A TREATISE IN ORGANIC GEOCHEMISTRY
Eugene Desmond McCarthy
(Ph.D. Thesis)
August 1967

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A TREATISE IN ORGANIC GEOCHEMISTRY
Eugene Desmond McCarthy
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and

TO MANDY
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Abstract

This treatise has concerned itself with a study of the major problems in organic geochemistry and how they relate to the more fundamental scientific questions of chemical evolution and the origin of life. The significant advances in organic geochemistry, which have been brought about within the last fifteen years, have been achieved as a result of the development of analytical techniques capable of handling microgram quantities of organic material -- in particular, gas-liquid chromatography and mass spectrometry. In the experimental approach adopted in this treatise, almost all of the analytical techniques of modern organic chemistry have been utilised in the structural identification of the organic components isolated from a series of crude oils and sediments. Since the major part of the work has concentrated on the hydrocarbons, there has been a particular emphasis on capillary gas chromatography and mass spectrometry. The recent acquisition of a combined gas chromatograph-mass spectrometer suggests that this instrument will be the most powerful analytical technique available to organic geochemists.
The isoprenoid hydrocarbons, generally considered to be derived from the biological precursor phytol, have been extensively studied in a series of geological samples of different ages. This class of compounds has been found in shales as recent as the Green River Shale ($52 \times 10^6$ years), as well as in a series of shales of Precambrian age. In general, the acyclic saturated isoprenoid hydrocarbons are not found in recent sediments where degradation processes do not seem to have taken place to any detectable degree. Other homologies, including the iso-, anteiso- and cyclohexynormal alkanes have also been characterised in crude oils.

Among the isoprenoid hydrocarbons, we have isolated and identified the $C_{17}$ isoprenoid, 2,6,10-trimethyldodecane, from the Antrim Shale. Its structure was confirmed by capillary gas chromatography coinjection techniques with a standard hydrocarbon that was synthesised from farnesol, followed by mass spectrometry. This represents the first report of this compound in the literature. In keeping with the diagenetic scheme we have postulated, in which the $C_{17}$ isoprenoid is considered to derive from a $C_{20}$ isoprenoid by a two-carbon cleavage, this isoprenoid hydrocarbon is found in significantly smaller amounts than the other isoprenoid hydrocarbons. The value of having synthetic standards available is stressed. Two $C_{19}$ isoprenoid hydrocarbons, isomeric with pristane, were synthesised and the mass spectra of the three isomers compared. The close similarity of the mass spectra emphasises the danger of identifying individual components from a hydrocarbon mixture on the basis of mass spectrometry alone. Adopting a similar approach, the $C_{21}$ isoprenoid
hydrocarbon, which had been isolated from several geological samples, was assigned the structure 2,6,10,14-tetramethylheptadecane, and it was considered to derive from a C₄₀ compound, such as lycopene, or from some higher homologue. Diagenetic pathways are discussed in detail.

Serious consideration is given to the fundamental premise upon which the organic geochemical approach rests -- that isoprenoid compounds are necessarily indicative of biological origin. The studies of Natta, however, have shown that natural rubber can result by the polymerisation of isoprene using a specific catalyst. This lends credence to the idea that isoprenoid compounds could arise by nonbiogenic processes. We have considered this question from both the thermodynamic standpoint and the biosynthetic standpoint. Although the overwhelming proportion of the evidence still indicates a biogenic origin for the organic components from crude oils and certain shales, ambiguities do arise when the identity of the hydrocarbon material is less clearly understood. An attempt is made to establish criteria which will distinguish between those hydrocarbons derived from biological sources and those derived from non-biogenic sources. It is concluded that the acyclic isoprenoid structure by itself cannot be regarded as an unequivocal marker of biological origin, a factor which may be of some significance when the first samples are returned from the Moon.
CHAPTER 1
INTRODUCTION

