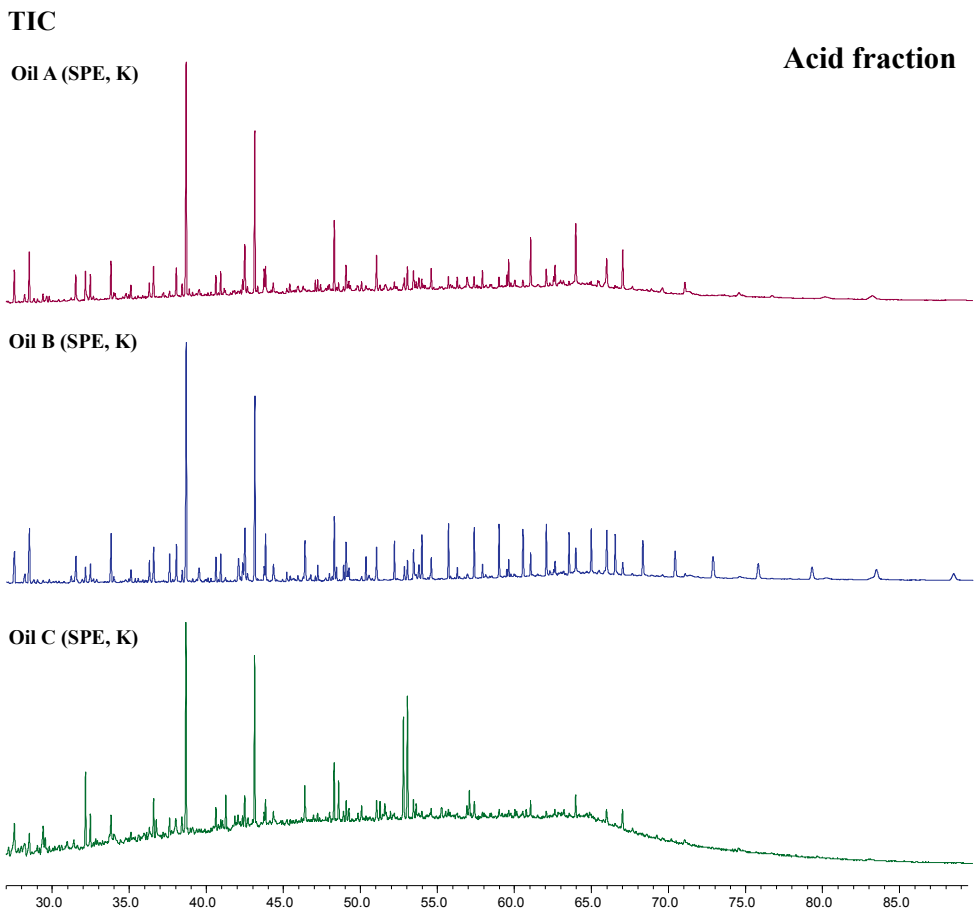


Figure 49. TIC for acid fractions, analyzed as TMS derivates, for A, B, and C samples extracted using solid-phase extraction (SPE) with KOH-modified silica.

The occurrence of these compounds, especially in sample B, which is not biodegraded, could indicate contamination introduced during sample preparation steps, as suggested by MEREDITH; KELLAND; JONES (2000). However, since samples exhibit low levels of

thermal evolution, as indicated by biomarkers in both saturated and aromatic fractions, the presence of these acids may reflect (i) OM original composition, (ii) *de novo* synthesis from microorganisms who reworked OM during diagenesis or, in the case of A and C samples, (iii) *de novo* products from organisms who biodegraded the oil (JAFFÉ; GALLARDO, 1993; KOIKE et al., 1992).

When inspected in detail, *n*-alkanoic acids distribution reveals dodecanoic (lauric, *n*-$C_{12:0}$), docosanoic (behenic, *n*-$C_{22:0}$), and triacontanoic (melissic, *n*-$C_{30:0}$) acids as the most abundant when the *n*-$C_{16:0}$ and *n*-$C_{18:0}$ homologues are disregarded.

The high C_{16} and C_{18} fatty acid concentrations may reflect contamination during the acid extraction procedure. There is not only one source of contamination for these compounds, so it is practically impossible to determine the origin of the contamination and even more difficult to exclude these sources completely. Some publications show how much the concentration of these compounds varies in geological samples. For example, in the paper published by Jones and co-workers (Jones et al., 2001), the fatty acids C_{16} and C_{18} are in the range of 5000-7000 µg/g in oil samples. On the other hand, Thiel and Hoppert (2018) assess that these compounds are present in white analyses in a concentration equivalent to 0.01-1.0 µg/g in the Jurassic sedimentary rock sample (Thiel and Hoppert, 2018).

Overall *n*-alkanoic acid distribution is characterized by a predominance of homologues with even number of carbon atoms for all three samples, which reflects the low thermal evolution of OM. Despite the high abundance of *n*-$C_{16:0}$ and *n*-$C_{18:0}$ homologues, the proportion of *n*-C_{20+} components indicates somewhat similarity with OM sourced from higher plants to the depositional environment, suggesting OM contributions from both autochthonous (algae and bacteria) and allochthonous (higher plants) sources (BARAKAT; RULLKÖTTER, 1995).

The calculated TAR parameter was adapted from the original formula of the TAR parameter that is used for *n*-alkanes. For carboxylic acids, the signal area corresponding to the respective fatty acid referring to *n*-alkane was used. For all samples, the values were below 0.05. These values suggest a high contribution of marine OM even for samples A and C, which should have higher values, characteristic of the environment with some contribution of terrestrial OM. However, as well as when applied to *n*-alkanes, the TAR parameter should be used with caution when applied to fatty acids because it uses *n*-C_{16} and *n*-C_{18} acids that can originate from external sources, thus interfering in the parameter interpretation.

1.4.1.2 Acyclic isoprenoic acids

The relative abundance of acyclic isoprenoid acids, mainly pristanic and phytanic acids is significantly different between samples. A sample shows a low abundance of these

compounds, while B sample shows high levels. C sample shows even lower levels of these compounds.

Although there are other phytol sources to sediments, the main one is chlorophyll a. In productive aquatic environments, photosynthesis into the water column is the dominant source of chlorophyll-a derivatives, indicating a mainly autochthonous planktonic origin for phytanic and pristanic acids. Thus, the higher proportion of phytanic acid relative to pristane in sample B agrees with the higher proportion of phytane relative to pristane in the saturated hydrocarbon fraction and suggests deposition under anoxic conditions favoring MO preservation. The low abundance/absence of pristanic and phytanic acids in A and C samples can be explained by the removal of acids with lower molar weight through biodegradation, which would also explain the high abundances of *n*-$C_{16:0}$ and *n*-$C_{18:0}$ by *de novo* synthesis in these samples (JAFFÉ; GALLARDO, 1993; KOIKE et al., 1992).

1.4.1.3 Bicyclic terpanoic acids

Some compounds whose structures are consistent with a labdane backbone were identified in the *m/z* 123 mass chromatogram (**Figure 50**). The higher abundance of these structures in C following A samples, those with evidence of biodegradation processes, agrees with the expected preferential removal of *n*-alkanoic and acyclic isoprenoids acids, thus concentrating the samples on labdanic acids (BEHAR; ALBRECHT, 1984; NASCIMENTO et al., 1999). These acids and their hydrocarbon equivalents are related to diterpenoids commonly found in higher plants, and their occurrence can be used as an indicator of contribution, to some extent, of OM from higher plants (JIANG; GEORGE, 2018).

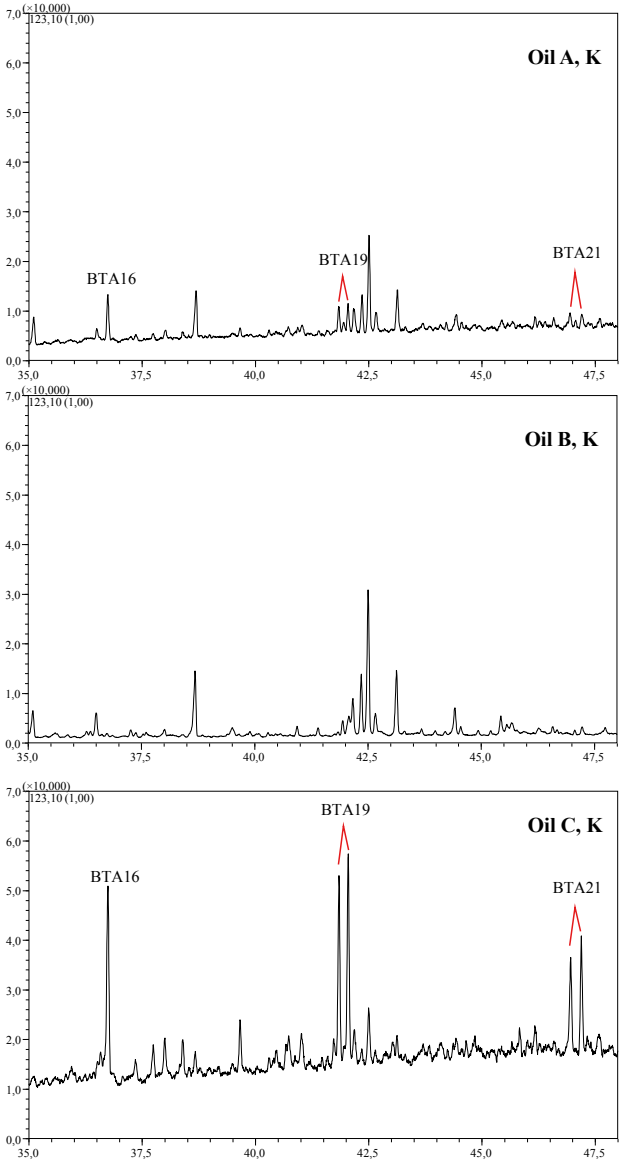


Figure 50. *m/z* 123 Mass chromatograms showing bicyclic terpanoids acid (BTA, TMS derivatives) distribution, identified as labdanic acids. Number at each abbreviation indicates carbons total atoms for each structure.

