Aliphatic hydrocarbon biomarkers of Sekar Kurung Gresik crude oil

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INTRODUCTION

Petroleum is a complex compound of hydrocarbons that are naturally occurring in the gas phase (natural gas), liquid (crude oil) and solid (asphalt). Petroleum was derived from a variety of organic materials that were chemically converted over a long period (hundreds of millions of years) under various heat and geological conditions. Crude oil is a mixture of solid and liquid that is made of carbon and hydrogen, but also a small amount of sulfur, oxygen, nitrogen, and small amount of metal (such as nickel and vanadium) [1].

Petroleum geochemistry is an essential science in understanding a petroleum system. Source rocks may be directly sampled and analyzed to reveal their source potential and thermal maturity. Extracted bitumens from naturally and artificially matured source rocks are then compared with oils, providing a direct correlation [2].

Assessment of geochemical organic was conducted based on the behavior of the fossils compound (biomarker). Biomarker is a complex biological molecular compounds derived from living organisms that have died. These compounds retain the basic framework of the source of biosynthesis origin in living organisms, but has undergone a change of its functional groups, break of the bond and changes of stereochimistry that occur as a result of conditions in the bowels of the earth, such as the presence of thermal and pressure changes [3]. Many compounds in petroleum and other fossil fuels are unambiguously biomarkers. The most common and widely studied are acyclic isoprenoids, selected branched alkanes, cyclic terpenoids and steroids.

Immature bitumens consist of unsaturated and functionalized compound that frequently present under thermal conditions. Biomarker may also contain heteroatoms such as sulfur which may become incorporated into the above mentioned biomarker during early diagenesis under anoxic, marine conditions. Acid by-products from microbial degradation, and porphyrins containing nitrogen and metals are common in low maturity oils [2].

The existence of this biomarker deliver information about the complex molecules that occurs in source rocks, which are obtained from organic compounds precursor biomarker contained in various living organisms. The compound also provides information on the maturity, their biodegradation, migration in sediments and petroleum [4], the origin of organic material, depositional environment and quality of oil [5]. Biological precursor compounds, may also provide the information about the picture of the geochemical processes that take place in the sediment at different stages of maturation [6].

In general, the presence of biomarkers in an oil can be distinguished over aliphatic and aromatic hydrocarbons groups. Biomarkers on aliphatic hydrocarbons can be found in the form of n-alkanes, acyclic isoprenoids, bicyclic sesquiterpene and polycyclic terpenoids. The distribution of n-alkanes biomarker compounds in petroleum sample can deliver information about the types of sources and the maturity level of petroleum [7]. Pristane and phytane ratio which is specifically isoprenoid compounds can potentially be used to determine the environmental conditions i.e. oxidative or reductive deposition during the deposition process of organic material [8]. The existence of polycyclic terpenoids compounds indicate the origin of the organic matter and the degree of biodegradation of petroleum marker [9].

Aliphatic hydrocarbons are the most important group of biomarker compounds. This group of compounds can provides information regarding the characteristics of a crude sample, for example an oil-forming precursor formed from naftagelates has high abundance of the alkane short chain [7]. The type of oil that originated from the marine environment was indicated by the unimodal distribution of n-alkane short chain ([n-C19 - n-C25]). Deposition environment of oil derived from reductive environments was by the present of pristane and phytane compounds [10].

The purpose of this study was to identify and characterize aliphatic hydrocarbon fraction biomarkers Sekar Kurung crude oil Gresik. Sekar Kurung Gresik is situated in the East Java Basin, north region of...
Rembang which spread from Tuban eastward through Lamongan, Gresik, and almost the entire island of Madura. This basin formed in the Late Oligocene trending east - west parallel to Java. The hallmark of this Rembang zone is the deposition from shelf to the rich in carbonates and material mainland (terrisgenteous), as well as the deposition of sediments from the shallow sea not far from shore at a depth of the seabed is not uniform [11].

EXPERIMENTAL

Materials
Samples of crude oil from Sekar Kurung Gresik, aquabidest, acetone p.a., dichloromethane p.a., n-hexane p.a., cotton, silica gel, sand, cellite, chloroform p.a., isopropanol, potassium hydroxide, diethyl ether p.a., formic acid p.a. chloroform, methanol p.a, dibenzoanthracene, nitrogen gas, copper powder, paraffin and aluminum foil.

