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CARBON SKELETONS OR CHEMICAL FOSSILS

A Lecture by

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Carbon Skeletons or Chemical Fossils:

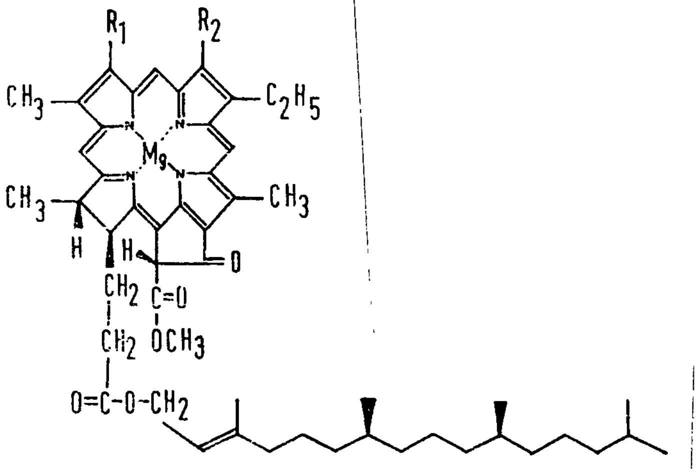
The Oxford English Dictionary defines a fossil as anything dug up. Curiously the word is first used in a book on Meteors by W^{m} Fulke.. Fossils therefore, need not be the remains of animals or plants which belonged to the past, however, that definition is the one most commonly in use. They need not be exactly representative of what went before because geological processes can act to replace what was formerly soft tissue with mineral matter and thus fossils can be just an imprint in the rock of previously living material. The further one goes back into the fossil record of course the much less well preserved the tangible evidence of pre-existing plant and animal remains.

Fossils can take many forms both large and small so that a stromatolite, a large mass of mineralised algae is every bit as much the remains of a former organism as the skeleton of a dinosaur. The size of fossils can diminish out of sight of the naked eye or even the light microsope and into the realms of elecron microscopy where magnifications of 10000 times are required to see forms interpreted as once living species. There is, however, a lower limit to how small one can go since an organism, to replicate itself, must be capable of carrying the necessary amount of genetic information to do so. The genetic code is contained in rather large molecules, double helical spirals of DNA, so the minimum size of even a single celled organism is larger than one might think. The putative nanofossils observed in a martian meteorite ALH 84001, are appoaching the theoretical limit of life.

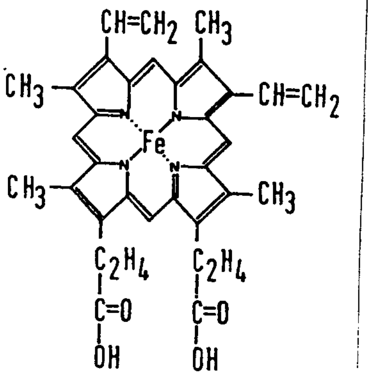
Despite the caveats above, methods for detecting life can go on at levels far below what can be viewed microscopically and infact after geology has done its best to obliterate the visible evidence that life on Earth has been around for a long time. This is because of what are called "chemical fossils" or "biological markers"; the study of these entities being referred to as molecular palaeontology.

Chemical fossils have structures every bit as recognisable as conventional fossils and what is more they can retain them over vast periods of geological time. The whole idea of molecular palaeontology comes about because biology is very specific concerning the kinds of chemical compounds it uses for different biochemical activities. Nucleic acids are the sub-units of DNA (and RNA) as has already been mentioned. Another well known class of compounds are the amino acids which make up not only the muscle content of animals but constitute enzymes, the catatysts which help all the chemical processes of life to happen. Unfortunately large molecules like DNA and proteins are not all that persistent geologically speaking, and neither are their component sub-units very robust. However, other types of compound essential to life, the lipids, which can be found in the fatty parts of animals and plants, are extraordinary long lived. The skeletons of chemical fossils are made of carbon atoms; they are not seen with microscopes and the like but, are analysed and detected by the use of gas chromatography, to afford a separation, and mass spectrometry, which gives molecular weight and structural information.

