

Application of Biomarker Data in Oil Characterization

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Abstract

Biological markers (biomarkers) are a large group of hydrocarbons that are readily employed to assess the nature of petroleum oil samples. While the chemical applications of biomarkers are vast, this study used selected acyclic and cyclic, polycyclic terpenoids, and tetraterpenoids to understand the nature of twenty Williston Basin oils. The analysis also used the BioTool, an updated database that was developed for this particular study. Some of the reliable biomarker ratios included saturated hydrocarbon pristane/phytane ratio, diasteranes/steranes, C_{24}/C_{23} tricyclic terpane, $C_{24}TeT/C_{30}$ hopane, and C_{31} 22R homohopane/ C_{30} hopane. Overall, a biomarker analysis using the BioTool is efficient, effective, and user-friendly. Further work should be carried out developing constraints on the biomarker ratios for certain characteristic oil Basins.

Introduction

1.0 Overview of biological markers

Biological markers (biomarkers)—coined as “chemical fossils” by Eglinton et al. (1964)—are a group of complex organic compounds composed of carbon, hydrogen, oxygen, nitrogen, and sulfur (Peters et al., 2005) and they are primarily found in oils and rock extracts of oil and gas production operations. They derive from lipid components of previously living organisms (Peters et al., 2005; Killops and Killops, 2013). Biomarkers are commonly found in crude oils and petroleum source rocks, and are measured to understand its respective source organic matter, its thermal maturity, and the paleoenvironment during diagenesis and catagenesis (Peters et al., 2005). Their nickname "chemical fossils" is derived from the fact that after undergoing through a thermal alteration, they remain structurally similar to compounds produced by living organisms (Figure 1).

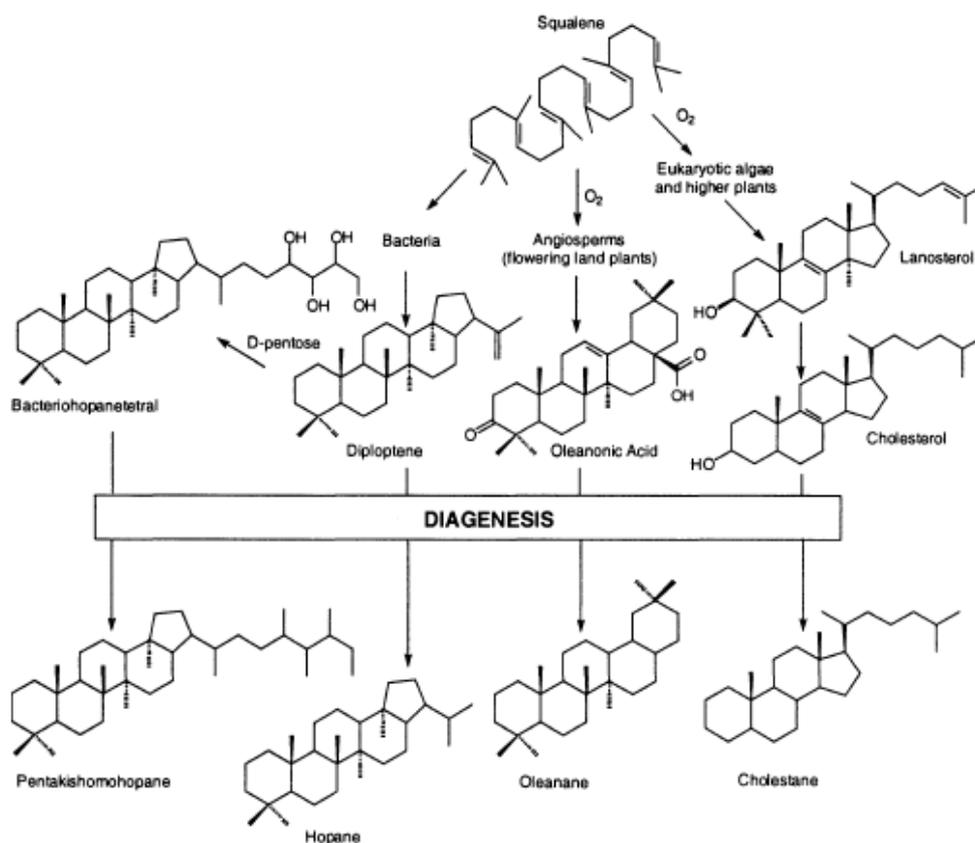


Figure 1: Biosynthesis of chemicals produced by living organisms and their equivalents that are produced through diagenesis (Hsu et al. 2011).

1.1 Organic chemistry and classification of biomarkers

Biological markers are complex organic compounds that originate mainly from lipid membranes of animals (Peters et al., 2005). Lipids have a wide range of chemical structures and functions, and can occur as Acetogenic lipids and Isoprenoid lipids. Acetogenic lipids are comprised of acetate (CH_3CO_2) units derived from acetyl coenzyme-A (De Niro and Epstein, 1977; Hayes, 2001). The second class of lipids comprises of polyisoprenoids, composed of two or more five-carbon units of isoprene (or methylbutadiene) units (Figure 2). Isoprenoids are compounds that compose two or more isoprene units are called terpenoids, isoprenoids, or isopentenoids; terpenoid is the most common term used in literature. According to the isoprene rule, the biosynthesis of terpenoids requires polymerization of 5-carbon (C_5) isoprene molecules. Thus, the number of carbon atoms in a terpenoid is always a multiple of 5. The linkages between isoprene subunits could occur in three ways: 1) head-to-head (1-1; considered regular); (2) head-to-tail (1-4; considered irregular); and 3) tail-to-tail (4-4; considered irregular). Most isoprenoids follow the isoprene rule, but there are some exceptions (e.g. pristane).

Isoprenoids could be further classified into three classes, acyclics and cyclics, polycyclic terpenoids, and tetraterpenoids. The first two categories will be the main focus of this study.

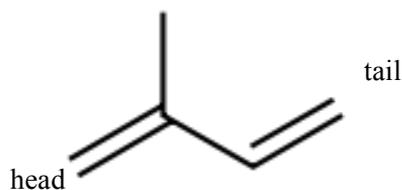
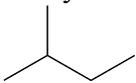
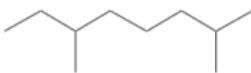
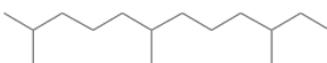
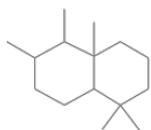
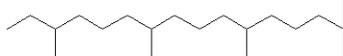
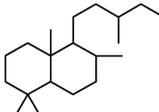
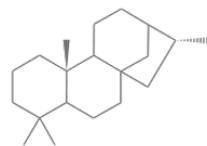
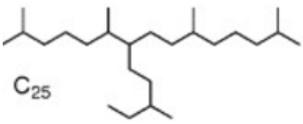
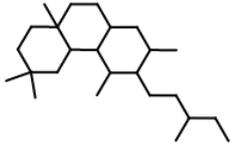
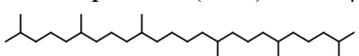
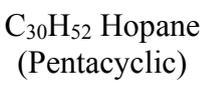


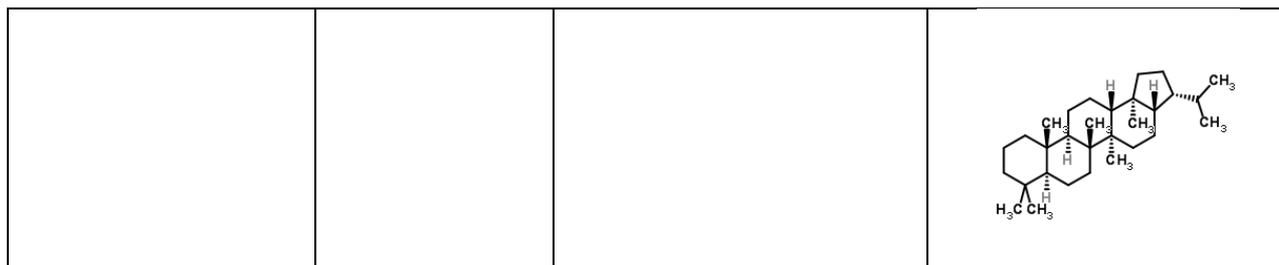
Figure 2: Chemical structure of one biosynthesized isoprene unit. The “head” corresponds to the Carbon 1 (first carbon), and the “tail” is Carbon 4 (last carbon).

1.2 Terpanes

Terpanes are the saturated form of terpenoids and are comprised of a wide range of acyclic and cyclic chemical structures. These structures are based on the number of isoprene units (Table 1). In general tricyclics, tetracyclics, hopanes, and steranes biomarkers contribute to the terpane diagnostic “fingerprint” and have been extensively used to characterize petroleum oils and source rocks (Seifert, 1986; Peters et al., 2005).

Table 1: Terpane structures, isoprene units and their corresponding acyclic and cyclic examples.

Terpane Name	Isoprene Units	Acyclic Example	Cyclic Example
hemiterpane (C5)	1	2-Methylbutane 	
monoterpane (C10)	2	2,6-Dimethyloctane 	
sesquiterpane (C15)	3	Farnesane 2,6,10-Trimethyldodecane 	Drimane (Bicyclic) 
diterpane (C20)	4	Phytane 	Labdane (Bicyclic)  Phyllocladane (Tricyclic) 
sesterterpane (C25)	5	Highly branched isoprenoid (C25 HBI)  C ₂₅	C25 Tricyclic Terpene 
triterpane and steranes (30)	6	Squalane (C30) 	C ₃₀ H ₅₂ Hopane (Pentacyclic) 



1.2.1a Tricyclic terpanes (C_{19} - C_{30})

Tricyclic terpanes are aliphatic cyclic hydrocarbons that are characterized based on five-carbon isoprene units (Peters et al., 2005). They comprise of three rings and an alkyl chain, and are a part of the terpenoid biomarker group (Peters et al., 2005).

Tricyclic terpanes (TT) were first discovered by Anders and Robinson (1971) and structurally (chemical) described by synthesis data (Ekweozor and Strauz, 1982; Aquino Neto et al., 1982). Its series extend from C_{19} to C_{54} and are widely documented in petroleum source rocks and crude oils (Aquino Neto et al., 1983; Moldowan et al., 1983; DeGrande et al., 1993; Peters et al., 2005; Fazeela et al., 2011; Tao et al., 2015). To date, the biological sources of tricyclic terpanes remain unclear. Their precursors are thought to be C_{30} tricyclohexaprenol, a possible anaerobic product of hexaprenol and constituent of prokaryotic membranes (Ourisson et al., 1982; Aquino Neto et al., 1982; Peters et al., 2005). High concentrations of tricyclic terpanes and their aromatic counterparts, however, have been widely-correlated with Tasmanites rocks, suggesting a source from these algae (Aquino Neto et al., 1983, 1992; Greenwood et al., 2000). Isotope analyses of kerogen oxidation and Raney Ni reduction products carried out by Simoneit et al. (2005) have also concurred with an algal source. Despite this, Talyzina et al. (2000) observed few tricyclic terpanes in Tasmanites-rich shale; Dutta et al. (2006) reported traces of tricyclic terpenoids in pyrolysates of the *Leiophaeidia*, not Tasmanites; and Farrimond et al. (1999) found tricyclic compounds in a wide range of sediments and multi-aged oils. This suggests that tricyclic terpenoids may not be solely found in Tasmanites; there could be other sources or factors that are in play for their formation.

Despite the controversy surrounding the origin of tricyclic terpanes in sediment and crude oil, they provide extremely valuable information for the petroleum industry. Due to their widespread abundance and distribution in oils, tricyclic terpanes are heavily applied in petroleum exploration to assess organic matter input, thermal maturity, depositional environment and correlation of crude oils (Peters et al., 2005). Other tricyclic terpanes, such as tricyclic diterpanes (C_{19} - C_{20}), have been used as terrigenous indicators (Alexander et al., 1983; Peters et al. 2005). The most common tricyclic terpane is the C_{23} $13\beta,14\alpha(H)$ stereochemical series homolog, an indicator in crude oils derived from saline lacustrine and marine sources (Aquino Neto et al., 1983; Peters et al., 2005; Tao et al., 2015).

The presence of tricyclic terpanes in m/z 191 mass chromatograms are used for determining organic matter input, assessing oil/oil and oil/source-rock correlations, and identifying depositional environments and thermal maturity (Seifert et al. 1980; Hanson et al., 2000; Peters et al., 2005).

1.2.1b Biodegradation effect on tricyclic terpanes

A nine-level scale for assessing the levels of biodegradation of any crude oil was first proposed by Volkman et al. (1983b). Since then, multiple studies have been conducted to refine the biodegradation scale (e.g. Seifert and Moldowan, 1979; Connan, 1984; Williams et al., 1986; Wenger et al., 2002; Larter et al., 2006). The first five levels of biodegradation are focused and based on n-alkanes, alkylcyclohexanes, isoprenoids.

