# EVIDENCE OF STEROID BIOMARKERS OVER THE DIFFERENT GEOLOGICAL TIME SCALE - A REVIEW

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## Abstract

Molecular biological markers are naturally occurring, stable organic lipid molecules with high taxonomic specificity and potential for preservation, which can be considered as a particular biosynthetic origin. The original term applied to chemical biomarkers was "chemical fossils"[1], which evolved to "biological markers" (Hunt, 1979), and now to the currently popular term "biomarker."(1)

In this paper I have reviewed five papers on particular steroid biomarker, which can be assigned to a particular eucarya class and marine environment specificity from neoproterozoic period onwards. Samples are basically from shallow marine sediments and were tested by GC-MS. In this paper the main findings are the geological age wise distribution of steroid biomarkers which has been used to infer the antiquity of oxygenic photosynthesis. Sterols are features of eukaryotic membranes, and evolution of steroid biomarker proves the development common ancestor of eukaryotes. Sterens allow the investigation of the fossil record for Palaeozoic algal diversification and evolution. Sterens are important drugs used in medicinal industry now a days, so the biosynthetic pathways of sterols from sedimentary hydrocarbons can be a important source of investigation..

**Key words**: Steroid biomarker, Eucarya, Marine sediment, GC-MS, Neoproterozoic period.

## Introduction

Biomarkers from fossils are stores of molecular information. There are several biomarkers available after a prolong research of different scientists. One of them is Steroid Biomarker. Mainly they all are different lipid molecules that can stay for millions of years in environment. It is used even to reconstruct the past. These lipids are molecular fossils of natural products highly resistant against further degradation. They often retain the diagnostic carbon skeleton of their biological precursors and may endure billions of years enclosed in sedimentary rocks. These molecular fossils can be extracted from rocks (the antiquity of which can be established by radiometric isotopic techniques) and identified using mass spectrometry. In this paper I have shown that hydrocarbon biomarkers that are indicative of sterol distribution allowed the scientists to deduce specific sterols at defined quantities and hence figure out where exactly the taxa sits on the phylogenic tree.

This review paper deals with the distribution of mainly steroid biomarkers over the geologic timescale and its relation with the evolution. These biomarkers encode information about ancient biodiversity, trophic associations, and environmental conditions. Therefore, biomarkers offer a powerful means to study life and its interaction with the environment as recorded in rocks of Geologic age.

**Review of Related Literature**

Biomarkers extracted from sedimentary rocks have the potential to give information on the historical events vis-à-vis specific microbial metabolisms. Biomarkers have yielded the oldest dates for angiosperms [1,2] and rhizosolenid diatoms [1] in the Phanerozoic, and for the first occurrence of green sulfur bacteria (Chlorobiaceae) and purple sulfur bacteria (Chromatiaceae) in the Proterozoic [1].

The earliest identifications of molecular indicators for life were reported by Prof. Treibs (1934), who characterized the porphyrins (geological derivatives from chlorophyll) in sedimentary rocks and bitumens. Steroid biomarkers are known from 1690 million-year-old rocks of Australia [3]and also a large and diversified biomarkers are known from the Proterozoic, including eukaryote, bacterial, and archaen biomarkers as well as a number of 'orphans'[1,2] biomarkers in younger sediments are much useful in evolutionary studies as well. As for examples: dinosteranes, biomarkers unique to dinoflagellates, are now known in the Cambrian rocks , nearly 300 million years before the oldest definite dinoflagellate fossils [1].24-n-propylcholestanes are diagnostic markers for marine algae of the order Sarcinochrysidales and brown tide algae [4]. 24-norcholestanes have been found in crude oils associated with the proliferation of diatoms from the Jurassic to the Tertiary [3] and 24-norsterols have been reported in both diatoms and dinoflagellates [1,2].

Pelagophyte algae ('brown tides' and sarcinochrysidales) are almost representative of marine environments. Both 24-n-Propylcholestanes and 24-isopolycholestanes are found with ααα and αββ stereochemistry( both20 S and 20R) exclusively for marine and deltaic environments. The relative amounts of C27-C29 steranes can be used to give indication of source differences .For example, predominance of C28, C29 and C30 steranes indicate an origin of the oils derived mainly from mixed terrestrial and marine organic sources [5], whereas oils showing slightly low abundance of C28 and C29 and relatively higher concentrations of C27 steranes indicate more input of marine organic source.

