Article

Geochemical Characteristics of Expelled and Residual Oil from Artificial Thermal Maturation of an Early Permian Tasmanite Shale, Australia

Xiaomin Xie 1,* , Ye Wang 2 , Jingwen Lin 1 , Fenting Wu 1 , Lei Zhang 1 , Yuming Liu 1 and Xu Hu 1

1 Key Laboratory of Oil and Gas Resources and Exploration Technology, Ministry of Education, College of Resources and Environment, Yangtze University, Wuhan 430100, China; linjingwen2020@sina.com (J.L.); wufenting_w@sina.com (F.W.); degun_wuxi@sina.com (L.Z.); 201707852@yangtzeu.edu.cn (Y.L.); huxu990908@sina.com (X.H.)
2 Key Laboratory of Western Mineral Resources and Geological Engineering, Ministry of Education, Chang’ an University, Xi’an 710061, China; yewang2008@outlook.com
* Correspondence: xiaominxie2019@sina.com

Abstract: Lipid biomarkers play an important role in defining oil-source rock correlations. A fundamental assumption is that composition (or ratios) of biomarkers in oil is not significantly different from that in bitumen in the source rock. In order to compare the geochemical characteristics of expelled oil and residual oil, a Permian Tasmanite oil shale was used for an artificial maturation experiment to simulate the oil generation period. The results show that the Tasmanite oil shale generated high amounts of hydrocarbons (731 mg HC/g TOC) at low maturation temperatures (340 °C). The hydrocarbon (HC) group compositions are different between the expelled oil (with more aromatic HC and saturated HC) and the residual oil (with more resin fraction and asphaltene). The Pr/Ph ratio (up to 4.01) of the expelled hydrocarbons was much higher than that in residual oil (<1.0). Maturity-related biomarkers Ts/(Ts + Tm), and αααC29-20S/(20S + 20R) and C29-αββ/(ααα + αββ), also showed complicated variations with pyrolysis temperature, especially at post peak oil generation. C27-, C28-, and C29- sterane distributions showed variations with pyrolysis temperature. Therefore, without considering the influence of maturity on the abundance of compounds, either source, maturity and/or organic matter type from the chemical characteristics may not be correct.

Keywords: biomarkers; Tasmanite oil shale; artificial maturation experiment; expelled oil; residual oil; geochemical parameters; oil-source correlation

1. Introduction

Oil-source rock correlation is important for petroleum exploration and development, and one needs to consider both the hydrocarbon generation and hydrocarbon expulsion processes. Hydrocarbon generation from source rocks is controlled mainly by thermal maturation and the kerogen type [1,2]. There are two approaches to analyze the kerogen type: one is a geochemical classification as developed by Tissot and Welte [3] who classified the organic matter with H/C and O/C atomic ratios, as well as Rock-Eval data presented as HI–OI and HI–Tmax diagrams. Another approach uses organic petrology which determines the sources of organic matter through the morphology of macerals observed under the microscope [4,5]. Significant amounts of hydrocarbons can only be generated from kerogen when the rocks reach a suitable thermal maturity. Different kerogens generate different distributions of hydrocarbons which vary with maturity and source, so that geochemical parameters based on lipid biomarkers and isotope values can also be used for oil-source correlation analysis [6–9].

An underlying assumption for oil-source correlation analysis is that the composition (or ratios) of geochemical compounds in the migrated oil is not significantly different from
those of the bitumen remaining in the source rock [10,11] and that they provide an accurate
guide to the types of organic matter in the original source rocks. A large number of proxies
have now been developed including the distribution of C27-, C28-, and C29- steranes
derived from the corresponding sterols), the ratios diahopane/C30 hopane vs. gammacer-
ane/C31 22R-homohopane; C29- norhopane/C30- hopane vs. C31 22R-homohopane/C30-
hopane; C27- Ts/Ts + Tm trisnorhopanes (17α(H)-22,29,30-trisnorhopane/(18α(H)-22,29,30-
trisnorhopane + 17α(H)-22,29,30-trisnorhopane), C27- rearranged/regular steranes (dia-
cholestane/cholestan) vs. steranes/hopanes, to name just a few [1,10,12]. However, many
of these ratios vary with maturity, and careful attention must be applied when using these
compounds for assigning organic matter sources. For example, Boreham et al. [12] used
biomarker ratios for oil-source correlation but restricted their study to mature potential
source rocks for comparisons with the oils.

One might expect a close relationship between the compositions of geochemical com-
pounds in the kerogen and the generated hydrocarbons from the kerogen. However, factors
such as biodegradation and diagenesis during deposition and subsequent burial can lead to
major differences between the original biolipids and those incorporated into the sediment
record [10]. Additionally, the kerogen may show a preponderance of polymeric material
including selectively preserved algaenans (biopolymers of algal origin) and diagnostically
formed macromolecular organic matter. There can also be mismatches between the organic
petrology and geochemical analyses in the sedimentary rocks. For example, Grice et al. [13]
detected the biomarkers of Botryococcus braunii (B. braunii) in an ancient hypersaline euxinic
system, while “SEM of the isolated kerogens showed a high degree of morphological alter-
ation and the B. braunii colonies were not recognisable”. Xie et al. [14] found large amounts
of diatoms under SEM in the Eocene Huadian oil shales, but diagnostic diatom biomarkers
were below detection. The organic matter identified by organic petrology method focuses
on the kerogen, while molecular compound analyses typically focus on the solvent-soluble
hydrocarbons. The two methods focus on different parts of the organic matter in rocks, and
a further complication is determining which of the identified macerals is a major source
of hydrocarbons [15]. Besides, organic matter in source rocks is complicated, and the
generated hydrocarbon could be mutually influenced by different types of organic matter
during the process of hydrocarbon generation [16,17]. Due to the complexity of organic
matter composition in source rocks, it is difficult to ascertain which hydrocarbons were
generated from which type of organic matter.

Oil shales with a predominant source of hydrocarbons from a single maceral provide
a way to study the effects of generation and expulsion on hydrocarbon distributions.
Artificial maturation experiments can reproduce the thermal transformation process of
kerogen and oil-gas generation based on natural maturity trends [18]. The Tasmanite oil
shale from the Woody Island Formation in the Tasmania Basin is abnormally rich in a single
alga Tasmanites punctatus with low maturity (%Ro = 0.5), providing an ideal sample for
artificial maturation experiments. In our study, hydrocarbons generated from a Tasmanite
shale have been collected both as expelled oil (i.e., hydrocarbons expelled from the shale)
and residual oil (remaining hydrocarbons that can be extracted from the shale) after each
artificial thermal maturation experiment. Geochemical parameters in the expelled oil and
residual oil have been compared to test possible effects on these ratios with increasing
thermal maturity and how this might affect oil-source correlations.