Instrumentation
A set of equipment i.e. soxhlet apparatus, analytical balance, tulips funnel, flask, rotary evaporator, heating mantle, TLC plate, beaker glass, separating funnel, TLC chamber, vial bottle, spray bottle, spatula, pipette, pasteur pipette and a set of distillation equipment, column chromatography, gas-chromatography mass spectrometry (GC-MS).

Extraction and fractionation
Crude oil (2 g) was separated from asphaltum by adding excess hexane (at ratio of 1:40), resulting in malten and asphaltum fractions. Malten was fractionated by column chromatography method to separate neutral fraction, acid fraction and polar fraction. The first elution was done using diethyl ether to obtain neutral fractions. Elution was continued using 2% formic acid in diethyl ether to obtain acid fraction. The last elution was done using a mixture of methanol: chloroform: water (65: 25: 4) to obtain the polar fraction.

The neutral fraction was refractored by Thin Layer Chromatography (TLC) to separate hydrocarbon, alcohol and ketone fraction using dichloromethane eluent. The hydrocarbon fraction obtained was refractored by TLC method using n-hexane to separate the aromatic and aliphatic hydrocarbon fraction. The biomarkers in aliphatic hydrocarbon fraction were analyzed and identified using gas chromatography-mass spectrometry (GC-MS) Shimadzu QP2010S conducted at 50 °C (5 min), 50 °C – 300 °C (10 °C / minute) and isothermal temperature at 290 °C for 25 minutes [6].

RESULTS AND DISCUSSION

Biomarkers of aliphatic hydrocarbon
Analysis and identification of aliphatic hydrocarbon fraction of Sekar Kurung Gresik crude oil sample indicate the presence of n-alkane compounds, isoprenoid alkanes, bicyclic sesquiterpene, eudesmane, cadinane, hopane, bicanadane, gammacarane and diasterane. Identified biomarkers are used to determine the origin of organic matter, the deposition environment and the crude oil maturation.

The result of the GC-MS analysis is shown as total chromatograms in Figure 1.

Biomarkers of n-alkane
n-alkane hydrocarbons are the most abundant biomarker found in all types of oil. n-alkane hydrocarbons are not biodegradable. One of n-alkanes commonly found is n-hexadecane (nC16) [12].

The distribution of n-alkanes in crude oil can be used to determine the source of organic compounds. Mass spectra of the n-alkane hydrocarbon compound was identified had a base peak of m/z 57 and a pattern of decreasing intensity linearly at m/z 71, 85, 99 and so on with addition of 14 (as the release of methane) (-CH2) (Herod et al., 1995). The presence of n-alkanes identified by mass fragmentogram at m/z 57, which is the base peak of alkanes compound. The base peak at m/z 57 is identified as a result of the release of the butyl group (-C4H9).

General information that can be obtained from the distribution of n-alkane compounds is the origin of geological samples that are often found as geological guidance [13]. The presence of short chain n-alkanes (C<20) compounds (Figure 1 at peak 1.A) provide information regarding the contribution of organic matter in the sediments that are derived from algae and bacteria [14], while the long-chain n-alkane (C>20) (Figure 1 at peak 1.B) indicates that the sediment is derived from the wax cuticle on terrestrial higher plants (terrestrial) [15]. The existence of long-chain n-alkanes biomarkers compounds were from the mainland of higher plants, wax and woody deposited into the sea was generally carried by the flow of water (river) or through windborne dust flying [16]. It is also stated in the research of Bakar et al (2011), who reported the distribution of long-chain n-alkanes in the range of C21-C27 indicates contribution of organic compound land plants.

The homologous hydrocarbons of n-C14-n-C28 (Figure 1 at peak 1.A and 1.B) indicates that the organic compounds derived from the terrestrial environment and the contribution of microorganisms to the formation process of petroleum organic compounds.

n-alkanes in the ranges of C14 – C20 is an indicator of terrestrial (land) plant derived from higher plant. However, the compound with C<20 are less dominating. Sediment that contain homologous n-alkanes with carbon range between C14 to C20 and C17 at the maximum peak indicate that the source of the sediment organic matter formed from marine phytoplankton and algae [17]. The contribution of marine phytoplankton and algae as a source of organic matter on Sekar Kurung crude oil is possible because of the geological of Sekar Kurung region that is near the sea.

Distribution of long-chain n-alkanes are dominated by C27-C28, show that the source of sediment’s organic matter derived from vascular plant, microalgae, cyanobacteria and aquatic microorganism, especially macrophytes, Tissot and Walte (1984) also stated that the homologous compound of n-alkanes C25-C35 is a marker compound for sedimentary organic matter source derived from the wax coat of higher plants.

