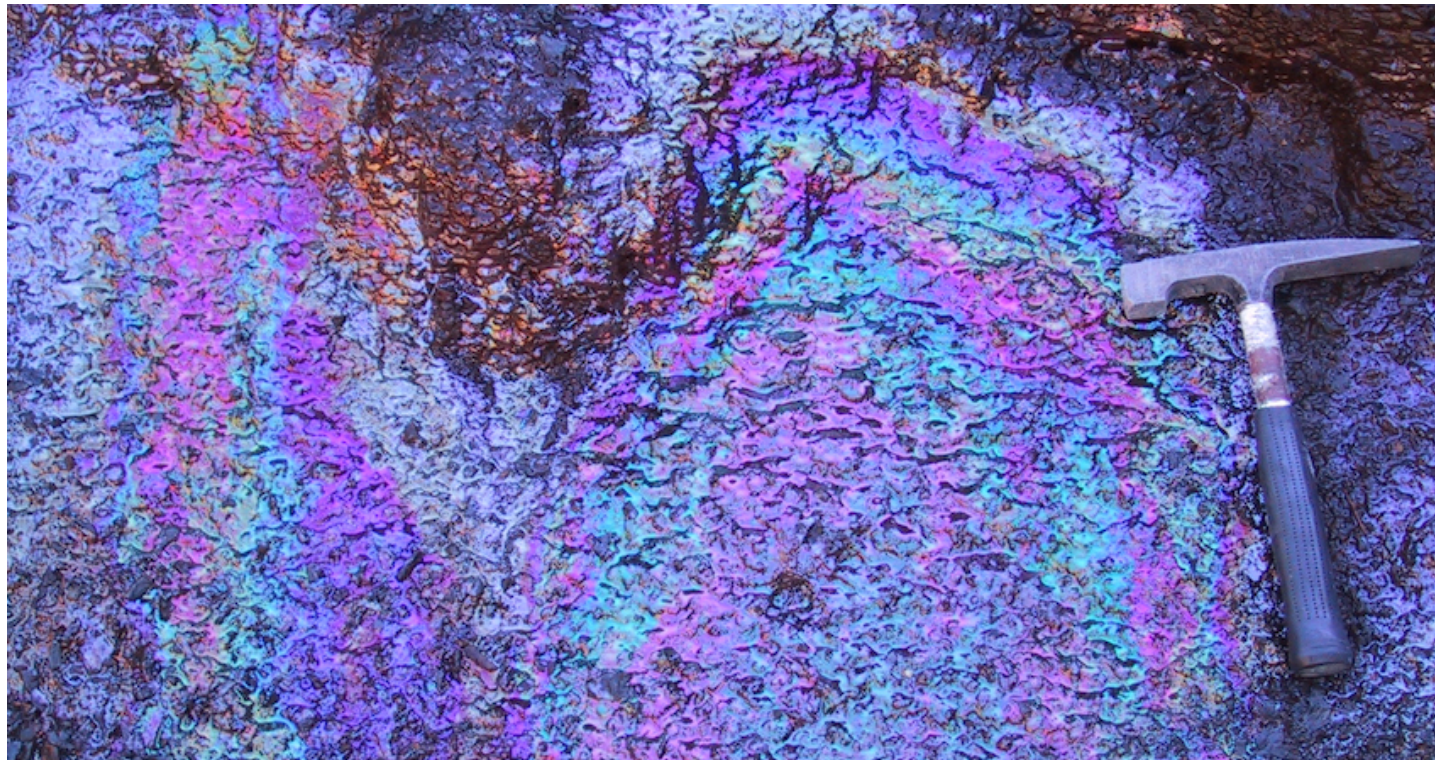


# Crude oil: evidences for its biological origin

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*"Will the defendant please rise! Please state your first and last name, age and origin?"*

*Petra Oleum, Old and Biological would answer all the crude oil in the world if they had to appear before such an imaginary court. Subjected to the question of scientists, oil has been seen by some as a fluid of mineral origin, therefore having an abiotic origin... However, there are many indications of its biological origin, that is, its filiation with what was alive, even if it was millions of years ago. Oil itself confesses by leaving traces everywhere that connect it to life. This article reviews the various proofs of the biological origin of oil by situating them in a historical context.*

## 1. Ideas and facts at the root of a controversy

### 1.1. From ancient philosophers to Renaissance scholars

# GEORGII AGRICOLAE

De ortu & causis subterraneorum  
De natura eorum quae effluunt ex terra  
De natura fossilium  
De ueteribus & nouis metallis  
Hermannus, siue De re metallica Dialogus.  
Interpretatio Germanica uocum rei metallicae,  
addito Indice fecundissimo.

Lib. v  
Lib. iiii  
Lib. x  
Lib. ii



BASILEAE M D XLVI

Cum privilegio Imp. Maiestatis ad quinquennium.