2. Geological Setting and Samples

The onshore Tasmania Basin is located in the state of Tasmania, south of mainland
Australia. The basin contains a glaciomarine (Lower Parmeener, Supergroup) to terrestrial
sequence (Upper Parmeener, Supergroup) of Late Carboniferous to Late Triassic age [19]
(Figure 1). The Tasmanite oil shale was deposited at the bottom of the Woody Island
Formation, Lower Parmeener Supergroup, during the Early Permian. The Woody Island
Formation is mainly composed of siltstone and mudstone. The oil shale occurs at several
sites in Tasmania, but it is mainly found in north-west Tasmania where it is immature [20,21].
The depositional environment was glacial, and glendonites and scattered ice-rafted pebbles have been found in these rocks [19].

Figure 1. The sampling location and maturity distribution of the Tasmanite shales (modified from Reid and Burrett [19]).

Four samples (including two oil shale samples and two mudstone samples) were collected from outcrops near the Mersey River, Latrobe. The oil shale samples were obtained from an abandoned mine, and the two mudstones were collected in the outcrop near the river (Figure 1). According to Revill et al. [21], the oil shale at Latrobe shows both small-scale and large-scale lensing, suggesting a fluctuating depositional environment.

3. Experimental Methods

All samples were analyzed using Rock-Eval pyrolysis, organic petrology analysis, and one Tasmanite oil shale (Aus-2) sample was selected for X-ray diffraction measurements and artificial thermal maturation experiments. All experiments were conducted at the State Key Laboratory of Shale Oil and Gas Enrichment Mechanisms and Effective Development in Wuxi, China.

3.1. Kerogen Isolation and Sink-Float Separation

Shale sample Aus-2 (200 g) was crushed into small particles (~150 µm), and then extracted with chloroform in a Soxhlet apparatus for 48 h. Rock particles were treated with 20% HCl for 12 h to remove carbonates, washed with distilled water, and then with 48% HF for 2 days to remove silicates. The non-digestible residues were kerogen with some
pyrite, which were prepared for density separation as described in detail in Xie et al. [16]. The kerogen residues were suspended in the heavy liquid using a sonicator and then centrifuged in an Optima XE-100 centrifuge at 20,000 rpm (~160,000 × g) for 150 min with room temperature. The Aus-2 kerogen residues were separated into density fractions from <1.06 g/mL to >1.14 g/mL.

3.2. Organic Petrology, X-ray Diffraction (XRD), Rock-Eval Pyrolysis and Carbon Isotope Analysis

Rock samples were analyzed using organic petrology, XRD analysis and Rock-Eval pyrolysis. The kerogen sample was used for carbon isotope analysis. Detailed descriptions of these experimental methods have been included in Xie et al. [14,16,22,23].

Organic petrographic analyses (including the maceral composition and reflectance analysis) were conducted on rock samples with a Leica DM 4500P microscope with J&M photometer. Rock-Eval pyrolysis was performed on a Rock-Eval 6 pyrolyzer (Vinci Technologies) [24]. Stable carbon isotope analyses were made using a MAT-253 mass spectrometer, with an analytical precision of ±0.02‰, and a replicate sample precision of ±0.1‰.

3.3. Kinetics of Hydrocarbon Generation under an Open System

Kinetics of hydrocarbon generation under an open system is based on the single cold trap pyrolysis chromatography analysis. The analysis was conducted on a Vinci Rock-Eval 6 coupled with an American Weatherford single cold trap pyrolysis chromatograph. Three heating rates, 5 °C/min, 15 °C/min and 25 °C/min were used for the pyrolysis experiments. The pyrolysis products were collected using a liquid N2 trap, followed by chromatographic analysis of the products. The kinetics parameters were calculated using Kinetics 05 software developed by the Lawrence Livermore State Laboratory in the United States.

3.4. Artificial Thermal Maturation Experiment

An artificial thermal maturation experiment was conducted using a purpose-built instrument developed by Sinopec [25]. The sample Aus-2 (500 g) was separated into six parts. Each part sample was put into a stainless-steel vessel, and the overlying lithostatic pressure, liquid pressure, and confined (i.e., water-filled) pore spaces could be adjusted according to the geological conditions. In this experiment, the vessel was first heated to 300 °C directly and then to each final temperature (320 °C, 340 °C, 350 °C, 375 °C, 400 °C) with a 1 °C/min heating rate. The final temperature was held for 72 h, and the reason for setting the final temperature at 400 °C for maturation was for expelled oil and residual oil comparison analysis. When the fluid pressure during the heating process exceeded a set value, the products were collected through a pressure release valve and weighed [25]. The volume and composition of released gases was measured using a Hewlett Packard 6890 gas chromatograph. The oil expelled from the vessel and in the tubes was washed out with chloroform to gravimetrically quantify the expelled oil. Chloroform extraction of the samples remaining after the experiments was used to measure the residual oil. Compound group analyses were conducted using an Iatroscan thin-layer chromatography-flame ionization detector (TLC-FID) system. The separation and identification of saturated and aromatic hydrocarbons, other polar compounds and asphaltenes was based on Mohnhoff et al. [26].

The residual rocks from each thermal maturation experiment were analyzed for TOC content and Rock-Eval analysis. Residual and expelled oil samples were analyzed using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). GC analyses were carried out using an Agilent 7890A gas chromatograph with ultrahigh purity helium as a carrier gas. An Agilent 6890 gas chromatograph with an Agilent 5973 mass spectrometer were used for the GC–MS analyses. All analyses were conducted in simultaneous SIM (selected ion monitoring) and scan modes (m/z 50–800) at 70 eV and the biomarker ratio results were calculated based on the SIM data.
4. Results

4.1. Organic Petrology and Mineral Composition

Inorganic petrographical microscopy analysis using XRD showed that the minerals in the Aus-2 sample were dominated by quartz (53%) and clay (37%), with some carbonate, feldspar and siderite (Figure 2a,b).

