

**Application of Biomarker Data in Oil Characterization**

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**Submitted to Mineral Resources Engineering Department  
of the Technical University of Crete  
in partial fulfillment  
for the requirements for the degree of  
Master of Science**

**Professor Nikos Pasadakis, Masters Thesis Advisor**

## **Acknowledgements**

I am most grateful for my advisor, Nikos Pasadakis, for his support and guidance throughout this project. I enjoyed my overall experience working with Eleni in the Hydrocarbon Laboratory, and I thank you for taking the time to show me how to analyze samples. I would like to thank the students of the 2015-2016 Petroleum Engineering class for their helpful discussion throughout the course year. I would like to especially thank Vassileios Papakostas not only for his never-ending support and motivation this year, but also for making my time in Crete memorable.

It was an honor being a student at the Technical University of Crete, where I was able to learn from excellent professors in the field of Petroleum Engineering. I would like to also thank all of my friends and family, all who supported me through my decision to complete a Master's degree in Greece. There are no words to express my gratitude for your love and support.

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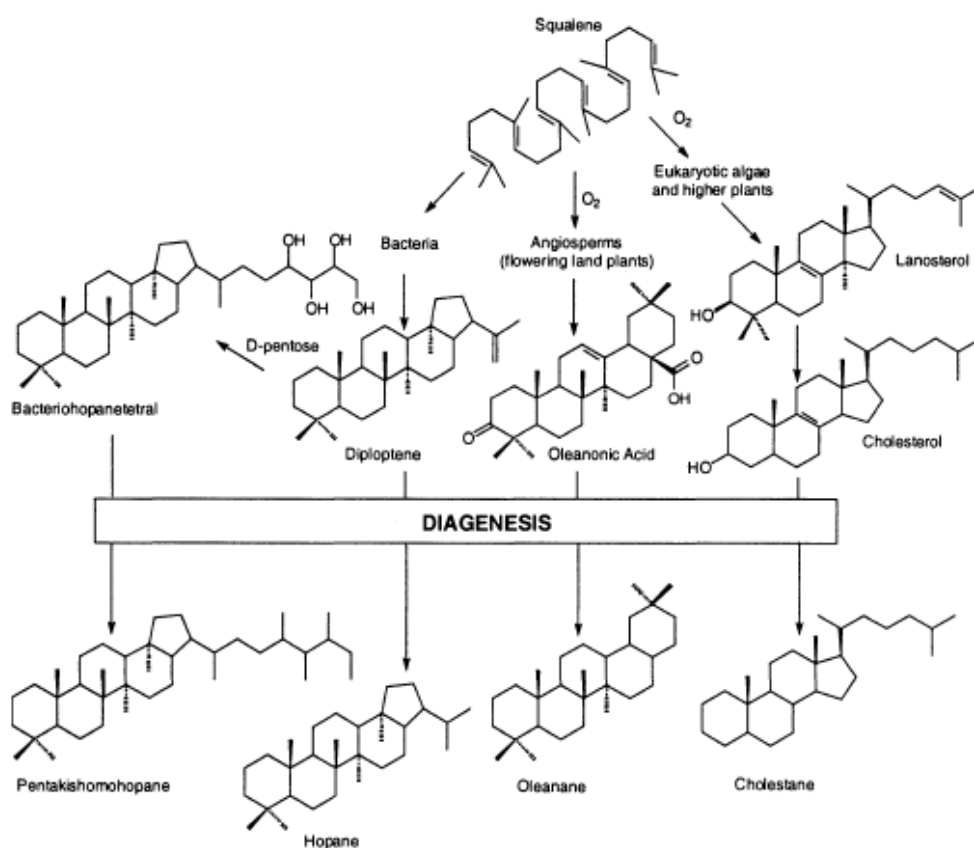
## **Abstract**

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).

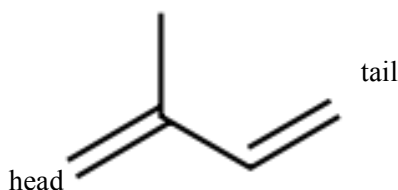


**Figure 1:** Biosynthesis of chemicals produced by living organisms and their equivalents that are produced through diagenesis (Hsu et al. 2011).

## 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 ( $\text{CH}_3\text{CO}_2$ ) 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 ( $\text{C}_5$ ) 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.



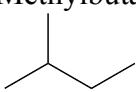
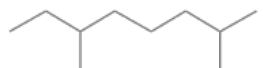
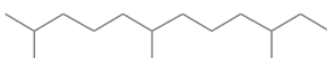
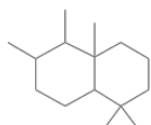
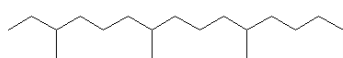
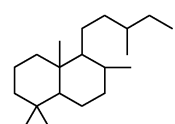
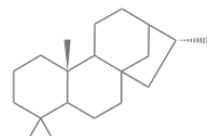
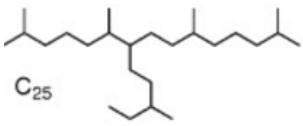
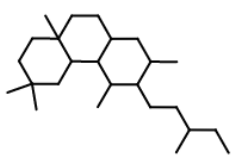
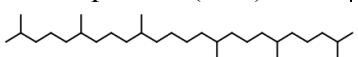
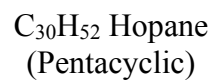
**Figure 2:** Chemical structure of one biosynthesized isoprene unit. The “head” corresponds to the Carbon 1 (first carbon), and the “tail” is Carbon 4 (last carbon).

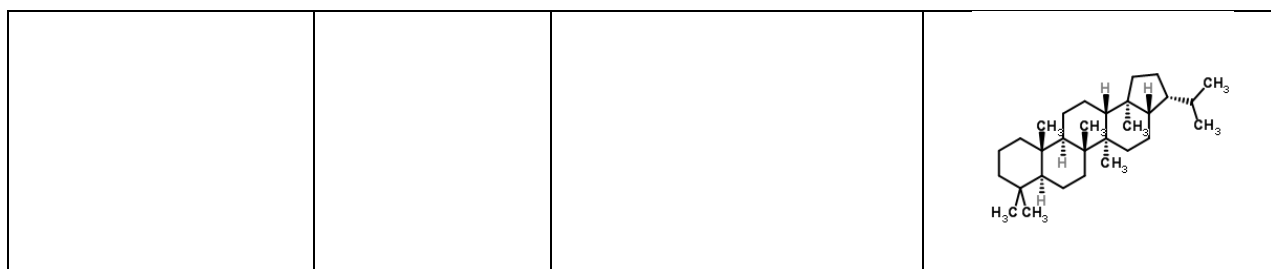


## 1.2 Terpanes

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<br>   |   |
| monoterpane (C10)            | 2              | 2,6-Dimethyloctane<br>  |   |
| sesquiterpane (C15)          | 3              | Farnesane<br>2,6,10-Trimethyldodecane<br>                   | Drimane (Bicyclic)<br>  |
| diterpane (C20)              | 4              | Phytane<br>   | Labdane (Bicyclic)<br><br>Phyllocladane (Tricyclic)<br> |
| sesterterpane (C25)          | 5              | Highly branched isoprenoid (C25 HBI)<br><br>C <sub>25</sub> | C25 Tricyclic Terpane<br>  |
| triterpane and steranes (30) | 6              | Squalane (C30)<br>  | C <sub>30</sub> H <sub>52</sub> Hopane (Pentacyclic)<br>   |



### 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 Strausz, 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 Leiophaeridia, 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 lead 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. C<sub>20</sub> and C<sub>21</sub> are most resistant, but C<sub>28</sub> is less resistant than C<sub>29</sub>). 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.