1.4.1.4 Tricyclic terpanoic acids

Tricyclic terpanoic acids (TTAs) are present in acidic fractions of A and C samples (**Figure 51**). These compounds are commonly reported in acidic fractions of crude oils (BEHAR; ALBRECHT, 1984; JAFFÉ; GALLARDO, 1993; SCHMITTER; ARPINO; GUIOCHON, 1981). Unlike the hydrocarbon fraction, where these compounds appear as

a pseudo-homologous series of $C_{20–30}$, only C_{21}, C_{24}, and C_{26} pseudo-homologues were detected, the last two as diastereoisomeric pairs (*R* and *S* at C-22). The C21 homologue is the most abundant in the A sample, while in the C sample, C_{24} is the most.

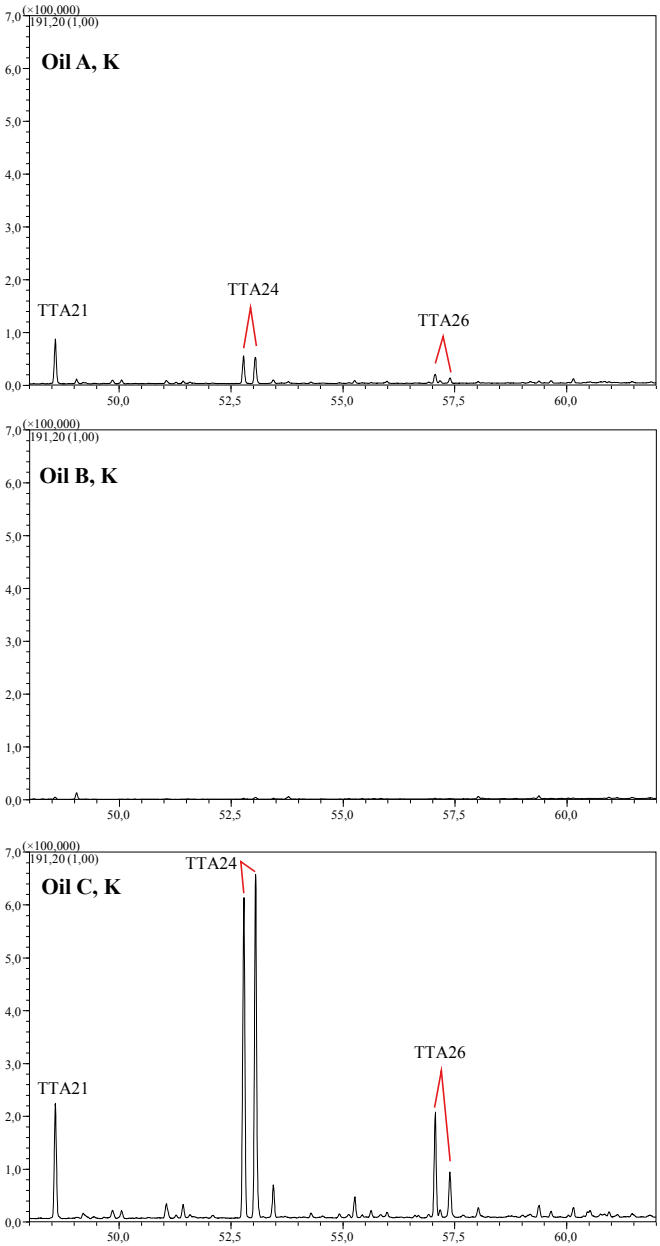


Figure 51. *m/z* 191 Mass chromatograms showing de tricyclic terpanoids acids (TTA, TMS derivatives) distributions, identified as cheilanthanoic acids. Number at each abbreviation indicates total number of carbon atoms for each structure.

The increase in tricyclic terpanoic acids with the degree of biodegradation is believed to be due to preferential removal of other classes during this process (BEHAR; ALBRECHT, 1984). Far less likely are (i) neoformation from corresponding alkanes or (ii) incorporation by *de novo* synthesis by bacteria or (iii) effect to thermal evolution. These processes could have a lesser effect, given that (i) tricyclic terpanes distribution is almost identical between A and C samples (**Figure 51**), and biodegradation would be expected to preferentially remove some homologues from the series, affecting the distribution of tricyclic terpanes and causing samples of different biodegradation levels to show different distributions of these compounds. Furthermore, (ii) no tricyclic terpanes (or some logical precursor to the series) have been found in organisms able to contribute significantly to sedimentary OM so far. At the same time, (iii) A and C samples have the same degree and thermal evolution, differing only in the extent of biodegradation as indicated by biomarkers. Thus, tricyclic terpanoic acids presence in acid fractions suggests that they are already originally present in the samples and are enriched as biodegradation proceeds.

1.4.1.5 Tetracyclic terpanoic acids

A couple of tetracyclic terpanoic acids (TeTA) were detected in all three samples. They were identified as 3-carboxyalkylesteranes or, alternatively, ω-(steran-3-yl)formic and ω-(steran-3-yl)acetic acids, e.g. carboxylic acid acids that have carboxyl or carboxyalkyl group attached at the C-3 position of sterane backbone.

Overall, the abundance of these compounds is low. B sample shows the highest abundance of them (**Figure 50**), besides a complete distribution among the three samples studied, possibly due to the marine sourced OM, as indicated by biomarker parameters in saturated and aromatic fractions.

Only one isomer of each pseudo-homologous series was identified, presumably the one with biological configuration 14α(H),17α(H) 20*R*, with the most stable form 5α(H). However, other minor signals are observed in the chromatogram, which could represent epimers of 5β(H) configuration, as also observed previously in the acidic fraction of kerogen samples from the Monterey Formation (California) (BARAKAT; RULLKÖTTER, 1994), from lacustrine sediments in Nördlinger Ries, Germany (BARAKAT; RULLKÖTTER, 1995) and from marine-evaporitic oils from Potiguar Basin (LOPES et al., 1999).

In all samples with the highest abundance in B sample, signals consistent with a dinosterane-3-carboxylic acids (or 3-carboxydinosteranes) structure were observed. Detection of these compounds is in full agreement with the detection of dinosteranes in saturated fraction (**Figure 43**) and triaromáticos dinosteranes in aromatic fraction, demonstrating early diagenetic stages of OM, as well as the contribution of OM from marine dinoflagellates to sediments of marine origin in case of B sample and marine incursions into

the saline lacustrine environment, in case of A and C samples.