Figure 2. Photomicrographs of Tasmanites in oil shale samples (a–c) and mudstone samples (d–f). (a1–f1) were taken under transmitted light, while (a2–f2) were taken under reflected fluorescence light. Panel e1 is the photo under reflected white light. Scale bar = 50 µm.

Tasmanites fossils were abundant in the oil shale and exist as either spherical or flattened disk patterns (Figure 2a–c). The flattened disk pattern was common in both the oil shale and mudstones, while the spherical Tasmanites were mostly restricted to the mudstones, tending to be filled with frambooidal pyrite (Figure 2d–f). The flattened disks probably resulted from the compaction of the spheroidal form [21]. The disk was typically 100–300 µm in length and 10–50 µm in width with compaction of two cell walls. The main difference between the oil shales and surrounding mudstones was the abundance of Tasmanites algae in the oil shales indicative of major fluctuations of microalgal production over time. The algal remains were assigned to the unicellular alga *Tasmanites punctatus* [27], with affinities to the extant green algae *Pachysphaera* [28].

4.2. Rock-Eval Pyrolysis and Carbon Isotope Analysis

The TOC contents of two Tasmanite oil shales were considerably higher (6.6% and 12.0%) than the two mudstone samples (0.71% and 1.04%) (Table 1). The hydrogen index
(HI) reached 909 and 929 mg HC/g TOC (Table 1) in the two oil shale samples with low oxygen index (OI) values, indicating that the kerogen had hydrocarbon-rich Type I organic matter [3], consistent with abundant Tasmanites observed under the microscope (Figure 2a–c). The mudstones had relatively low HI values indicating Type II kerogen with some Tasmanites algae (Figure 2d–f). The bitumen reflectance was 0.52%, indicative of low maturity, consistent with the results of Cook [29] and Reid and Burrett [19].

Table 1. Rock-Eval results of oil shale and mudstone samples from the Early Permian in Tasmania, Australia.

| Sample | Lithology | S1  | S2   | Tmax | TOC  | HI   | OI   | δ13C_kerogen (%) |
|--------|-----------|-----|------|------|------|------|------|-----------------|
| AUS-1  | Oil shale | 2.9 | 112.3| 438  | 12.26| 929  | 10   |                 |
| AUS-2  | Oil shale | 1.03| 60.2 | 440  | 6.58 | 909  | 9    | −14.0           |
| AUS-3  | Mudstone  | 0.02| 1.9  | 437  | 0.71 | 262  | 31   |                 |
| AUS-4  | Mudstone  | 0.02| 1.5  | 429  | 1.04 | 144  | 12   |                 |

TOC = total organic carbon (wt%), S1 = amount of free hydrocarbons volatilized at 300 °C (mg/g rock), S2 = Amount of hydrocarbons produced between 300 °C and 650 °C (mg/g rock), Tmax = temperature of maximum hydrocarbon generation (°C), HI = Hydrogen Index = S2 × 100/TOC, OI = Oxygen Index = S3 × 100/TOC.

The carbon isotope value of the bulk kerogen in the oil shale sample Aus-2 was −14.0‰, which is much less depleted than that of most marine sediments or other lacustrine shales. This is possibly due to the special sedimentary background where the Tasmanite oil shale was deposited. Domack et al. [30] and Revill et al. [21] demonstrated that the Tasmanite oil shale was deposited in a sea-ice environment, as indicated by the occurrence of glacial sediments and dropstones. The carbon cycle in the sea-ice environment was a closed system, isolated from atmospheric CO2, and the fractionation of carbon isotopes was limited, resulting in enriched δ13C values in the kerogen [21].

4.3. Artificial Thermal Simulation Experiments

The amounts of oil generated from the artificial thermal simulation experiments are shown in Table 2 and Figure 3. Hydrocarbon generation showed a narrow peak with increasing temperature with the highest generated hydrocarbon yield of 731 mg HC/g TOC at 340 °C. The sample was over-mature when the pyrolysis temperature was 400 °C, and the HI value was only 56 mg HC/g TOC, suggesting almost all possible hydrocarbons had been generated. For comparison, Xie et al. [14] conducted artificial maturation experiments on two Eocene Huadian oil shale samples from a lacustrine system, and they found the peak oil generation (a yield of 909 mg HC/g TOC) for Botryococcus spp. and diatom-rich sample (HD-21) occurred at 425 °C. In contrast, the oil generation reached a peak (427 mg HC/g TOC) at 400 °C for sample HD-20 (which contained abundant benthic macroalgae). Therefore, Tasmanites might generate hydrocarbons at lower temperatures than other algae.

Table 2. Yields of hydrocarbons of oil shale sample Aus-2 during the artificial thermal simulation experiments over the temperature range from 300 °C to 400 °C.

| Temperature (°C) | EASY%Ro | Hydrocarbon Gases (mg/g TOC) | Residual Oil (mg/g TOC) | Expelled Oil (mg/g TOC) | Total Oil (mg/g TOC) | Total Hydrocarbons (mg/g TOC) |
|------------------|---------|-----------------------------|------------------------|------------------------|---------------------|-----------------------------|
| 300              | 0.62    | 7.05                        | 50.85                  | 10.48                  | 61.33               | 68.39                       |
| 320              | 0.75    | 12.93                       | 132.90                 | 45.56                  | 178.46              | 191.39                      |
| 340              | 1.08    | 28.78                       | 592.15                 | 109.70                 | 701.85              | 730.63                      |
| 350              | 1.13    | 48.85                       | 424.30                 | 113.19                 | 537.49              | 586.34                      |
| 375              | 1.56    | 113.29                      | 188.10                 | 110.98                 | 299.08              | 412.37                      |
| 400              | 1.72    | 234.44                      | 55.10                  | 91.30                  | 146.41              | 380.84                      |

Note: EASY%Ro obtained by using an Arrhenius first-order parallel-reaction approach.
5. Discussion

5.1. Can Additional Sources of OM Be Recognized?

Organic matter in source rocks usually originates from complex mixtures of macerals [31] having multiple origins from phytoplankton, bacteria, archaea, terrigenous and aquatic plants, and secondary producers such as zooplankton and other animals [32]. Therefore, bulk geochemical analyses represent weighted arithmetic averages of the component macerals in the rocks. In such cases it is difficult to infer the yields and characteristics of hydrocarbons generated from a specific type of organic matter.