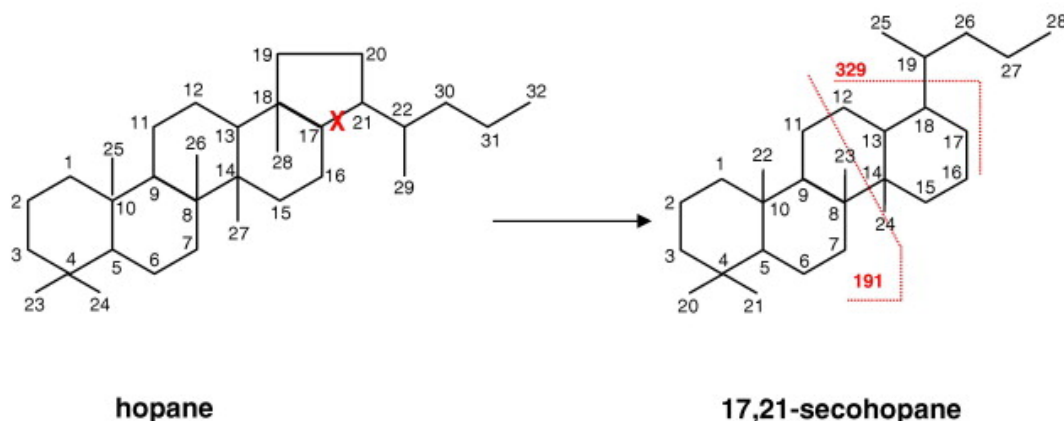
Aquino Neto et al. (1983) observed the generation of tricyclic terpanes from kerogens, suggesting that it might be strongly linked with macromolecular organic matter by multiple covalent bonds. Bowden et al. (2006) observed a larger amount of tricyclic terpanes relative in resin and asphaltene fraction 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 terpene (TT) ratios

$C_{22}TT/C_{21}TT$ ,  $C_{24}TT/C_{23}TT$ , and  $C_{26}TT/C_{25}TT$  tricyclic terpene 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 terpene 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 terpenes 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 terpene 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 terpenes. 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  $C_{24}$  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  $C_{24}TeT/C_{30}$  Hopane,  $C_{24}TeT/C_{23}TT$ ,  $C_{24}TeT/C_{26}TT$ . These ratios are commonly used to assess source of deposition. The  $C_{24}TeT/C_{30}$  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  $C_{24}TeT/C_{26}TT$  values ( $>1$ ) indicate carbonate organic matter (Peters and Moldowan, 1993); low to medium values suggests marine shale source; and low  $C_{24}TeT/C_{26}TT$  values have been observed in deltaic shale sources (Peters et a., 2005).

### *1.2.3a Hopanes and steranes in petroleum ( $C_{27}$ 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  $17\alpha(H)$ ,  $21\beta(H)$  structure, which are more thermodynamically stable. Other stereoisomeric structures include  $17\alpha(H)$ ,  $21\beta$ - and  $17\beta$ ,  $21\beta$ -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  $17\alpha(H)$ -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  $C_{29}$  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  $C_{29}$  and  $C_{27}$  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 C<sub>28</sub> steranes and C<sub>27</sub> 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 (C<sub>31</sub>>C<sub>32</sub>>C<sub>33</sub>>C<sub>34</sub>>C<sub>35</sub>) 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<sub>27</sub> > C<sub>28</sub> > C<sub>29</sub> > C<sub>30</sub> (Peters et al., 2005).

### 1.2.3c Hopanes (H) and steranes ratios

Some of the common hopane ratios used as source parameters are are C<sub>24</sub>TeT/C<sub>30</sub>H, tricyclics/hopanes, C<sub>29</sub>H/C<sub>30</sub>H, C<sub>35</sub>/C<sub>34</sub> homohopane, and C<sub>33</sub>/C<sub>32</sub> homohopane, (C<sub>28</sub>+ C<sub>29</sub>TT)/C<sub>30</sub>H. Common maturity parameters include Tm/C<sub>30</sub>H, Ts/C<sub>30</sub>H, and C<sub>29</sub>Ts/C<sub>29</sub> 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   |
|-------------------------------|--|-------------------------------|--|
| <b>Saturated Hydrocarbons</b> | 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/Hopanes                             | Increase                      | Useful in late oil window; also increases at high levels of biodegradation.  |
| <b>Aromatic Hydrocarbons</b>  | 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. |
|                               | 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).

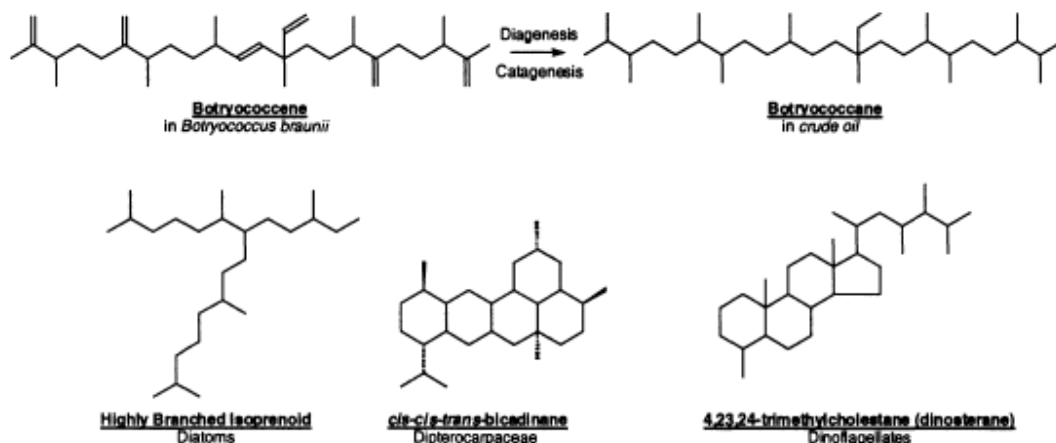
| Characteristics                          | Shales             | Carbonates          | References         |
|--|--------------------|---------------------|--------------------|
| <i>Non-biological marker parameters</i>  |                    |                     |                    |
| API, gravity                             | 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)        | $\geq 1$           | $\leq 1$            | 1, 2, 6, 9         |
| <i>Biological marker parameters</i>      |                    |                     |                    |
| Pristane/phytane (Pr/Ph)                 | High ( $\geq 1$ )  | Low ( $\leq 1$ )    | 1, 2, 6, 9, 10, 12 |
| Phytane/ <i>n</i> C18                    | Low ( $\leq 0.3$ ) | High ( $\geq 0.3$ ) | 2, 6               |
| Steranes/17 $\alpha$ -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).