In general terms, organic geochemistry refers to that scientific discipline which concerns itself with the organic chemistry of the geological environment. This discipline took its beginning in the earlier part of this century in the controversy that was involved with the origin of petroleum. Indeed, to have referred to organic geochemistry in the context of the origin of petroleum prior to this time would have seemed a contradiction in terms. Inorganic theories of petroleum formation prevailed until the turn of the last century. Berthelot (1866, 1870) had proposed that water in the earth's crust reacted with metallic carbides to produce hydrocarbons. Mendelieff (1904) and other eminent chemists supported the same hypothesis. Although the biogenic theory of the origin of petroleum has gained increasing acceptance since the last decade or so, such eminent scientists as Professor Hoyle (1955) and Sir Robert Robinson (1964) maintained that biogenic processes do not contribute significantly to hydrocarbon production. This aspect of the treatise is emphasised at the beginning, since it is a theme which will continue to recur in different contexts throughout this thesis. The importance and significance of this aspect will be apparent when consideration is given to the subject of chemical evolution and the origin of life, and again when one considers the possibility of life existing, or having existed, on other planets. Furthermore, any organic geochemical approach to an understanding of the earth's evolutionary history must establish at the outset by what criteria one might recognise hydrocarbons produced from a biogenic source, from hydrocarbons produced abiogenically.
Today organic geochemistry no longer concerns itself solely with the problem of petroleum formation. Of course this problem still remains, and the crux of this problem still centers around two phenomena: a) the genesis of the hydrocarbons of petroleum, and b) the migration and subsequent accumulation of petroleum. In order to trace the development of organic geochemistry since the decline of the inorganic theories of petroleum formation and to gain an understanding into other fundamental problems to which the organic geochemical approach now addresses itself, three aspects merit some consideration and discussion in this introductory chapter.

1. **Historical or classical organic geochemistry**

2. **Chemical evolution and the origin of life**

3. **The organic geochemical approach to the origin of life and the concept of the "biological marker"**

Each of these aspects serves in vindicating the organic chemist's approach to the search for life forms at the earliest period of geological time.

1. **Classical Organic Geochemistry**

We return to the origin of petroleum. Several excellent reviews are available which discuss this subject in great detail (Meinschein, 1959, 1961, 1963a; Robinson, 1964; Philippi; 1965). These provide an historical presentation of the development of various theories for the origin of petroleum. In addition they present a critical assessment of all the evidence now available which bears on this problem. It is intended here to give only a brief resume of the historical development of organic geochemistry, inasmuch as it is relevant to the subject matter of this thesis.

In the early 1900's a knowledge of the geological environment in which petroleum was found weakened any theories which demanded high temperatures and pressures for the formation of hydrocarbons. Oil was being found almost
exclusively in the much milder environments of sedimentary basins. Such a discovery finally discredited theories which involved the destructive distillation of biological detritus, a theory proposed by Engler (1893) after he had distilled hydrocarbons from fish oil. Gradually, what has become to be known as the classical theory of the origin of petroleum -- namely, that oil is the accumulation of organic compounds, principally of the hydrocarbon type, derived from biological precursors -- began to gain its followers. They supported the idea that organic matter was slowly transformed into hydrocarbons and they endeavoured to reproduce such transformations in the laboratory. This experimental approach, which had been adopted by such workers as Zelinsky (1927, 1928, 1931) and Trask (1930, 1942) was aggravated by the geological time factor involved in such transformations and many criticisms of the classical theory rested on the chemists not paying sufficient adherence to the exact geological conditions in their laboratory experiments.

In 1935, the German chemist, Treibs, carried out experiments which represent the first of their kind in the field of organic geochemistry. His findings brought about the final demise of the high-temperature theories and provided, for the first time, clear, unequivocal evidence from a chemical standpoint for the biogenic origin of petroleum. His experimental approach laid the foundations of organic geochemistry as it is known today. Treibs (1934, 1935, 1936) in a series of analyses established the presence of certain pigments whose structural characteristics could be related to present day animal and plant pigments. These pigments were recognised by Treibs as porphyrins and are structurally similar to chlorophyll and hemin. The porphyrins were found in mineral oils complexed with the transition metals vanadium and nickel. Since porphyrins are unstable compounds at temperatures even below 250°C it was felt that the environment in which oil was found
could not have exceeded such temperatures, and was probably very much lower than 250°C. These compounds represented the first fossil porphyrins. Since that time Blumer (1950, 1951, 1952, 1956, 1961, 1962) has continued the work of Treibs and many more fossil pigments have been characterised. Vanadium porphyrins were generally associated with marine sediments, whereas nickel was found more frequently in fresh water sediments. Figure 1 shows the structure of a typical fossil porphyrin (vanadyl deoxyphylloerythrin etioporphyrin) and the biological compound, chlorophyll a, from which it is probably derived.