In order to confirm the maceral composition, two approaches based on maceral separation and kinetic analysis, were conducted on the Tasmanite oil shale Aus-2. The results of sink-float separation of isolated kerogen showed that 93.6 wt% Tasmanites algae were separated with fluid of density 1.06–1.14 g/cm$^3$, and <7.0 wt% of the material in the kerogen is heavier than 1.14 g/cm$^3$, which is mostly pyrite with some amorphous organic matter (Figure 4). Thus, the palynomorphs of the kerogen are dominated by Tasmanites (50–200 µm in diameter) with yellow fluorescence, and the organic matter in this sample is almost entirely composed of Tasmanites.

Hydrocarbon generation is a progressive transformation process as a function of time and temperature, which can be described using chemical kinetics. The kinetic characterization shows that Tasmanite oil shale Aus-2 displays an extremely narrow distribution of activation energies, which is as low as 220 kJ/mol (52 kcal/mol) with a frequency factor (A) of $3.3 \times 10^{13}$ S$^{-1}$ (Figure 5). Revill et al. [21] also showed a narrow activation energy distribution with similar values of approximately 54 kcal/mol at a frequency factor of $8.9 \times 10^{13}$ S$^{-1}$. Such a narrow activation energy distribution suggests that the organic matter belongs to a single type, which is consistent with the results from the sink-float separation experiment.
Figure 4. The sink-float separation results of the kerogen isolated from Tasmanite oil shale Aus-2, showing most of the organic matter (93.6 wt%) is Tasmanites algae. Upper panel shows microphotographs of palynomorphs. Microphotographs of (a–c,e) were taken using transmitted light, while (d,f) are fluorescence light, corresponding to (c,e), respectively. Scale bar = 50 µm.

Figure 5. Activation energy distribution of Tasmanite oil shale Aus-2. (a) Kerogen transformation ratio vs Temperature comparison with different heating rate of 5 °C, 15 °C and 25 °C per minute. (b) The activation energy frequency distribution of oil shale Aus-2.
5.2. Variation in Group Constituents with Different Temperatures

The constituents of the major lipid types in the residual oil, expelled oil and total oil are shown in Figure 6. The content of expelled oil is much lower than that of the residual oil (Table 2, Figure 3), and the group components show different trends in these two fractions of oil (Figure 6). In the residual oil, the observed trend is aromatic hydrocarbons (Aro-HC) >> Resin fraction ≈ asphaltene > saturated hydrocarbons (Sat-HC). However, the trend in expelled oil is Aro-HC > Sat-HC > Resin fraction > asphaltene. These result in the trend for total oil being Aro-HC >> Resin fraction > asphaltene > Sat-HC.

Figure 6. Relative composition of group components (saturated hydrocarbons, aromatic hydrocarbons, non-hydrocarbons, and asphaltenes) in (A) residual oil, (B) expelled oil and (C) total oil from artificial maturation of the Aus-2 sample.
Abundant aromatic compounds have previously been noted in samples of the Tasmanite oil shale [20]. In more mature samples from southern Tasmania, Revill et al. [20] noted a high abundance of 1,2,8-trimethylphenanthrene presumably derived from tricyclic hydrocarbons that are abundant in the oil shale [33]. In less mature samples similar to Aus-2, a range of alkylated naphthalenes and phenanthrenes was detected. Greenwood et al. [34] conducted laser pyrolysis of Tasmanite shales and found an abundant series of parent and alkyl aromatic hydrocarbons. Pyrolysis-induced aromatization is well documented [35], and flash pyrolysis in the presence of minerals can induce secondary processes (e.g., loss of hydrocarbon and cleavage of alkyl moieties), resulting in an enhanced abundance of the parent aromatics, but the artificial maturation experiments described here were run at much lower temperatures and the kerogen was mostly mineral-free.

The saturated hydrocarbons are of low abundance from the artificial maturation experiments from 300 °C to 400 °C. At the lower temperature range (300–340 °C), aromatic hydrocarbons and saturated hydrocarbons decrease, while asphaltene and resin fraction increase in the amounts generated. Interestingly, the contents of aromatic and saturated HC are lowest, and non-hydrocarbon and asphaltene are highest during the oil generation peak temperatures from 340 °C to 350 °C. After 350 °C, resin fraction and asphaltene decrease, saturated hydrocarbons increase and then decrease, while aromatic hydrocarbons increase substantially.

Piedad-Sánchez et al. [18] carried out artificial maturation of a bituminous coal from Asturias (NW Spain) in a confined pyrolysis system and found the aliphatic and polar content decreased, and aromatics of residual oil increased with increasing maturation. In contrast to previous experiments with other types of organic input, the aromatics were very abundant at the beginning of the current study, suggesting that the organic matter tends to generate aromatic hydrocarbons readily.

GC–MS analysis of extracts (residual oil) from the artificial maturation temperature at 300 °C illustrates a high abundance of tricyclic terpenoids (cheilanthanes: T19 to T24, Figure 7). A correlation between Tasmanites algae and tricyclic compounds has been widely identified [21,36]. Greenwood et al. [34] analyzed the tricyclic terpenoid composition of Tasmanites protokerogen by pyrolysis GC–MS, leading them to suggest that there is an inherent relationship between Tasmanites and tricyclic terpenoid production.

5.3. Characterization of Saturated Compounds and Its Significance for Interpretation of Geochemical Parameters

5.3.1. Characterization of Saturated N-Alkanes and Their Environmental Significance

The relative compositions of the expelled oil and residual oil are shown in Figure 8; derived parameters are shown in Table 3 and Figure 9. The compositions show systematic variations with increasing temperature from 300 °C to 375 °C (especially for the expelled oil), and long chain n-alkanes are abundant in the low temperature products. At 400 °C, the long chain n-alkanes became more abundant. The Pr/n-C17 and Ph/n-C18 ratios decreased with increasing maturation temperature: these parameters were much higher in the residual oil than in the expelled oil (Table 3, Figure 9). In contrast, Pr/Ph ratios in the expelled oil were higher than those in the residual oil. The Pr/Ph in the residual oil ranged from 0.57 to 1.44, but the ratio in the expelled oil ranged from 1.4 to 2.1 at 300 °C to 375 °C, with a big jump to 4.01 at 400 °C. The chemical structures of Pr and Ph were nearly identical suggesting that the thermal stability of these compounds should be similar. Additionally, the small difference in boiling points of these compounds (296 °C and 322 °C) is not likely to result in a large change in the compound ratios between pyrolysis at 375 °C and 400 °C. However, while it is possible to derive Pr from Ph through the removal of a methyl group, methylation of Pr to generate Ph from Pr is not very likely. The increase in Pr/Ph ratio with increasing thermal stress thus suggests that residual precursor moieties are available for Pr but not for Ph.
Figure 7. GC–MS analysis of extract (residual oil) with artificial maturation temperature at 300 °C, showing (a) TIC, (b) m/z 123 (tricyclic alkanes), (c) m/z 191 (mainly tricyclic alkanes labelled T).