The potential of molecular palaeontology can be demonstrated by reference to the geological fate of chlorphyll. Chlorophyll is the green pigment which allows the process of photosynthesis to take place, converting or fixing carbon dioxide from the atmosphere into larger moleucles which non-photosynthetic organisms can utilise as energy sources. One half of cholophyll is very similar to haem, which is the pigment that gives blood its red colour, so the fate of these two compounds can be considered together.



chlorophyll

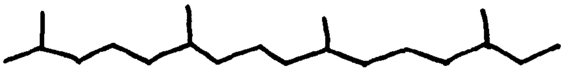


haem

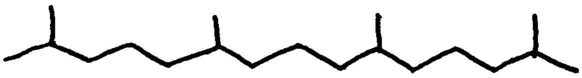
Once any cholorphyll (or haem containing organism) dies, the pigment begins to under go irreversible chemical reactions. First of all comes removal of the metal magnesium (or iron) from the centre of the ring; once in the geosphere replacement with nickel or vanadium occurs. Following demetallation, in the case of chlorophyll, the long side chain of carbon atoms called the phytyl group is cleaved off and the two halves go their separate ways. The multi-ring nucleus, called the porphyrin entity, undergoes further modification, mainly reduction processes, until two different kinds of stable (see scheme) prophyrin result, one with a five rings (deoxyphylloetioporphyrin, DPEP) and one four rings (etioporphyrin, etio). It was the finding of these two compounds in petroleum and a variety of sedimentary rocks during the 1930's by Alfred Triebs, which gave the impetus for the whole subject of organic geochemistry. The discovery also sounded the death knell for the idea that petroleum might be a complete hotch potch of abiogenically produced organic molecules .

Originally, it was thought that the ratio of DPEP to etio might reflect the relative contributions of plants and animal sources to petroleum but infact this is not so; careful structural analysis is able to show that the DPEP can undergo further reactions to give rise to etio by rupture of the five membered ring. More sophisticated methods, than those available to Triebs, were also able to reveal that the molecules, which he thought were the end products of chlorophyll and haem breakdown were more complicated. Instead of having a single compound of specific molecular weight, a wide range of compounds with weights differing by units of fourteen in mass were encountered. This is interpreted as it being possible to have a variety of substituents around the macrocyclic ring; the unit fourteen corresponds to the insertion of $-CH_2-$ groups into the structure. As a result of the work of this type,it was realised that these different species use "chlorophylls" of different structure for their photosynthetic processing.

In considering the porphyrin nucleus we temporarily set aside the fate of the phytyl side chain. This too undergoes extensive geologic processing.



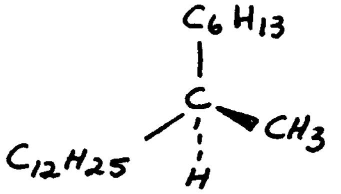
phytane C_{20}

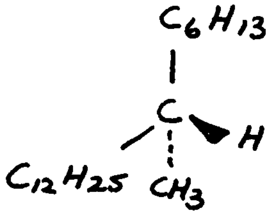


pristane C_{19}

The reactions which go on include hydrogenetion, dehydration, oxidation and decarboxylation, with two clear routes possible. The end products, to give them their systematic names are 2,6,10,14 tetramethylhexadecane and 2,4,6,10 tetramethylpentadecane or to use trivial names phytane and pristane respectively. These two long chain hydrocarbons are extraordinarily stable even more so than the porphyrin half) and theoretically could survive anything other than extensive metamorphic alteration on Earth for the entire geological time scale. For this reason it was believed that they could be used to establish when photosynthesis began on our planet. During the 1960's and 1970's, phytane and pristane were searched for (and found) in sediments of increasing age back to the very earliest rocks then known $3.5 \text{ x } 10^9$ years old. The problem, however, was that these medium molecular weight molecules were geologically mobile and therefore might represent contamination from more recent formations which had migrated in fluids to the older rocks. The question of how old life is on Earth, is now addressed by a technique which is not succeptible to the problem just described. (see below)

Mention of the isoprenoid species like phytane allows an introduction to be made to the concept of stereochemistry or chirality, thought by some to be the ultimate test of a biological origin. Chirality exists because chemical bonds available to individual carbon atoms are tetraherally oriented in space. This is depicted as follows:





Where bonds are marked (—) they are in the plane of the paper, those (- - -) are pointing into the page and those (◄) above it. It should be possible to see that that if a single tetrahedral carbon atom has four different groups around it then phytane for example can have two forms (actually more but for simplicity we consider only two here). These molecules are like hands, existing as non-superimposable mirror images; the central atom is said to be chiral, the name meaning handedness. Individual mirror image molecules, when subjected to plane polarised light, will rotate it exactly equally and opposite. A pair of molecules like this will be called enantiomers; mixed together in equal amounts they will cancel each other out and be optically inactive, a situation which is termed a racemic mixture. There is another class of optically active compounds called diastereomers; these are optical isomers which are not mirror images.

Because biology is a very specific process, it only ever produces or uses a single enantiomer. Geology, or other non-biogenic activities, are not so fussy (i.e.not stereospecific). Enantiomeric molecules transferred to the geosphere begin to change from one stereoisomer into the other (racemise) and a knowledge of the rates at which this happens can be used as a chronometer.