Figure 1. Cover page of Georgius Agricola's book, in which he discusses the origin of oil. [Source: Public domain, via Wikimedia Commons]

If crude oil or its derivatives have been used by man since the Neolithic [1], its origin has long remained mysterious. In his treatise *Opus Tertium*, published in 1268, **Roger Bacon** [2] comments on the absence of discussion on the origin of oils and bitumen by **Aristotle** [3] and other philosophers of Ancient History. It was during the **Renaissance** that **two contradictory hypotheses** about the origin of oil emerged. In 1546, **Georgius Agricola** [4] published *De Natura eorum quae Effluunt ex Terra* (Figure 1), a work in which the **first mention of the word oil** appears, from the Latin *petra oleum* (stone oil). Agricola extends Aristotle's concept of exhalations from the depths of the Earth and proposes that bitumen be the **condensation** of sulphurous vapours. **Andreas Libavius** [5] theorized in 1597 in his book *Alchemia* that bitumen is formed from the **resin of old trees**. Since then, a **long scientific debate** has continued between those who support the abiotic origin of oil and those who see it as a product derived from the transformation of fossilized organic matter.

## 1.2. Opposite views of the first scientists

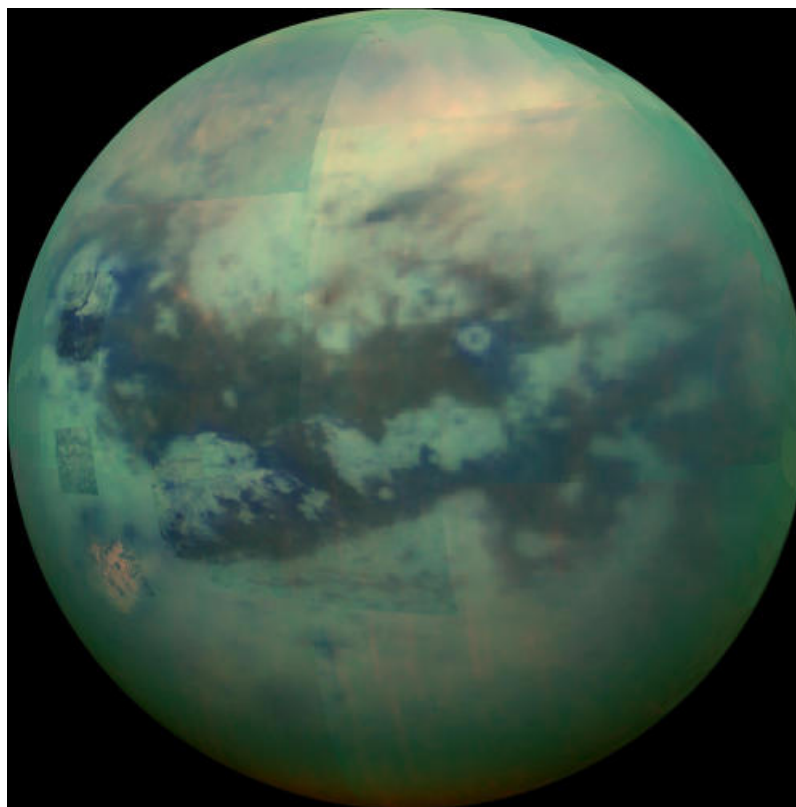


Figure 2. Fossil fern and horsetail footprints on a carbonaceous shale from carboniferous rocks (-300 million years) in the Massif Central. The scale bar is 1 cm long. [Source: Photo © A. Lethiers-F. Baudin]



It is interesting to note that the biological origin of coals has never been disputed because the remains of fossil plants they contain provide undeniable proof of this (Figure 2) [6]. Making a link between these two types of carbonaceous fossil fuels, and taking up Libavius' idea, the Russian scientist Mikhail Lomonossov [7] formulated in 1757 the hypothesis that **liquid oil** and solid **bitumen** would originate from **plant waste transformed** in the subsoil under the **effect of temperature** and **pressure**. This **hypothesis** was **rejected** at the beginning of the 19<sup>th</sup> century by the German geologist and chemist Alexander von **Humboldt** [8] and the French thermodynamicist Louis Joseph **Gay-Lussac** [9], both of whom made oil a primordial compound of the Earth rising from great depths through cold eruptions. This purely chemical hypothesis is not without argument and a **Russian school** of thought has widely disseminated this theory of an **abiotic origin of oil** since the middle of the 20<sup>th</sup> century; an idea that regularly resurfaces to suggest that there would be much more oil than we think in the depths of the Earth.