Tricyclic terpanes are thought to be highly resistant to biodegradation, thus their common use to correlate severely degraded oils (Seifert and Moldwan, 1979; Peters et al., 2005). However, the effect of biodegradation on the distribution of tricyclic terpanes is uncertain as very little is known about this area of research (Cheng et al., 2016). Several studies have found that tricyclic terpanes are unaffected in various crude oils and oil sands, where steranes and hopanes were biodegraded at different scales or removed (Reed, 1977; Seifert and Moldowan; 1979; Connan, 1984, Peters et al., 2005; Larter et al., 2012). However, severe terpane biodegradation in crude oils (e.g. Colombian and Venezuelan oils) has led to difficulties in characterizing them, leaving investigators to find alternative prediction tools (Spigolon et al., 2010; Garcia et al., 2015). Our understanding of the role of microorganisms in alteration of tricyclic terpanes is also limited. It is believed that demethylated tricyclic terpanes (17-nor-tricyclic terpanes) are generated via microbial demethylation of its C-10 position within the reservoirs (Jiang et al., 1990, Cassani and Eglinton, 1991; Alberdi et al., 2001).

Thus far, studies have found that tricyclic terpanes are: altered at varying biodegradation levels, ranging from level 6 to 10 on the Peters and Moldowan (1993) scale (PM); biodegraded through methylation at the C-10 position as well as other pathways; and varying in resistance to biodegradation (e.g. C₂₀ and C₂₁ are most resistant, but C₂₈ is less resistant than C₂₉). In Cheng et al. (2016), the behavior of tricyclic terpanes in a wide variety of heavy to severe biodegraded crude oils from China was investigated. The study observed biodegradation of tricyclic terpanes in the most severely degraded crude oils, and those without any hopanes and steranes present. Moreover, biodegradation occurred without the microbial demethylation. Based on results, Cheng et al. (2016) postulates that the biodegradation pathways of tricyclic terpanes occur in two steps. Oxidation from a methyl to a carboxyl group first occurs and forms tricyclic terpanoic acids, which are then demethylated at the C-10 position to create demethylated tricyclic terpanoic acids.

Aquino Neto et al. (1983) observed the generation of tricyclic terpanes from kerogens, suggesting that it might be strongly linked with macromolecular organic matter by multiple covalent bonds. Bowden et al. (2006) observed a larger amount of tricyclic terpanes relative in resin and asphaltene fraction than in kerogen free hydrocarbon products in a Jet Rock sample. This further suggests a connection between macromolecular fractions and tricyclic terpanes in kerogen fractions (Bowden et al., 2006). Moreover, kerogen fractions could potentially serve as an additional source of geochemical information when performing oil biomarker analyses.

1.2.1c Tricyclic terpane (TT) ratios

$C_{22}TT/C_{21}TT$, $C_{24}TT/C_{23}TT$, and $C_{26}TT/C_{25}TT$ tricyclic terpane ratios are generally used to identify oil sources, from marine to evaporitic (Peters et al., 2005). Oil from carbonate source rocks commonly show elevated $C_{22}TT/C_{21}TT$ coupled with lower $C_{24}TT/C_{23}TT$ ratios, facilitating identification from other sources (Peters et al., 2005). The $C_{26}TT/C_{25}TT$ tricyclic terpane ratio plotted against $C_{31}R/C_{30}$ hopane is also used to identify lacustrine from marine oils (Peters et al., 2005).

Terrestrial oils are commonly enriched with C_{19} and C_{20} tricyclic terpanes while the C_{23} member is most dominant in marine source oils (Aquino Neto et al., 1983; Peters et al., 2005). High concentrations of $C_{19}TT/C_{23}TT$ and $C_{20}TT/C_{23}TT$ ratios have been found in terrestrial organic matter (Hanson et al., 2000; Peters et al., 2005; Volk et al., 2005). Huang et al. (2015) suggested an increasing stability of $C_{19}TT$ in more thermally mature samples of the Tarim Basin, China. The study observed values of $C_{19}TT/C_{20}TT$ increasing with maturity (Huang et al., 2015).

To date, few studies have concentrated on tricyclic and tetracyclic terpane distributions within different depositional environments. Tao et al. (2015) investigated crude oils from NW China and developed new source dependent parameters of tricyclic and tetracyclic terpanes. The study associated $C_{25}TT/C_{26}TT$ ratios <1 with terrigenous oils (0.36-0.38) as well as saline lacustrine (0.29-0.65) and freshwater oils (0.31-0.45). Previous work reliably used $C_{25}TT/C_{26}TT$ ratios to distinguish marine (>1) and non-marine (<1) oils of West Africa (Burwood et al., 1992).

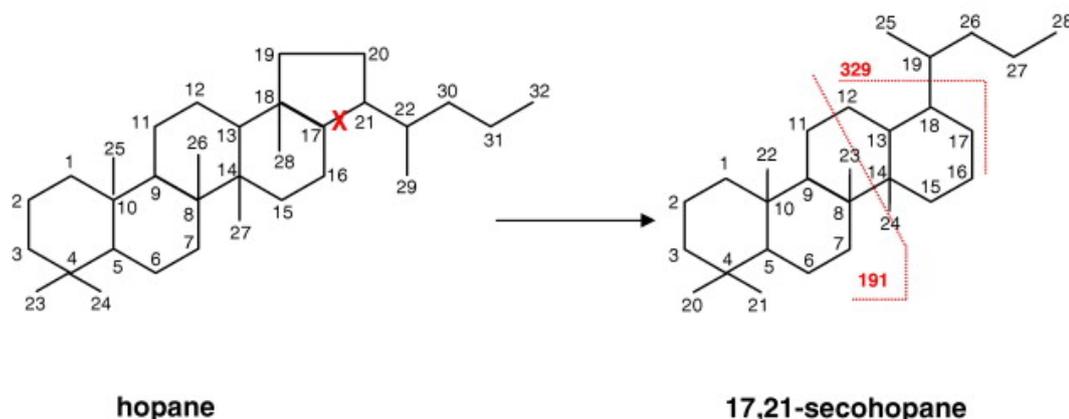


Figure 3: Chemical structures tetracyclic terpanes, which are believed to occur through the microbial opening of ring-E, C-C bond between C_{17} to C_{21} .

1.2.2a Tetracyclic Terpanes

Tetracyclic terpanes (C_{24} - C_{27}), or 17,21-secohopane series, are thought to derive from the degradation of pentacyclic hopanes (Trendel et al., 1982; Peters et al., 2005). This may occur through the thermal or microbial opening of ring-E, carbon/carbon bond between C_{17} to C_{21} , of hopanoids (Figure 3) (Trendel et al., 1982; Aquino Neto et al., 1983; Peters et al., 2005). The microbial opening of the ring may occur at early stage of diagenesis (Trendel et al., 1982; Aquino Neto et al., 1983). Similarly with tricyclic terpanes, tetracyclic terpanes occur in oil and rock extract, and are more resistant to degradation than hopanes (Aquino Neto et al., 1983; Peters et al.,

2005). High concentrations of C₂₄ tetracyclic terpanes in oils have been linked to both carbonate/evaporitic (Palacas et al., 1984, Connan et al., 1986; Connan and Dessort, 1987; Peters et al., 2005) and terrestrial (Philip and Gilbert, 1986; Grice et al., 2001; Peters et al., 2005) oil samples.

1.2.2b Tetracyclic terpane (TeT) ratios

The most commonly used tetracyclic terpane ratios used to characterize oils are C₂₄TeT/C₃₀ Hopane, C₂₄TeT/C₂₃TT, C₂₄TeT/C₂₆TT. These ratios are commonly used to assess source of deposition. The C₂₄TeT/C₃₀ Hopane ratio increases with increasing thermal maturity and increasing biodegradation (Palacas et. al, 1984, Connan et. al, 1986, Connan and Dessort, 1987). Tetracyclic terpanes are more resistant to biodegradation than the hopanes, facilitating the differentiation between carbonate and evaporitic source rock settings (Palacas et. al, 1984; Connan et al., 1986; Peters Moldowan, 1993). High C₂₄TeT/C₂₆TT values (>1) indicate carbonate organic matter (Peters and Moldowan, 1993); low to medium values suggests marine shale source; and low C₂₄TeT/C₂₆TT values have been observed in deltaic shale sources (Peters et a., 2005).

1.2.3a Hopanes and steranes in petroleum (C₂₇ and onward)

Hopane and sterane biomarkers are commonly used to help identify hydrocarbon sources. Hopanes are the most common pentacyclic terpenoids comprised of a chemical structure of a degraded hopanoid (seen in Table 1 and Figure 3). Hopanes are comprised of four cyclohexane rings and one pentacyclic ring, and are found in higher plants and prokaryotes (Ourisson et al., 1979; Peters et al., 2005). Most hopanes contain 27-35 carbons that form the 17 α (H), 21 β (H) structure, which are more thermodynamically stable. Other stereoisomeric structures include 17 α (H), 21 β - and 17 β , 21 β -hopanes. Hopanes that have more than 30 carbon atoms are generally called homohopanes.

The use of hopanes to characterize oils is common in many biomarker studies. Hydrocarbon products of 2-methylbacteriohopanepolyols, biomarkers for cyanobacteria have been used to understand the inception of oxygenation via photosynthesis (Summons et al., 1999; Brocks et al., 2003; Peters et al., 2005). High content of diahopanes (especially 17 α (H)-diahopane) is believed to indicate the catalytic effect of clay minerals under oxic-suboxic environments (Sinninghe et al., 1995; Zhu Yangming et al., 2007; Zeng et al., 2013). Oils from the Gangxi oil field, China had high abundances of tricyclic and pentacyclic terpanes (hopanes), suggesting a prokaryotic and algal source (Yao et al., 2014). Carbonate sourced rocks are often dominated with C₂₉ hopanes (Peters et al., 2005).

Steranes are thought to originate from sterols within eukaryotic organisms (Mackenzie et al., 1982a; de Leeuw et al., 1989; Peters et al., 2005). Like hopanes, most stable configurations of steranes are also comprised of multiple cyclohexanes and a single cyclopentane. In some oils occurring the Monterey Formation, steranes are isotopically uniform while hopanes were found varying laterally and vertically (Schoell et al., 1992; Peters et al., 2005).

The C₂₉ and C₂₇ sterane homologues are used to differentiate organic matter

sources from higher plants (terrigenous origin) and zooplankton, respectively (e.g. Huang and Meinschein, 1979; Zeng et al., 2014). The addition of C₂₈ steranes and C₂₇ often masks and complicates an interpretation, since both homologues are derived from algae.

1.2.3b Biodegradation of hopanes and steranes

Hopane and sterane biomarkers are generally more thermodynamically stable and resistant to biodegradation, and are commonly used to assess microbial transformation of crude oils (i.e., Peters and Moldowan, 1993; Peters et al., 1996b; Frontera-Suau et al., 2002; Prince and Walters, 2007). Several biodegradation studies using bacterial cultures have successfully biodegraded hopanes and homohopanes (Frontera-Suau et al., 2002). Lighter homohopanes (C₃₁>C₃₂>C₃₃>C₃₄>C₃₅) and the R epimers (over S epimers) seemed to be preferential to biodegradation (Frontera-Suau et al., 2002). Homohopane degradation is believed to occur via oxidization from a bacterial attack, favoring heavier homologues (Frontera-Suau et al., 2002; Peters et al., 2005).

Hopanes are removed before steranes in less biodegraded oils; the opposite is observed in heavily biodegraded oils. According to several studies, microbial degradation of steranes occurs as follows: $\alpha\alpha\alpha\ 20R \gg \alpha\beta\beta\ 20R \geq \alpha\beta\beta \geq 20S \alpha\alpha\alpha\ 20S \gg$ diasteranes and $C_{27} > C_{28} > C_{29} > C_{30}$ (Peters et al., 2005).

1.2.3c Hopanes (H) and steranes ratios

Some of the common hopane ratios used as source parameters are are C₂₄TeT/C₃₀H, tricyclics/hopanes, C₂₉H/C₃₀H, C₃₅/C₃₄ homohopane, and C₃₃/C₃₂ homohopane, (C₂₈+ C₂₉TT)/C₃₀H. Common maturity parameters include Tm/C₃₀H, Ts/C₃₀H, and C₂₉Ts/C₂₉ norH.

Diasterane/sterane ratios are commonly used to identify carbonate-rich from clay-dominant source rocks (Zeng et al., 2014). In some studies, this ratio has been used to characterize sulfur-rich oils, which is a typical clay-poor source rock (Peters et al., 2005) High gammacerane and low diasteranes were observed in a biomarker analyses from Egyptian asphalts, indicating a petroleum source from a clay-poor carbonate or an evaporite (Rullkötter and Nissenbaum, 1988).

1.3 Biological markers in petroleum

Biomarker traces found in oil samples are commonly used by petroleum geochemists as a means to better understand its characteristics. Biological markers can be used for correlations between source rocks and oil samples and in cases where source rocks are not available. The distribution of a biomarker in an oil sample can be used to infer the characteristics of the rock that generated the oil. Information that can be retrieved via this process are: the age of the rock, the relative amount of gas prone organic matter and oil prone organic matter in the source kerogen, the lithology of the source rock and its thermal maturity. Furthermore, the deposition environment can be defined (marine, lacustrine, fluvio-deltaic or hypersaline).

Specific biomarker parameters can be calibrated against specific kerogen quality parameters in a given basin. Characteristics of the source rock can be quantitatively predicted then after a measurement of the biomarker ratios in an oil sample and their projection onto calibration curves. This technique can show whether the oil was generated primarily from an oil-prone or from gas-prone organic facies (Dahl et al., 1994; McCaffrey et al., 1994). Information concerning the source type, age, maturity, and kerogen quality is of high importance for the GOR and oil reserves estimation thus it is crucial for decision making in the industry.