Table 3. Isoprenoid and n-alkane ratios from GC in expelled oil and residual oil of different temperature pyrolysis products from Aus-2.

| HC        | T(°C) | Major Carbon | OEP | Pr/n-C17 | Ph/n-C18 | Pr/Ph | $\frac{\Sigma n-C21}{\Sigma n-C22}$* | Sat-HC/Aro-HC | (Sat-HC + Aro-HC)/(Non-HC + Asphaltene) |
|-----------|-------|--------------|-----|----------|----------|-------|-----------------------------------|----------------|----------------------------------------|
| Aus-2     | -     | 16           | 0.88| 0.89     | 1.05     | 0.99  | 1.36                              | 1.15           | 0.89                                    |
| Residual oil | 300  | 24           | 0.68| 0.50     | 0.71     | 0.70  | 1.03                              | 0.25           | 2.63                                    |
|           | 320  | 24           | 0.75| 0.39     | 0.59     | 0.57  | 0.95                              | 0.19           | 1.51                                    |
|           | 340  | 19           | 1.01| 0.12     | 0.09     | 1.01  | 1.13                              | 0.21           | 0.82                                    |
|           | 350  | 19           | 1.04| 0.17     | 0.17     | 0.88  | 1.40                              | 0.17           | 0.71                                    |
|           | 375  | 18           | 1.05| 0.10     | 0.06     | 1.44  | 2.14                              | 0.22           | 1.97                                    |
|           | 400  | 14           | 1.05| 0.12     | 0.15     | 0.59  | 1.38                              | 0.25           | 2.62                                    |
| Expelled oil | 340  | 14           | 1.96| 0.82     | 0.46     | 1.87  | 2.83                              | 0.97           | 1.79                                    |
|           | 350  | 14           | 2.11| 0.71     | 0.45     | 2.14  | 4.98                              | 0.71           | 1.65                                    |
|           | 375  | 13           | 1.04| 0.27     | 0.23     | 1.43  | 4.80                              | 0.72           | 0.79                                    |
|           | 400  | 15           | 0.85| 0.22     | 0.13     | 1.87  | 5.21                              | 0.77           | 1.14                                    |

OEP: odd/even predominance of n-alkanes.
Figure 8. The relative composition of n-alkanes and isoprenoids from gas chromatography of expelled oil (a) and residual oil (b) of Aus-2 as a function of temperature in the artificial maturation experiments. Pr: pristane; Ph: phytane.

Figure 9. Plots of Ph/n-C17 vs Pr/n-C17 (a) and Pr/Ph vs Pr/n-C17 (b) in expelled oil and residual oil in the different temperature pyrolysis products from Aus-2.

Paleoenvironment analysis using geochemical indicators (e.g., Pr/n-C17 and Ph/n-C18, Pr and Ph) is important for source rock evaluation. Revill et al. [21] suggested different chemical environments prior to, during and after Tasmanite shale deposition since the Pr/Ph ratio was quite high (3.1) in the siltstone above and below the Tasmanite shales, while the ratio was around 1 or less for the shales. In fact, the extract from the siltstone may contain hydrocarbons expelled from the Tasmanite shales. In the current study, the expelled hydrocarbon had a much higher Pr/Ph ratio (up to 4.01). Commonly, the Pr/Ph ratio is used for oil-source correlations: for example, marine low-wax oils have relatively lower Pr/Ph values (1–3), while the ratio in non-marine high-wax oils are higher (typically 5–11; [37]). The ratio should be used with caution because it can increase with increasing
However, in our maturation experiments, Pr/Ph in the expelled oil has no relationship with temperature, but the ratio in the residual oil has a positive relationship ($R^2 = 0.82$), at least when the temperature is lower than 400 °C (Figure 10a). At a maturation temperature of 400 °C, the ratio in the expelled oil increased dramatically, while the opposite was true for the residual oil, which could be due to the large amount of hydrocarbon gas generation. There is an obvious effect of maturation temperature, with the Ph/$n$-C18 and Pr/$n$-C17 ratios showing negative relationships with artificial maturation temperature at low temperature (<400 °C) (Figure 10b,c), so that the original ratios could be calculated using these relationships. These results indicate substantial changes in the Pr/Ph ratio with maturity with obvious implications for its use as a geochemical proxy.

Figure 10. Pr/Ph (a), Pr/$n$-C17(b), and Ph/$n$-C18 (c) vs artificial maturation temperature for residual oil and expelled oil from the Tasmanite oil shale.
A diagram showing the relationship between Pr/\(n\)-C17 and Ph/\(n\)-C18 is widely used for evaluation of the paleoenvironment of the source rock [10]. The ratios of residual oil and expelled oil are plotted in Figure 11. For the residual oil, the interpretations from the diagram are transitional to close to algal marine, and the data plot in the transitional area for higher artificial maturation temperatures (higher maturities). For the expelled oil, the paleo-environment could be transitional to close to terrestrial, and the data plot as more terrestrial at high maturities. In this case, it is clear that these biomarker ratios are not well suited to evaluate the paleo-environment using either extracts or oils in the reservoir rocks, and the compounds with high maturities are even less appropriate for this purpose.

![Figure 11](image-url)

**Figure 11.** Pristane/\(n\)-C17 vs phytane/\(n\)-C18 plot of residual oil and expelled oil of Tasmanite oil shales during artificial maturation experiments (diagram cf. Peters et al. [10]).