### 1.3. The arguments of the proponents of an abiotic origin of oil



*Figure 3. Composite image (mosaic) of Titan, one of Saturn's moons, as seen by the Cassini probe. Greenish areas are clouds of methane and other organic molecules, while the surface contains methane lakes. [Source: © NASA/JPL/University of Arizona/University of Idaho]*

The **presence of hydrocarbons on gaseous planets**, where life is not supposed to have prospered, is **evidence** that **abiotic processes** lead to the **formation** of simple **hydrocarbon molecules**. This is the case, for example, on Titan (Figure 3), one of Saturn's moons, whose atmosphere and surface are rich in methane and ethane. Hydrocarbons, and even fairly complex and high molecular weight organic macromolecules, are also found in meteorites that have fallen to Earth.

**Methane is formed during the alteration of volcanic rocks** in the Earth's mantle where dihydrogen ( $H_2$ ) produced during the weathering of some iron-rich minerals reacts with carbon dioxide ( $CO_2$ ) during a so-called Sabatier reaction. This reaction takes place at high temperatures and pressures in the presence of a nickel catalyst to produce methane and water. Other Fischer-Tropsch reactions -which combine carbon monoxide ( $CO$ ) and dihydrogen to convert them into hydrocarbons- could occur when cooling magma and produce hydrocarbons by assembling  $CH_2$  units. Finally, the **thermal decomposition of iron carbonate** ( $FeCO_3$ ) in the presence of water **can also produce simple hydrocarbons**.

While it cannot be denied that abiotic processes lead to the formation of hydrocarbons, **these mechanisms can hardly account for the quantity, diversity and location of oil occurrences on Earth**. The idea that oil originates from sedimentary organic matter, *i.e.* organisms that have been living, is widely demonstrated by natural observations, laboratory analyses and experiments.

## 2. Evidence through natural observations

### 2.1. Oil accumulations in the heart of sedimentary basins

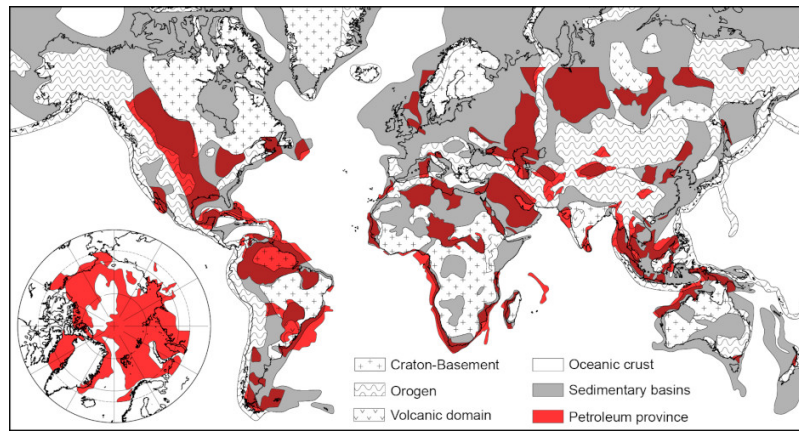


Figure 4. Simplified geological map of the World showing the main domains of granitic or metamorphic basements, orogens (= mountain ranges), volcanic provinces and sedimentary basins. The superimposed red zones correspond to the major oil provinces, which are almost all located in sedimentary basins. [Source: © F. Baudin].

**Sedimentary rocks are essential for a petroleum system to exist** since they constitute in the vast majority of cases the **source rock**, the reservoir and the cover of this system. **More than 99% of the world's oil reserves** are located in **sedimentary basins** and occurrences in magmatic or metamorphic rocks are rare (Figure 4). When oil is trapped in granite or other basement rocks, it is fairly easy to demonstrate that it has migrated from the adjacent sedimentary basin. No oil evidence has ever been found along faults in non-sedimentary continental rocks and extremely rare are oil seeps in the tens of thousands of mines dug in non-sedimentary rocks. The exploration of rocks associated with the impact of a meteorite crater in Sweden in the early 1990s, while it did reveal some traces of oil, did not reveal any deposits of commercial interest. The abiogenic origin of these oils is refuted by several of the geochemical arguments developed below.

## 2.2. The evidence by the deviation of light

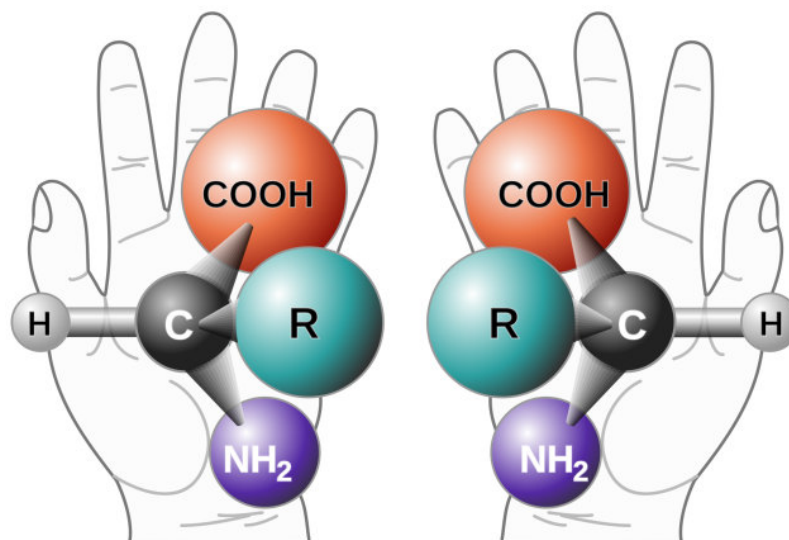


Figure 5. Representation of levorotatory and dextrorotatory isomers of a chiral compound, in this case an amino acid. Source: *περήλιο* © (Chirality with hands.jpg) [Public domain], via Wikimedia Commons.