1.3.1 Thermal maturity of source rocks

Depending on the source rock thermal maturity, certain biomarkers can be found in different relative abundances. A simple lab analysis of the migrated oil can show the stage of the source rock's thermal maturity then since those biomarkers act as indicators (Peters and Moldowan, 1993). Table 2 presents a small selection of biomarkers that are commonly used to gain insight on the processes that prevail during source rock maturation.

Several considerations must be kept in mind when using petroleum biomarkers to assess source rock thermal maturity. The most important is the concentration of biomarkers in an oil sample, which usually decreases as thermal maturity increases. Heating rate, source lithofacies and kerogen type are decisive factors for the relation between biomarker characteristics and source rock maturity. This means that for different basins, different correlations exist for the same values due to the non-linear relation between source rock and biomarker maturities. Another important observation is that a given biomarker parameter is applicable only over a specific maturity range due to the fact that after a maturity point of the source rock, specific indicators can reach their terminal values.

In biodegraded oils, biomarker maturity can be used for the calculation of their API gravity before biodegradation (Moldowan, et al. 1992). More suitable for such applications are parameters based on highly resistant to biodegradation compounds such as Triaromatic/(Monaromatic+Triaromatic steroids).

Table 2: Selected petroleum biomarkers that are indicative of source rock maturity.

Compound class	Biomarker Parameter	Effect of Increasing Maturity	Comments
Saturated Hydrocarbons	C29 Steranes [20S/(20S+20R)]	Increase	Useful in early to mid oil window. Decreases at very high maturity levels.
	C29 Steranes [abb/(abb+aaa)]	Increase	Useful in early to mid oil window.
	Moretane/Hopane	Decrease	Useful in early oil window.
	C31 Hopane [22S/(22S+22R)]	Increase	Useful in immature rocks to onset of early oil window.
	Ts/(Ts+Tm)	Increase	Also influenced by source lithology.
	Tricyclic Terpanes/Hopanenes	Increase	Useful in late oil window; also increases at high levels of biodegradation.
	Diasteranes/Steranes	Increase	Useful in late oil window; also affected by source lithology (low in carbonates, high in shales); also increases at high levels of biodegradation.
Aromatic Hydrocarbons	Monoaromatic Steroids: (C21+C22)/ [C21+C22+C27+C28+C29]	Increase	Useful in early to late oil window; resistant to effects of biodegradation.
	Triaromatic Steroids: (C20+C21)/ [C20+C21+C26+C27+C28]	Increase	Useful in early to late oil window; resistant to effects of biodegradation.
	Triaromatic /(Monoaromatic + Triaromatic Steroids)	Increase	Useful in early to late oil window; resistant to effects of biodegradation.

1.3.2 Use of biomarkers to characterize depositional environments

Biomarkers are commonly used to evaluate and reconstruct past depositional environments (Connan, 1981, 1986; Seifert and Moldowan, 1981, Peters et al. 2005). In order to make an efficient evaluation, the biomarkers should be in a state of conservation that permits their linking with their biochemical precursor molecule (Brassell and Eglinton, 1986). Distribution of this parent molecule in the present flora and fauna is important also under the assumption that the same distribution existed in ancient organisms also (Tissot and Welte, 1984). The characteristic that makes biomarkers suitable as indicators for depositional environments is that particular compounds are present in areas where specific animals or plants exist (Philp, 2004). One of the challenges of using biomarkers to determine depositional environments is the effect of maturation and biodegradation (Jiamo et al., 1990). Thus, most

biomarker analyses are carried out on samples that have similar maturation levels and are lithologically alike.

Numerous studies have used biomarker data to characterize and distinguish carbonate and shale basins. A combination of non-biomarker and biomarker parameters could be used to distinguish carbonate from a shale source rock (Table 3).

Table 3: Some of the numerous characteristics of petroleum that are used to distinguish carbonate and shale source rocks (modified from Peters et al., 2005).

Characteristics	Shales	Carbonates	References
<i>Non-biological marker parameters</i>			
API, gravity	Medium-high	Low-medium	1, 2, 3
Sulfur, wt %	Variable	High (marine)	1, 2, 3, 6, 9
Thiophenic sulfur	Low	High	1
Saturate/aromatic	Medium-high	Low-medium	1, 2, 3
Naphthenes/ alkanes	Medium-low		1, 3
Carbon preference index (C22-C32)	≥1	≤1	1, 2, 6, 9
<i>Biological marker parameters</i>			
Pristane/phytane (Pr/Ph)	High (≥1)	Low (≤1)	1, 2, 6, 9, 10, 12
Phytane/nC18	Low (≤0.3)	High (≥0.3)	2, 6
Steranes/17α-hopanes	High	Low	7, 9
Diasteranes/steranes	High	Low	1, 15, 16
C22 tri-/C21 tricyclic terpanes	Low (<0.25)	High (>0.5)	2, 13, 14
C24 tetra-/C26 tricyclic diterpanes	Low-medium	Medium-high	2, 7
C29/C30 hopane	Low	High (>1)	10, 11
C35 homohopane index	Low	High	4, 10
Hexahydrobenzohopanes and benzohopanes	Low	High	5
Dia/(Reg + Dia) monoaromatic steroids	High	Low	8
Ts/(Ts + Tm)	High	Low	4
dibenzothiophene/phenanthrene (DBT/Phe)*	High (>1)	Low (<1)	12
C20 monoaromatic steroids	Low	High	9

References associated with Table 1: 1) Hughes, 1984; 2) Palacas, 1984; 3) Tissot and Welte, 1984; 4) McKirdy et al., 1983; 5) Connan, 1981; 6) Connan, 1981; 7) Connan et al., 1986; 8) Riolo et al., 1986; 9) Moldowan et al., 1985; 10) ten Haven et al., 1988; 11) Fan Pu et al., 1987; 12) Hughes et al., 1995; 13) Hill et al., 2007; 14) Zumberge, 1984; 15) Mello 1988a; 16) Mello 1988b.

The pristane to pythane ratio acts as an indicator of toxicity of the depositional environments. It was noted (Brocks and Summons, 2004) that isoprenoid pristane and phytane is present in crude oils, which evolved a theory (Philp, 2004) that high concentrations of pristane can be found in oxic environments while high concentrations of phytane in anoxic environments (Peters et al., 2005).

Specific animals can be indicated by certain biomarkers also. For example, botryococcane is a biomarker derived from the Chlorophycean alga *Botryococcus braunii*, and found in freshwater and brackish water environments. Highly branched

isoprenoids derived from diatoms, such as bicadinanes indicate Dipterocarpaceae and dinosteranes which indicate dinoflagellates (Figure 4) (Hsu *et al.*, 2011). Hopanoids are the most common biomarker serving as proxies for certain types of bacteria and bacterial metabolisms.

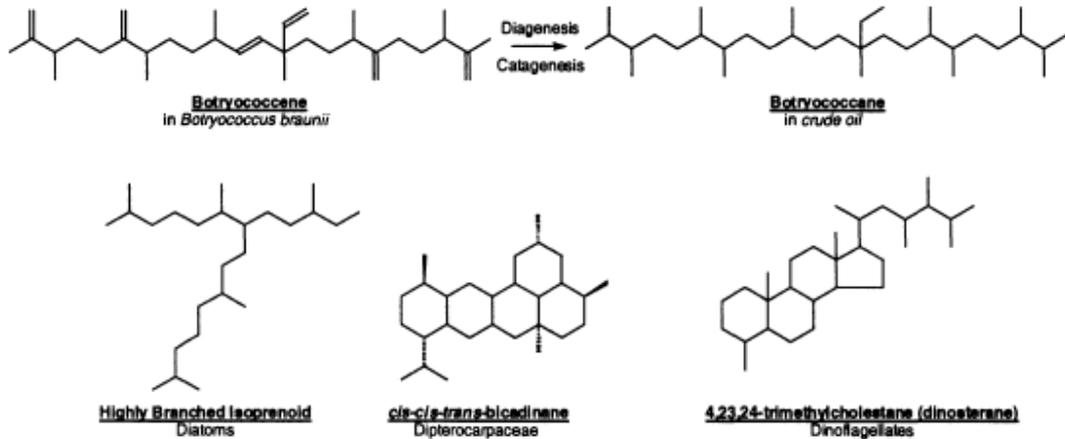


Figure 4: Biomarkers as indicators of specific organisms (Hsu *et al.* 2011).

Algal steroids show lacustrine conditions. High relative concentrations of gammacerane indicate the existence of hypersaline lakes. Diasteranes in general show clastic sediments while hopanoides are more common in hydrocarbons derived from shales). Biomarkers of warm water organisms, ones that exist in sediments with high concentrations of carbonates, can be found mostly in low latitude environments (Brooks and Summons 2004). Hopane to sterane ratio can distinguish marine sources (low values) from lacustrine environments (high values) (Figure 5).

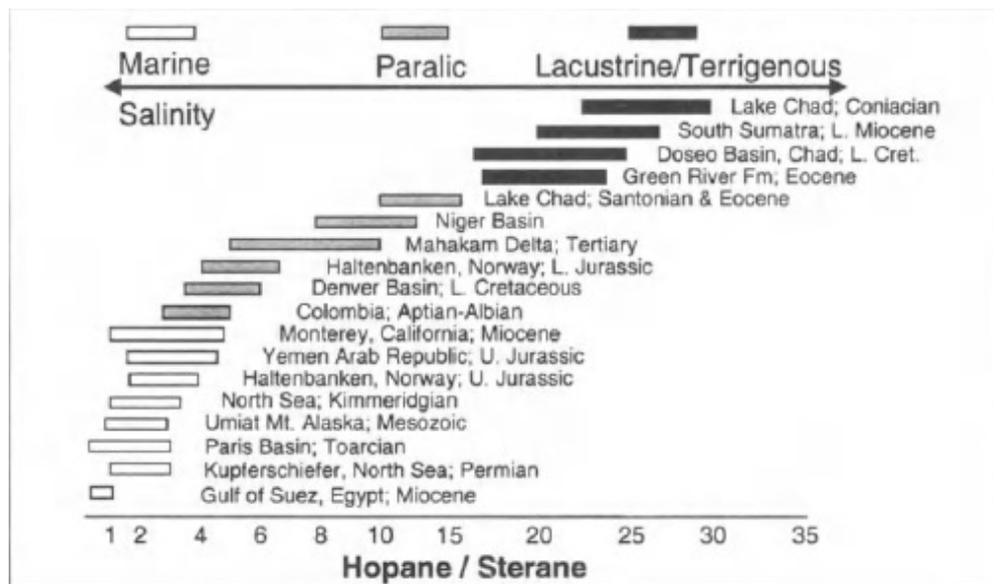


Figure 5: Correlation of marine and lacustrine sources to the hopane to sterane ratio (Hsu *et al.* 2011).

1.3.3 Use of biomarkers to constrain time of deposition

Biomarkers can act as age diagnostics. Norcholestanes derived from diatoms come from Cretaceous and Tertiary periods. Oleananes that come from angiosperm land plants indicate Late Cretaceous. Figure 6 shows examples of age diagnostic biomarkers.

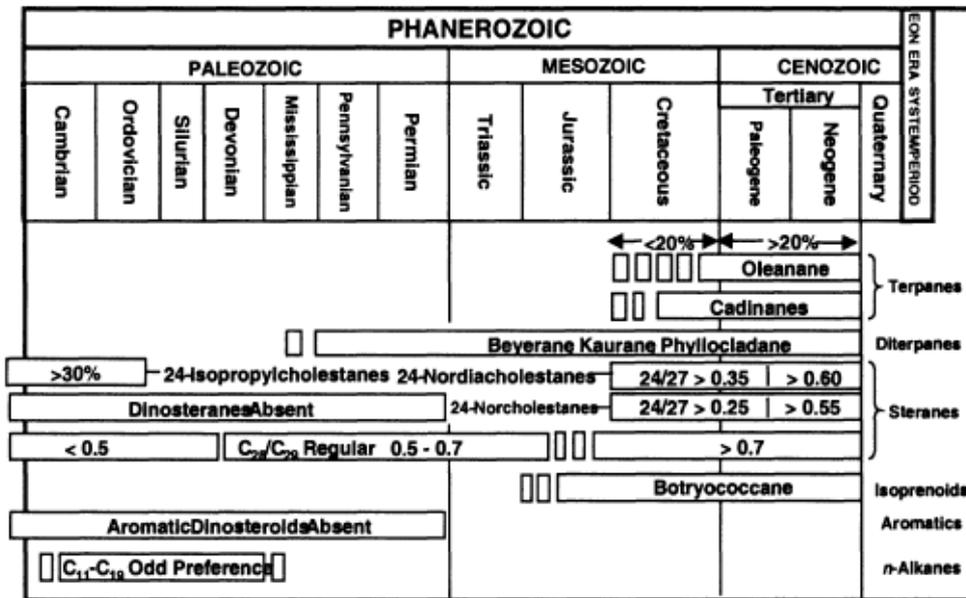


Figure 6: Age diagnostic biomarkers (Hsu et al. 2011)

1.4 Biomarker analyses using Biotool

The technological advance of the gas chromatography-mass spectrometry (GC-MS) system has allowed the extensive development of biological markers. The use of hopane and sterane biomarkers to characterize petroleum oils is well documented (e.g. Aquino Neto et al., 1983; Moldowan et al., 1983; Zumberg, 1984; Palacas et al., 1984; Seifert et al., 1984; Waples and Machichara, 1990; DeGrande et al., 1993; Peters et al., 2005; Fazeela et al., 2011; El Nady et al., 2014; Hu et al., 2015). These studies generally apply modern correlations and ratios that rely on well-established hopane and sterane biomarkers to understand the depositional environment, age, and maturity of oil or source rock. Generally, most biomarker analyses use hopane and sterane biomarker ratio constraints found in Peters et al. (2005). Since then, several studies have proposed novel biomarker ratios (e.g., Tao et al., 2015; Huang et al., 2015). The purpose of this study is to develop a thorough, up-to-date library (database) of saturate, terpane, and sterane biomarker ratios in the form of a Biotool. This tool not only allows one to properly assess petroleum samples, but also carry out the biomarker analyses in an efficient matter. The Biotool will then be used to characterize oils from the Willinstin Basin, North America.