Despite this discovery of Treibs of porphyrins in mineral oils, organic geochemistry received no further impetus until the early 1950's. Further studies were hampered by experimental difficulties in having to isolate very small quantities of individual compounds from complex organic mixtures. Treibs was probably able to isolate porphyrins from mineral oil because of the ease of extraction and isolation of pigments from the remainder of the organic matter. No further progress was made until the advent of chromatographic methods for the analysis of complex organic mixtures. In the period following the development of chromatographic techniques, and especially gas-liquid chromatography, considerable evidence began to accrue that the
DIAGENETIC ROUTE TO THE PETROPORPHYRINS

CHLOROPHYLL α

PHYTYL SIDE CHAIN
(C_{20})

VANADYL DEOXYPHYLLOEERYTHROETIOPORPHYRIN

Figure 1.
hydrocarbons in petroleum originated from biological precursors, a hypothesis that had been advanced by Whitmore as early as 1943. Smith (1954) was the first to report hydrocarbons in recent marine sediments. The age and optical activity of these hydrocarbons provided evidence for the biogenic theory. Later studies indicated that the hydrocarbon extract from the recent marine sediments showed considerable differences from ancient oils and sediments. Chibnall and Piper (1934) had found that odd-carbon number normal hydrocarbons were more abundant than the even-carbon homologues in plant and insect waxes. The prominence of the n-C\textsubscript{27}, n-C\textsubscript{29} and n-C\textsubscript{31} hydrocarbons is particularly striking. A similar distribution to that found by Chibnall and Piper was observed by Evans \textit{et al}. (1957) in recent marine sediment extracts, and has been used as evidence for a biogenic origin of some sediment hydrocarbons. This argument was based on an established biosynthetic pathway in nature.

The most recent developments in modern instrumentation, especially gas-liquid chromatography (GLC) and mass spectrometry (MS), and the combined GLC-MS instrument, has greatly facilitated the characterisation of individual hydrocarbons in petroleum fractions. These techniques have enhanced our current knowledge of the constituents of petroleum. The evidence points overwhelmingly to hydrocarbons derived from biological precursors. General agreement exists today on the hypothesis proposed by Whitmore as early as 1940.

Many of the major difficulties concerning the origin of petroleum still remain, however. No satisfactory explanation has been put forward to explain how biological material is transformed into crude oil, the so-called diagenetic pathway of hydrocarbon genesis. Many models have been proposed. In a recent review, G. T. Phillippi (1965) has emphasised the importance of the geothermal gradient in the formation of petroleum, a gradient which
indicates a direct correlation between hydrocarbon content and increasing temperature. Phillipi concludes that petroleum is formed from sediment organic matter essentially by thermal, non-biological processes. The other major problem, that of hydrocarbon migration, remains even more controversial, and no acceptable theory is available. A definitive solution to the problem of the origin of petroleum is awaited.

The abiogenic protagonists for the origin of petroleum have recognised that this question is bound up with the more fundamental problem of how life originated on the earth. Robinson (1964) draws attention to the well-known Fischer-Tropsch process for the synthesis of hydrocarbons in which carbon monoxide and hydrogen react to give water and hydrocarbons:

\[ x \text{CO} + y \text{H}_2 \xrightarrow{300^\circ\text{C/pressure\ catalyst}} \text{hydrocarbons} + \text{water} \]

Abiogenic syntheses of fundamental biological building units such as amino acids have also been demonstrated. In Robinson's own words, "The whole question (of the origin of petroleum) is bound up with the origin of life on earth" (Robinson, 1964). It is to this question that we now turn.

2. Chemical Evolution and the Origin of Life

The term chemical evolution is used to describe that period of the evolutionary history of the earth which spans the geological time scale from the time when the earth is believed to have taken its present form, approximately 4700 million years ago, until the advent of the first living organism. The exact position in geological history of this latter event is as yet indeterminate, but evidence today points to the existence of living organisms at least as old as three billion years. The idea that living organisms might derive in an evolutionary sense from the simple chemical molecules which are their constituents is but a logical extrapolation of
Darwin's evolutionary theory. That evolution should be continuous, not only in the domain of living organisms, was a concept that Darwin had clearly recognised himself, as is evident from this address he gave to the Royal Society. He says:

"You expressed quite correctly my views where you said that I had intentionally left the question of the Origin of Life un canvassed as being altogether ultra vires in the present state of our knowledge, and that I dealt only with the manner of succession. I have met with no evidence that seems in the least trustworthy in favour of so-called spontaneous generation. I believe that I have somewhere said (but cannot find the passage) that the principle of continuity renders it probable that the principle of life will hereafter be shown to be a part, or consequence, of some general laws..." The statement to which Darwin refers, and which he had forgotten, was written earlier, before 1871:

"It is often said that all the conditions for the first production of a living organism are now present, which could ever have been present. But if (and oh, what a big if!) we could conceive in some warm little pond, with all sorts of ammonia and phosphoric acid salts, light, heat, electricity, etc, present, that a proteine compound was chemically formed ready to undergo still more complex changes, at the present day such matter would be instantly devoured or absorbed, which would not have been the case before living creatures were formed." [Taken from Calvin, (1965)]

With these remarks Darwin clearly anticipated the types of experiments known as the "Primordial Atmosphere Experiments" which have produced the protein compounds to which he refers. Inadequate chemical knowledge at that time about the nature of molecules and their interactions prevented further development of these ideas.

Today there are two possible approaches to gaining an understanding of what the evolutionary history of the earth might have been. One of these is to extend the classical approach which was part of the approach adopted by Darwin. The classical approach to the understanding of the evolutionary history of the earth has been to examine the fossil record, in which morphologically recognisable entities can be placed in a chronological perspective which traces this evolutionary path from earliest times. Before a certain
period of geological time, approximately 600 million years ago and at the start of the Precambrian, morphological remains become virtually extinct. Today, the increase in our biochemical knowledge and the advent of analytical techniques which are capable of describing the intimate molecular architecture of individual molecules in acute detail, have provided an outlet from this impasse. The search for life forms at the earliest periods of the earth's history has been continued, not only at the morphological level, but rather at the molecular level. This approach, now adopted by the organic geochemist, has been used to provide evidence for the existence of life forms in Precambrian sediments as old as three billion years.

The other approach has endeavoured to reconstruct the evolutionary development of the earth by trying to simulate, in the laboratory, the types of chemical reactions that could have taken place and given rise to the simple organic molecules that constitute living organisms today. This has met with considerable success. By analogy with the atmospheres of other planets the atmosphere of the primitive earth is considered to be essentially reducing, consisting of methane, ammonia and water. Such a mixture of gases has been subjected to various sources of energy and it has been possible to show the formation of simple molecules upon which present day living organisms are based, molecules such as acetic acid, succinic acid, glycine and other amino acids, purines, and even small-chain peptides. It is hoped that such experiments might lead ultimately to the structures and reactions which are essential to the function of the living organism. Figure 2 is a summary in diagrammatic form of the geological time scale and the concomitant evolutionary developments in a chronological perspective. In addition, the major organic geochemical findings are marked on the time scale and the interval in geological time during which they have been found.
## Figure 2

### Geological Time Scale

<table>
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<tr>
<th>Samples</th>
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<th>Chemical Fossils So Far Found</th>
<th>Geologic Era</th>
<th>Events</th>
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<tr>
<td>MUD LAKE, FLORIDA</td>
<td>0</td>
<td>NUCLEIC ACIDS</td>
<td>CENOZOIC</td>
<td>MAN MAMMALS</td>
</tr>
<tr>
<td>GREEN RIVER SHALE (30 x 10⁶)</td>
<td>2 x 10⁶</td>
<td>CARBONIC ACIDS</td>
<td>TERRESTRIAL</td>
<td>TERRESTRIAL PLANTS</td>
</tr>
<tr>
<td>ANTRIM SHALE (350 x 10⁶)</td>
<td>3 x 10⁶</td>
<td>POLYPEPTIDES, AMINO ACIDS</td>
<td>EARLIEST Vertebrates</td>
<td>EARLIEST VERTEBRATES</td>
</tr>
<tr>
<td>NONESUCH SHALE</td>
<td>4 x 10⁶</td>
<td>FATTY ACIDS</td>
<td>PROTEROZOIC</td>
<td>EARLIEST MULTICELLULAR FOSSILS</td>
</tr>
<tr>
<td>GUNFLINT CHERT (1.9 x 10⁹)</td>
<td>4 x 10⁹</td>
<td>STEARIC ACIDS &amp; TRITERPENES</td>
<td>ARCHEZOIC</td>
<td>BLUE-GREEN ALGAE (FOSSILS)</td>
</tr>
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<td>SOUDAN SHALE (2.7 x 10⁹)</td>
<td>3 x 10⁹</td>
<td>LUPENYRANE ALKANES</td>
<td>MICROFOSSILS</td>
<td>FORMATION OF EARTH</td>
</tr>
<tr>
<td>FIG-TREE SYSTEM (3.1 x 10⁹)</td>
<td>4 x 10⁹</td>
<td>ETHANE, PROpane, PROPYLANE</td>
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**Chemical Evolution**
- **PRIMORDIAL ATMOSPHERE** ([CH₄, NH₃, H₂O])
- **Amino Acids, Polypeptides**
- **Purines, Pyrimidines, Nucleotides**
- **Formation of Earth**
- **Microfossils**
- **Earliest Multicellular Fossils**
- **Earliest Vertebrates**
- **Terrestrial Plants**
- **Man Mammals**
- **Blue-Green Algae (Fossils)**