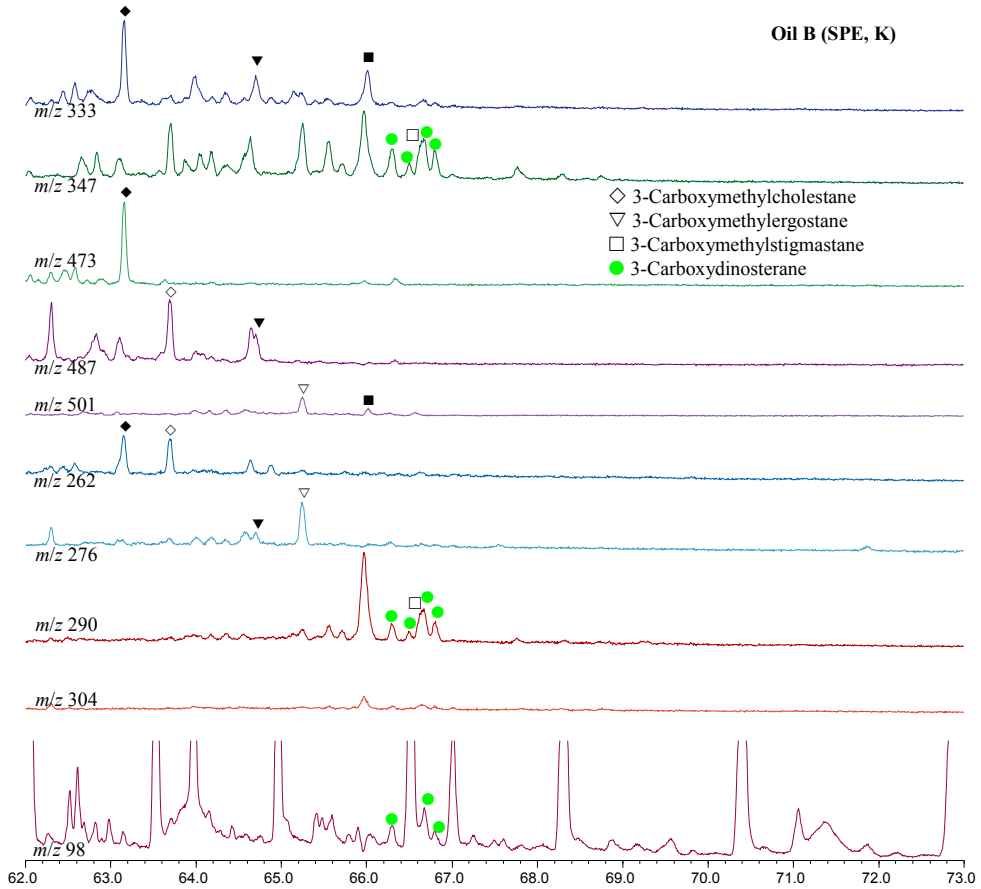


Figure 52. *m/z* 333, 347, 473, 487, 501, 262, 276, 290, 304, and 98 Mass chromatograms used as carboxyl group diagnostic position between 3-carboxyalkylsteranes homologues series for acid fraction from B sample. 3-Carboxyldinosterane signals are indicated.

1.4.1.6 Pentacyclic terpanoic acids

A pentacyclic terpanoic acids (PTA) pseudo-homologous series was detected in all three samples, and their distributions are presented in **Figure 53**–**Figure 55**. The compounds, identified in all three samples as hopanoic acids, range from C_{30} to at least C_{33}, in the isomeric ββ, βα and αβ forms in C-17 and C-21 and *R* and *S* in C-22. ββ isomers of higher homologues (C_{31+}) were observed only in the 22*R* configuration. The relative abundance between isomers varied among samples, with the C_{32} homologues being the most abundant.

In addition to the hopanoic acids, two C_{31} 25-norhopanoic acids were also identified, in lower relative abundance in A sample and higher relative abundance in C sample (**Figure**

53). No 25-norhopanoic acids were identified in B sample. A similar trend was observed in saturated fraction (**Figure 47**).

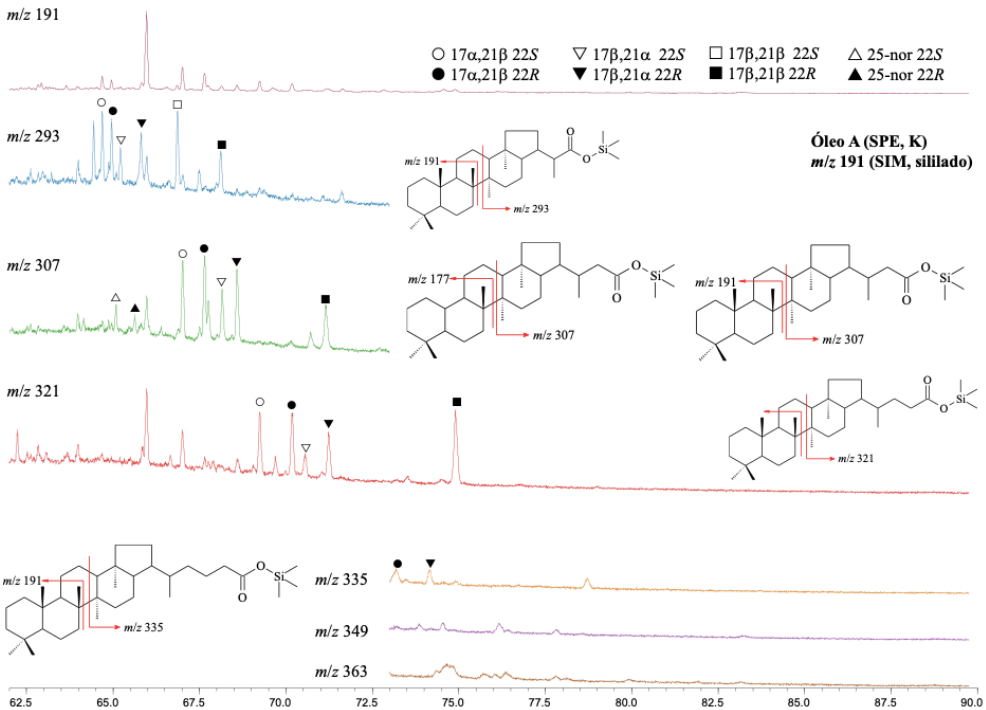


Figure 53. *m/z* 191, 293, 307, 321, 335, 349, and 363 Mass chromatograms showing pentacyclic terpanoic acids (TMS derivatives) distribution, identified as hopanoic acids, for A sample. Some 25-norhopanes are indicated.

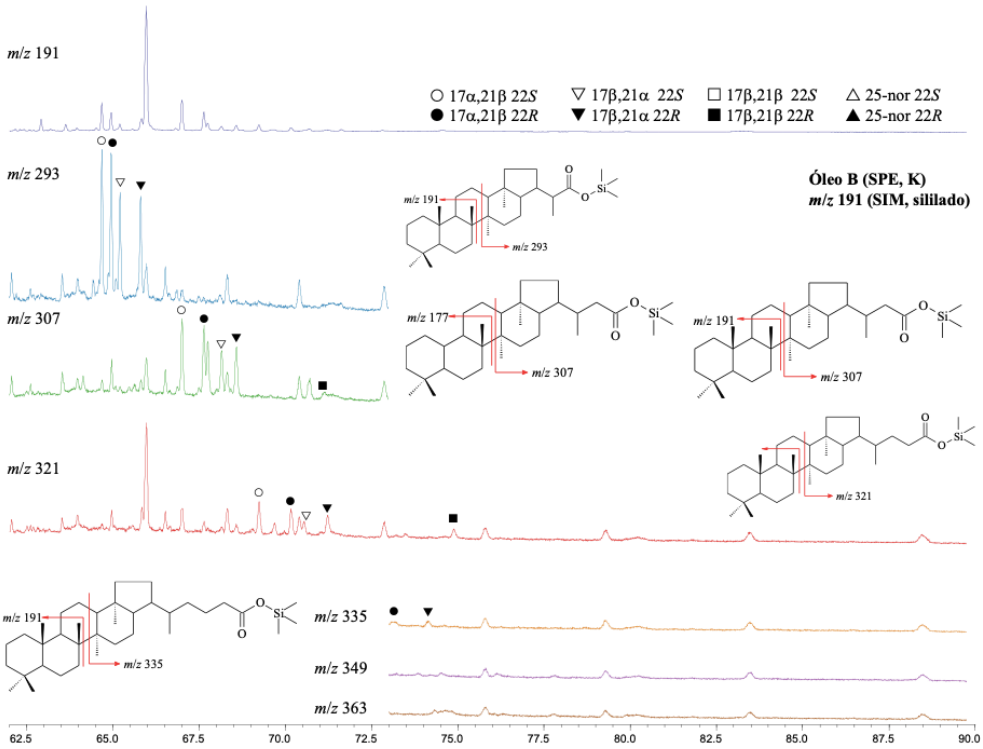


Figure 54. *m/z* 191, 293, 307, 321, 335, 349, and 363 Mass chromatograms showing pentacyclic terpanoic acids (TMS derivatives) distribution, identified as hopanoic acids, for B sample. Notice low abundance of ββ isomers

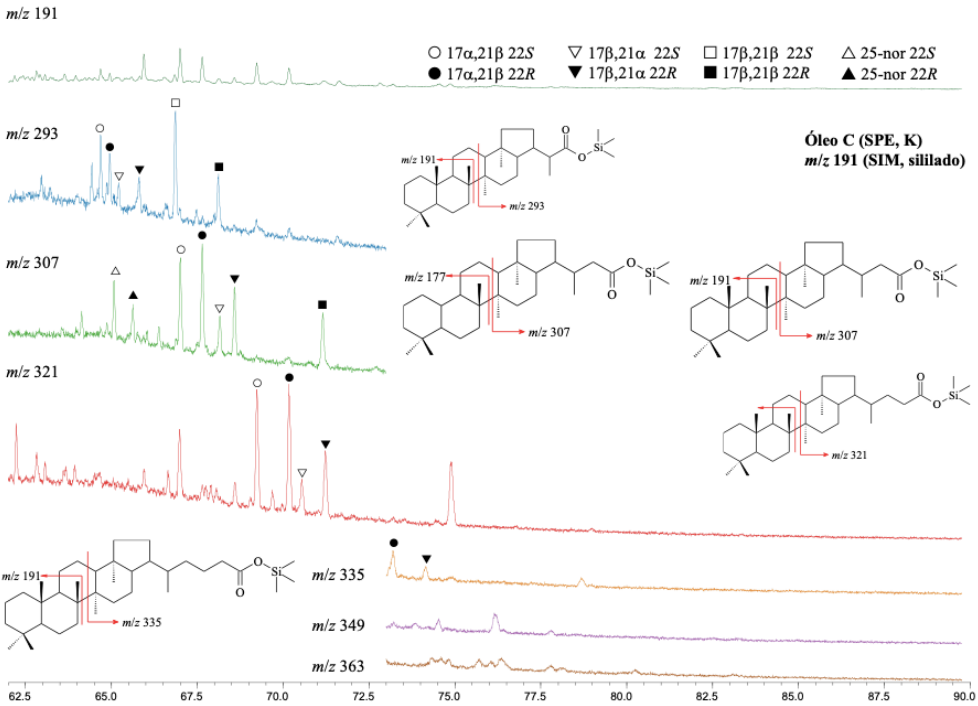


Figure 55. *m/z* 191, 293, 307, 321, 335, 349, and 363 Mass chromatograms showing pentacyclic terpanoic acids (TMS derivatives) distribution, identified as hopanoic acids, for C sample. Some 25-norhopanes are indicated.