Materials and Methods

2.1 Geological Setting of the Williston Basin

The Williston basin is a large circular basin that is 800 kilometers in diameter, covering western North and South Dakota, eastern Montana, and the Canadian provinces of Manitoba and Saskatchewan (Figure 7). It is part of a North American craton with a geologic history of carbonate deposition throughout the Paleozoic and clastic foreland deposition in the Mesozoic and Cenozoic (Porter et al., 1982; Ricketts, 1989).

The Williston basin is widely known for its large oil and potash deposits that occur throughout the 5-kilometer Phanerozoic (600mya to present) succession of the basin (Figure 8). According to Dow (1974), the Williston basin oils can be characterized into two categories. The Ordovician and Silurian oils are thought to be from a lower Paleozoic Winnipeg shale source (Type I) (Williams, 1974). The latter is a Mississippian Bakken shale-sourced oil (Type II) that is found in the Madison reservoir (Williams, 1974).



Figure 7: Map of Williston Basin. Major cities are denoted by a black dot.

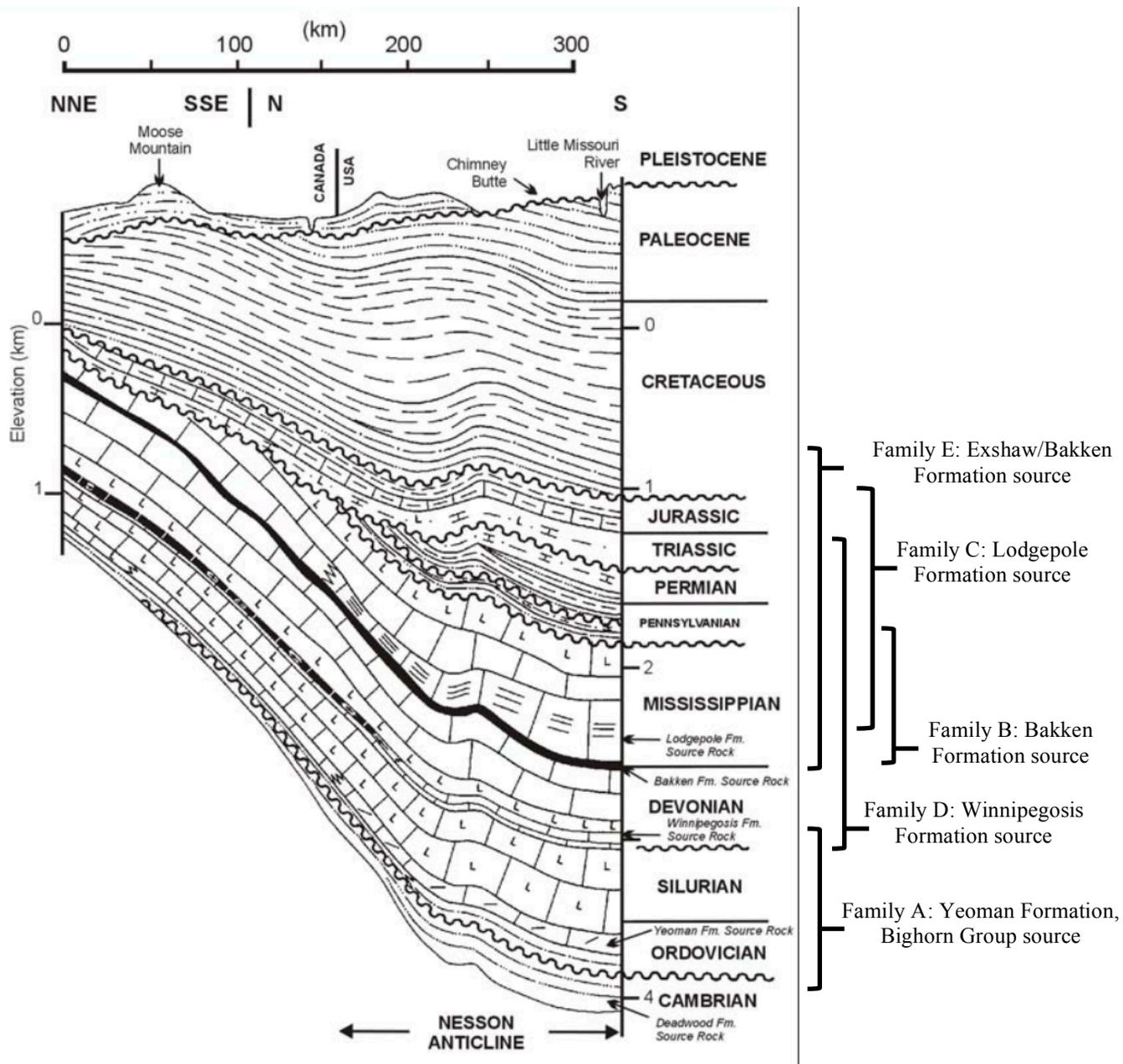


Figure 8: Geologic succession of the Williston basin. Oil families A, B, C, D, and E are based on Osadetz et al. (1992, 1994). Modified from Pasadakis et al. (2004).

2.2 Brief overview of oil samples

The Williston basin is a well-known site that has been extensively studied since its discovery in the early 1970s (e.g. Williams, 1974; Dow, 1974; Thode, 1981; Thompson, 1983; Leenheer and Zumberg, 1987; Zumberg, 1983; Leenheer, 1984). This study used data from 20 oil samples of Families A-E (Table 4). The Williston Basin oil family classification is briefly summarized in Figure 8 and Table 4 (detailed description in Osadetz et al., 1992 and 1994).

Table 4: Williston Basin oil family characterization, based on: * denotes characterization by Williams, (1974); **Osadetz et al. (1992; 1994). Modified from Pasadakis et al. (2004); ***Lillis, 2012).

Sample	Family	Oil Type*	Oil Type**	Source Rock***	Source Rock Age	Lithology**
L00549 L00920 L00550	A	Type I	Red River Oils	Red River Formation***; Winnipeg Formation**; Bighorn Group	Middle-Upper Ordovician	light brown shale type (kukersite)
L00554 L00515	B	Type II	Bakken Oils	Bakken Formation	Upper Devonian - Mississippian	dark shale
L00732 L00672 L00753 L00559	C	Type II	Mississippian and Jurassic Oils	Lodgepole Formation	Lower Mississippian	lime mudstone
L00558 L01312 L01276 L00842 L00756 L00755	D	Type III	Winnipegosis Oils	Winnipegosis Formation	Middle Devonian	laminite; limestone; dolostone
L00829 L00833 L00839 L00820 L00811	E	Type III	Bakken Oils	Exshaw/Bakken Formation	Upper Devonian - Mississippian	dark shale

2.1.1 Family A oils

Osadetz et al. (1992; 1994) described Family A oils as Ordovician Red River oils originating from kukersite source rocks. These kukersites deposits are kerogenites dominated by the marine alga *Gloecapsomorpha prisca*, and believed to have been deposited in a subtidal marine water setting (Reed et al., 1986; Hoffman et al., 1987; Longman and Palmer, 1987; Stasiuk and Osadetz, 1990; Lillis, 2012). In Sippel, (1998), Family A oils are mostly produced from the A-D zones of the upper Red River Formation. Jarvie, (2001) also characterized Red River oils as rich in normal paraffins and also varied in sulfur content and pristine/phytane ratios. The Red River

Formation is mainly composed of marine limestone and dolomite (Lillis, 2012). Distinct kukersite layers have been observed in the upper portions of the Red River Formations and in Bighorn Group within the Yeoman, Herald, and Stony Mountain Formations (Kohm and Loudon, 1982; Osadetz and Snowden, 1995; Osadetz et al., 1992; Fowler et al., 1998).

2.1.2 Family B and C oils

Williams (1974) and Osadetz et al. (1992) described the Bakken oils to be Type II, Family B oils from the Upper Devonian-Mississippian Bakken Formation. According to Lillis (2012), most of the early Bakken oil was produced from shales; more recent production has come from sandstones, siltstones, and dolostones of the Bakken Formation. The composition of these oils is known to be from a marine source (Osadetz et al. 1992) (Table 4). Bakken oils are also highly mature and low in density.

Family C oils are sourced from a Lower Mississippian Lodgepole Formation source rock (Table 4). These oils generally have low pristane/phytane values (<1) and C₃₅ hopane predominance (Osadetz et al., 1992). Like Family B oils, Family C oils are mature and from carbonate source rock, and T_m/C₃₀ hopane ratios are higher than those of Family E. Diasteranes/steranes are also lower than those of Family B values.

2.1.3 Family D and E oils

Family D oils were first discovered in the Canadian Williston basin (Osadetz et al., 1991). They are considered Type III oil occurs in the well-known Middle Devonian Winnipegosis Formation (Williams, 1974; Lillis, 2012). These oils are also low in tricyclic/hopane ratios, and can be subdivided into D1 and D2, oils from Elkhorn Point Group and Winnipegosis reefs (Osadetz et al., 1992).

Family E oils are described as Type III, Paleozoic marine source oils from the Exshaw and Bakken Formation (Osadetz et al., 1994). These oils are also known as mature Bakken oils from the Upper Devonian to Mississippian (Williams, 1974). In less biodegraded Family E samples, diasterane to regular sterane ratios are >1 (Osadetz et al., 1994).

2.3 Biotoool

The Biotoool that was developed for this study is an updated database of relevant saturates, terpane, hopane, and sterane ratios used to assess different types of oil samples. The database is in the form of hyperlinked files in an Excel file (see figure 9 for an example). While most of the biomarker ratios were employed, this study focused on a selected suite of biomarkers to characterize Williston Basin oils.

46	Hopanes ratios
47	19tri/20tri
48	22tri/21tri
49	(19tri+20tri)/(23tri+24tri)
50	(19tri+20tri)/C23tri
51	24tri/23tri
52	19tri/23tri
53	24tet/30hop
54	24tet/23tri
55	ETR
56	26tri/25tri
57	24tet/26tri
58	Ts/Ts+Tm
59	Ts/30hop
60	Tm/Hopane
61	C29nor/C30 Hop
62	BNH/Hop
63	C31-homo
64	C32-homo
65	C33-homo
66	C34-homo
67	C35-homo
68	C31R/C30
69	C29Ts/C29 nor-hopane)
70	C31 S/S+R
71	C32 S/S+R
72	C32 S/R
73	C35/C34 homo
74	C33/C32 homo
75	moretane
76	oleanane
77	gammacerane
78	23tri/C30-Hop
79	C28+C29tri/C30hop

Figure 9: Example of the Biotoool excel file for Terpane, Hopane ratios.

Results and Discussion

All biomarker data of Family A-E saturates, hopanes, and steranes are presented in Appendix I, II, and III, respectively.

3.0 Saturate hydrocarbons

Pristane and phytane are primarily derived from the chlorophyll's phytol side chain (Didyk et al., 1978). The pristane/phytane (Pr/Ph) ratio is commonly used as an indicator of redox conditions of the depositional environment (Didyk et al. 1978). High Pr/Ph (>1) ratios usually indicate a shale source rock; low (<1) indicate a carbonate source (Connan et al., 1981; Hughes, 1984; Palacas, 1984; Moldowan et al., 1985; ten Haven et al., 1988) (Table 3). In non-biodegraded crude oils difference source rock organic matter, ratios <2 indicate a marine setting; >3 terrestrial source; and $\sim 1-3$ lacustrine environment (Peters et al., 2005) (Table 3). Oil samples that were assessed in this study fall under a marine setting (<2) (Table 5). High Pr/Ph in Family A, B, and E oil indicate a shale source rock, which is in agreement with the described lithology (Table 4-5). Similarly, Pr/Ph ratios for Family C-D were <1 , corresponding to a carbonate source rock.

The isoprenoid to normal alkane ratios Pr/nC_{17} and Ph/nC_{18} are used together for environment and maturation (Hunt, 1995; Peters et al., 2005). These two ratios generally decrease with increasing thermal maturity (Hunt 1995). Marine oils are usually thermally mature. With the exception of 4 samples in Family A (2 samples) and D (2 samples), Pr/nC_{17} and Ph/nC_{18} values were less than 1, suggesting mature, marine oils. These values directly reflect the known thermal maturity of the Williston Basin oils.