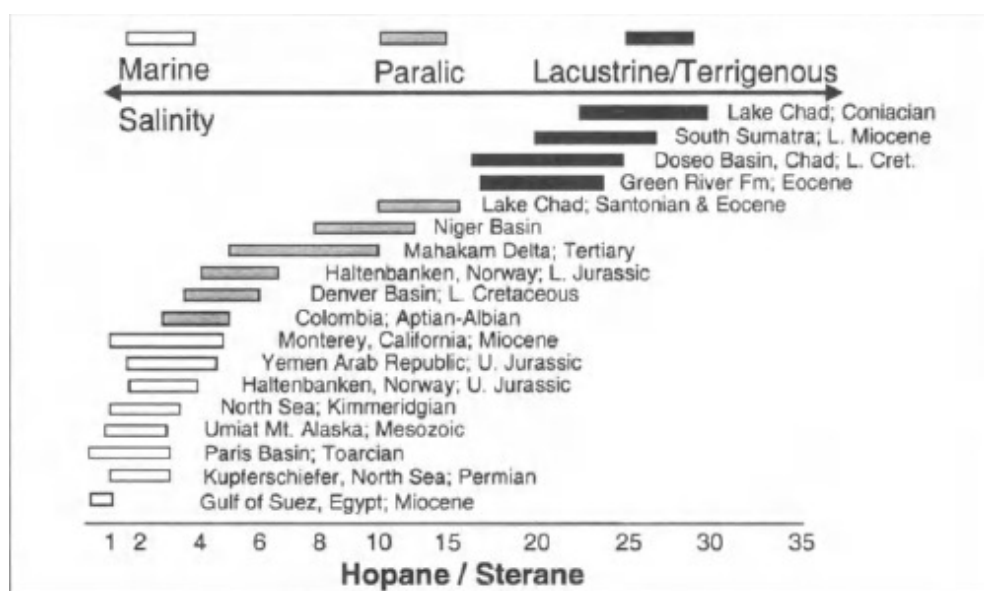
Specific animals can be indicated by certain biomarkers also. For example, botryococcane is a biomarker derived from the Chlorophycean alga *Botryococcus braunii*, and found in freshwater and brackish water environments. Highly branched

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).



#### 1.4 Biomarker analyses using Biotoool

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 Biotoool. This tool not only allows one to properly assess petroleum samples, but also carry out the biomarker analyses in an efficient matter. The Biotoool will then be used to characterize oils from the Willinstin Basin, North America.

## Materials and Methods

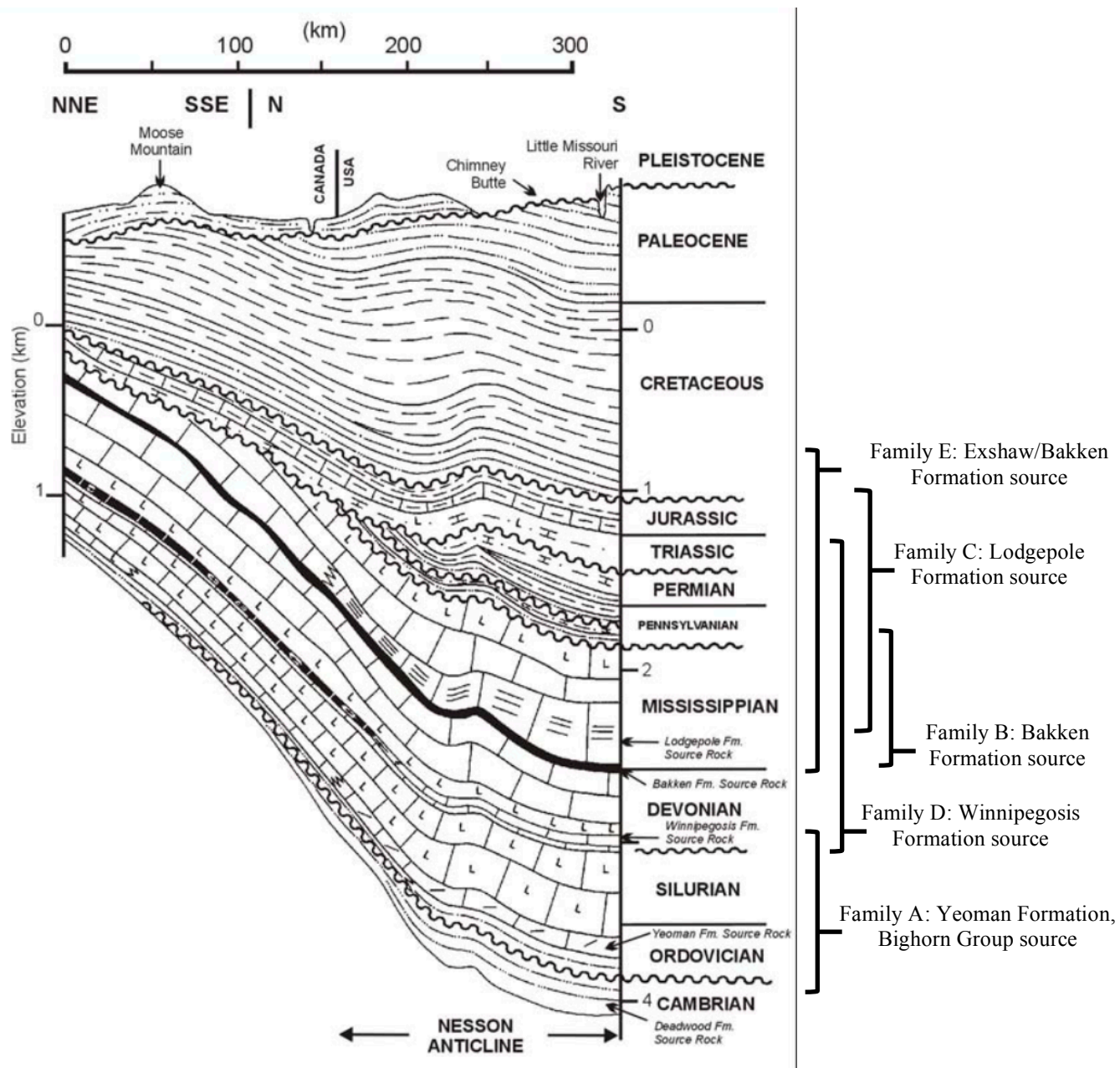
### 2.1 Geological Setting of the Williston Basin