**Review Analysis and Findings:**

The basic findings of the review work are being depicted here in the following tables precisely as follows:

- Mostly all biomarkers were found after the archean period, i.e. from Proterozoic period onwards.
- C-30 steroid and stigmestane are found for a certain period
- All of them are of eukaryotic class
- All of them from marine ecosystem

- Mainly algae ,diatoms and sponge taxa specific
- Main source materials from sedimentary rock and shallow sediments.
- Detection of steroid hydrocarbons far back in Earth history has been used to infer the antiquity of oxygenic photosynthesis. Sterols are features of eukaryotic membranes, and evolution of steroid biomarker proves the development common ancestor of eukaryotes.
- Furthermore, their patterns of occurrence over billion year time-scales correlate strongly with environments of deposition. Accordingly, biomarkers are excellent indicators of environmental conditions even though the taxonomic affinities of all biomarkers cannot be precisely specified.
- The identification of this molecule at high abundances in Neoproterozoic rocks has been interpreted to reflect the presence of multicellular life prior to the rapid diversification and radiation of life during the Cambrian explosion.[2][3] In this transitional period at the start of the Phanerozoic, single-celled organisms evolved to produce many of the evolutionary lineages present on Earth today.
- Steroids are important constituents of eukaryotic cell-membranes and are preserved in sediments as steranes. C28- and C29-steranes are indicators for the presence of green and C27-steranes for the presence of red algae, respectively. The relative abundance of steranes allows the investigation of the fossil record for Palaeozoic algal diversification and evolution.

**TABLE -1**: **Distribution of Steroid Biomarkers over geologic timescale**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HADEAN EON | PRECAMBRIAN EON | | PHANEROZOIC EON | | | | | | | | | | | | | |
| | ARCHEAN | PROTEROZOIC | PALEOZOIC ERA | | | | | | MESOZOIC ERA | | | CENOZOIC ERA | | | | |
| | | | Cambrian | Ordovician | Silurian | Devonian | Carboniferous | Permian | Triassic | Jurassic | Cretaceous | Paleogene | Neogene | Quaternary | | |
| | | | | | | | | | | | | | | | | |
| 24-Isopropyl cholestane | | | 24-Isopropyl cholestane | | | | | | | | | | | | | |
| Cholestane | | Cholestane | | | | | | | | | | | | | | |
| Ergostane | | | Ergostane | | | | | | | | | | | | | |
| 24-norcholest | | | | | | | | | | | 24-norcholestane(C-30) | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| ane | | | | Steroids. | |
| Stigmastane | | | Stigmastane | | |

**Table 2: Specificity of the respective Biomarkers**

| BIOMARKER | CLASS SPECI-FIC | AGE SPECIFIC | ENV. SPECI-FIC | TAXA SPECIFIC | REMARKS |
|---|---|---|---|---|---|
| 24-norcholestane (C-26) | Eucarya | Considered to be an age-diagnostic marker for post-Jurassic oils and bitumens | Marine | Diatoms | Steranes, diasteranes and aromatic steroids with 26 to 30 carbon atoms are abundant in most oils and bitumens from the Cenozoic to the Paleoproterozoic and possibly the Archean . |
| Cholestane (C-27 steroids) | Eucarya | Proterozoic period. | Marine | From red algae | In aquatic sources probably almost exclusively derived from diverse eukaryotes. In organic matter from terrestrial sources (e.g. paleosols) input from soil bacteria of the order Myxococcales conceivable. |
| Ergostane(C-28 steroids) | Eucarya | Phanerozoic period. onwards | Marine | Exclusively eukaryotic, but usually no distinct sources discernible. | precursors of ergostane (C28) are frequently found in yeast and fungi, diatoms (Bacillariophyceae) and several other classes of |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | microalgae. |
| Stigmastane (C-29 steroids) | Eucarya | Neoproterozoic period onwards | Marine | Higher plants and in many microalgae. | stigmastane (C29) typically occur in higher plants but are also the major sterols in many microalgae, such as several freshwater eustigmatophytes and chrysophytes, and green algae of the class Chlorophyceae |
| 24-n-propylcholestane (C-30 steroids) | Eucarya | Late archean onwards | Marine | Pelagophyte algae ('brown tides' and sarcinochrysidales); | Its potential biological precursors have only been detected in five marine algae of the class Pelagophyceae.<br>These include the "brown tide" algae Aureoumbra, Aureococcus, three species of the order Sarcinochrysidales |
| 24-isopropylcholestane<br>(C-30 steroids) | Eucarya | Neoproterozoic period to ordovician | Marine | sponges and possibly the sponge-related stromatoporoids, Demosponges | Traces of $C_{30}$ steranes have been found in fluvio-deltaic source rocks, although this may reflect marine incursions to peat swamps . |

**Methodology**

Gas chromatography is the main technique here used to separate out the individual components of very complex mixtures. High resolution gas chromatography using fused silica capillary columns is the ideal method to obtain maximum separation of compounds over a wide range of

molecular weights. Compounds must be volatile in order for this to work. Therefore, in the case of complex lipids from soil and environmental samples, we often must hydrolyze and derivatize in order to obtain the core components in volatile form. These lipid subunits include fatty acid methyl esters and trimethyl silyl ethers of sterols and hopanoids. We often work on petroleum samples, which are composed of many hundreds of thousands of different compounds. Computerized gas chromatography – mass spectrometry (GCMS) is the principal method used to detect and identify compounds by exploiting the mass variations of different compounds. A typical GCMS system performs six functions.