It must be noted that Pr/nC_{17} and Ph/nC_{18} could not completely differentiate shale from carbonate source rocks for every oil sample. According to Peters et al. (2005), low Ph/nC_{18} indicates a shale source and high values indicate a carbonate setting (Table 3). For Pr/nC_{17} ratios, high (>0.6) and low (<0.5) values indicate terrestrial and marine sources, respectively. In Diasty et al. (2016), limestone carbonate rocks had Pr/nC_{17} and Ph/nC_{18} ranging from 0.44-0.76. The isoprenoid to normal alkane ratios for Family C-D (known limestone sourced oils) ranged from 0.25-1.77.

Table 5: Biomarker parameters of the Williston basin oils, Family A-E. Note that the (-) represent a no value for the corresponding value.

Sample Name	Family	Pr/Ph	Pr/nC₁₇	Ph/nC₁₈
L00549	A	-	1.29	0.06
L00920	A	1.40	0.06	0.13
L00550	A	-	1.26	0.07
L00554	B	1.39	0.65	0.53
L00515	B	1.55	0.64	0.50
L00732	C	0.64	0.50	0.72
L00672	C	0.60	0.43	0.66
L00753	C	0.61	0.42	0.61
L00559	C	0.59	0.42	0.65
L00558	D	0.95	0.42	0.44
L01312	D	0.68	0.77	1.49
L01276	D	0.72	0.25	0.50
L00842	D	0.61	0.97	1.77
L00756	D	0.66	0.35	0.71
L00755	D	0.59	0.59	1.12
L00829	E	0.93	2.31	2.51
L00833	E	0.87	2.88	5.59
L00839	E	0.93	0.37	1.08
L00820	E	0.44	0.58	3.88
L00811	E	0.72	0.69	1.85

3.1 Distribution of tricyclic terpanes

The percentage of tricyclic terpanes in each oil family was assessed using the averages of C₁₉-C₂₃ diterpanes (Figure 10). These tricyclic terpanes are commonly used alongside other ratios, such as pristane/phytane, hopane/sterane, pristane/*n*C₁₇, gammacerane and oleanane/C₃₀ hopane to identify the depositional environment of oils (i.e. marine or terrigenous) (Fu Jiamo et al., 1990; Peters and Moldowan, 1993; Peters et al., 2005).

Overall, C₁₉ (36.2 ± 3.42%) and C₂₀ (24.19 ± 12.4%) tricyclic terpanes were more abundant than C₂₁ (19.65 ± 2.58%) and C₂₃ (19.96 ± 11.84%) in Family A (Figure 10). An abundance of C₁₉ and C₂₀ tricyclic terpanes usually indicate a terrestrial source of organic. This interpretation, however, is in conflict with geological setting of Family A oils. Sample size or instrumental error might be factors affecting this. Values of C₁₉ and C₂₀ tricyclic terpanes were more abundant than C₂₁ and C₂₃ in Family B-E, indicating a marine source setting (Figure 10). Overall, the average abundance values directly correspond to the known source and lithology of Family B-E oils.

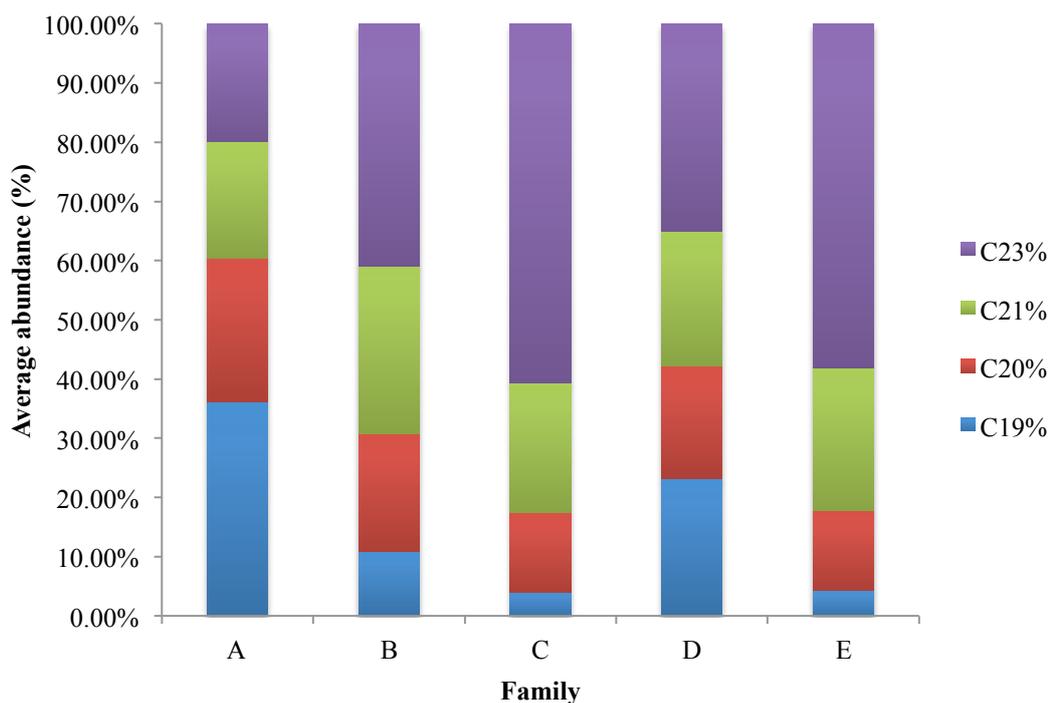


Figure 10: Distribution (in percent %) of averaged C₁₉, C₂₀, C₂₁, C₂₃ tricyclic terpanes for Families A-E.

3.2 Selected terpenoid ratios

Tetracyclic terpanes commonly occur in petroleum samples and are thought to originate from the microbial opening of the hopanes (Peters et al., 2005). A combination of tetra- and tricyclic parameters differentiates carbonate from evaporite depositional environments. Cross plots of selected ratios were used to assess the sources of Williston Basin oils, including $(C_{19}+C_{20})/C_{23}TT$ vs. $C_{23}TT/C_{21}TT$, $C_{24}TeT/C_{26}TT$ vs. $C_{19}TT/C_{20}TT$, $C_{24}TT/C_{23}TT$ vs. $C_{22}TT/C_{21}TT$, $C_{24}TeT/C_{20-26}TT$ vs. $C_{24}TeT/C_{30}$ Hopane, and $C_{26}TT/C_{25}TT$ vs. $C_{20}TT/C_{23}TT$ (Figure 11-14; Table 6). Using these plots, distinct differences could be identified.

Family A $C_{23}/C_{21}TT$ and $(C_{19}+C_{20})/C_{23}TT$ values were 0.29-1.54 and 1.89-11.35, respectively (Table 6). All other oil samples had $C_{23}/C_{21}TT$ and values >1 ; most other oils had $(C_{19}+C_{20})/C_{23}TT$ values <1 (Figure 11a-b; Table 6). Overall, combination of elevated $C_{23}/C_{21}TT$ values and low $(C_{19}+C_{20})/C_{23}TT$ indicates that Family A-E oil are of high marine organic matter input from carbonate a source.

An abundance of C_{24} tetracyclic terpene (TeT) is most indicative of carbonate environments (Peters et al., 2005). Depleted C_{24} TeT suggests a shale source. Low $C_{24}TeT/C_{26}TT$ (majority <0.5) and low to medium $C_{19}TT/C_{20}TT$ ratio values (<2) of most Family A, B, and D oils suggest marine shale source (Figure 12; Table 6). Medium to high $C_{24}TeT/C_{26}TT$ (~ 0.48 to <3) and low $C_{19}TT/C_{20}TT$ (<0.6) indicates a marine carbonate source.

High C_{24} tricyclic terpene is dominant in phosphate rich depositional environments, and dominant in deep-water marine depositional environments (Zumberge, 1987). The $C_{24}TT/C_{23}TT$ parameter less than 1 suggests marine-sourced oil. $C_{24}TT/C_{23}TT$ and $C_{22}TT/C_{21}TT$ ratios have been used to distinguish oils generated from lacustrine, marine shale, and paralic shale source rocks (Peters et al., 2005; Tao et al., 2015). Overall low $C_{24}/C_{23}TT$ (<1) of all samples indicates a marine source (Table 7; Figure 13). Family A, B, and E shale samples have moderately low $C_{22}TT/C_{21}TT$ values (0.15-0.72) indicate a marine source with low carbonate source. The low $C_{22}TT/C_{21}TT$ values for Family C and D, however, do not directly correlate with their respective limestone and lime mudstone lithology (Table 7; Figure 13).

The $C_{24}TeT/C_{20-26}TT$ ratio was developed in Tao et al. (2015) to identify any crude oils from a terrestrial or higher plant source. This parameter was used alongside with $C_{24}TeT/C_{30}$ to distinguish anoxic from oxic environments. $C_{24}TeT/C_{20-26}TT$ values of all oil samples were extremely low and close to 0 (~ 0.024 -0.071). This directly corresponds to the marine geologic settings of the samples. $C_{24}TeT/C_{30}$ Hopane ratios for Family A (0.013-0.04), B (0.292-0.610), and E (<0.2) difficult to interpret as anoxic, shale samples (Table 6). Family C lime mudstone (0.087-0.124) and D limestone and dolostone (<0.04) values were observed to be less than 0.125.

Crude oils from marine shale, carbonate, and marl source rocks usually have C_{31} 22R homohopane/ C_{30} hopane that are greater than 0.25 (Peters et al., 2005). All Williston Basin samples have C_{31} 22R homohopane/ C_{30} hopane ratios between 0.25-0.65, directly corresponding to a carbonate or shale source (Table 7; Figure 15). $C_{24}TeT/C_{30}$ hopane ratio was used alongside C_{31} 22R homohopane/ C_{30} hopane, with values ranging from 0.013 to 0.610. In Tao et al. (2015), $C_{24}TeT/C_{30}$ hopane ratios

were used to distinguish marine, saline lacustrine, freshwater lacustrine, and terrigenous sourced oil samples. The marine samples in that study ranged from 0.15-0.23. Based on ratio values generated from this study, it is possible that the $C_{24}TeT/C_{30}H$ hopane ratio could have a wide range of values that are below 1.

Table 6: Selected biomarker ratios of the Williston basin oils, Family A-E. Note that the (-) represent a no value for the corresponding value.

Sample Name	Family	C_{19}/C_{20} TT	C_{20}/C_{23} TT	$C_{25}/C_{26}T$ T	$C_{24}TeT/C_{26}TT$	$C_{24}TeT/C_{20-26}TT$	$C_{24}TeT/C_{30}H$	C_{23}/C_{21} TT	$(C_{19}+C_{20})/C_{23}TT$
L00549	A	1.42	0.91	0.27	0.08	0.025	0.013	1.54	2.21
L00920	A	3.33	0.44	0.15	0.08	0.034	0.013	1.36	1.89
L00550	A	0.95	5.82	0.20	0.20	0.074	0.040	0.29	11.35
L00554	B	0.63	0.47	3.11	0.93	0.042	0.292	1.35	0.78
L00515	B	0.48	0.49	0.57	0.49	0.071	0.610	1.55	0.73
L00732	C	0.25	0.27	0.94	0.50	0.046	0.123	2.64	0.33
L00672	C	0.53	0.22	1.34	0.62	0.043	0.122	1.97	0.34
L00753	C	0.22	0.25	0.96	0.53	0.046	0.124	2.61	0.31
L00559	C	0.23	0.15	1.66	1.13	0.047	0.087	4.54	0.19
L00558	D	0.97	0.69	0.26	0.18	0.052	0.035	1.57	1.37
L01312	D	0.67	0.53	0.19	0.14	-	-	1.88	0.89
L01276	D	3.58	0.37	0.11	0.07	0.024	0.013	1.15	1.70
L00842	D	0.56	0.46	0.29	0.21	-	-	1.95	0.73
L00756	D	1.06	0.84	0.22	0.15	0.049	0.082	1.30	1.74
L00755	D	1.99	0.40	0.12	0.09	-	-	1.40	1.19
L00829	E	0.45	0.23	0.98	0.48	0.042	0.121	2.26	0.34
L00833	E	0.38	0.21	3.79	2.86	0.064	0.177	2.31	0.29
L00839	E	0.07	0.25	1.31	0.60	0.049	0.140	2.50	0.27
L00820	E	0.38	0.22	2.35	1.42	0.057	0.135	2.40	0.30
L00811	E	0.36	0.24	0.49	0.81	0.050	0.119	2.67	0.33