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<br>L00920<br>L00550                               | A      | Type I    | Red River Oils                  | Red River Formation***;<br>Winnipeg Formation**;<br>Bighorn Group | Middle-Upper Ordovician        | light brown shale type (kukersite) |
| L00554<br>L00515   | B      | Type II   | Bakken Oils                     | Bakken Formation  | Upper Devonian - Mississippian | dark shale                         |
| L00732<br>L00672<br>L00753<br>L00559                     | C      | Type II   | Mississippian and Jurassic Oils | Lodgepole Formation   | Lower Mississippian            | lime mudstone                      |
| L00558<br>L01312<br>L01276<br>L00842<br>L00756<br>L00755 | D      | Type III  | Winnipegosis Oils               | Winnipegosis Formation  | Middle Devonian                | laminite; limestone; dolostone     |
| L00829<br>L00833<br>L00839<br>L00820<br>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  $C_{35}$  hopane predominance (Osadetz et al., 1992). Like Family B oils, Family C oils are mature and from carbonate source rock, and  $T_m/C_{30}$  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 | <b>Hopanes ratios</b>                       |
| 47 | <a href="#">19tri/20tri</a>                 |
| 48 | <a href="#">22tri/21tri</a>                 |
| 49 | <a href="#">(19tri+20tri)/(23tri+24tri)</a> |
| 50 | <a href="#">(19tri+20tri)/C23tri</a>        |
| 51 | <a href="#">24tri/23tri</a>                 |
| 52 | <a href="#">19tri/23tri</a>                 |
| 53 | <a href="#">24tet/30hop</a>                 |
| 54 | <a href="#">24tet/23tri</a>                 |
| 55 | <a href="#">ETR</a>                         |
| 56 | <a href="#">26tri/25tri</a>                 |
| 57 | <a href="#">24tet/26tri</a>                 |
| 58 | <a href="#">Ts/Ts+Tm</a>                    |
| 59 | <a href="#">Ts/30hop</a>                    |
| 60 | <a href="#">Tm/Hopane</a>                   |
| 61 | <a href="#">C29nor/C30 Hop</a>              |
| 62 | <a href="#">BNH/Hop</a>                     |
| 63 | <a href="#">C31-homo</a>                    |
| 64 | <a href="#">C32-homo</a>                    |
| 65 | <a href="#">C33-homo</a>                    |
| 66 | <a href="#">C34-homo</a>                    |
| 67 | <a href="#">C35-homo</a>                    |
| 68 | <a href="#">C31R/C30</a>                    |
| 69 | <a href="#">C29Ts/C29 nor-hopane)</a>       |
| 70 | <a href="#">C31 S/S+R</a>                   |
| 71 | <a href="#">C32 S/S+R</a>                   |
| 72 | <a href="#">C32 S/R</a>                     |
| 73 | <a href="#">C35/C34 homo</a>                |
| 74 | <a href="#">C33/C32 homo</a>                |
| 75 | <a href="#">moretane</a>                    |
| 76 | <a href="#">oleanane</a>                    |
| 77 | <a href="#">gammacerane</a>                 |
| 78 | <a href="#">23tri/C30-Hop</a>               |
| 79 | <a href="#">C28+C29tri/C30hop</a>           |

**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 Saturated 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  $\text{Pr}/\text{nC}_{17}$  and  $\text{Ph}/\text{nC}_{18}$  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),  $\text{Pr}/\text{nC}_{17}$  and  $\text{Ph}/\text{nC}_{18}$  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  $\text{Pr}/\text{nC}_{17}$  and  $\text{Ph}/\text{nC}_{18}$  could not completely differentiate shale from carbonate source rocks for every oil sample. According to Peters et al. (2005), low  $\text{Ph}/\text{nC}_{18}$  indicates a shale source and high values indicate a carbonate setting (Table 3). For  $\text{Pr}/\text{nC}_{17}$  ratios, high ( $>0.6$ ) and low ( $<0.5$ ) values indicate terrestrial and marine sources, respectively. In Diastay et al. (2016), limestone carbonate rocks had  $\text{Pr}/\text{nC}_{17}$  and  $\text{Ph}/\text{nC}_{18}$  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.

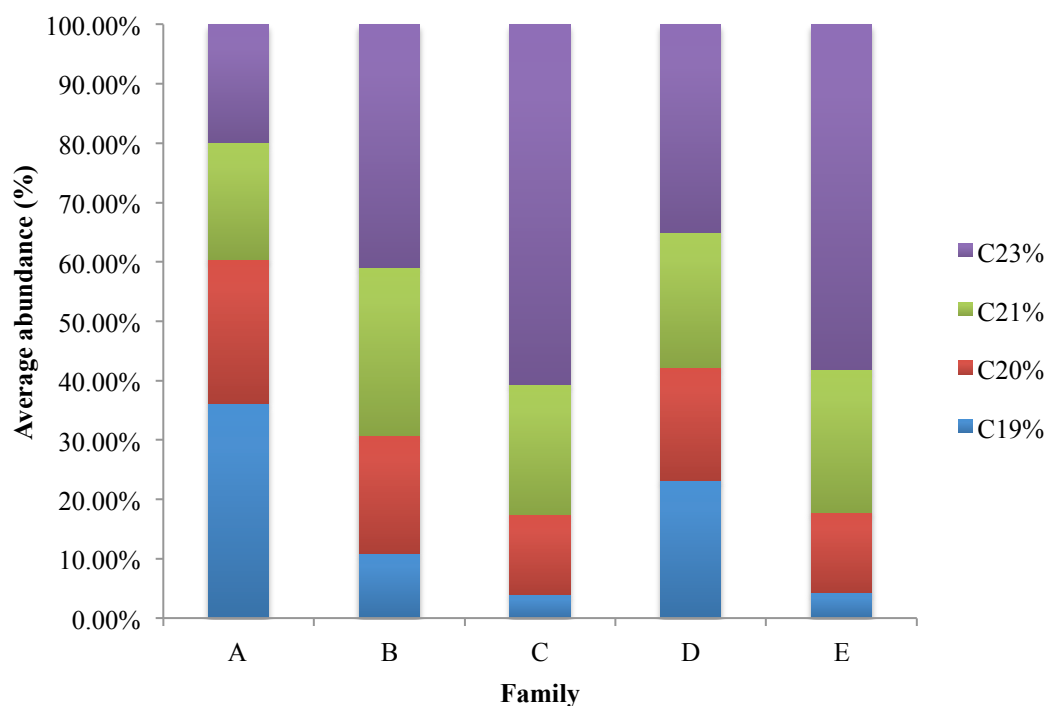
**Table 5:** Biomarker parameters of the Williston basin oils, Family A-E. Note that the (-) represent a no value for the corresponding value.