**Biological Evolution**
- **Earliest Multicellular Fossils**
- **Earliest Vertebrates**
- **Terrestrial Plants**
- **Man Mammals**
It is but a logical extension to apply the criteria that have been established in the search for life forms in the Precambrian to the search for life in space. The possibility exists, within the near future, that explorations of extraterrestrial sites will take place. Hitherto our only information about extraterrestrial life has been provided by samples of meteorites which from time to time have plunged through the earth's atmosphere to the surface of the earth. Although extensive research work has been carried out on these objects, the conclusions have been aggravated by two serious problems: the contamination problem, and the uncertainty of the meteorites' previous history. The analysis of samples brought back from other planets seeks to overcome these difficulties and to establish whether life forms do exist, or could have existed, in such an environment.

3. The Concept of the "Biological Marker"

With this background it is now possible to examine the organic geochemical approach in greater detail and to introduce the concept of the "biological marker". The organic geochemist's approach rests on the following premises: that certain molecules, possessing a characteristic structural skeleton, show a reasonable stability to degradation over long periods of geological time; that their structural specificity is understandable in terms of known biosynthetic sequences, and that their formation by any non-biological means is of low probability. Such compounds have commonly been referred to as "biological markers" or "chemical fossils". The various compounds which have been looked for are the ones that are typical constituents of living organisms today: the nucleic acids, the proteins, the amino acids, the organic pigments, the carbohydrates and the lipids. All these fulfill the structural specificity requirement stated above but only the lipids, and to a lesser extent the class of organic pigments known as the porphyrins,
survive from the earliest period of time to be related to their original form today.

Geochemical studies have been carried out on almost all of these classes of organic compounds, which can be regarded as the basic building units of all living matter. Some organisms are rich in proteins (algae), others are rich in carbohydrates (trees). The type of protein, carbohydrate or lipid may vary from one biological species to the other. However, a similarity is maintained in the basic pattern and in general one can say that the distribution of these classes of organic compounds in plants and animals remains uniform. The purpose of the geochemical study is to investigate the fate of these organic materials in the course of deposition and diagenesis.

Proteins are considered to give rise to the amino acids which have been characterised in sediments. Structurally intact proteins do not survive under geological conditions for any significant period of time; the occurrence of intact proteins has been reported from shells and bones from the Pleistocene upwards (Abelson, 1959; Piez, 1962; Hare, 1961). Amino acids, however, are much more stable and there are claims that they have been found in sediments as old as the Precambrian (Abelson, 1959; Swain, 1961; Harrington, 1962).

Both the carbohydrates and the nucleic acids have been studied to a lesser extent from a geochemical point of view. These compounds are considerably less stable under geological conditions and have been characterised only in the most recent sediments. In addition, the absence of analytical techniques capable of handling very small quantities of material has directed attention to those classes of compounds which are amenable to analysis in such amounts with modern analytical instrumentation. These compounds are the pigments and lipids.
Among the pigments considerable work has been carried out on the porphyrins. The early work on this class of compounds was carried out by Treibs (1935), as mentioned earlier. Since that time porphyrins have been characterised in a variety of oils and sediments of differing ages. The stable tetrapyrrole portion of the molecule has been identified in sediments dating into the Precambrian (Barghoorn et al., 1965).