**Many biological compounds are optically active**, *i.e.* they deflect a light beam passing through them to the right or left when facing light. These compounds are respectively referred to as dextrorotatory (e.g. sucrose) or levorotatory (e.g. fructose). Virtually all amino acids in living organisms are levogyrous. Abiotic compounds do not have this rotational power of light. They are optically inactive because they have an equal proportion of the levorotatory and dextrorotatory isomers of a chiral compound (Figure 5). Life tends to preferentially select one isomer over the other, hence the optical property of biological compounds. However, **oil is most often optically active**, suggesting its biological origin. However, this property tends to disappear with the degree of thermal maturation of the oils, but it can also be amplified when microbes degrade the oil.

## 3. Geochemical evidence

### 3.1. Carbon isotopes

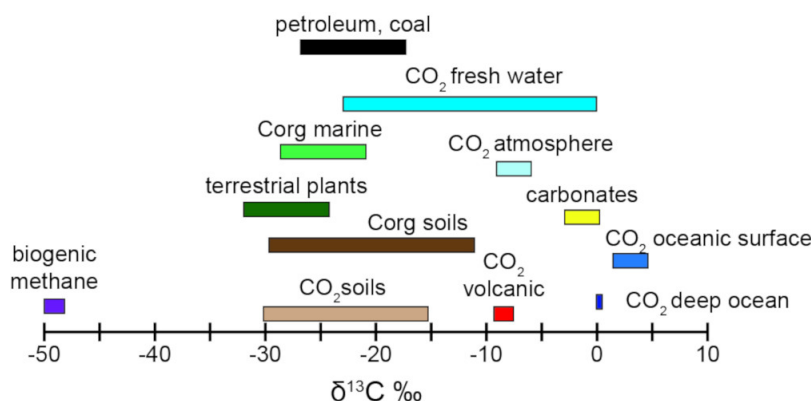


Figure 6. Range of variation in the isotopic ratio <sup>12</sup>C/<sup>13</sup>C of different terrestrial materials. The CO<sub>2</sub> of seawater is taken as a reference; negative values correspond to a <sup>13</sup>C depletion which is expressed here at ‰ Corg: Organic carbon. [Source: Adapted from Trumbore & Druffel (ref. [10])]

Carbon has two naturally stable isotopes: <sup>12</sup>C and <sup>13</sup>C [10]. The **organic matter formed during photosynthesis** from atmospheric CO<sub>2</sub> or HCO<sub>3</sub><sup>-</sup> dissolved in water is **much depleted in <sup>13</sup>C** because plants preferentially fix <sup>12</sup>C. In most plants this depletion is in the order of 0.02‰ (which is also noted at δ<sup>13</sup>C = - 20 ‰) compared to the inorganic carbon source. **Oil and fossil organic matter have the same <sup>13</sup>C depletion as organic matter in living tissues** (Figure 6) [11]. This isotopic deficit is systematic, whether we consider individual molecules or total organic matter. No known inorganic processes lead to the formation of high molecular weight molecules with such a <sup>13</sup>C deficiency. For gases, the proportion of <sup>12</sup>C decreases from methane to propane and in the different forms of butane; this is consistent with the kinetics of isotopic fractionation during thermal cracking of sedimentary organic matter (also called kerogen) or petroleum. The opposite trend, *i.e.* an increase in <sup>12</sup>C from methane to butane, characterizes the polymerization products of the Fischer-Tropsch reaction. This singular isotopic signature is not found in any gas field in the world.

### 3.2. The discovery of porphyrins in oil

The German chemist **Hans Fischer** [12] was awarded the Nobel Prize for Chemistry (1930) for his research on **organic pigments**, particularly in blood (**hemin**) and plants (**chlorophyll**).