5.3.2. Biomarker Evaluation of Thermal Maturity and Variation with Temperature

Maturity related biomarkers (e.g., Ts/(Ts + Tm), C29- sterane \(\alpha\beta\alpha\beta/(\alpha\alpha\alpha + \alpha\beta\beta)\) ratio and \(\alpha\alpha\alpha C29-20S/(20S + 20R)\)), have been widely applied in petroleum geology [35,39–41]. 18 (H)-22,29,30-trisnorneohopane (Ts) is more stable than 17 (H)-22,29,30-trisnorhopane (Tm) with increasing maturity [42]; thus, the Ts/(Ts + Tm) ratio is normally regarded as a maturity parameter [1,10]. Through comparison with vitrinite reflectance and HI vs. Tmax values, some studies have shown that Ts/(Ts + Tm) is likely to be the best maturity-related biomarker parameter for the maturity range from 0.5 %Ro to 0.9 %Ro (vitrinite reflectance) [42–44].

In this study, the Ts/(Ts + Tm) ratios of residual oil ranged from 0.24 (at 350 °C) to 0.73 (at 300 °C), and the lowest ratio occurred at 350 °C with the highest expelled oil yield (Figure 12a). The ratios of expelled oil were from 0.18 to 0.40, showing a positive relationship (R² = 0.79) with the artificial maturation temperatures (Figure 12a). To this extent, the Ts/(Ts + Tm) ratio was still related to the hydrocarbon occurrence in rocks, but additional work is necessary to confirm that the ratio is an effective maturity indicator in different kind of rocks.
Figure 12. (a) Plots of $T_s/(T_s + T_m)$ vs. pyrolysis temperatures; (b) Variations in $\alpha\alpha\alpha C_{29\text{-}20S}/(20S + 20R)$ vs. $C_{29\text{-}20R}/(\alpha\alpha\alpha + \alpha\beta\beta)$ for both residual oil and expelled oil.

The $C_{29\text{-}20R}/(\alpha\alpha\alpha + \alpha\beta\beta)$ ratio is an important parameter for maturity evaluation: Peters et al. (2005) found that this ratio increased up to 0.71 as maturity increased. The ratios for the residual oil ranged from 0.26 (at 300 °C) to 0.46 (at 350 °C), while the ratios (0.24–0.39) were more stable in the expelled oil. The highest ratios both in residual oil and expelled oil occurred at 350 °C, coincident with the highest expelled oil yield. The $\alpha\alpha\alpha C_{29\text{-}20S}/(20S + 20R)$ ratio can be used for maturity evaluation because the 20R epimer is less stable than the 20S epimer [10], and a ratio value of 0.4 is equivalent to ca. 0.8 %Ro (vitrinite reflectance) [45].

The $\alpha\alpha\alpha C_{29\text{-}20S}/(20S + 20R)$ vs. $C_{29\text{-}20R}/(\alpha\alpha\alpha + \alpha\beta\beta)$ ratios are shown in Figure 12b, and most points are in the area corresponding to low maturity. There was a positive relationship between $\alpha\alpha\alpha C_{29\text{-}20S}/(20S + 20R)$ and $C_{29\text{-}20R}/(\alpha\alpha\alpha + \alpha\beta\beta)$ in the residual oil. However, the relationship was not correlated with pyrolysis temperature. The ratios of $\alpha\alpha\alpha C_{29\text{-}20S}/(20S + 20R)$ and $C_{29\text{-}20R}/(\alpha\alpha\alpha + \alpha\beta\beta)$ have an irregular variation after the highest hydrocarbon generation yield at 340 °C, both in residual oil and expelled oils. Therefore, this maturity-related biomarker parameter needs to be carefully applied in petroleum geology, especially when the maturity is post peak oil generation.

5.3.3. Characterization of $C_{27\text{-}}, C_{28\text{-}}, C_{29\text{-}}$ Steranes and Variation with the Pyrolysis Temperature

Biomarkers have been used to determine organic input characteristics in source rocks and oils and for oil-source correlations. $C_{27\text{-}}, C_{28\text{-}}$ and $C_{29\text{-}}$ steranes have been widely detected in source rocks and crude oils, and the steranes derived from sterols and can provide information of sedimentary environment in source rocks [46,47]. Generally, the $C_{27\text{-}}$ sterols are predominant in zooplankton and some algae in marine systems, the $C_{28\text{-}}$ sterols mainly exist in chlorophyll c-containing marine phytoplankton and lake algae (e.g., diatoms, coccolithophorids and dinoflagellates), and $C_{29\text{-}}$ sterols have been found mainly in terrigenous organic matter, some freshwater microalgae and marine green algae [10,48–50]. A ternary diagram of $C_{27\text{-}}, C_{28\text{-}}$ and $C_{29\text{-}}$ steranes was proposed by Huang and Meinschein [48] for differentiating depositional environments. While an oversimplification [51], such diagrams have been widely used since then [47,52,53].

Biomarker parameters from GC–MS analysis of expelled oil and residual oil at different Ratios were calculated from peak areas in $m/z$ 217 and $m/z$ 191 mass fragmentograms. The peak widths for the $C_{29\text{-}}\alpha\alpha\alpha$-sterane was considerably wider than that for the $C_{27\text{-}}$ sterane and so peak heights can give a false impression of abundances (e.g., as in Figure 13).
Figure 13. The C27-, C28-, C29- sterane distributions in residual oil and expelled oil, showing variations with pyrolysis temperatures. Note that the peak width of C29- steranes was greater than that of C27- steranes and so C27- steranes seem more abundant than is actually the case.

In this study, we have shown that the organic matter is mainly derived from Tasmanites. Compared to C27- and C28- steranes, the C29- steranes both in residual oil and expelled oil show the highest relative abundances, suggesting that Tasmanites contained mainly C29- sterols. The distributions of C27-, C28-, and C29- steranes varied with temperature (Figure 13). The distribution of C27-, C28-, and C29- steranes at pyrolysis temperatures at 340 °C or lower showed similar distributions typical of immature organic
matter with C29- > C27- > C28- steranes with the 20R isomers dominant (Figure 13). Similar distributions of steranes have been reported from the immature Tasmanite shale at Oonah in northern Tasmania [21]. The 340 °C to 375 °C pyrolysis samples correspond with the highest overall hydrocarbon yields but very low abundances of the regular steranes. The distributions in these samples are not well defined and almost certainly compromised by other, coeluting pyrolysis products (Figure 13). At 400 °C, the yields of residual and expelled oils are low (Table 2), but the sterane distribution is clear (Figure 13) and shows a higher proportion of C27- steranes (Table 4). The C27-/C29- sterane ratio is similar in the residual oil at all temperatures apart from 400 °C (Table 4). However, in the expelled oil ratio is distinctly higher at the three higher temperatures (Table 4). In addition, the abundances of the geological isomers 20S and ββ20S + R had increased relative to those steranes with the original biological 20R stereochemistry. The m/z 217 mass chromatograms were also much cleaner with sterane distributions easily seen suggesting that at these pyrolysis temperatures additional steranes have been produced by the cleavage of covalently bound steroidal molecules or released as occluded compounds by the rupture of surrounding cages [54]. A shift in the predominance from C29 to C27 would be consistent with these steranes having a contribution from different precursor moieties.