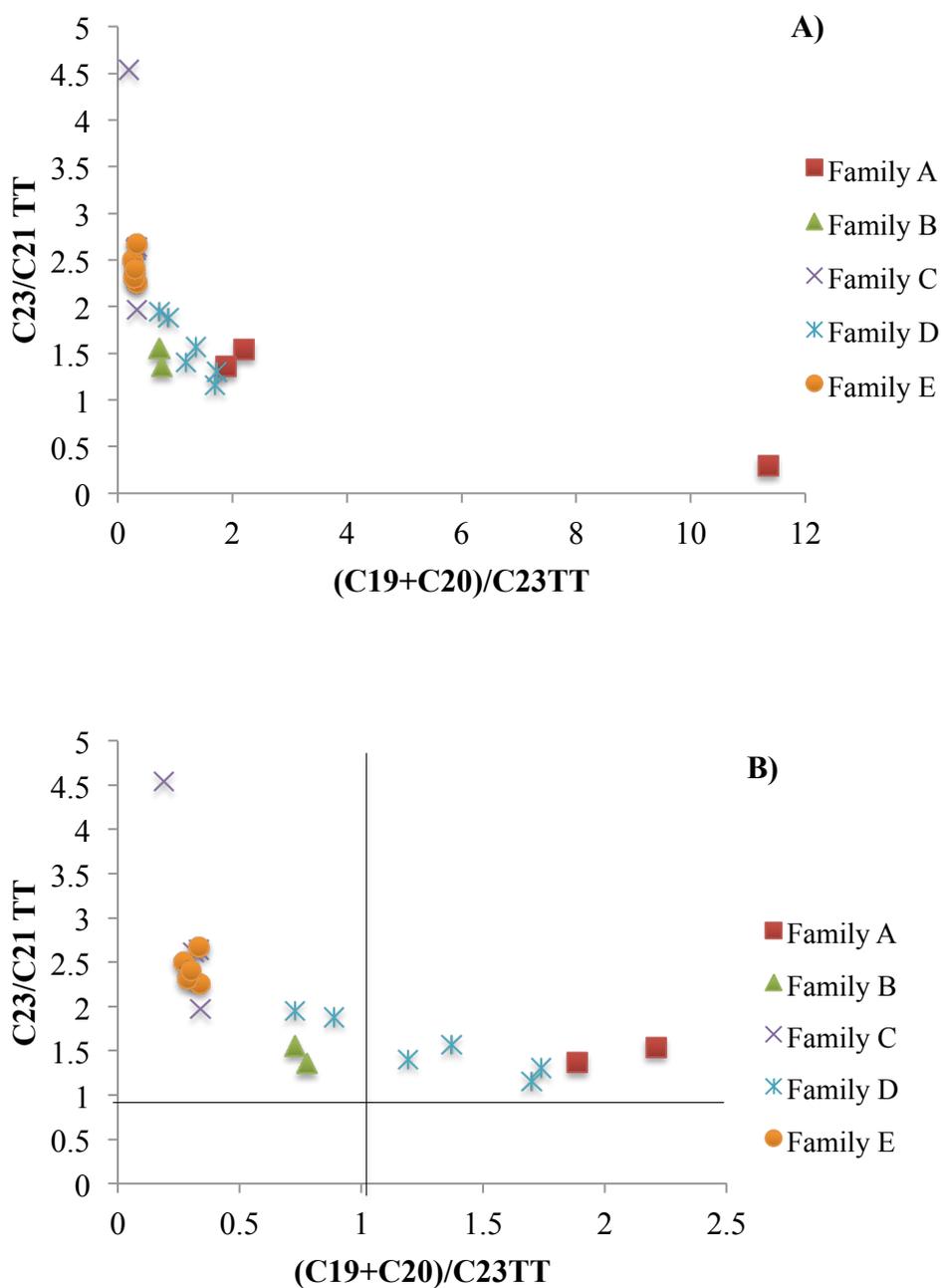


Figure 11: Cross plot of $C_{19}+C_{20}/C_{23}TT$ ratio. A) Cross plot with all Williston Basin samples. Note that there is one outlier sample from Family A that distorts the plot; B) cross plot from A without the one outlier. Note that TT is a notation for tricyclic terpanes.

Table 7: Selected biomarker ratios of the Williston basin oils, Family A-E. Note that the (-) represent a no value for the corresponding value.

Sample Name	Family	C ₂₃ TT/C ₃₀ Hopane	C ₂₂ /C ₂₁ TT	C ₂₄ /C ₂₃ TT	C ₃₁ R/C ₃₀ H
L00549	A	0.09	0.27	0.22	0.36
L00920	A	0.05	0.49	0.65	0.28
L00550	A	0.02	0.15	1.28	0.33
L00554	B	1.34	0.72	0.64	0.51
L00515	B	1.57	0.25	0.69	0.27
L00732	C	0.61	0.56	0.57	0.35
L00672	C	0.64	0.52	0.58	0.38
L00753	C	0.62	0.60	0.53	0.33
L00559	C	0.55	1.38	0.28	0.42
L00558	D	0.09	0.42	0.80	0.30
L01312	D		0.27	0.60	
L01276	D	0.06	0.44	1.10	0.25
L00842	D		0.28	0.51	
L00756	D	0.21	0.17	0.82	0.65
L00755	D		0.20	0.70	
L00829	E	0.64	0.42	0.60	0.31
L00833	E	0.68	0.34	0.58	0.35
L00839	E	0.62	0.54	0.66	0.31
L00820	E	0.56	0.38	0.63	0.28
L00811	E	0.57	0.42	0.59	0.31

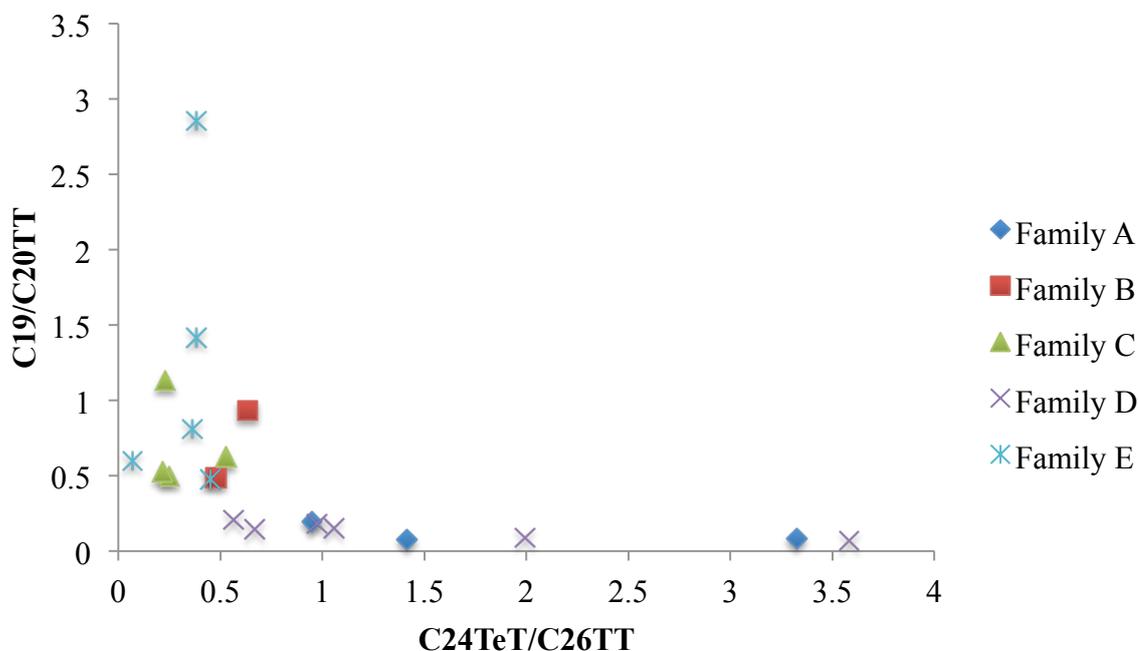


Figure 12: Cross plot of C₂₄TeT/C₂₆TT vs. C₁₉TT/C₂₀TT ratios. These two ratios are common source parameters. Note that TT is a notation for tricyclic terpanes.

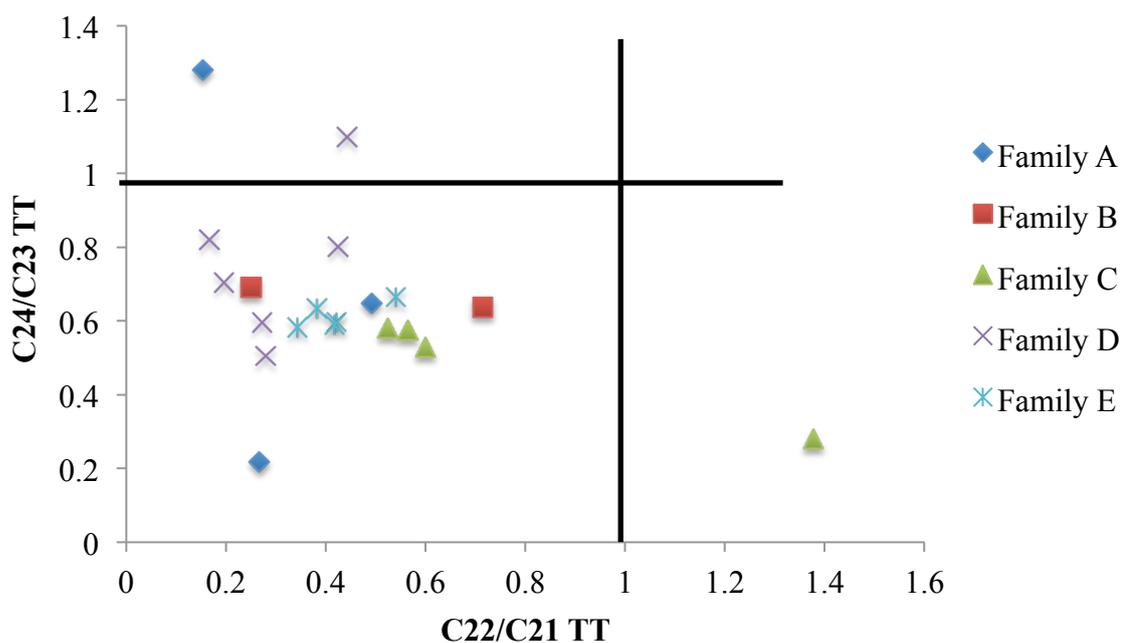


Figure 13: Cross plot of $C_{24}TT/C_{23}TT$ vs. $C_{22}TT/C_{21}TT$ ratios. These two ratios are generally used to differentiate oils from carbonate rocks, which usually have high $C_{22}TT/C_{21}TT$ and low $C_{24}TT/C_{23}TT$ (Peters et al., 2005).

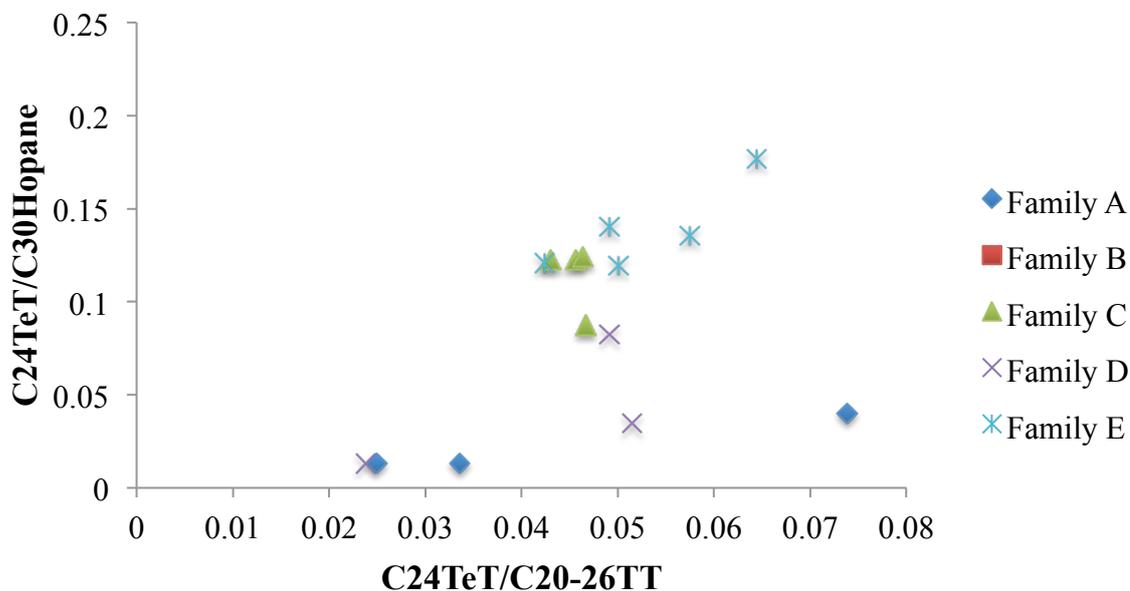


Figure 14: Cross plot of $C_{24}TeT/C_{20-26}TT$ vs. $C_{24}TeT/C_{30}Hopane$ ratios.

3.3 Correlation of terpane and hopane ratios

In Tao et al. (2015), $C_{24}TeT/C_{30}$ hopane ratios were correlated with $C_{23}TT/C_{30}$ hopane and observed a correlation between ratio proportions that was source specific. Upper Devonian Family B oils from the Bakken Formation are clearly distinguished from the rest of the oils (Figure 15). It must be noted that this correlation cannot differentiate shale from carbonate lime stone sources (i.e. Family C and Family D).