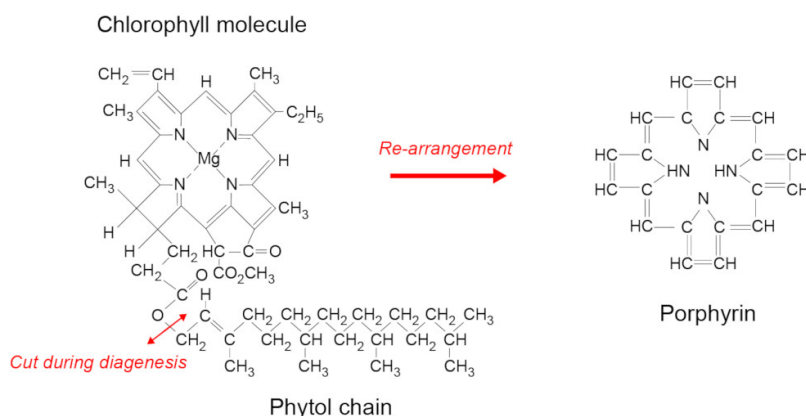


Figure 7. Chemical structure of chlorophyll consisting of a tetrapyrrole core and a phytol side chain. After plant death, the chlorophyll molecule splits in two and each part evolves differently according to the conditions of the sedimentation medium. The tetrapyrrole core partially re-organizes itself to give a molecule called porphyrin. There are dozens of types of porphyrins and all oils contain them, which is undeniable proof of their biological origin. [Source: Scheme © F. Baudin]

Chlorophyll is the main pigment in **green plants**, with which it absorbs part of the solar energy, making **photosynthesis** possible. At the molecular level (Figure 7), it consists of two parts : a polar (*i.e.* water-soluble) "head" formed of four pyrrole core, symmetrically surrounding a magnesium atom, and a phytol "tail"; a long alcohol chain comprising 20 carbon atoms that is non-polar (*i.e.* soluble in lipids). There are five forms of chlorophyll (a, b, c, d, e), each with its own absorption spectrum.

After plants death, the **tetrapyrrole nucleus** and the **phytol side chain** separate and evolve differently. The tetrapyrrole nucleus reorganizes very slightly - in particular magnesium is replaced by nickel or vanadium oxide - thus giving rise to a family of biomarkers: **porphyrins**.

**Alfred Treibs** [13], a student of Hans Fischer, highlighted in 1936 the **ubiquity of porphyrins** in crude **oils** and kerogen-rich clays and the ways in which they are transformed from chlorophyll, thus providing clear **evidence** of their **plant origin**. Alfred Treibs is therefore considered to be the father of organic geochemistry.

### 3.3. More and more biomarkers

Since this discovery, we no longer count the number of molecules detected in oils that are derived from or even strictly identical to a molecule known in living organisms (Figure 8). These molecules, called **biomarkers**, are true **geochemical fossils** because they have a structure very close to the biomolecules of living organisms.

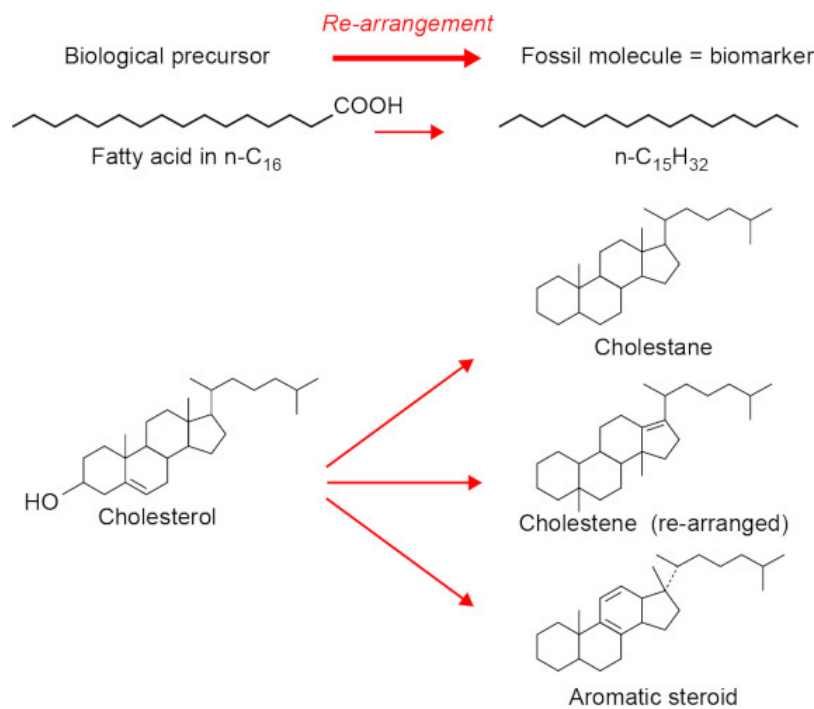


Figure 8. Example of biomarkers (= geochemical fossils) and their biological precursors. [Source: Scheme © F. Baudin]

Indeed, a large number of cell membranes of prokaryotes and eukaryotes, but also leaf cuticles, pigments or resins, are made of **resistant biomolecules** that are **preserved in sediments** where they alter little and **remain identifiable**. In general, only functional groups (-OH or -COOH for example) and double bonds disappear under about ten to a hundred metres of burial. But beyond that, the hydrocarbon skeleton - which is finally preserved - often makes it possible to identify its biomolecular precursors (Figure 8). These characteristic molecules, which can be recovered in the extractable fraction of sedimentary organic matter or in kerogen, perfectly meet the definition of a fossil as are leaf prints or mineralized remains of organisms.