| <b>Sample Name</b> | <b>Family</b> | <b>Pr/Ph</b> | <b>Pr/nC<sub>17</sub></b> | <b>Ph/nC<sub>18</sub></b> |
|--------------------|---------------|--------------|---------------------------|---------------------------|
| <b>L00549</b>      | <b>A</b>      | -            | 1.29                      | 0.06                      |
| <b>L00920</b>      | <b>A</b>      | 1.40         | 0.06                      | 0.13                      |
| <b>L00550</b>      | <b>A</b>      | -            | 1.26                      | 0.07                      |
| <b>L00554</b>      | <b>B</b>      | 1.39         | 0.65                      | 0.53                      |
| <b>L00515</b>      | <b>B</b>      | 1.55         | 0.64                      | 0.50                      |
| <b>L00732</b>      | <b>C</b>      | 0.64         | 0.50                      | 0.72                      |
| <b>L00672</b>      | <b>C</b>      | 0.60         | 0.43                      | 0.66                      |
| <b>L00753</b>      | <b>C</b>      | 0.61         | 0.42                      | 0.61                      |
| <b>L00559</b>      | <b>C</b>      | 0.59         | 0.42                      | 0.65                      |
| <b>L00558</b>      | <b>D</b>      | 0.95         | 0.42                      | 0.44                      |
| <b>L01312</b>      | <b>D</b>      | 0.68         | 0.77                      | 1.49                      |
| <b>L01276</b>      | <b>D</b>      | 0.72         | 0.25                      | 0.50                      |
| <b>L00842</b>      | <b>D</b>      | 0.61         | 0.97                      | 1.77                      |
| <b>L00756</b>      | <b>D</b>      | 0.66         | 0.35                      | 0.71                      |
| <b>L00755</b>      | <b>D</b>      | 0.59         | 0.59                      | 1.12                      |
| <b>L00829</b>      | <b>E</b>      | 0.93         | 2.31                      | 2.51                      |
| <b>L00833</b>      | <b>E</b>      | 0.87         | 2.88                      | 5.59                      |
| <b>L00839</b>      | <b>E</b>      | 0.93         | 0.37                      | 1.08                      |
| <b>L00820</b>      | <b>E</b>      | 0.44         | 0.58                      | 3.88                      |
| <b>L00811</b>      | <b>E</b>      | 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 C<sub>19</sub>-C<sub>23</sub> diterpanes (Figure 10). These tricyclic terpanes are commonly used alongside other ratios, such as pristane/phytane, hopane/sterane, pristane/*n*C<sub>17</sub>, gammacerane and oleanane/C<sub>30</sub> 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, C<sub>19</sub> (36.2 ± 3.42%) and C<sub>20</sub> (24.19 ± 12.4%) tricyclic terpanes were more abundant than C<sub>21</sub> (19.65 ± 2.58%) and C<sub>23</sub> (19.96 ± 11.84%) in Family A (Figure 10). An abundance of C<sub>19</sub> and C<sub>20</sub> 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 C<sub>19</sub> and C<sub>20</sub> tricyclic terpanes were more abundant than C<sub>21</sub> and C<sub>23</sub> 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 C<sub>19</sub>, C<sub>20</sub>, C<sub>21</sub>, C<sub>23</sub> 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  $C_{24}TeT/C_{26}TT$  ( $\sim 0.48$  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).

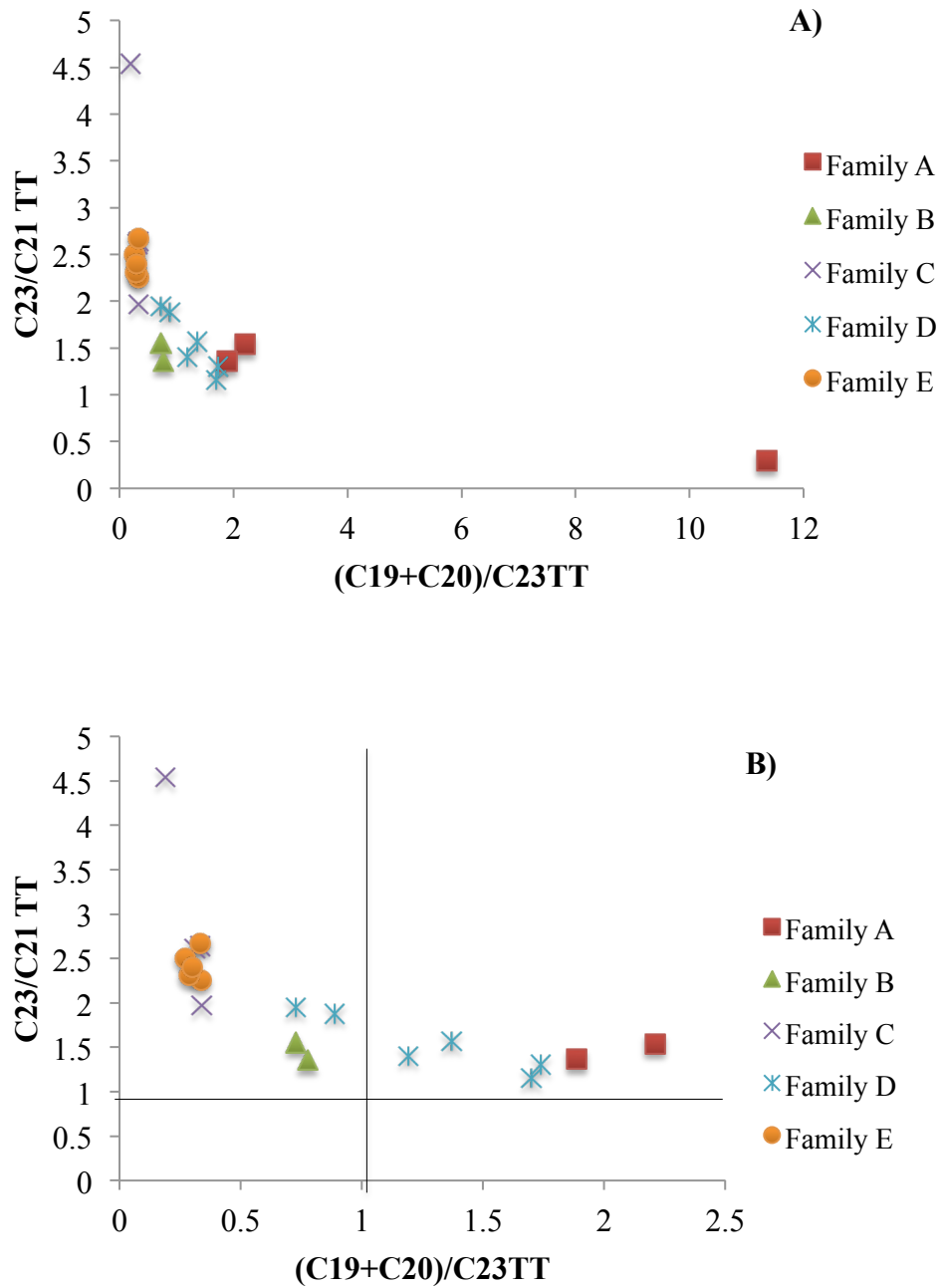
The  $C_{24}TeT/C_{20-26}TT$  ratio was developed in Tao et al. (2015) to identify any crude oils from a terrestrial or higher plant source. This parameter was used alongside with  $C_{24}TeT/C_{30}$  to distinguish anoxic from oxic environments.  $C_{24}TeT/C_{20-26}TT$  values of all oil samples were extremely low and close to 0 ( $\sim 0.024$ -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.