These compounds are derived by (i) preferential removal of other biomarkers with a consequent concentration of 25-norhopanoic acids already present in the samples or by (ii) removal of methyl-25 group by oxidation and decarboxylation by microbial transformations of hopanes into 25-norhopanes during biodegradation. The latter hypothesis finds support in detecting 25-carboxyhopanes in biodegraded asphalts (TRENDEL et al., 1990).

1.4.1.7 Aromatic carotenoids acids

A series of C_{12}-C_{15} aryl isoprenoid acids (AIA) was detected in Sample C, supposedly with the carboxyl group attached to the side chain. A representative profile of AIA is shown in **Figure 56**. This class of compounds is presented both as TMS derivatives (**Figure 56** A) and methyl esters (**Figure 56** B). Analyzing the profile of TMS derivatives, only the AIA C_{12}, $C_{13,}$ and C_{15} were detected. Apparently, there are two isomers with C_{13} that would be indicative of aryl isoprenoids with a different substitution pattern on the aromatic ring. In the profile of derivatives such as methyl esters, the detected range is greater, in addition to those detected as TMS, there are also C_9, C_{11} and C_{14}. If the origin of aryl isoprenoid acids from GSB in the samples studied here is confirmed, it would be the first time that this report

has been made in any study already carried out.

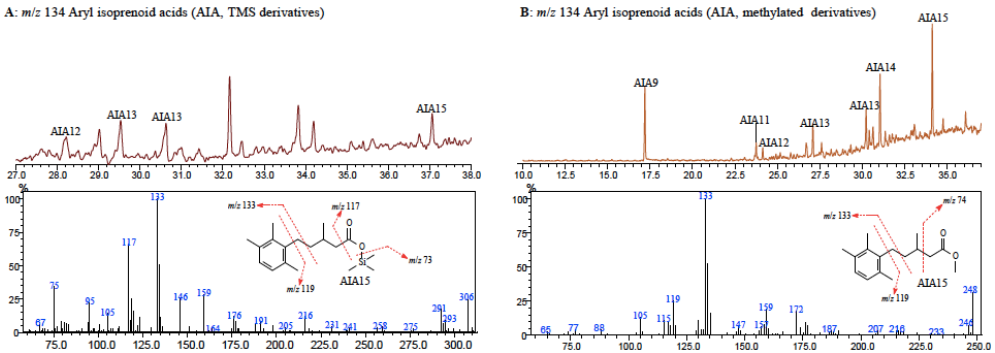


Figure 56. Figure **A**: *m/z* 134 Mass chromatograms showing aryl isoprenoid acids (AIA, TMS derivatives) distribution. Figure **B**: *m/z* 134 Mass chromatograms showing aryl isoprenoid acids (AIA, methylated derivatives) distribution Number at each abbreviation indicates carb ons total atoms for each structure.

1.4.1.8 Acid compounds quantification

Table 4 presents the normalized quantification results for some acidic compounds identified. **Table 5** presents some key relationships used in interpreting of distribution of carboxylic acids.

Table 4. Quantification results for few compounds identified on acid fraction.

Compound	Oil A	Oil B	Oil C
n-C_{16} + n-C_{18}	66.24%	190.36%	60.31%
n-C_{17} + n-C_{19} + n-C_{20}	19.72%	39.83%	3.15%
Acyclic isoprenoids acids	6.55%	57.57%	0.12%
Tricyclic terpanoic acids	71.60%	2.33%	96.60%
25-Norhopanoic acids	0.44%	0.00%	0.06%
αβ-Hopanoic acids	0.96%	0.20%	0.03%
βα-Hopanoic acids	0.43%	0.09%	0.02%
ββ-Hopanoic acids	0.31%	0.00%	0.03%

Table 5. Key-ratios, based on acid fractions distribution compounds, used on geochemical interpretation.

Biomarker ratio	Oil A	Oil B	Oil C
25-Nor/(25-Nor + αβ-Hop)	0.31	0.00	0.66
ββ-Hop/(ββ-Hop + αβ-Hop)	0.24	0.00	0.45
Tric/(Tric + *n*-alkanes)	0.80	0.07	0.97
(25-Nor+ ββ-Hop)/(25-Nor + ββ-Hop + *n*-alkanes + αβ-Hop)	0.04	0.00	0.03

The low abundance of *n*-$C_{17,18,19}$, associated with lower concentrations of acyclic isoprenoid acids and higher tricyclic and 25-norhopanoic acid concentrations, suggest higher levels biodegradation which agrees with information obtained from saturated and aromatic fractions.

1.5 General Considerations: Characterization and Extraction Processes

Concerning neutral fraction analysis, elution solvents were changed to avoid chlorinated solvents aimed at an effective separation between saturated and aromatic fractions. A sample-to-silica ratio was used, without alumina or silver nitrate use, resulting in a better separation between such classes as shown using *n*-alkanes/*n*-alkylbenzenes and triaromatic steranes.

An additional factor displaying the complex nature of the evolution of the sample, and hence the composition of its oils, is the detection of biomarkers associated with dinoflagellates of marine origin in both the saturated fraction (dinosteranes), and the aromatic fraction (triaromatic dinosteranes) in all three samples.

Since dinoflagellates commonly bloom due to ocean eutrophication, the presence of dinosterols-derivatives in oil B, which clearly shows characteristics of a sample deposited under marine conditions, this is of no surprise.

However, for oils A and C, the biomarkers indicate a lacustrine depositional environment, sometimes fresh and sometimes saline, dinosterols-derivatives presence suggests some marine influence on lacustrine-origin OM. In all cases, the presence of biomarkers associated with dinoflagellates suggests dinoflagellates were important primary producers, and their OM was deposited under eutrophic conditions.

Regarding biodegradation, samples A, B and C were classified as PM3, PM0, and PM5, respectively, on the Peters and Moldowan scale. 25-Norhopanes were detected only at low concentrations, and biomarker-based geochemical parameters indicate that biodegradation did not change the distribution of steranes and hopanes.

The low abundance of tricyclic terpanoic acids in oil B acidic fraction agrees with the low abundance of tricyclic terpanes into the corresponding neutral fraction. 25-Norhopanoic acids detection is also in agreement with the detection of 25-norhopanes in the A and C oils, and the ratio 25-norhopanoic acid/(25-norhopanoic acids+ αβ-hopanoic acids) supports the same order of biodegradation, C > A >> B, inferred based on saturated hydrocarbons.

Unlike hopanes, hopanoic acids distribution shows the ββ-isomers, of biologically and thermally less stable configuration, and the ratio ββ-hopanoic acid/(ββ-hopanoic acid + αβ-hopanoic acids) is higher for the C oil, which may be derived from the bacteria responsible for oil biodegradation.

Especially in oil B, series of 3-carboxylsteranes, 3-carboxymethylsteranes were observed, the isomers with biological configuration (ααα*R*), behavior like that observed

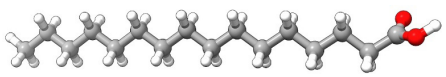
inthe saturated hydrocarbon fraction predominate, where steranes are abundant, and the ααα 20*R* and 20*S* isomers predominate.

In summary, acidic fractions data from three samples indicate that acidic biomarkers provide information parallel and complementary to that obtained from the hydrocarbon biomarkers. The extraction, derivatization, and analysis of carboxylic acids from oil samples proved efficient, leading to quantitative recoveries and good yields.

CHAPTER 4

ISOLATION OF NEUTRAL AND ACIDIC BIOMARKERS FROM SEDIMENTARY ROCKS OF THE CODÓ FORMATION – CRETACEOUS

Sedimentary rocks are formed by biological detritus and are an essential source of fossil material, such as oil and coal. Geobiologists use crucial evidence to understand ancient life on Earth through organic remains preserved in rocks. Specific lipids are very resistant to degradation, and under the right geologic conditions, can be preserved in recognizable form for billions of years. This chapter presents the process of extraction and analysis of organic matter from outcrop rocks and cores of the Codó Formation - Cretaceous.