Transfer of separated compounds to the ionizing chamber of the mass spectrometer, Ionization, Mass analysis, Detection of the ions by the electron multiplier, Acquisition, processing and display of data by computer and Compound separation by gas chromatography.

A syringe injects a known amount of the saturate fraction dissolved in hexane into the GC column, which is a long, thin capillary tube 0.32 mm wide and 60 metres long. The inner surface of the column is coated with a film of nonvolatile liquid, called the stationary phase. The temperature of the column is gradually raised, using a temperature-programmed oven, causing the compounds to move. Each injected sample is vaporized and mixed with helium, an inert carrier gas, which is also called the mobile phase.

The larger molecules are retained by the stationary phase at the head of the column, a process called ‘cold trapping’. The mobile phase and sample mixture move along the column, while components are separated as they are repeatedly retained by the stationary phase and released into the mobile phase depending on their volatility and affinity for each phase. The compounds elute, or are washed out from the end of the column, at different rates and are analyzed by the mass spectrometer.

**Table 3 Sample Source and Methodology**

| **SOURCE MATERIAL** | **METHODOLOGY** |
|---|---|
| oils and bitumens<br><br>recent shallow marine sediments | GC-MS |
| shallow marine sediments | GC-MS |
| shallow marine sediments | GC-MS |
| shallow marine sediments | GC-MS |
| shallow marine sediments | GC-MS |

## Conclusion

From the chart analysis it is clear that steroid biomarkers are responsible for reconstruction of paleo environments as well as evolution of life on Earth. For example the abundance of 24-norcholestanes in crude oils increases considerably from the Jurassic to the Cretaceous and again

in the Tertiary, a distribution that appears to coincide with diatom radiation and deposition of major diatomaceous sediments. The abundant sedimentary 24-isopropylcholestanes, produced by marine demosponges, record the presence of Metazoa in the geological record before the end of the Marinoan glaciation (~635 Myr ago). They currently represent the oldest evidence for animals in the fossil record, and are evidence for animals pre-dating the termination of the Marinoan glaciation. This suggests that shallow shelf waters in some late Cryogenian ocean basins (>635 Myr ago) contained dissolved oxygen in concentrations sufficient to support basal metazoan life at least 100 Myr before the rapid diversification of bilaterians during the Cambrian explosion.

The rigorous stratigraphic and geochronologic placement of the samples in this study constrains the first appearance of sponge biomarkers and suggests that sponges were continuously prevalent in a wide range of Neoproterozoic environments before the known record of other animal fossils, including megascopic animal body fossils, 575 Mya.

Steroids are the most important diagnostic biomarkers for marine depositional environments including deltaic environments. Age diagnostic markers are specifically applicable to diagnose the ages,for example C-30 is considered to be an age-diagnostic marker for post-Jurassic oils and bitumens. Steroids can also be used to reconstruct the past. Presence of algal steranes and bacterial triterpenoids suggested that photosynthesis was strongly reduced possibly less than a century after the impact followed by a rapid resurgence of carbon fixation and ecological reorganization.

**Future Prospects**

Certain biomarkers like Stigastane and C-30 Steroids, only available for certain periods of time in the geologic scale. This is very unusual. They indicate the presence of different algae. This can be a matter of investigation that why this type of biomarkers were not recorded after this period. Palaeozoic algal diversification and evolution can also be studied further from this steroid study. This study indicates the paleo sterol synthesis and origin of biomolecules on Earth. So our near future field if huge and with immense scope of research.

**Limitations**

Lack of proper samples sometimes is a problem for this type of investigation. Sometimes its difficult to locate proper source material distinctly for a particular biomarker, like ergosterane. Stigmastane generally available for higher plants but several freshwater eustigmatophytes and chrysophytes, and green algae of the class Chlorophyceae also denotes the presence of this biomarker. So, sometimes it is difficult to mark a particular organic molecule for a specific taxa.

**References:**

1. Brocks, J.J.,and Summons, R.E., 2004,"Sedimentary Hydrocarbons,Biomarkers for Early Life",In: Treatise on Geochemistry., Vol 8, Biogeochemistry.Schlesinger WH (ed)., Elsevier-Peragamon,Oxford,pp. 63-115.

2. Brocks, J.J., Logan ,G.A., Buick, R., and Summons, R.E., 1999,"Archean molecular fossils and the early rise of eukaryotes",Science,pp. 1033–1036.

3. Holba, A. G., et al.1998"24‑norcholestanes as age sensitive molecular fossils". Geology,(26), pp.783–786.

4. Ourisson,G.,1994"Biomarkers in the Proterozoic record", In: Bengtson S (ed.), Early Life on Earth, New York: Columbia University Press,pp. 259–269.

5. Summons, R.E., Bradley, A.S., Jahnke, L.L., Waldbauer, J.R.,2006"Steroids, triterpenoids and molecular oxygen", In:Philos Trans R SocLond B Biol Sci. Jun 29;361(1470):951-68.