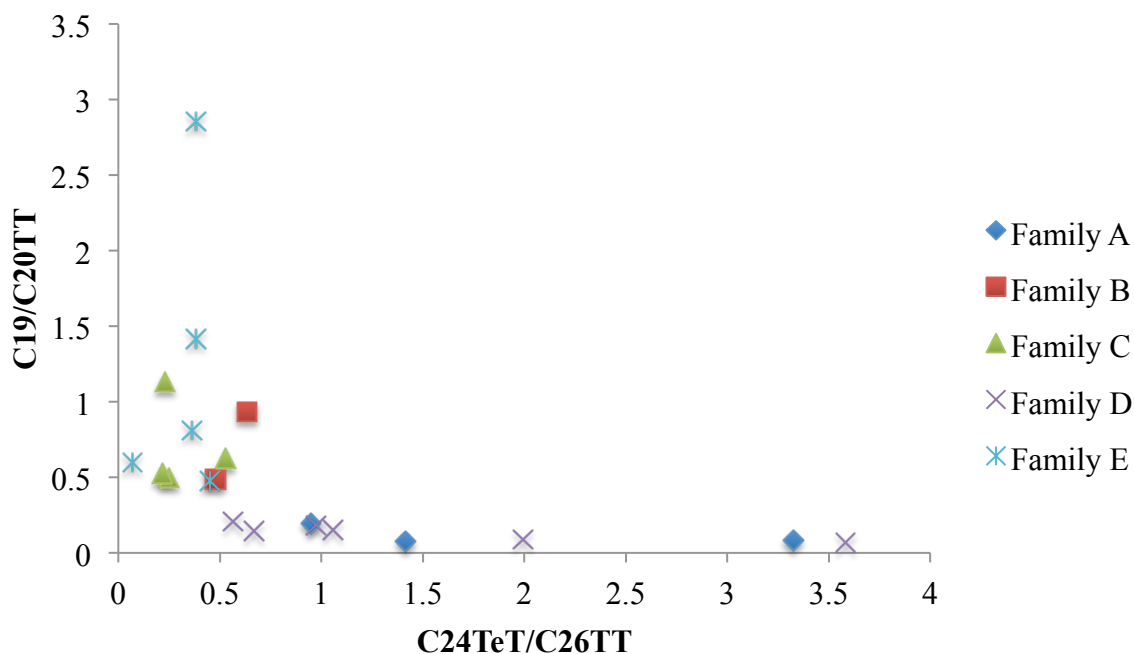
| Sample Name | Family | $C_{19}/C_{20}$<br>TT | $C_{20}/C_{23}$<br>TT | $C_{25}/C_{26}T$<br>T | $C_{24}TeT/C_{26}TT$ | $C_{24}TeT/C_{20-26}TT$ | $C_{24}TeT/C_{30}H$ | $C_{23}/C_{21}$<br>TT | $(C_{19}+C_{20})/C_{23}TT$ |
|-------------|--------|-----------------------|-----------------------|-----------------------|----------------------|-------------------------|---------------------|-----------------------|----------------------------|
| 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                       |



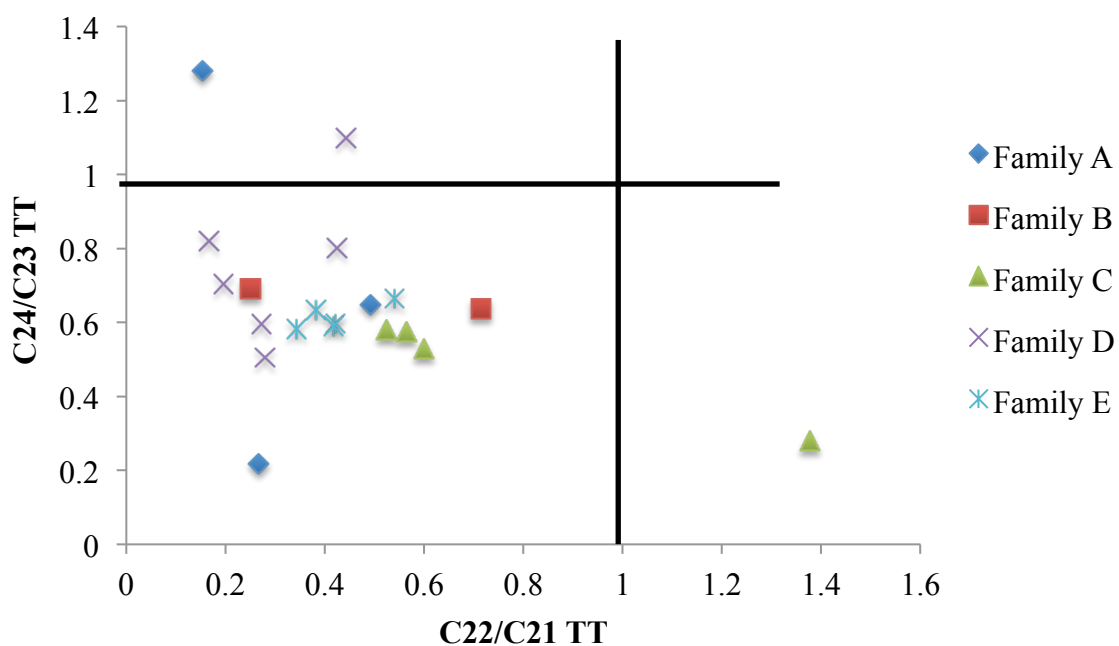
**Figure 11:** Cross plot of  $C_{19}+C_{20}/C_{23} TT$  ratio. A) Cross plot with all Williston Basin samples. Note that there is one outlier sample from Family A that distorts the plot; B) cross plot from A without the one outlier. Note that TT is a notation for tricyclic terpanes.