Aliphatic hydrocarbon fraction analysis for the distribution of homologous n-alkanes and isoprenoid alkane in fragmentogram m/z 57 is shown in Figure 2.

Biomarkers of isoprenoid alkanes
The presence of branched alkane compounds in the aliphatic hydrocarbon fraction is indicated by the increased intensity of M + -15 and M + -29 fragments (Figure 3), indicating the presence of a methyl branch in the C2 (isoo-alkane) or C3 (anteiso-alkane) alkane chain. There is an increase in abundance in certain fragments (especially at branch termination) resulting in no longer forming a linear pattern, indicating the isoprenoid pattern [18]. The biomarkers of isoprenoid compounds in the presence of geological samples were used as an indicator to determine the origin of organic materials, characterization and correlation studies to obtain information about the deposition environment. The structure of isoprenoid compounds found in variety
of old sediments and petroleum with a structure that is still in good condition [12].

![Image](https://example.com/image1)

**Fig. 2** Fragmentograms of Sekar Kurung Gresik crude oil aliphatic hydrocarbon fraction with m/z 57.

The existence of isoprenoid biomarkers is identified by fragmentogram at m/z 57. The analysis result of isoprenoid biomarkers indicate that distribution of alkane isoprenoid compounds are in C15-C30 carbon range. The existence of isoprenoid biomarkers confirmed that the organic matter was derived from phototrophic organisms [19]. The results of Soudan, Minnesota and Antrim sediment as well as Nonesuch oil also found the presence of isoprenoid alkane compounds in the range of C16 and C18-C20. The existence of this compound was originate from the phytol compound which is a side chain of chlorophyll in phototrophic organisms.

Pristane and phytane compounds existance can provide the information regarding the deposition environment. If the ratio of Pr/Ph > 1, the deposition environment is oxic, whereas if Pr/Ph < 1, then the depositional environment is anoxic [8]. The formation of compounds derived from the phytane saturation and dehydration reaction are due to diagenetic process. While the oxidation reaction, decarboxylation and saturation of the compounds could produce pristane compound [20]. The ratio of Pr/Ph 5.3 (> 1) indicates that oil samples were formed under oxic environment conditions. The mass spectra of pristane shown in Figure 3 below:

![Image](https://example.com/image2)

**Fig. 3** Mass spectra of pristane (C19H38) from aliphatic hydrocarbon fraction.

**Biomarkers of sesquiterpenoid**

Biomarkers of bicyclic sesquiterpoids are the largest part of terpenoids group. These compounds have been widely studied in crude oil and sediment from various parts of the world but they were not stable [21]. This compound is first reported by Bendoraitis (1974) with the presence of a bicyclic sesquiterpene in Loma Nove biodegrade crude oil from the Gulf Coast of Texas. Furthermore, Philp et al. (1981) and Richardson (1982) reported the distribution of these compounds in the crude oil in Australia and Indonesia. The existence of sesquiterpene indicate the source of the organic compound in oils was originate from higher plants.

The presence of sesquiterpoid biomarkers is identified by mass fragmentogram at m/z 109 and 123 that shown in Figure 4 below:

![Image](https://example.com/image3)

**Fig. 4** Mass fragmentogram m/z 109 aliphatic hydrocarbon fraction.

Some of the identified biomarkers such as C15-bicyclic sesquiterpene, 4β (H) -eudesmane and trans cadinane deliver information that petroleum organic compounds are produced by typical terrestrial plants [22] of the Tertiary Period [23]. 4β (H) -eudesmane provide information that petroleum organic compounds were produced by terrestrial plants [22] that were characteristic of the Miocene Period [24]. These compounds have also been found and used as an indication of the high level of plant organic matter in the Middle Miocene Epoch oil sample of Brunei Darussalam [23].

C15 bicyclic sesquiterpene, was elucidated from fragment ions at m/z 193 and 208. The peak of ion fragments were the typical fragment for C15-bicyclic sesquiterpene compounds published by Philp (1985). The detection of fragment ions at m/z 193 was the base peak due to the loss of the (CH3)2 group. The molecular ion value detected at m/z 208 (Fig. 4) shows 2 degrees of unsaturation. This is suitable for the structure of C15 bicyclic sesquiterpene compound having 2 cyclic rings. Thus, the compound obtained is C15 bicyclic sesquiterpene (C31H32).