1 | ISOLATION OF NEUTRAL AND ACIDIC BIOMARKERS FROM SEDIMENTARY ROCKS OF THE CODÓ FORMATION - CRETACEOUS

Many biomarkers are highly stable and preserved in sedimentary rocks over billions of years. As seen in the previous chapter, although the fraction of carboxylic acids in old sediments and oils are not routinely used in petroleum biomarker studies, they have proven to be very useful in thermal evolution studies, in biodegradation-related studies, and in establishing diagenetic pathways, as well as in characterizing recent sediments and oils. Thus, it is expected that these biomarkers will soon be used in routine analyses in exploration and correlation studies.

In terms of molecular composition, OM extracted from sedimentary rocks is far less complex than oil samples, and extraction methods give better results, especially for samples with high total organic carbon contents.

Because of its great relative abundance, most work on sedimentary rocks is limited to the analysis of hopanoic acids, while few are related to analysis steranoic acids. Their presence has been attributed to fractionation due to geochromatographic effects, or they may be incorporated during oil migration ("washing" effects), leading to the incorporation of acids of different maturities; oils with different levels of thermal evolution. Oil contamination during migration through immature sediments or formation during biodegradation, either by bacterial oxidation or from the biomass of degrading bacteria, is reported by different authors (FARRIMOND; GRIFFITHS; EVDOKIADIS, 2002; JAFFE; ALBRECHT; OUDIN, 1988; JAFFÉ; GALLARDO, 1993; MEREDITH; KELLAND; JONES, 2000; WATSON et al., 2002). In immature samples, hopanoic acids may be more abundant than hopanes (BENNETT; ABBOTT, 1999), but lupanoic acids may be absent or present only in low relative abundance (JAFFE; GARDINALI, 1990). Like hopanes, hopanoic acids occur as 17β(H), 21β(H), 17β(H),21α(H) and 17α(H),21β(H) (22S and 22R) isomers, with progressive loss of the biological βββ isomers with increasing maturity.

In this section, only the chromatographic profiles obtained will be presented without a detailed discussion of outcropping rock samples and cores from the Codó Formation - Cretaceous. The overall objective is NOT to perform a detailed sample characterization but to evaluate (i) the extraction capability of carboxylic acids in rock samples using the proposed stationary phase, (ii) the derivatization procedure and detection of these acids besides (iii) observing the general distribution of biomarkers.

1.1 Parnaíba Basin – Codó Formation

The intracratonic Parnaíba Basin (**Figure 57**) is filled with Paleozoic and Mesozoic rocks, covering an area of more than 600. 000 km^2 in Northern Brazil (MILANI; ZALAN, 1999), bounded by the Guaporé, São Francisco and São Luis cratons, with a total thickness of its rocks of 3.500 m (GÓES; SOUZA; TEIXEIRA, 1990; VAZ et al., 2007). The origin of

the Mesozoic record is related to pure, transcurrent shear, associated with the installation of an abandoned intracratonic rift system that developed along the Brazilian equatorial margin during the separation of the African and South American continents in the Neo-Jurassic/Eo-Cretaceous (AZEVEDO, 1991; ROSSETTI; GÓES; TRUCKENBRODT, 2001).

The sedimentary record of the Parnaíba Basin is divided into five supersequences: Silurian (Serra Grande Group), Mesodevonian-Eocarboniferous (Canindé Group), Neocarboniferous-Eotriassic (Balsas Group), Jurassic, and Cretaceous, whereby the same is delimited by discordances that extend throughout the basin or cover extensive regions (CARDOSO et al., 2017; FERNANDES; DA FONSECA; PONCIANO, 2012; VAZ et al., 2007).

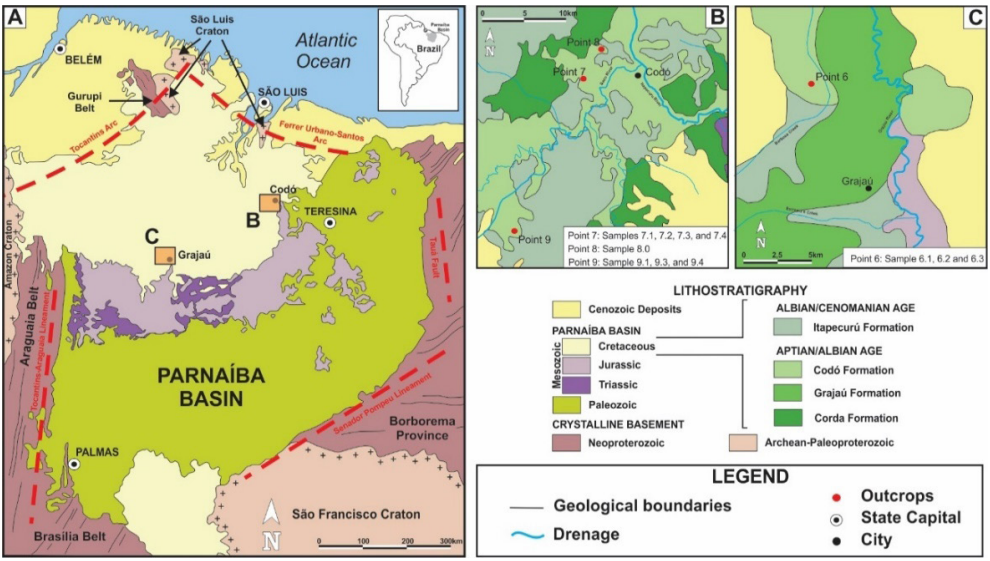


Figure 57. Location and schematic geological map of the Parnaíba Basin. This Figure was reproduced with permission from Sousa et al. 2019

The Cretaceous supersequence is composed of the Corda, Grajaú, Itapecuru, and Codó Formations; it was formed during the movement of the depocenters to the northern and northwestern extremities of the basin, as a reaction to the opening of the Atlantic Ocean, leading to transgressions and regressions in the initial stage of the formation of the super sequence forming its main marine deposits (VAZ et al., 2007).

The Codó Formation was extensively studied between 1979 and 1982 by PETROMISA, a subsidiary of PETROBRAS. This formation includes black and bituminous shales, with carbonate intercalations and anhydrite; white and greenish sandstones, and highly fossiliferous deposits - Aptian-Albian (REIS; CAPUTO, 2007).

Paleoenvironmental interpretations of the Codó Formation (CF) are not yet consensual in the scientific community. The discussion about the marine influence and the existence

of hypersaline lakes in the deposits has always been a point of divergence among the various authors who have studied this unit (ANTONIOLI; ARAI, 2002; BAHNIUK et al., 2014; BASTOS et al., 2014; PAZ; ROSSETTI, 2001; RODRIGUES, 1995). Recent studies suggest that the evolution of the Codó Formation occurred in a closed lacustrine paleoenvironment with alternating episodes of shrinking and expanding lake levels (BAHNIUK et al., 2015).

There is better documentation of the Codó Formation from studies of the Parnaíba Basin, which is adjacent to the São Luís Basin in the south.

Rodrigues (1995), from biomarker data, suggested the division of this Formation in 5 chemostratigraphic units, with different depositional characteristics: units 1 and 5 - siliciclastic intervals with low Total Organic Carbon (TOC), oxic conditions, marine environment (normal marine to oligohaline); unit 2 - intercalations of limestone, marl, and shales with higher TOC, in a restricted aerobic, dioxic to anoxic hypersaline environment; unit 3 - with high TOC and hydrogen index values, an indication of an anoxic depositional environment; and unit 4 - evaporitic sedimentation (anhydrite, limestone, and marl), low TOC and with a predominance of type III organic matter, suggesting oxic/dissoxic conditions.

Gonzalez et al. (2020) suggested six types of subdivisions during sedimentation of the Codó Formation. Two similar stages, 1 and 3 (2630 - 2617 m and 2595.8 - 2608.45 m), have amorphous organic matter, with an absence of marine and stratification indications in the water column with high % TOC; stage 2 (2617 - 2608.45 m), with high % TOC, reductive conditions and detection of gammacerane; stage 4 (2595.8 - 2567m) lacustrine environment (palynofacies), with marine contribution (stratification in the water column); stage 5 (2558 - 2540m) freshwater environment, poorly restricted, possibly coastal (marine contribution), with arid conditions and stage 6 (2494 - 2472m) which corresponds to the upper part of the Formation, with increasing marine deposition.

1.2 Process of separation of neutral and acids, an overall analysis of the data by GC-MS

The rock samples were collected in August 2014, Codó and Grajaú cities in the maranhão state. The rock samples were cut, cleaned, and crushed (DE SOUSA et al., 2020; SOUSA et al., 2019). The crushed rock was subjected to extraction in a Soxhlet-type system for 24 h using the azeotropic mixture dichloromethane/methanol 12%. The extract was filtered, concentrated, and fractionated using column chromatography (CC). The fractions were concentrated and prepared for GC-MS analysis, according to Sousa et al. (2019). The neutral fraction was obtained (**Figure 58**).