When kerogen undergoes natural thermal cracking to form oil as a result of increasing temperature at depth and over time, some of these biomarkers remain unchanged and even form part of the oil. It is therefore difficult to imagine so many complex molecules present in all oils that would only be produced by abiotic processes.

### 3.4. A chemical signature of marine oils that co-evolves with phytoplankton diversification



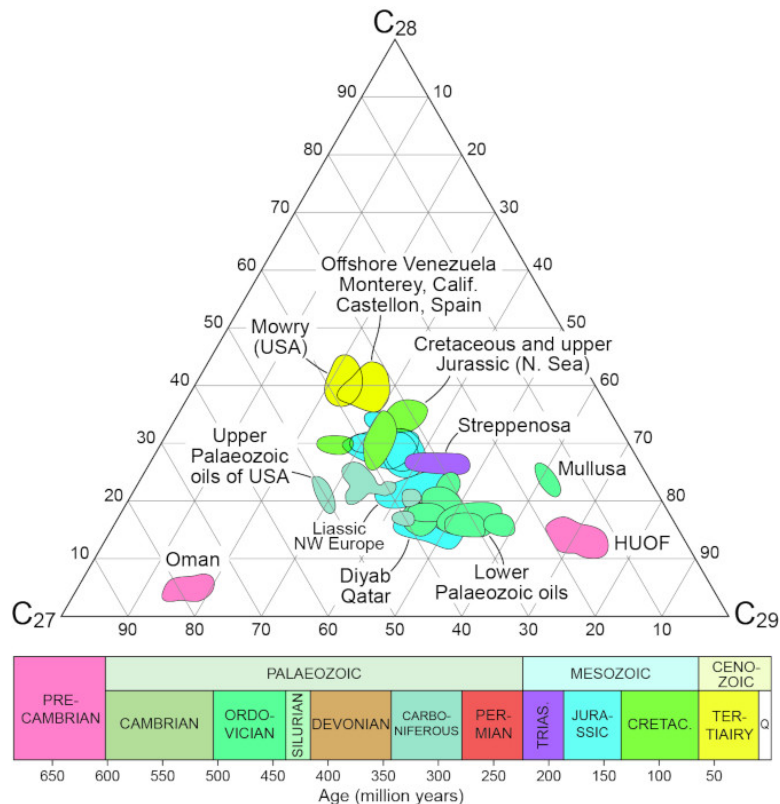


Figure 9. Ternary diagram of the relative proportion of steranes in 414 crude oils as a function of their age. With the exception of a very old oil (600 million years old), there has been an increase in the proportion of C<sub>28</sub> sterane at the expense of C<sub>29</sub> over geological time. [Source: Adapted from Grantham & Wakefield; ref. [13]]

Among biomarkers, steranes are an important class. These molecules are **derived from sterols** that play a role in plants and animals in maintaining the structural and functional integrity of cell membranes. A large number of steranes with 27, 28, 29, 29 or even 30 carbon atoms are **identified in crude oils**.

In the late 1980s, Shell geochemists analyzed more than 400 crude oils generated by 650 to 45 million year-old marine rocks and extracted and identified the different types of steranes and grouped them by increasing number of carbon atoms. It appears that the **proportion of steranes with 28 carbon atoms increases over geological time** while the proportion of steranes with 29 carbon atoms decreases; that of steranes with 27 carbon atoms remains almost stable (Figure 9) [14].