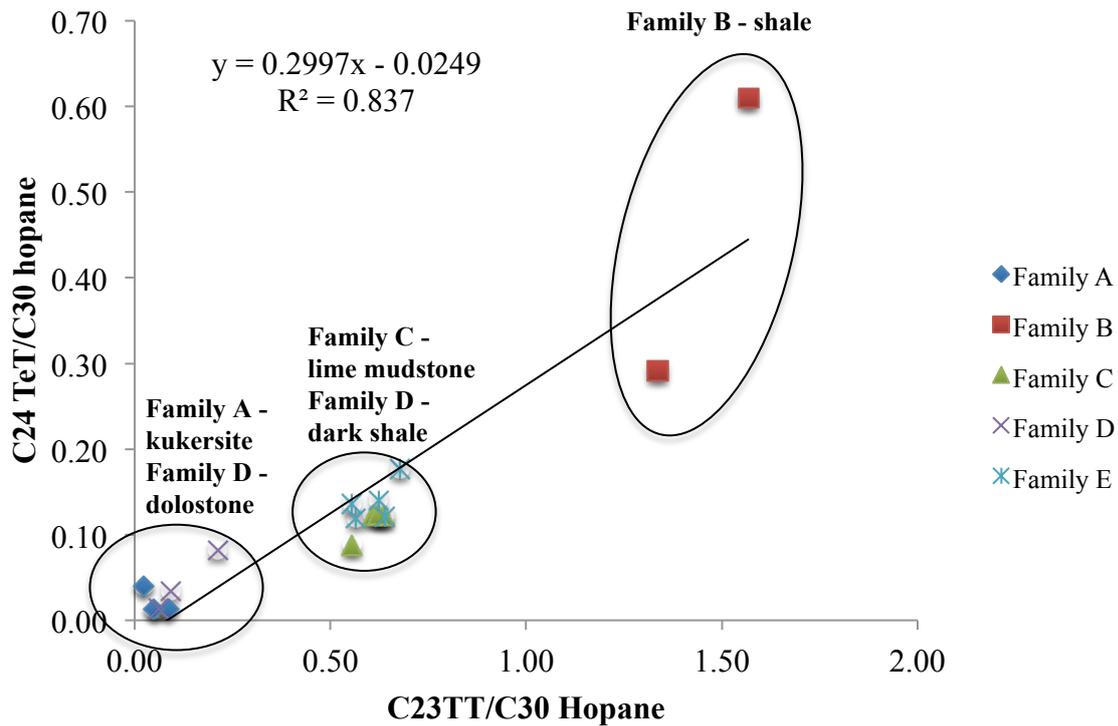


Figure 15: Cross plot of $C_{24}TeT/C_{30}$ Hopane vs. $C_{23}TT/C_{30}$ Hopane ratios along with a regression line. These two ratios are generally used to differentiate oil sources. For the Williston Basin, this cross plot seems to distinguish Family B Bakken Formation oils from the rest of samples.

3.4 Distribution of steranes

Sterane ternary diagrams are generally used to show relationships between oils and sourcerocks (Peters et al., 2000). A C₂₇–C₂₈–C₂₉ sterane ternary diagram is used to distinguish groups of crude oils from different source rocks or different organic facies of the same source rock. With the exception of one sample, oils from this study plotted similarly (Figure 16). Based on the diagram, oil samples from Family A-E are from an open marine setting (triangular diagram found in Waples and Machichara, 1990).

High diasterane/sterane ratios have been as a source parameter. In this study, marine shale and carbonate source oils are clearly identified using diasterane/sterane and Tm/C₃₀ hopane cross plot (Figure 17). High values of diasterane/sterane (>1) are found in marine shale oils (Figure 17; Peters et al., 2005). A low abundance of diasterane (<1) indicates a carbonate source. Overall, diasterane/sterane values seem to serve as good indicators of source rocks.

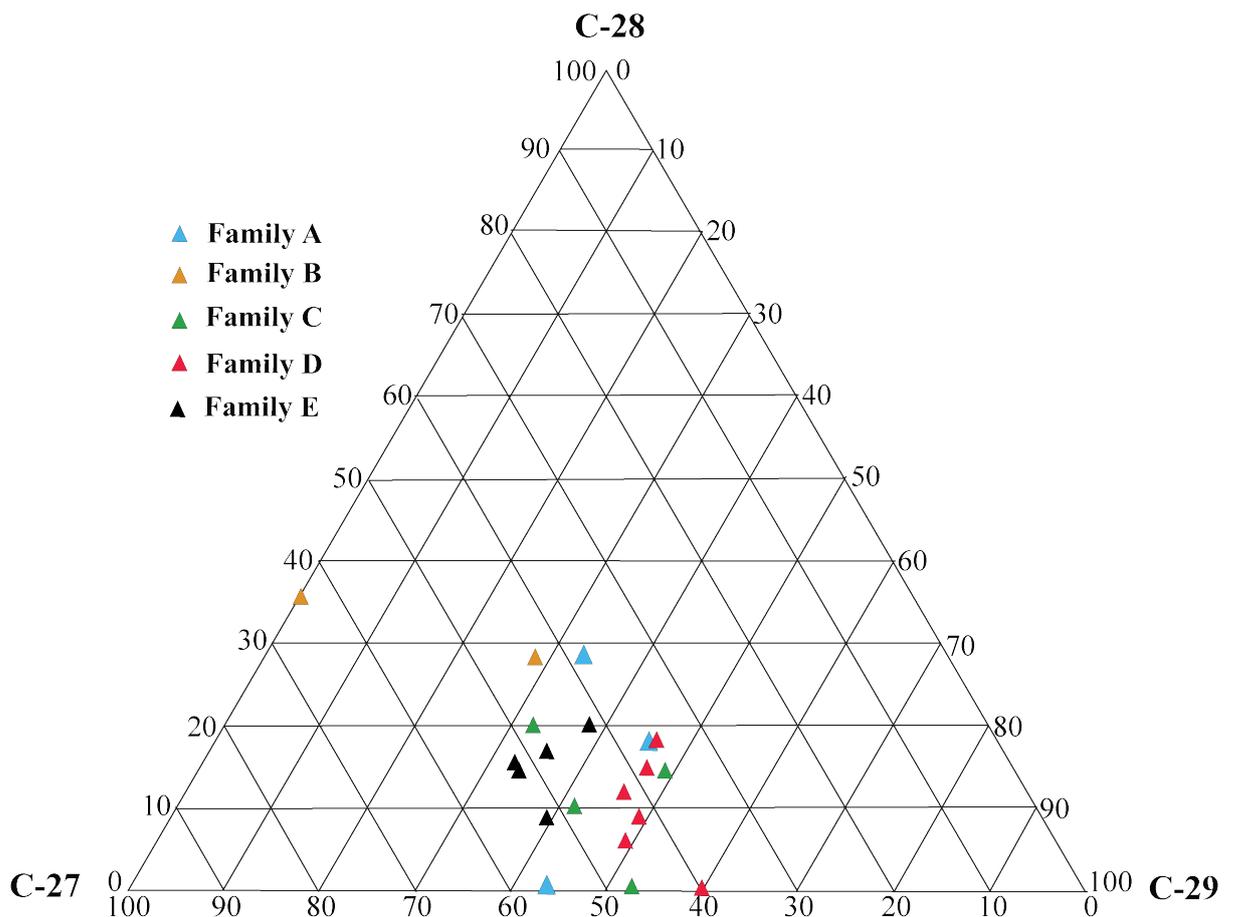


Figure 16: Ternary plot for C₂₇-C₂₈-C₂₉ steranes.

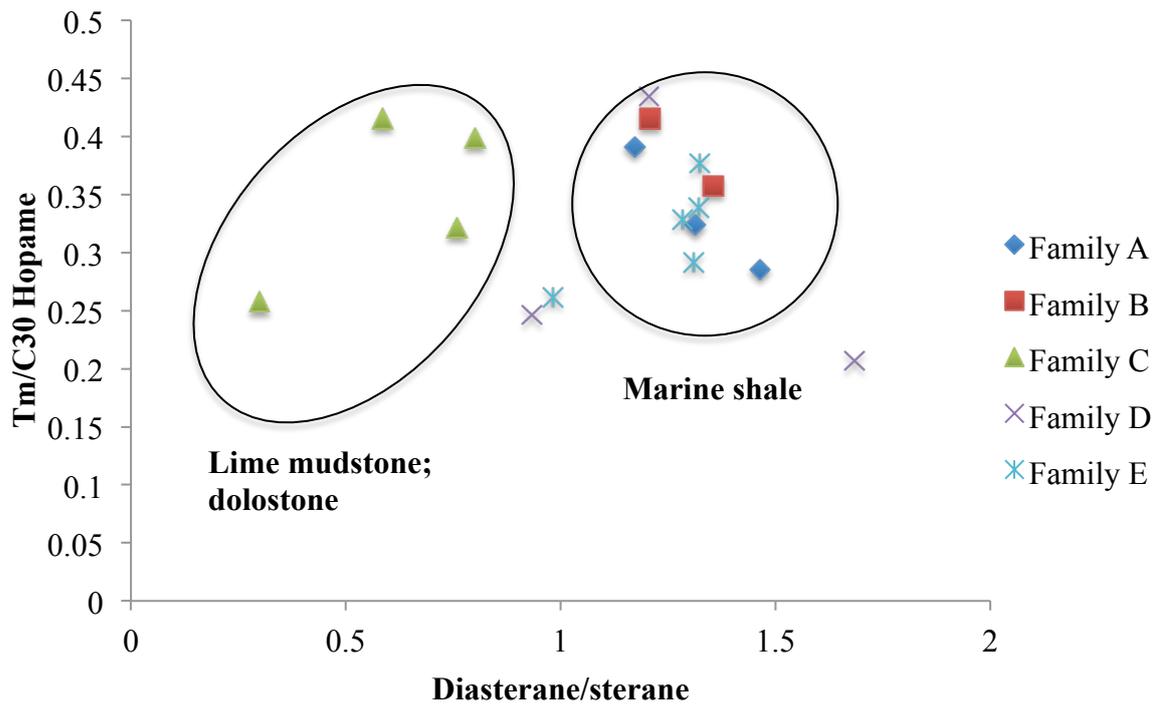


Figure 17: Tm/C₃₀Hopane vs. Diasterane/sterane ratio crossplot.

4. Conclusions

1. Twenty Williston Basin oils, from Family A-E, were characterized selected saturate, terpane, hopane, and sterane ratios from the Biotoool database. In general, the C₂₃ tricyclic terpane homologue prevailed in Family B-E oils and confirmed the marine nature of the samples. The marine-sourced Family A had elevated abundances of C₁₉ and C₂₀, classifying it as a higher plant sourced rock. Multiple parameters should be used when analyzing oil samples.
2. Saturate biomarker ratios pristane/phytane were effective in assessing the source and maturity of Williston Basin oils. This study confirms the reliability of pristane/phytane ratios for any oil sample.
3. Selected tricyclic and tetracyclic terpane biomarkers varied in effectiveness and reliability. Most of the tri- and tetracyclic terpane ratios employed differentiated a portion of the oils (e.g. C₂₄TT/C₂₃TT and C₂₂TT/C₂₁TT), especially those samples that were shale-sourced. Some of the ratios that were developed by Tao et al. (2015) could be used to assess oils, but may not be as effective as other well-established ratios.
4. Sterane ternary plot (C₂₇-C₂₈-C₂₉) along with the Tm/C₃₀ Hopane vs. diasterane/sterane crossplot were the most reliable and accurate in this study. The C₂₇-C₂₈-C₂₉ plot placed all Williston Basin oils in the open marine category, with one sample in the planktonic region. The Tm/C₃₀ Hopane vs. diasterane/sterane crossplot was successful at differentiating the shale-sourced oils from the carbonate-sourced ones.
5. It is critical to employ multiple parameters when assessing oil samples from a specific site. While some of the biomarker ratios are not fully developed, they can serve as a powerful tool for understanding the nature of petroleum samples.