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

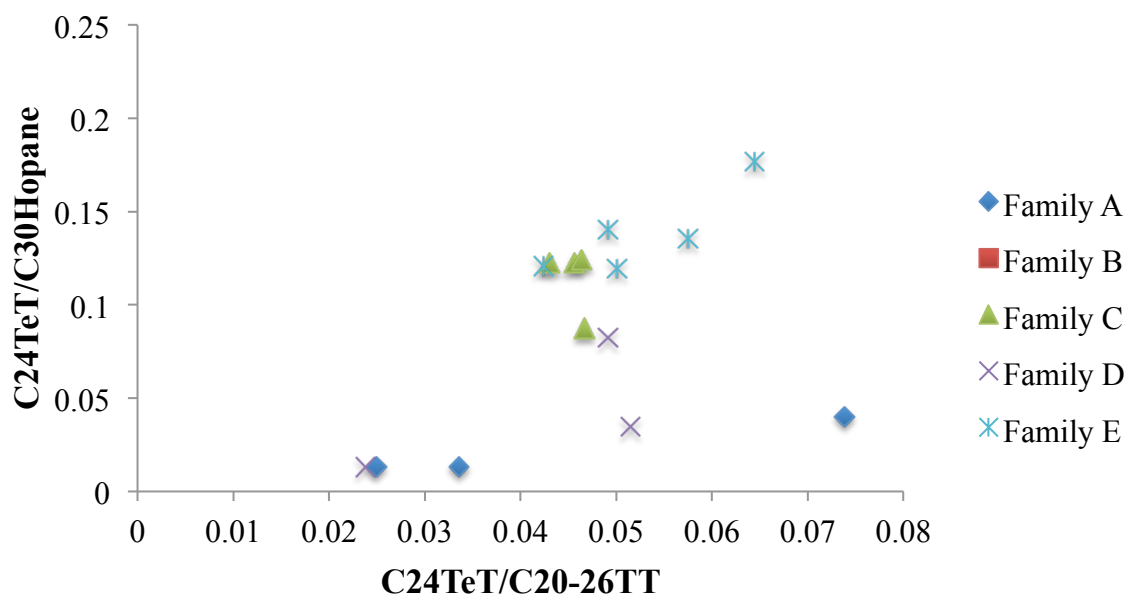
| Sample Name | Family | $C_{23}TT/C_{30}$<br>Hopane | $C_{22}/C_{21}$<br>TT | $C_{24}/C_{23}$<br>TT | $C_{31} R/C_{30}$<br>H |
|-------------|--------|-----------------------------|-----------------------|-----------------------|------------------------|
| L00549      | A      | 0.09                        | 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  $C_{24}TeT/C_{26}TT$  vs.  $C_{19}TT/C_{20}TT$  ratios. These two ratios are common source parameters. Note that TT is a notation for tricyclic terpanes.





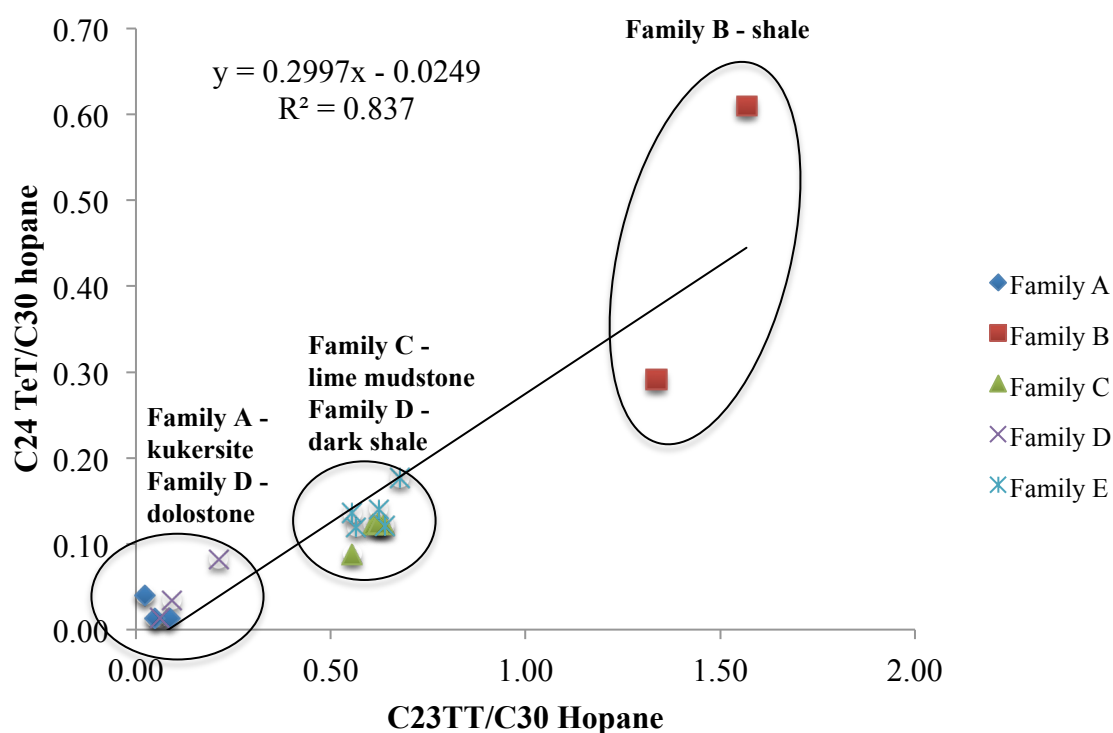
**Figure 13:** Cross plot of  $C_{24}TT/C_{23}TT$  vs.  $C_{22}TT/C_{21}TT$  ratios. These two ratios are generally used to differentiate oils from carbonate rocks, which usually have high  $C_{22}TT/C_{21}TT$  and low  $C_{24}TT/C_{23}TT$  (Peters et al., 2005).