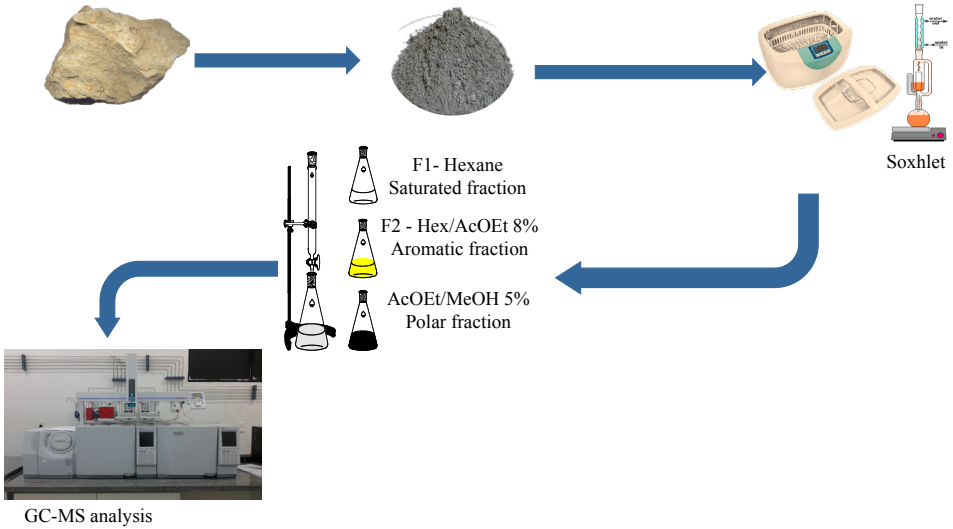


Figure 58. Classical extraction methodology, with modification of the elution system for separation of the neutral fractions.

Figure 59 and **Figure 60** show the distribution of neutral biomarkers in outcropping rocks of the Codó Formation - Parnaíba Basin and Codó Formation - São Luiz Basin, Brazil.

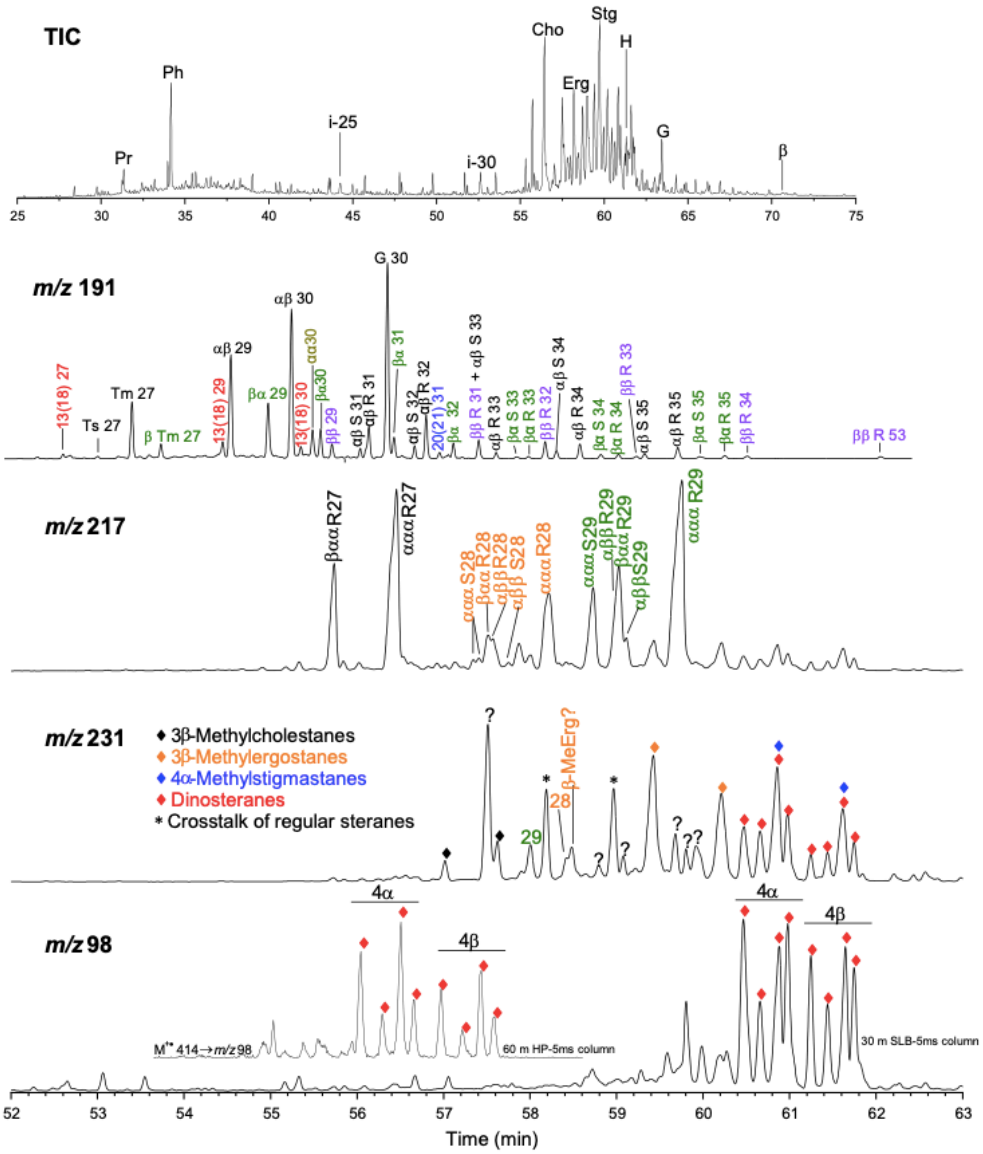


Figure 59. Distribution of saturated biomarkers in sedimentary rock outcrops of the Codó Formation, Parnaíba Basin, Cretaceous. Image adapted from Sousa et al., 2019.

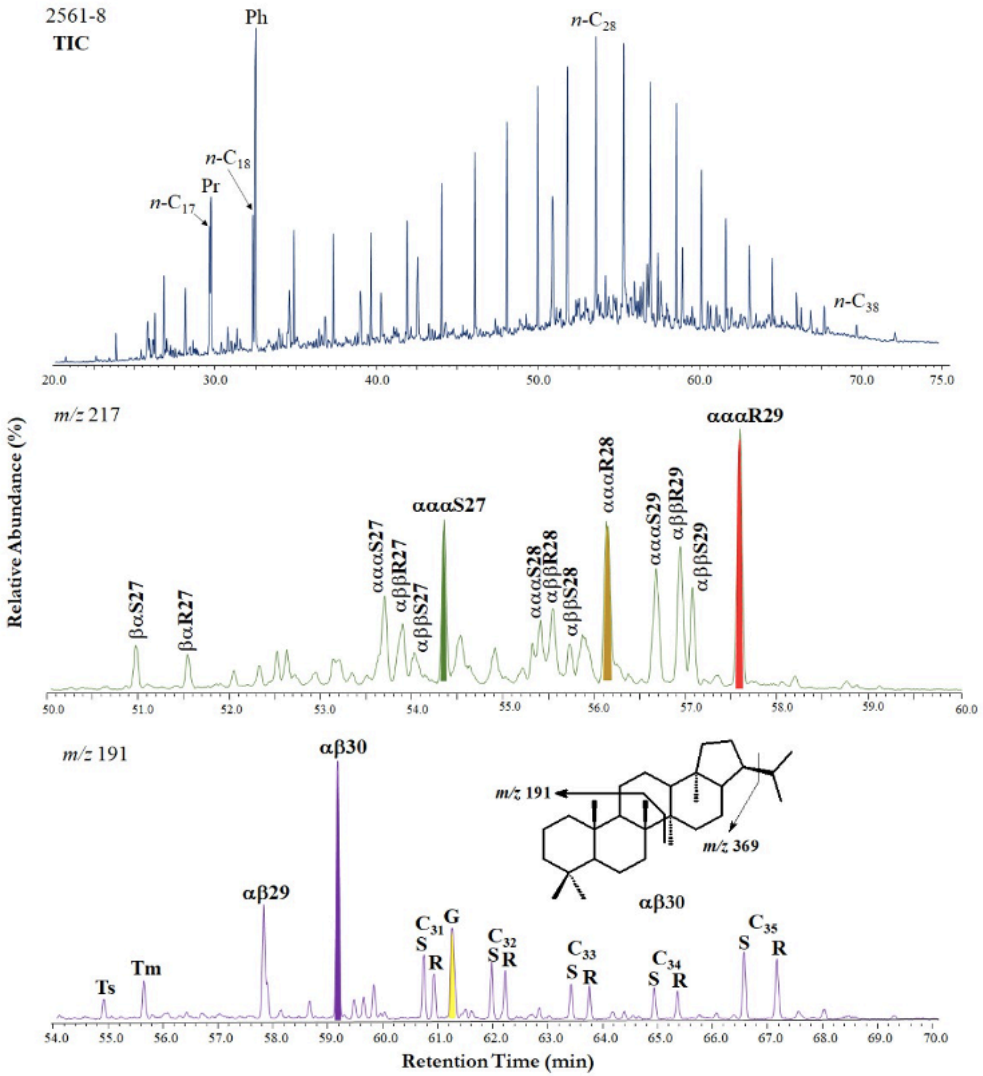


Figure 60. Distribution of saturated biomarkers in sedimentary rocks of the Codó Formation, São Luiz Basin, Cretaceous. Core sample from the Codó Formation - São Luiz Basin. The Codó Formation is considered one of the most important hydrocarbon-bearing rocks of the São Luís Basin, composed mainly of black carbonate shales. Image adapted from Silva *et al.* (2022).