Table 4. Temperature pyrolysis temperatures from sediment sample Aus-2.

| Oil       | T (°C) | αααC2920S /(20S + 20R) Steranes | C29ββ/(αα + ββ) Steranes | C27/C29 Steranes | Ts/(Ts + Tm) |
|-----------|--------|--------------------------------|--------------------------|-----------------|--------------|
| Residual oil | 300    | 0.18                           | 0.26                     | 0.47            | 0.73         |
|           | 320    | 0.22                           | 0.29                     | 0.53            | 0.56         |
|           | 340    | 0.28                           | 0.39                     | 0.61            | 0.53         |
|           | 350    | 0.37                           | 0.46                     | 0.43            | 0.24         |
|           | 375    | 0.29                           | 0.34                     | 0.66            | 0.44         |
|           | 400    | 0.40                           | 0.40                     | 0.82            | 0.51         |
| Expelled oil     | 300    | 0.15                           | 0.30                     | 0.52            | 0.18         |
|               | 320    | 0.26                           | 0.24                     | 0.57            | 0.28         |
|               | 340    | 0.36                           | 0.30                     | 0.54            | 0.23         |
|               | 350    | 0.43                           | 0.39                     | 0.73            | 0.32         |
|               | 375    | 0.34                           | 0.25                     | 0.92            | 0.40         |
|               | 400    | 0.37                           | 0.30                     | 0.73            | 0.38         |

For each pyrolysis temperature, the distribution of C27-, C28- and C29- steranes in the residual oil was essentially similar to that in the expelled oil indicating that oil-source rock correlations can be effective. However, given the change in sterane distribution as a function of pyrolysis temperature (and presumably geological thermal maturity), credible interpretation of this distribution with respect to similarity or differences among oils and with various source rocks is reliant on a constrained level of thermal maturity.

6. Conclusions

Tasmanite shale, composed of one main type of algal organic matter (Tasmanites punctatus), has been analyzed using artificial maturation experiments. The generated hydrocarbons from the Tasmanite shale contained surprisingly high amounts of aromatic hydrocarbons. The geochemical comparison of expelled oil and residue oil show that:

- Saturated hydrocarbons are likely to be expelled from the shale, and aromatic hydrocarbons, resin fraction and asphaltene are more likely to be retained in the shale.
- The geochemical compositions of Pr/Ph and Pr/n-C17 ratios in expelled oil are much higher than those in the corresponding residual oil, while the Ph/n-C18 ratio shows the opposite trend.

Maturity related biomarkers and sterane (C27, C28 and C29) distributions have been compared both in residual oil and expelled oil along with pyrolysis temperature. The results show that the maturity related biomarkers such as Ts/(Ts + Tm), αααC29-20S
/(20S + 20R) and C29-αββ/(ααα + αββ), showed irregular variations with the pyrolysis temperature, especially for post peak oil generation. C27-, C28- and C29- sterane distributions showed variations with maturity with a switch from C29 dominance to C27 sterane dominance at 400 °C thus complicating any interpretation of sterane sources based on sterane distributions alone.

All hydrocarbon ratios varied with maturation temperature, but the variations are complex. More experiments are needed to establish the mechanisms and systematic evolution of these geochemical compounds with maturity. We hope that these data will be useful for re-evaluating these compounds as biomarkers for assessment of depositional environment and maturity-related biomarker assessments and for oil-source correlation investigations.

Author Contributions: Conceptualization, X.X. and Y.W.; methodology, J.L.; software, F.W.; validation, L.Z., Y.L. and X.H.; formal analysis, X.X.; investigation, Y.W.; resources, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, J.L. and F.W.; supervision, X.X.; funding acquisition, X.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Natural Science Foundations of China, grant number: 41972163, 42173055 and 41603047.

Institutional Review Board Statement: The study did not involve humans or animals.

Informed Consent Statement: Not applicable.