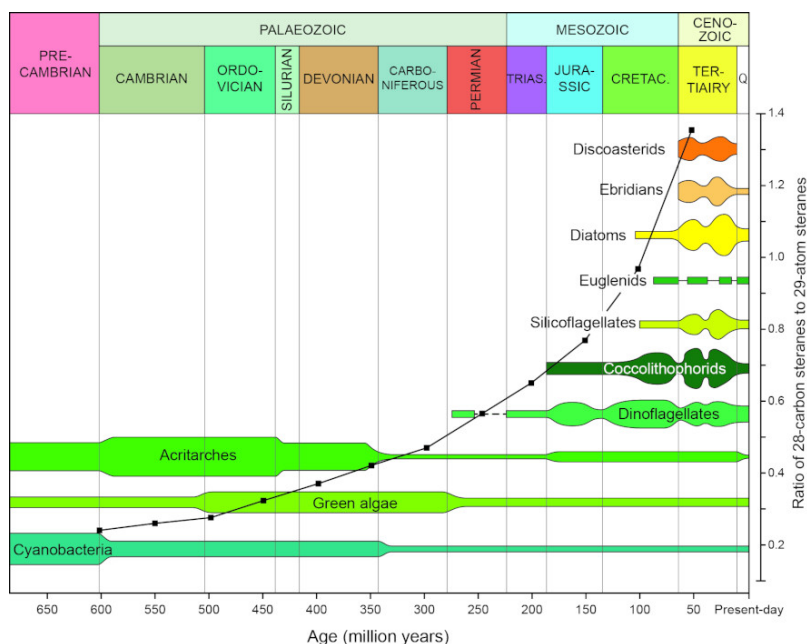
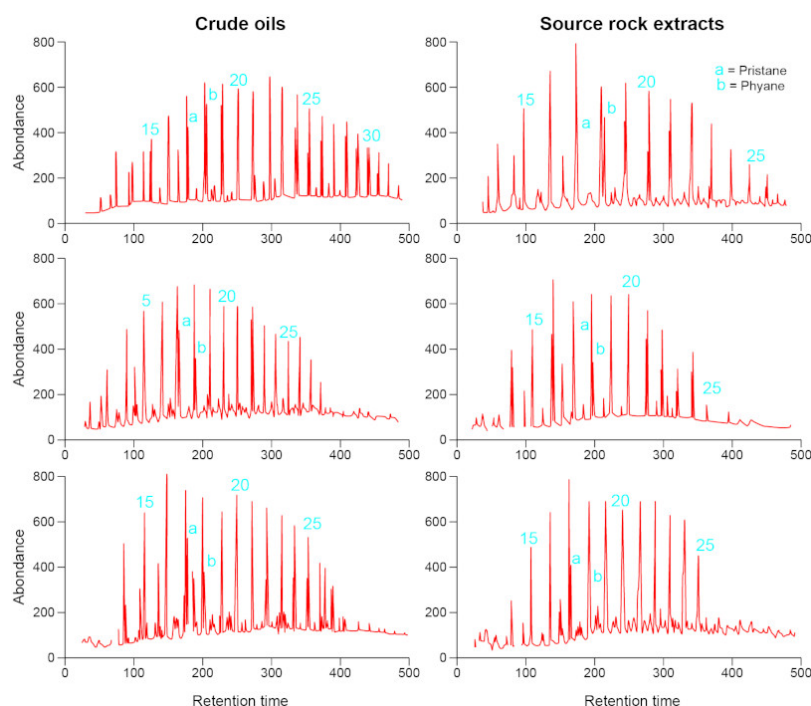


Figure 10. Comparison of the distribution of the most important phytoplankton groups over the past 600 million years and the ratio of steranes with 28 to 29 carbon atoms found in oils from marine source rocks. [Source: Adapted from Grantham & Wakefield; ref. [13]]

For these geochemists, these changes do not reflect the chemical evolution of sterols of a particular variety of a marine organism that would become more abundant over time, but is **related to the diversification of phytoplankton organisms**, recognized by paleontologists from their mineralized remains. This phytoplankton diversification accelerated significantly during the Jurassic and Cretaceous periods, between 180 and 65 million years ago. The sources of steranes with 28 carbon atoms should thus be linked to the appearance and diversification in the oceans of these periods of microalgae such as dinoflagellates, coccolithophorids (the origin of chalk) and diatoms (Figure 10).

This evolution of the chemical signature of marine oils in parallel with that of phytoplankton obviously suggests a cause-and-effect relationship and provides an element in favour of the biological origin of the oils.

## 4. Proof through experimentation



*Figure 11. Comparison of molecular signature, i.e. the distribution and abundance of different molecules, in crude oils (left) compared to that extracted from rocks (right). Each peak corresponds to a specific molecule and its height is proportional to its concentration in the mixture. Here the peaks numbered 15, 20, 25 correspond to linear alkanes containing 15, 20 and 25 carbon atoms respectively. Peaks a and b correspond to branched alkanes derived from the chlorophyll phytol chain. We can therefore see each distribution as a molecular fingerprint, like a fingerprint that is characteristic of each individual. The similarity between the molecular imprint of oils and source rock extracts is evidence of their filiation. [Source: © F. Baudin]*

At the end of the 18<sup>th</sup> century, and in parallel with the development of the coal industry, **petroleum products** were **manufactured by heating sedimentary rocks rich in kerogen**. The similarity of the products obtained with natural oils seeping at the surface, or those found later in deep pools, provided arguments in favour of the Lomonossov hypothesis (see section 1.2).

With the development of analytical techniques in the second half of the 20<sup>th</sup> century, it was possible to reconstruct in the laboratory the temperature and pressure conditions existing at depth in sedimentary basins. It is even possible to perform these experiments in the presence of water, a molecule so abundant in terrestrial rocks. The **products obtained** during these experiments are **physically** and **chemically** very **comparable to natural oils**. When analysed in terms of their molecular composition, there is a striking similarity between the molecules present in natural oil and those produced by heating kerogen, both in terms of the nature and abundance of the molecules (Figure 11). They are **so similar** that geochemists consider these molecular distributions as a bit **like a fingerprint** or DNA and use them to genetically link a natural oil to its source rock.