The organic matter can be extracted traditionally in a system by solvent recycling, or by ultrasound. In our laboratory, we have achieved results. The method of isolating the acid biomarkers was the same as applied to the oil from Campos Basin (previous chapter): SPE - KOH/Silica; starting with 20 mg extract. This procedure is comparatively faster and, according to preliminary tests, showed a recovery efficiency of over 90%. The profiles below result from the analysis of the acids as silylated derivatives because it is a much less complex sample than the oil. Nevertheless, It could be analyzed as methyl esters. The

advantage of analyzing them as esters is their stability.

Figure 61, **Figure 62**, and **Figure 63** show the linear, isoprenoic, and cyclic acid distribution, respectively, in a rock sample from the Codó Formation - Parnaíba Basin - Brazil. The neutral fraction was characterized in previous work by Bastos et al., (2020) and Sousa et al., (2019).

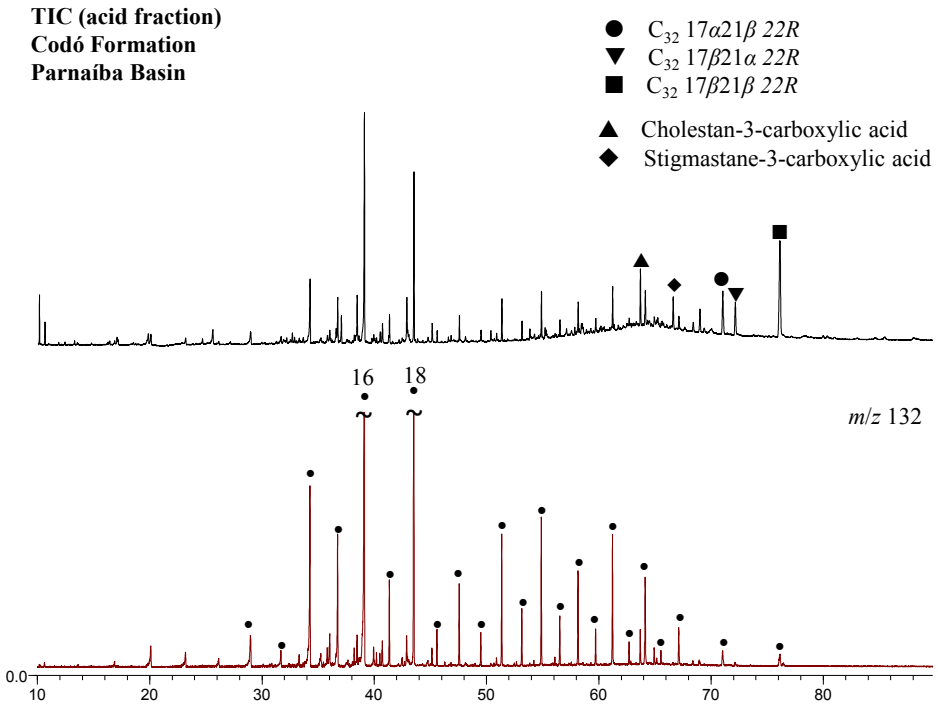


Figure 61. Total ion and *m/z* 132 mass chromatograms of the acidic fraction (as trimethylsilyl esters) of the organic rock extract from Codo Formation (Parnaiba Basin, Aptian) showing the presence of n-alkanoic acids.

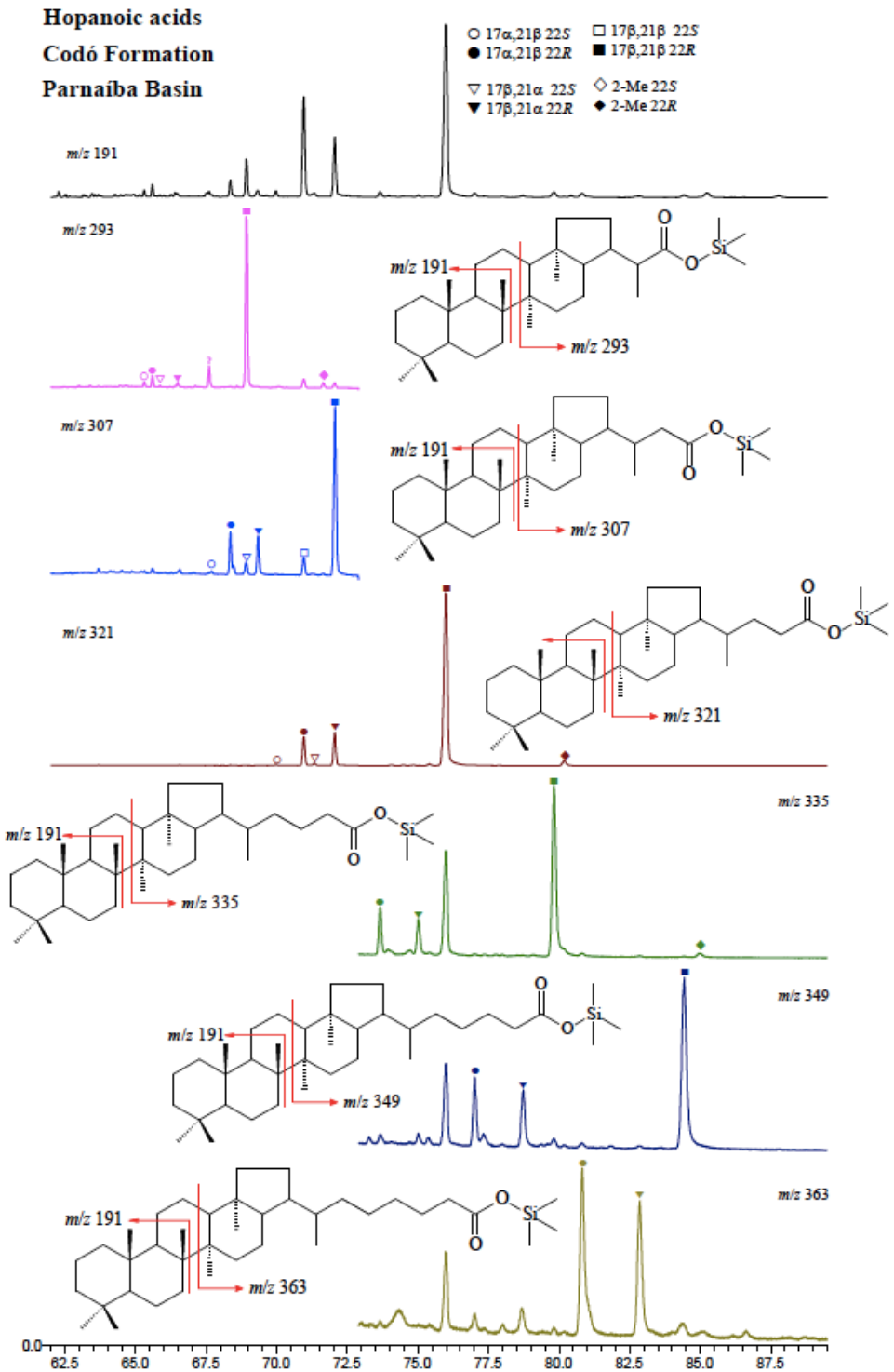


Figure 62. Distribution of hopanoic acids as silylated derivatives in a sedimentary rock extract from the Codó Formation - Parnaíba Basin - Brazil.