**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).

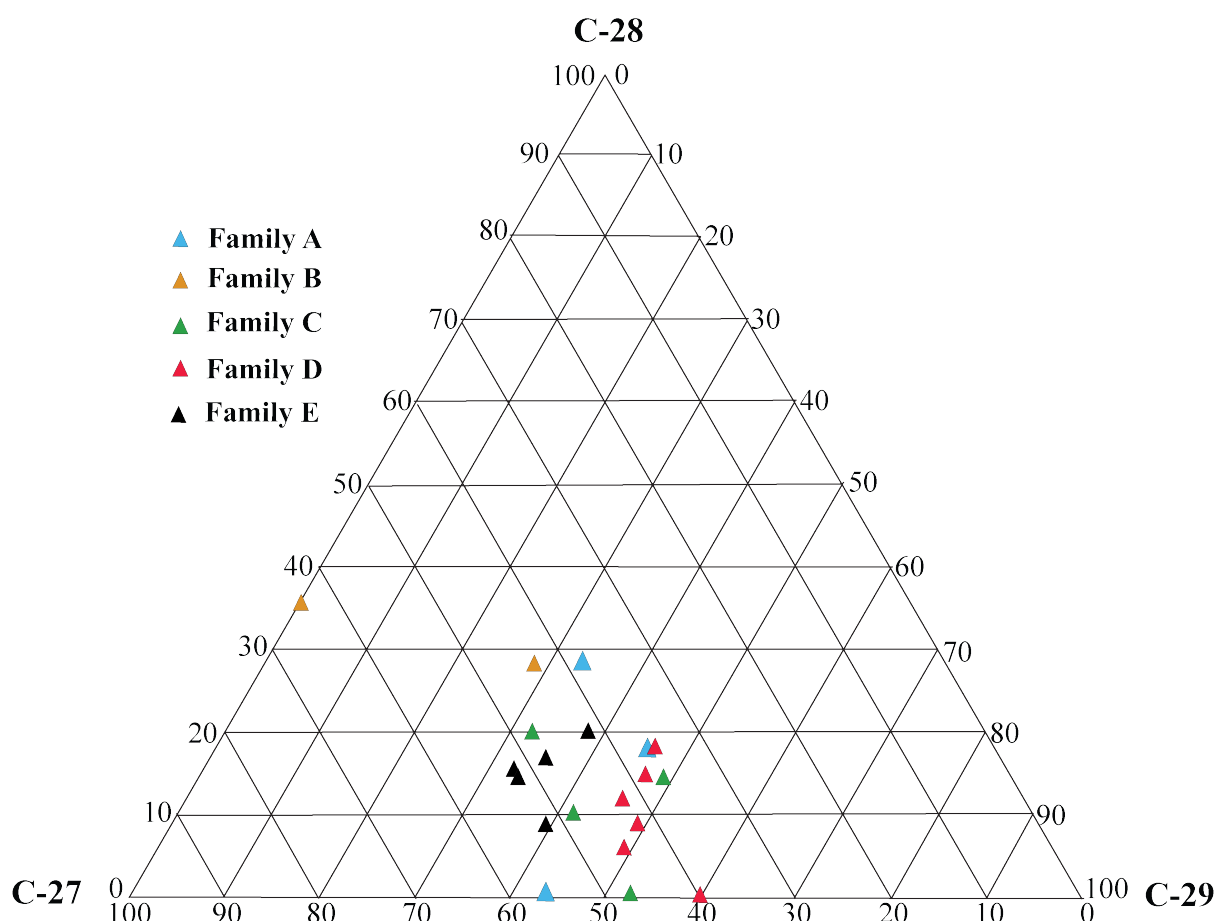


**Figure 15:** Cross plot of  $C_{24}TeT/C_{30}$  Hopane vs.  $C_{23}TT/C_{30}$  Hopane ratios along with a regression line. These two ratios are generally used to differentiate oil sources. For the Williston Basin, this cross plot seems to distinguish Family B Bakken Formation oils from the rest of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

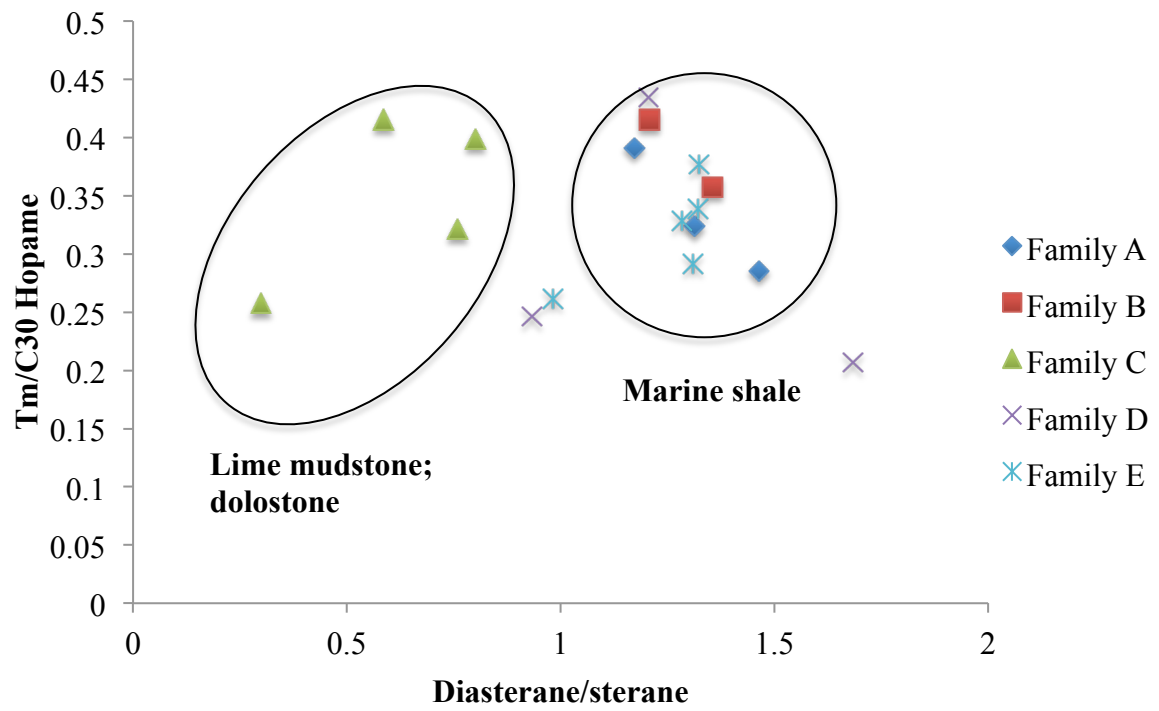
### 3.4 Distribution of steranes

Sterane ternary diagrams are generally used to show relationships between oils and sourcerocks (Peters et al., 2000). A  $C_{27}$ – $C_{28}$ – $C_{29}$  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  $Tm/C_{30}$  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  $C_{27}$ – $C_{28}$ – $C_{29}$  steranes.



**Figure 17:** Tm/C<sub>30</sub>Hopane 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  $C_{23}$  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  $C_{19}$  and  $C_{20}$ , 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.  $C_{24}TT/C_{23}TT$  and  $C_{22}TT/C_{21}TT$ ), 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 ( $C_{27}$ - $C_{28}$ - $C_{29}$ ) along with the  $Tm/C_{30}$  Hopane vs. diasterane/sterane crossplot were the most reliable and accurate in this study. The  $C_{27}$ - $C_{28}$ - $C_{29}$  plot placed all Williston Basin oils in the open marine category, with one sample in the planktonic region. The  $Tm/C_{30}$  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<br>bisnorhopane | 15130  | 13101   | 6641   | 3450   | 3834   | 12165  | 12172  | 39031  | 68259  | 41228   | 81448   | 21816  | 107441  | 16487  | 34261  | 11457  | 5452   | 6349   | 13642  | 29357  |
| C27,17b<br>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<br>A | L00920<br>A | L00550<br>A | L00554<br>B | L00515<br>B | L00732<br>C | L00672<br>C | L00753<br>C | L00559<br>C | L00558<br>D | L01312<br>D | L01276<br>D | L00842<br>D | L00756<br>D | L00755<br>D | L00829<br>E | L00833<br>E | L00839<br>E | L00820<br>E | L00811<br>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-<br>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         |