## 5. Messages to remember

There is a wealth of evidence for the biological origin of oils, including the presence of molecules that derive directly from molecules synthesized by living organisms, whether prokaryotes or eukaryotes.



Thus, porphyrins, ubiquitous molecules in oils, are derived from different types of chlorophyll.

Similarly, fossil molecules are found in crude oils that can easily be attached to other pigments (carotenoids for example) or molecules that make up the cell walls of prokaryotes or eukaryotes.

The greater proportion of  $^{12}\text{C}$  compared to  $^{13}\text{C}$  in petroleum molecules, the so-called isotopic signature, is also in favour of their biological origin since life selects the light isotope in the same proportions.

Finally, more than 99% of the oil fields are located in sedimentary basins, *i.e.* sediments deposited on the bottom of ancient seas or lakes where life was present, as shown by mineralized fossils.

Generally speaking, oil source rocks contain mainly organic matter derived from marine or lacustrine phytoplankton biomass, more or less modified by bacteria.

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## References and notes

**Cover image.** Natural oil seepage [Source: © F. Bergerat]

[1] We know of a Neolithic stone axe that was pressed together with bitumen as 'glue'.  
<https://www.franceculture.fr/emissions/lessai-et-la-revue-du-jour-14-15/le-bitume-dans-lantiquite-revue-archeopages> (radio program in french)

[2] Roger Bacon (1214 - 1294), an English philosopher, scientist and alchemist, is considered one of the fathers of the scientific method.

[3] Aristotle (384 BC - 322 BC), Greek philosopher of antiquity. He is one of the few to have covered almost all the fields of knowledge of his time: biology, physics, metaphysics, logic, poetics, politics, rhetoric and, occasionally, economics.

[4] Georgius Agricola, known as Agricola, by his real name Georg Pawer (1494 - 1555), a 16<sup>th</sup> century German scientist, considered the father of mineralogy and metallurgy.

[5] Andreas Libavius (1555 - 1616), real name Andreas Libau, German chemist and doctor. *Alchemia*, published in 1597, is the first book of systematic chemistry.

[6] The formation of coal deposits began in the Carboniferous with the accumulation of very large quantities of so-called higher plant debris (trees, ferns...) in shallow water layers poor in oxygen (peat-type environment). These conditions allowed some of the organic matter to escape the action of the decomposers. For several million years, the accumulation and sedimentation of these plant debris caused a gradual change in temperature, pressure and oxidation-reduction conditions in the coal layer, leading to the formation of compounds that are increasingly rich in carbon: peat (50 to 55%), lignite (55 to 75%), coal (75 to 90%) and anthracite (> 90%). Most coals have a low oil potential. On the other hand, they produce gas as they mature, in particular methane, which is the cause of firedamping in coal mines.

[7] Mikhail Vasilievich Lomonossov (1711 - 1765) is a chemist, physicist, astronomer, historian, philosopher, poet, playwright, linguist, Slavist, teacher and Russian mosaicist.

[8] Alexander von Humboldt (1769 - 1859), is a German naturalist, geographer and explorer. Associate member of the French Academy of Sciences and President of the Société de Géographie de Paris. Through the quality of the surveys he conducted during his expeditions, he laid the foundations for scientific exploration.

[9] Louis Joseph Gay-Lussac (1778 - 1850), is a French chemist and physicist, known for his studies on the properties of gases.

[10] Radioactive carbon  $^{14}\text{C}$  was initially present in the petroleum-originating compounds (formed from photosynthesis) and in the same proportions as in photosynthetic organisms living at the time. However, since the period of this element is relatively short on a geological scale (5700 years), the current oil no longer contains  $^{14}\text{C}$  and therefore cannot be dated by this technique. This property is currently used to distinguish between particles responsible for air pollution from those derived from petroleum products (gasoline, diesel) and those derived from wood combustion, for example.

[11] Trumbore S.E. & Druffel E.R.M. (1995) *Carbon isotopes for characterizing sources and turnover of nonliving organic matter*. In R. G. Zepp & C. Sonntag (Eds.), *Role of Nonliving Organic Matter in the Earth's Carbon Cycle* (pp. 7-22). Chichester: John Wiley & Sons Ltd.

[12] Hans Fischer (1881 - 1945), a German chemist specialising in organic chemistry.

[13] Alfred E. Treibs (1899 - 1983), a German organic chemist and pioneer in organic geochemistry.

[14] Grantham P.J. & Wakefield L.L. (1988) *Variations in the sterane carbon number distributions of marine source rock derived crude oils through geological time*. *Organic Geochemistry*, 12-1, 61-73.

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