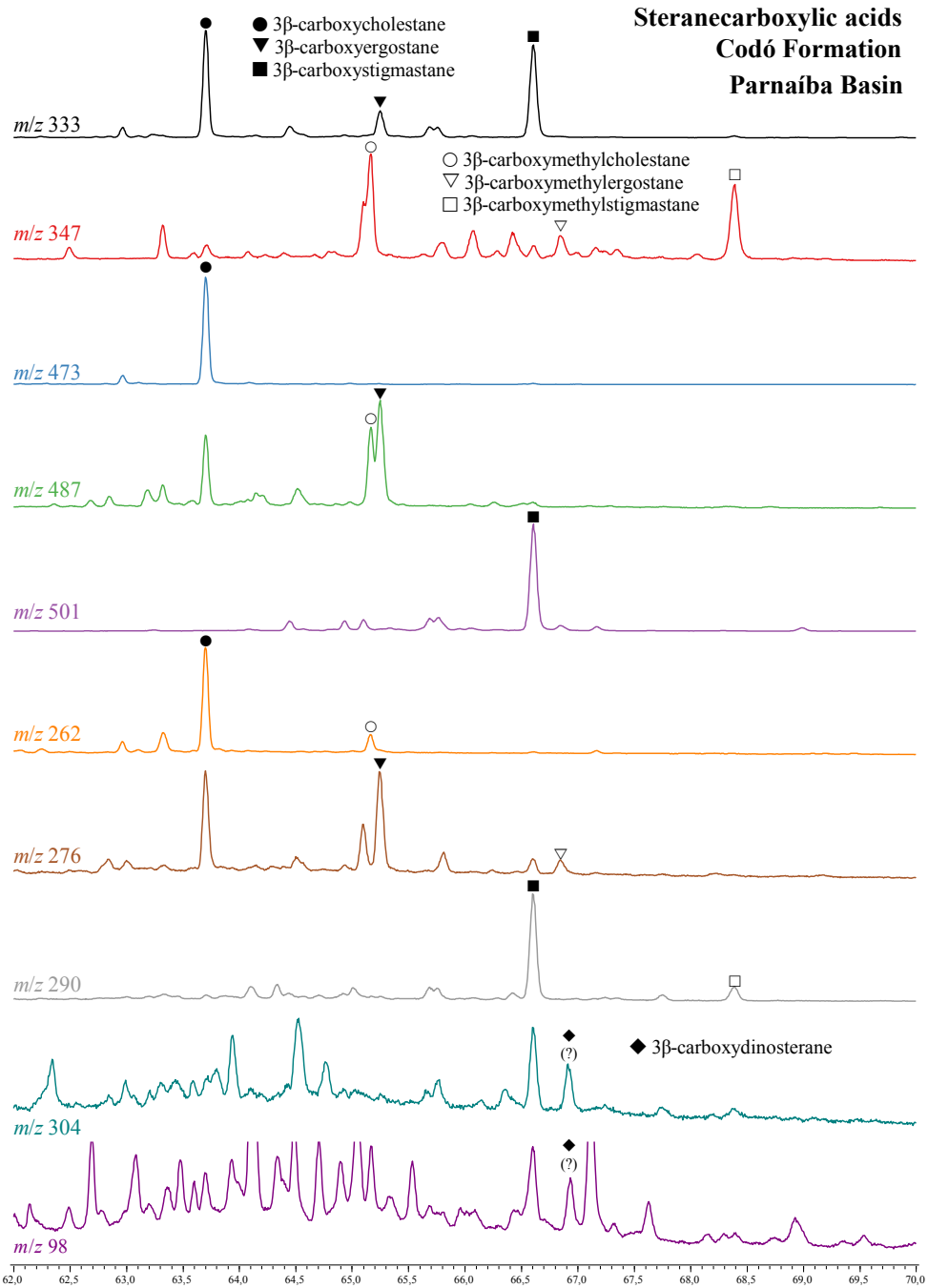


Figure 63. Distribution of steranoic acids as silylated derivatives in a sedimentary rock extract from the Codó Formation - Parnaíba Basin - Brazil.

1.3 General considerations about the Codó Formation

The great extension of the Codó Formation, associated with the occurrence of multiple marine incursions and regressions in the Aptian, creates several paleoenvironmental variations, favoring a great variety of organisms living in this area. This has generated many discussions regarding the origin, the predominant depositional environment.

The analysis of saturated and aromatic hydrocarbons in outcrop samples collected in the municipalities of Codó and Grajaú suggested the organic matter presents low thermal evolution, molecular indicators from samples selected indicating marine sources, an anoxic depositional paleoenvironment under euxinic conditions.

The results indicate that the extraction protocol employed - both the modified stationary phase and the extraction methodology employed - is very promising. This formation will be the object of study for future projects, and its characterization will be done later, with analytical details. The extraction, derivatization, and analysis of carboxylic acids from oil samples proved efficient, leading to quantitative recoveries and good yields.

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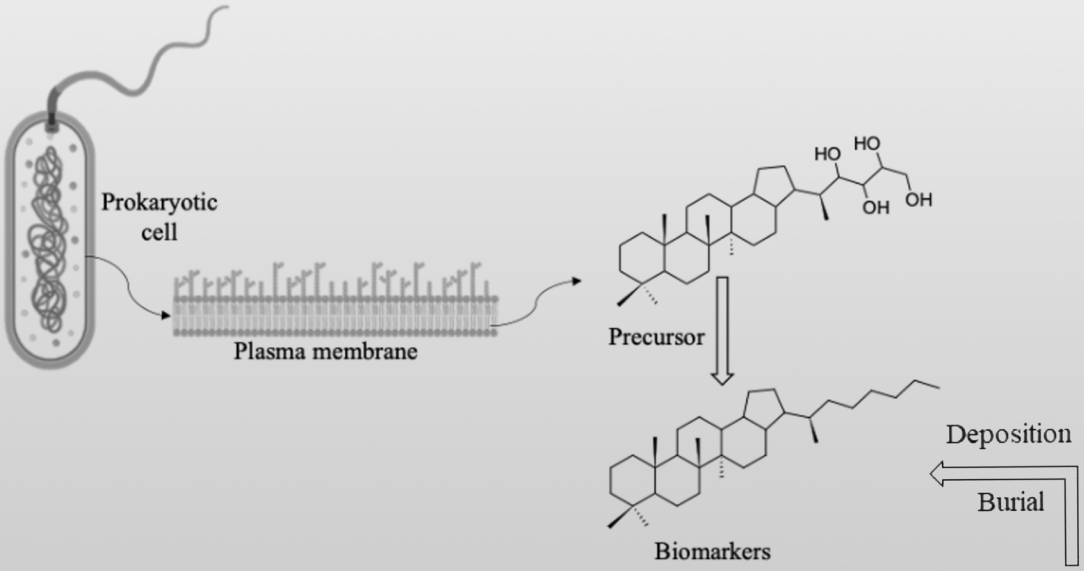

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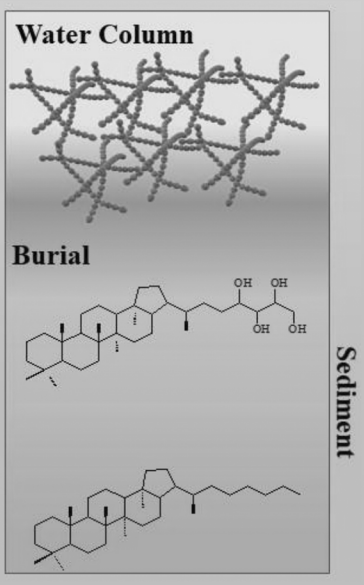

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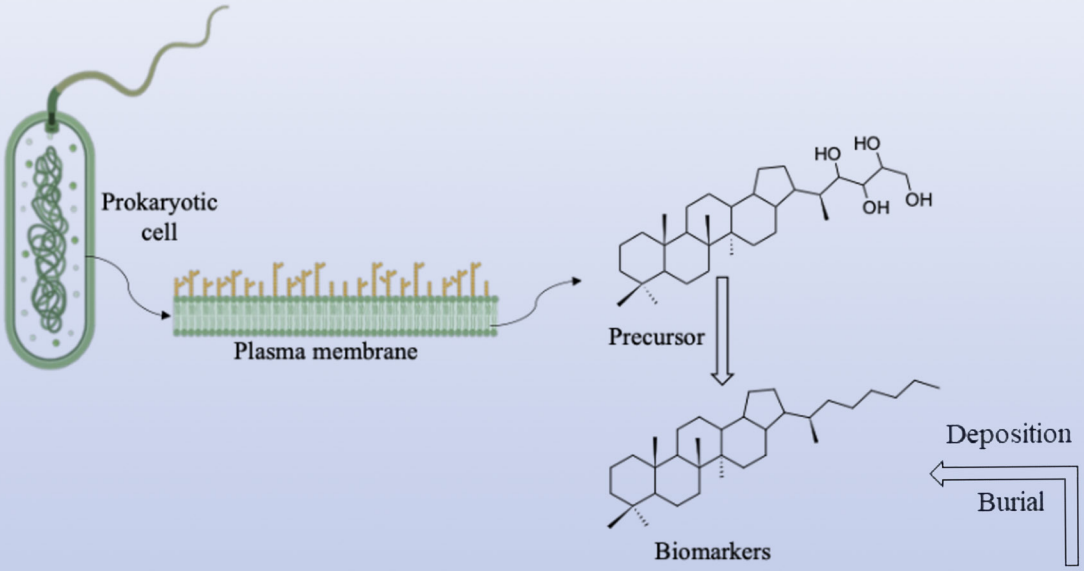

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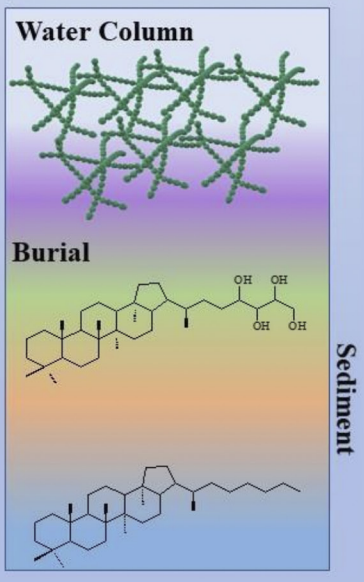

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