Acknowledgments: This research was supported by the National Natural Science Foundation of China. Lloyd Snowdon, John Volkman, Maowen Li are greatly thanked for their sampling assistance and constructive suggestions during conceptualization and editing. Jin Xu and Zhongliang Ma are thanked for their experimental assistance. Three anonymous reviewers are thanked for constructive comments which helped us to improve the manuscript largely.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Peters, K.E.; Moldowan, J.M. The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments; Prentice Hall: Hoboken, NJ, USA, 1993.
2. Balbinot, M.; Kalkreuth, W. Organic geochemistry and petrology of the Gomo Member, Recôncavo Basin, Brazil. Int. J. Coal Geol. 2010, 84, 286–292. [CrossRef]
3. Tissot, B.P.; Welte, D.H. Petroleum Formation and Occurrence; Springer: Berlin, Germany, 1984.
4. Hutton, A.C. Petrographic classification of oil shales. Int. J. Coal Geol. 1987, 8, 203–231. [CrossRef]
5. Hackley, P.C.; Walters, C.C.; Kelemen, S.R.; Mastalerz, M.; Lowers, H.A. Organic petrology and micro-spectroscopy of Tasmanites microfossils: Applications to kerogen transformations in the early oil window. Org. Geochem. 2017, 114, 23–44. [CrossRef]
6. Volkman, J.K.; Alexander, R.; Kagi, R.I.; Noble, R.A.; Woodhouse, G.W. A geochemical reconstruction of oil generation in the Barrow Sub-basin of Western Australia. Geochim. Cosmochim. Acta 1983, 47, 2091–2105. [CrossRef]
7. Gelin, F.; Boogers, I.; Noordeloos, A.A.M.; Sinninghe Damsté, J.S.; Hatcher, P.G.; de Leeuw, J.W. Novel, resistant microalgal polyethers: An important sink of organic carbon in the marine environment? Geochim. Cosmochim. Acta 1996, 60, 1275–1280. [CrossRef]
8. Brock, J.J.; Buick, R.; Summons, R.E.; Logan, G.A. A reconstruction of Archean biological diversity based on molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup, Hamersley Basin, Western Australia. Geochim. Cosmochim. Acta 2003, 67, 4321–4335. [CrossRef]
9. Zhang, M.; Liu, C.L.; Tian, J.X.; Lu, Z.D.; Pang, H.; Zeng, X.; Kong, H.; Yang, S. Geochemical characteristics of crude oil and oil-source correlation in the western Qaidam Basin, China. J. Nat. Gas Geoscienc. 2020, 5, 227–238. [CrossRef]
10. Peters, K.E.; Walters, C.C.; Moldowan, J.M. The Biomarker Guide: Biomarkers and Isotopes in Petroleum Systems and Earth History, 2nd ed.; Cambridge University Press: Cambridge, UK, 2005; Volume 2.
11. Peters, K.E.; Hostettler, F.D.; Lorenson, T.D.; Rosenbauer, R.J. Families of Miocene Monterey crude oil, seep, and tarball samples, coastal California. Am. Assoc. Pet. Geol. Bull. 2008, 92, 1131–1152. [CrossRef]
12. Boreham, C.J.; Hope, J.M.; Jackson, P.; Davenport, R.; Earl, K.L.; Edwards, D.S.; Logan, G.A.; Krassay, A.A. Gas-oil-source correlations in the Otway Basin southern Australia. In Proceedings of the PESA Eastern Australasian Basins Symposium II, Adelaide, Australia, 19–22 September 2004; pp. 603–627.
13. Greic, K.; Schouten, S.; Blokker, P.; Derenne, S.; Largeau, C.; Nissenbaum, A.; Sinninghe Damsté, J. Structural and isotopic analysis of kerogens in sediments rich in free sulphurised Botryococcus braunii biomarkers. Org. Geochem. 2003, 34, 471–482. [CrossRef]
38. Ten Haven, H.L.; de Leeuw, J.W.; Rullkötter, J. Restricted utility of the pristane/phytane ratio as a palaeoenvironmental indicator. Nature 1987, 330, 641–643. [CrossRef]
39. Requejo, A.G. Maturation of petroleum source rocks—II. Quantitative changes in extractable hydrocarbon content and composition associated with hydrocarbon generation. Org. Geochem. 1994, 21, 91–105.
40. Farrimond, P.; Bevan, J.C.; Bishop, A.N. Tricyclic terpane maturity parameters: Response to heating by an igneous intrusion. Org. Geochem. 1999, 30, 1011–1019. [CrossRef]
41. Böcker, J.; Littke, R.; Hartkopf-Fröder, C.; Jasper, K.; Schwarzbaumer, J. Organic geochemistry of Duckmantian (Pennsylvanian) coals from the Ruhr Basin, western Germany. Int. J. Coal Geol. 2013, 107, 112–126. [CrossRef]
42. Fang, R.H.; Littke, R.; Zieger, L.; Baniasad, A.; Li, M.J.; Schwarzbaumer, J. Changes of composition and content of tricyclic terpane, hopane, sterane, and aromatic biomarkers throughout the oil window: A detailed study on maturity parameters of Lower Toarcian Posidonia Shale of Hils Syncline, NW Germany. Org. Geochem. 2019, 138, 1–19. [CrossRef]
43. Farrimond, P.; Taylor, A.; Telnæs, N. Biomarker maturity parameters: The role of generation and thermal degradation. Org. Geochem. 1998, 29, 1181–1197. [CrossRef]
44. Mißbach, H.; Duda, J.P.; Lünsdorf, N.K.; Schmidt, B.C.; Thiel, V. Testing the preservation of biomarkers during experimental maturation of an immature kerogen. Int. J. Astrobiol. 2016, 15, 165–175. [CrossRef]
45. Huang, D.F.; Li, J.C.; Zhang, D.J.; Huang, X.M.; Zhou, Z.H. Maturation sequence of Tertiary crude oils in the Qaidam Basin and its significance in petroleum resource assessment. J. Southeast Asian Earth Sci. 1991, 5, 359–366.
46. Schwark, L.; Empt, P. Sterane biomarkers as indicators of Palaeozoic algal evolution and extinction events. Palaeogeogr. Palaeoclimatol. Palaeoecol. 2006, 240, 225–236. [CrossRef]
47. Asemani, M.; Rabbani, A.R.; Sarafdokht, H. Origin, geochemical characteristics and filling pathways in the Shadegan oil field, Dezful Embayment, SW Iran. J. Afr. Earth Sci. 2021, 174, 104047. [CrossRef]
48. Huang, W.Y.; Meinschein, W.G. Sterols as ecological indicators. Geochim. Et Cosmochim. Acta 1979, 43, 739–745. [CrossRef]
49. Gibb, S.W.; Cummings, D.G.; Irigoien, X.; Barlow, R.G.; Mantoura, C. Phytoplankton pigment chemotaxonomy of the northeastern Atlantic. Deep Sea Res. Part II 2001, 48, 795–823. [CrossRef]
50. Zhang, Y.; Jiang, A.; Sun, Y.; Xie, L.; Chai, P. Stable carbon isotope compositions of isoprenoid chromans in Cenozoic saline lacustrine source rocks from the Western Qaidam Basin, NW China: Source implications. Chin. Sci. Bull. 2012, 57, 1013–1102. [CrossRef]
51. Volkman, J.K. A review of sterol markers for marine and terrigenous organic matter. Org. Geochem. 1986, 9, 83–99. [CrossRef]
52. Moldowan, J.M.; Seifert, W.K.; Gallegos, E.J. Relationship between petroleum composition and depositional environment of petroleum source rocks. Am. Assoc. Pet. Geol. Bull. 1985, 69, 1255–1268.
53. Hatem, B.A.; Abdullah, W.H.; Hakimi, M.H.; Mustapha, K.A. Origin of organic matter and palaeoenvironment conditions of the Late Jurassic organic-rich shales from Shabwah sub-basin (western Yemen): Constraints from petrology and biological markers. Mar. Pet. Geol. 2016, 72, 83–97. [CrossRef]
54. Snowdon, L.R.; Volkman, J.K.; Zhang, Z.; Tao, G.; Liu, P. The organic geochemistry of asphaltenes and occluded biomarkers. Org. Geochem. 2016, 91, 3–15. [CrossRef]