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Appendix I – Hydrocarbon saturate biomarker data

	L00549	L00920	L00550	L00554	L00515	L00732	L00672	L00753	L00559	L00558	L01312	L01276	L00842	L00756	L00755	L00829	L00833	L00839	L00820	L00811
	A1	A2	A3	B1	B2	C1	C2	C3	C4	D1_1	D2_1	D2_2	D2_3	D2_4	D2_5	E1	E2	E3	E4	E5
C10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C11	0.2	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.0	0.0	-	-	0.0	-	0.0	0.0	0.0	0.0	0.0
C12	0.3	0.3	0.6	0.3	0.4	0.3	0.3	0.3	0.3	0.0		-	-	-	-	0.0	0.0		0.0	0.0
C13	1.1	1.1	2.9	1.2	1.2	0.9	1.0	1.0	0.8	0.5	0.6	0.6	0.7	1.7	1.1	0.6	0.7	0.4	0.5	0.4
C14	8.3	9.2	47.0	11.3	10.6	3.7	3.2	3.3	2.7	7.9	5.5	6.7	3.1	20.7	14.8	2.6	3.0	2.6	3.3	2.6
C15	63.1	60.7	157.7	41.7	33.5	11.0	10.1	7.8	14.9	27.4	21.7	36.2	11.2	54.8	41.1	2.8	2.7	2.9	3.4	3.4
C16	142.7	113.5	206.0	50.6	41.2	28.5	27.9	28.0	37.5	41.1	31.6	57.1	23.7	58.9	48.7	2.1	2.6	1.6	2.3	2.2
C17	248.6	182.2	290.0	46.9	41.9	35.5	37.4	40.1	49.9	46.5	46.4	81.6	45.1	67.8	53.4	2.0	2.0	1.0	1.5	1.5
Pr	319.4	10.0	364.1	30.6	26.9	17.6	16.2	16.8	21.2	19.7	35.6	20.6	43.7	24.0	31.4	4.7	5.8	0.4	0.8	1.0
C18	54.0	53.3	62.8	41.1	34.6	38.2	40.9	45.4	55.2	47.7	35.0	56.9	40.9	50.9	47.4	2.0	1.2	0.4	0.5	0.8
Ph	3.3	7.2	4.3	21.9	17.4	27.4	27.0	27.5	35.8	20.8	52.3	28.7	72.2	36.2	53.3	5.0	6.6	0.4	1.9	1.4
C19	120.1	104.5	113.0	33.9	29.0	33.7	37.1	40.8	48.9	44.6	30.6	52.2	34.4	41.3	35.2	1.5	1.6	0.6	0.8	0.7
C20	24.4	33.1	31.0	30.8	25.8	34.8	39.1	43.1	52.1	46.9	31.0	47.8	33.3	38.0	33.1	1.9	2.2	0.4	0.8	0.7
C21	17.3	27.4	23.7	24.8	21.1	29.2	34.3	37.1	44.9	44.9	33.5	47.9	35.1	38.1	33.7	0.9	0.9	0.5	0.6	0.3
C22	13.1	24.4	19.1	19.7	16.5	27.1	30.5	33.3	42.9	45.0	33.1	46.0	35.7	36.8	32.6	0.7	0.2	0.4	0.2	0.4
C23	11.0	23.1	16.4	16.5	13.7	23.8	27.0	29.5	37.2	42.3	35.0	47.0	36.4	36.6	32.2	1.1	1.1	0.5	0.5	1.0
C24	9.4	21.3	15.1	13.9	11.3	22.7	26.1	26.9	35.9	43.0	39.8	49.2	41.3	37.7	35.8	1.2	0.8	0.2	0.6	0.9
C25	9.0	19.8	14.1	13.0	10.6	20.3	23.6	24.4	32.7	41.2	46.2	51.5	46.3	38.9	38.7	1.1	1.4	0.4	0.8	1.2
C26	7.8	16.4	12.0	9.4	7.6	18.6	21.6	21.5	30.3	39.3	36.4	45.7	36.9	32.6	31.1	1.1	0.8	0.7	1.0	1.2
C27	8.2	14.4	11.8	8.3	6.5	17.5	18.9	19.2	25.7	35.0	35.0	43.9	34.5	29.8	28.1	0.7	0.4	0.2	0.9	1.4
C28	7.1	12.2	10.7	6.8	6.4	17.1	18.7	19.3	27.0	33.3	25.1	38.3	24.2	24.6	23.2	1.3	0.7	0.1	0.9	1.5
C29	6.2	9.9	7.7	6.5	4.7	14.5	15.2	15.6	20.3	25.3	20.8	30.3	19.6	18.7	16.4	1.0	1.0	-	1.0	1.6
C30	4.7	7.4	5.7	4.8	3.8	13.8	13.1	14.0	18.7	20.1	15.0	23.9	12.8	14.5	12.4	0.9	0.5	0.2	0.7	0.7
C31	4.8	6.9	5.5	4.2		12.4	11.9	12.5	15.7	15.4	13.0	20.3	12.4	11.8	10.1	0.8	0.4	0.2	0.9	0.9
C32	3.8	4.9	4.4	2.7	2.8	10.1	9.9	10.3	13.9	11.5	9.3	15.5	8.0	8.1	6.9	0.8	0.5	0.2	0.2	0.6
C33	3.4	-	3.6	2.6	2.7	7.8	8.6	9.3	10.3	9.3	10.2	13.7	7.6	6.6	5.2	0.2	0.3	0.4	-	0.6
C34	-	-	-	-	-	-	-	-	-	-	7.5	-	-	-	-	0.3	-	-	0.8	0.6
C35	-	-	-	-	-	-	-	9.1	-	-	-	-	-	-	-	0.5	0.7	0.5	0.4	-

Appendix II – Terpane/Hopane biomarker data

	L00549	L00920	L00550	L00554	L00515	L00732	L00672	L00753	L00559	L00558	L01312	L01276	L00842	L00756	L00755	L00829	L00833	L00839	L00820	L00811
Family	A	A	A	B	B	C	C	C	C	D	D	D	D	D	D	E	E	E	E	E
17a21b hopane	4472	21641	7435	366	896	9602	7629	9687	12863	24694	0	19418	0	17264	0	8897	9183	11176	10653	12181
C19-tri	24238	71843	42332	8655	6406	16374	27656	16361	11892	75384	64078	74021	49794	68649	83921	28872	23251	5821	25391	31333
C20-tri	17103	21605	44576	13672	13371	65700	52656	74689	51789	77379	95948	20659	88255	64887	42092	64096	61050	84803	66589	86524
C21tri	12145	36338	26475	21306	17492	93364	120154	112882	74422	71152	95956	48274	97571	58991	75327	122526	126985	134102	127876	132240
C22_tri	3246	17839	4070	15237	4371	52720	62990	67754	102476	30147	26144	21347	27280	9798	14759	51715	43615	72492	48741	55187
C23_tri	18720	49550	7656	28804	27123	246378	236432	294455	337878	111553	180113	55731	189856	76820	105818	276312	293156	335199	306752	353499
C24_tri	4068	32150	9801	18342	18706	141539	136883	155761	94745	89393	107335	61277	95959	63069	74516	164746	170605	222783	194442	208349
C25_triS	6787	12300	3141	16164	7926	40043	31351	54484	32181	28955	32220	13242	23548	11388	29489	31525	25863	66232	36948	28792
C25_triR	3563	12190	10159	4822	4372	53571	66898	52310	45825	30204	41410	6587	64469	31476	6742	75172	75397	98153	87232	16419
C26triS,R	38097	163786	65745	6749	21559	99781	73055	111588	46988	228999	377865	175309	302060	192486	298246	109359	26748	125944	52742	92230
C24_tetra	2899	13537	12981	6284	10555	49613	45422	58835	53280	41695	54079	11885	63230	29799	27244	52117	76447	75380	74650	74647
C28_triS	4358	4761	5801	8405	8001	40379	54938	58639	43879	23556	57427	8319	72908	23849	9429	66221	66964	76655	64455	82290
C28_triR	32244	73048	29029	17063	18084	50485	57197	98825	80884	80382	147578	47933	170945	54931	51269	86840	83130	99092	98240	99278
C29_triS	3922	3462	0	6025	0	40746	49481	51855	33716	23231	49890	16503	56163	4637	21450	60541	49490	70002	65056	87841
C29_triR	0	0	4027	2869	11223	0	0	0	15643	0	0	0	0	0	3106	0	0	0	0	0
Ts	39617	179867	92924	11846	7290	59278	66313	79990	88721	304028	380189	192977	381420	222582	372632	51164	82028	93524	95127	93732
25,28,30 TNH	312	266	337	182	198	247	234	238	228	284	465	323	553	216	501	0	241	374	257	339
Tm	84191	290079	104866	8948	6188	130099	154294	189365	156942	297414	713572	188748	755132	157442	304874	141996	126358	202837	144066	211849
C28,29,30 bisnorhopane	15130	13101	6641	3450	3834	12165	12172	39031	68259	41228	81448	21816	107441	16487	34261	11457	5452	6349	13642	29357
C27,17b trisnorhopane	0	18101	4485	3568	4968	15380	9536	5416	4734	10533	31711	6305	73941	5887	15900	10984	9530	8263	15978	9135
C29,30 norhopane	0	0	0	2990	2949	0	0	0	0	0	5952	4352	3856	2961	7055	0	4556	7496	4397	0
C29Ts	6064	120433	68880	7574	6772	4219	50767	56458	34857	317217	191971	44605	326977	63003	215772	4472	61476	48364	41600	94609
C29 moretane	11666	47925	15792	5255	7169	16311	14440	32020	50401	55626	147505	16139	178155	29926	3090	29790	42098	27603	21563	3185
Oleanane	3071	5270	6538	8418	3032	5069	5396	12023	0	5256	22094	4180	3223	2978	49017	2955	7838	6534	4410	9039
C30 hopane	215127	1016241	324287	21542	17304	404819	371333	474568	608955	1207459	0	913176	0	362439	0	432132	432926	537995	551608	624901
C30 moretane	16957	86024	41521	11110	6478	2814	6226	35886	32489	86183	278057	63676	393091	68790	149392	21508	29036	32208	41848	44124
C31S	108001	430663	132271	8677	3173	217505	191656	240736	356890	533131	1674822	326070	0	316396	642221	208058	226935	244791	249963	292124
C31R	78170	282716	106853	10984	4673	142706	142594	158535	256007	359146	551935	223960	1055439	234181	379213	133328	152598	168973	155912	193814
Gammacerane	141	395	0	203	229	295	332	356	322	537	770	201	952	227	420	364	306	208	319	381
C30bb_hop_C31_mor	3028	6252	2848	4047	3372	38762	47073	35555	79524	35248	277229	50996	445814	68360	234762	51444	4469	32442	70747	83335
C32_S	45543	236453	77071	6237	4760	110533	76383	76280	216029	352927	675038	202243	560068	193188	87785	123362	51744	140922	119838	164822
C32_R	3104	136550	64828	5580	8036	75449	60562	55148	149487	243771	475996	104114	490223	126574	123475	21026	77159	77295	95864	90847
C33_S	11397	72087	33094	4025	14742	45172	71374	84949	90628	250817	383852	59024	489695	103317	175612	54767	76886	88711	93928	82769
C33_R	21184	91321	28947	8318	5625	32993	23641	30714	78508	125303	113002	79046	162376	48001	119097	40384	13480	72761	12220	37381
C34_S	5207	112051	21498	4889	5083	21300	26483	29811	91233	138195	332151	127894	0	30080	145149	24257	26315	7970	30364	42493
C34_R	3695	68419	22879	9584	4847	13412	8710	17072	43648	82332	93181	56689	84011	83514	130455	8340	3813	23131	11919	28980
C35_S	3066	3984	6920	4235	6316	17993	8204	34685	67830	28012	88628	23163	8762	41053	43791	9354	8425	22984	3861	29024
C35_R	6542	4319	8928	0	3320	6091	8957	3741	41572	39964	84413	10766	7458	31857	13383	9161	12138	6922	4204	13157

Appendix III – Hydrocarbon sterane biomarker data

Family	L00549 A	L00920 A	L00550 A	L00554 B	L00515 B	L00732 C	L00672 C	L00753 C	L00559 C	L00558 D	L01312 D	L01276 D	L00842 D	L00756 D	L00755 D	L00829 E	L00833 E	L00839 E	L00820 E	L00811 E
C21 pregnane	251	468	398	517	565	1408	1410	1244	1152	1246	1197	568	954	529	842	877	1566	1656	1309	1735
C22 homo- pregnane	0	248	278	422	308	534	726	827	664	672	646	363	693	354	406	815	831	930	684	328
C27_ba_diaS	408	801	504	461	598	748	618	893	491	1036	1192	794	2036	943	1355	1484	1501	1665	1513	1827
C27_ba_diaR	328	599	432	237	364	563	578	637	460	597	722	501	1229	624	774	777	1053	1050	669	1360
C21_norcholestane	0	334	271	198	207	286	331	137	172	217	341	282	403	372	447	483	502	578	396	280
C27_aaaS	240	511	315	170	314	855	938	1019	1321	756	1670	228	2452	541	718	740	1100	775	945	1092
C27_abbR	311	1047	412	366	455	2133	1890	2639	3450	1674	3375	1267	4142	1399	1869	1926	1674	2330	2468	2903
C27_abbS	232	826	403	298	286	1905	1693	2284	2949	1829	3680	1185	4031	1614	1885	1883	1983	1920	2154	2759
C27_aaaR	388	445	398	408	396	873	1107	892	1864	994	1938	541	2835	760	1014	1021	849	1276	1278	1318
C28_aaaS	133	433	236	293	273	824	794	785	1076	761	1295	739	1402	740	1150	732	1002	990	1088	1344
C28_abbR	185	326	188	0	0	0	355	481	517	519	0	555	1174	440	0	0	535	0	482	546
C28_abbS	188	408	288	0	203	611	510	535	763	788	1459	565	1721	523	837	506	472	567	480	512
C28_aaaR	0	199	277	226	258	0	458	326	376	0	238	268	457	268	327	300	419	351	229	427
C29_aaaS	325	403	473	257	308	890	1015	976	1327	1452	2160	741	3823	588	0	906	760	1277	1194	1172
C29_abbR	379	1313	399	243	333	2459	1995	2694	2956	3762	6312	2355	6710	1959	3267	1959	2006	2300	2884	2973
C29_abbS	337	1084	498	345	0	1655	1931	2134	2640	2692	4999	1673	6203	1960	2327	1384	1835	1897	2175	2113
C29_aaaR	298	552	340	0	298	932	762	1188	1439	1503	2094	720	3687	915	1092	695	803	881	1002	995