There is an excellent example of the use of stereochemistry to demonstrate the origin of a particular group of compounds from the study of meteorites. Just as the presence of apparently biological markers in very old rocks was fiercely debated in the 1970's so too was the finding of extractable compounds in carbonaceous chondrites which had fallen from the sky. Amongst the species encountered were the molecules really fundemental to life i.e. amino acids. As soon as it became possible to determine the stereochemistry of these species, it was recognised that they were racemic mixtures. These proved beyond all doubt that they were indigenous to the meteorites and not recent contamination picked up on Earth; recent contamination would have been all one isomer. It was subsequently shown that the amino acids in meteorites were in proportions which suggest a production from small precursors by an abiogenic means i.e. the most abundant compounds in the group were the simplest structures which could be made, glycine being far and away the most abundant and the amounts of other larger amino acids decreasing rapidly as complexity increased. Very recent analysis shows that some of the very simplest amino acids in meteorites have a stereochemical imbalance; there is a school of thought growing up which says that it is possible to synthesis optically active material abiogenically (perhaps under the infuence of polarised star light).

The looking for evidence of building up molecules atom by atom is a diagnostic of abiogenicity is one which works rather well. For example there are ca 10^5 ways to putting together 20 carbon atoms. Biology as we have already seen in the example of the phytyl side chain, chooses to put them in very distinctive order. There is another preferred way, that is in a long chain in a straight sequence. Therefore in the geological environment, there is an abundance of straight chain hydrocarbons; petroleum for example has a plethora of hydrocarbons up to C_{40} and beyond. The distribution is gaussian about a mean which depends on the history of the oil. Petroleum is biologically produced material well processed by geology however in the early stages of its formation, i.e. the nearer one gets to the biological/geological interface the more likely one is to find not a gaussian distribution but one which is dominated hydrocarbons with odd numbers of carbon atoms over those with even ones. This is because straight chains are synthesised by biology not by adding single atoms but by inserting two atoms at a time. The building unit of biosynthesis is CH_3COCoA and the major product before alkanes are made is the even numbered fatty acids. Thus alkanes have to be produced by cleaving off a single C atom so they end up odd numbered.

Another important facet of the biosynthetic production from CH_3COCoA is that because one atom in reduced and the other oxidised (different chemistry involved) they tend to have different isotope compositions. So that a long chain hydrocarbon produced by biosynthesis would have an alternating pattern of isotopic abundance: a position enriched in ^{12}C, depleted in ^{12}C, enriched, depleted etc. This has been very elegantly demonstrated to be true by J M Hayes of the Wood's Hole Oceanographic Institute.

Measuring intramolecular isotopic composition is exceedingly difficult but it has been applied to low molecular weight hydrocarbons in meteorites. Here as size increases uniformly atom by atom the overall isotopic abundance of ^{12}C increases, as might be expected for an abiogenic process (^{12}C atoms react quicker than ^{13}C). Polyaromatic hydrocarbons from meteorites also show an internal isotopic composition suggestive of being built up by reactions which go faster for light isotopes, but the mechanism is more complicated than just adding single atoms.

So far, we have discussed only recognisable molecules found in rocks or meteorites which are extractable by solvents. Unfortunately these are in avery short supply, for the most part the biological material contributed to sediments does not survive as such. Instead it gets converted by geological processing into a huge intractable macromolecule called kerogen. The biologically structural information in kerogen can only be extracted by smashing the macromolecule with various techniques. Pyrolysis is the easiest and most popular, but others include hydrolysis, oxidation and hydrogenation. When kerogen is broken down it reveals sub-structures similar to the individual molecules which went in. However, the older and more processed the macromolecule the less the biological information survives since action in the geosphere produces methane and crosslinking. Geology, however, failes to obliterate the isotopic signature.

Biology confers organic matter produced by biosynthesis a clear isotopic value from a fractionation of some 30‰ in favour of the ^{12}C over ^{13}C. Manfred Schidlowski of the MPI Mainz has made a life time study of the isotopic composition of kerogens isolated from sedimentary formationation of all ages. Some ten thousand samples have been investigated from the sediment interfaces at the bottom of his back garden pond to the most ancient known rocks currently known on Earth. In each case the kerogen has been isolated and measured along with any carbonate found associated; the carbonate provides an indication of the isotopic composition of the atmosphere when the rock was formed. For all these samples the isotopic fractionation imposed by biology is still visible. Even the very oldest rocks which have been much altered by the geological events of nearly 4 billion years retain the evidence. The beauty of using the Schidlowski method of finding out when life began on Earth is that the macromolecular nature of kerogen means it is not mobile. In consequence it cannot be argued that recent contamination is involved. Interestingly, the organic matter and carbonates found in martian meteorites conform to Schidlowski's rules but many scientists still do not accept that this is evidence for life on Mars.

Further Reading

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