The mass spectra of C15 bicyclic sesquiterpene is shown in Figure 5 below:

![Image](https://example.com/image4)

**Fig. 5** The peak mass spectrum of C15 bicyclic sesquiterpene.
Hopanoid was known as pentacyclic triterpenoids fraction and maturity. Hopanoid compounds are found abundantly in marine organisms. The presence of hopane, bicadinane and gammacerane. The stage of its formation revolves around the sterane compound have base peak at m/z 191. The bicadinane compound has high abundance at m/z 349. The presence of bicadinane compounds is identified by m/z 191 fragments in Figure 6. The interpretation of the mass spectrum of this compound was compatible with the compounds that have been reported by van Aarsen et al. (1992). The bicadinane as well as hopane and sterane compound have base peak at m/z 191. The bicadinane compound has high abundance at m/z 369 and 412. According to Peters et al. (2005) that have studied the peak at m/z 369 and 412, but can be distinguished by looking at retention times, hopane compounds have higher retention time than bicadinane compounds.

Another triterpenoid compound that observed was gammacerane. Biomarkers of gammacerane were used extensively in petroleum geochemistry to determine the relationship and determination of the oils source. Sterane exist in fossil fuels were derived from a complex mixture of sterols produced by plants, animals and other organisms. Sterane formation from sterol requires a number of sterene reactions. Sterane formation from sterol requires a number of sterene reactions. Sterane is a component in the dammar resin Angiospermae family. The precursor of this compound was abundant in polycadinane polymer resin of Angiospermae plant [30]. The analysis of gammacerane biomarker compounds and their derivatives, confirm the previous biomarker studies, that the dammar resin of Angiospermae family, Dipterocarpaceae, contributes to organic compounds in Sekar Kurung Gresik crude oil. The stage of its formation revolves around the Miocene era.

Gammacerane is a pentacyclic triterpenoids biomarker, compiled a number of six-ring with C₃₀H₄₂ formula which is generally associated with high salinity environments such as sea or lake environments [28;29]. Gammacerane has been identified in shale, oil and sediment [31]. Gammacerane was derived from the reduction of tetrahymanol [32], and earlier studies suggested it was derived from protozoan tetrahymanola [27]. Later, researches found the source of tetrahymanol, the precursor of gammacerane. Gammacerane in relatively small quantities indicate that the environmental deposition conditions was on the ground with low salinity levels (<0.05%). This is because in these conditions, some organisms such as bacteria phototropic produce tetrahymanol [29].

Gammacerane compound was the contribution of protozoa [18]. Gammacerane is a typical biomarker for marine or terrestrial deposition environment with certain salinity levels, indicating that the origin of crude oil Sekar Kurung Field are formed from phototrophic bacteria in the deposition conditions with low salinity levels.

**Biomarkers of sterane**

Sterane in the range of C₂₇-C₃₀ were used extensively in petroleum geochemistry to determine the relationship and determination of the oils source. Sterane exist in fossil fuels were derived from a complex mixture of sterols produced by plants, animals and other organisms. Sterane formation from sterol requires a number of sterene and steradiene as an intermediate [18]. Sterane and sterene were originate from sterols and eukaryotic cell membranes, especially algae and higher plants. Its presence was more common in marine sediments but not in the source rock or crude oil. Therefore, sterane and diasterane also present in fossil fuels [28]. Sterane were considered as the primary source of eukaryotic organisms in sediments [27]. The distribution of sterane and diasterane...
is identified by mass fragmentogram at m/z 217 (Figure 7), which is
typical in the series of sterane [18].

CONCLUSION
Aliphatic hydrocarbon biomarker compounds e.g. n-alkanes (C_{13}-
C_{28}), acyclic isoprenoid (iC_{23}-iC_{20}), bicyclic sesquiterpene, eudesmane, cadinane, hopane compound (C_{30}),
bicadinane, gammacerane, and diasterane (C_{27}-C_{29}) were obtained from
analysis of aliphatic fractions in Sekar Kurung Gresik crude oil.

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Fig. 7 Mass fragmentogram m/z 217 aliphatic hydrocarbon fraction.

In this sample, compounds 13α(H) 17β(H)-diasterane, 24-ethyl-
5α(H)cholestan-24-ol were identified. The 13α (H) -17β (H) -diasterene compound was derived from sterols Cs formed from phytoplanton namely green algae and 24-ethyl-5α(H) cholesterol that derived from terrestrial plant [18].
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