Of all the classes of organic compounds the lipids have probably received most attention. Among the lipids the presence of a series of hydrocarbons, whose structures are based on the \( C_5H_8 \) isoprene unit, has been invoked as evidence for life-forms in Precambrian times. In particular, two specific hydrocarbons, phytane, thought to be derived from the phytol side chain of the chlorophyll molecule, the green pigment of plants, and pristane, derived from phytol and also present in marine organisms, have been sought.

\[
\text{Phytane} \quad \text{Pristane}
\]

Their architectural skeleton, which shows a methyl branch every four carbons, is stable over long periods of time. Hydrocarbons which can be related to the steroid and triterpenoid class of organic compounds have also been found in ancient sediments. Examples from this class are cholestane and gammacerane, whose structures are shown:

\[
\text{Cholestane (C}_{27}\text{ sterane)} \quad \text{Gammacerane (C}_{30}\text{ triterpane)}
\]
Finally, biogenically unaltered carotenoids have been extracted from terrestrial and marine sediments up to 100,000 years, but they do not survive in sediments which are older than this.

In this introduction, the fundamental premise upon which the organic-geochemical approach rests in gaining an understanding of the earth's evolutionary history has been described. It is essentially chemical in nature and utilises knowledge of the intimate molecular architecture of individual molecules and their biosynthetic pathway towards this objective. In the ensuing chapters, experimental work is described which relates to various aspects of this approach.

ADDENDUM

One of the more notorious theories for the origin of petroleum has been that of Velikovsky ("Worlds in Collision", by Immanuel Velikovsky, Delta Books, p. 53), in which he suggests that the tails of comets, consisting mainly of carbon and hydrogen when they pass through an atmosphere containing oxygen, will be set on fire and "rain" petroleum over the earth. Whereas this theory receives rather startling plausibility from the legends and traditions of primitive peoples, scientific evidence suggests that such an hypothesis is highly implausible.
CHAPTER 2
THE EXPERIMENTAL APPROACH TO ORGANIC GEOCHEMISTRY

INTRODUCTION

No single factor has contributed more to the expansion of our knowledge in the field of organic geochemistry than the development of analytical techniques capable of handling very small quantities of material (~1 μg) isolated from complex organic mixtures. In the early days of the biogenic theory of petroleum formation, many erroneous hypotheses arose due to the lack of sensitivity of analytical techniques at that time. The comprehensive analytical work of Trask (1930, 1942) was unable to detect the presence of hydrocarbons in sediments although his research efforts extended over numerous samples. Now Chibnall and Piper (1934) had already identified hydrocarbon constituents in plants and waxes, and to justify their apparent absence in sediments it was proposed that hydrocarbons were destroyed biogenically in the early stages of deposition. As a result, Whitmore's hypothesis (1946-47), that the hydrocarbons of living organisms were precursors to the hydrocarbons in petroleum, was long in gaining acceptance.

We have already referred to the classical work of Treibs on the isolation of porphyrins from natural bituminous materials including oil, shale, and coal. That Treibs was able to isolate the porphyrins from such material was probably due to the ease of extraction of the pigments as a class, a factor that has already been emphasised in the introduction. Recent work on porphyrins isolated from bituminous materials (Baker, 1966; Morandi and Jensen, 1966) have indicated that porphyrins in petroleum were not a single species but rather a series of compounds, whose molecular weights are considerably higher than that of vanadyl deoxyphyloerythrin etioporphyrin (DPEP). The work of Baker (1966) particularly, has confirmed that such porphyrins,
known as petroporphyrins, are certainly not homogeneous, and up to a dozen homologues are present. In the DPEP series of compounds the molecular weight may be written as $308 + 14M$, where $M$ is an integer ($2$ or greater) which represents the number of methylene groups attached to the porphyrin nucleus. 

This serves to illustrate two points: first, that Treibs, although he thought he was isolating an individual compound, was probably isolating a mixture, which he was unable to detect due to the unavailability of analytical techniques at that time which were capable of detecting such a homologous series (particularly mass spectrometry); and secondly, that although it has been possible to detect the presence of this series by mass spectrometry techniques, it is still not possible to separate the individual homologues from this series, which emphasises the analytical difficulties which continually face any studies in organic geochemistry, not only at the time of Treibs' work, but even today.

The development of chromatographic techniques during the 1950's as a standard analytical method for the analysis of complex mixtures, brought about a resurgence of interest and research effort in the field of organic geochemistry. This resurgence was very much overshadowed by the analytical efforts that were now being directed towards the biological fields. Most organic compounds found in sediments reflect to some degree their chemical structure in the living organisms from which they came. The analytical approach to organic geochemistry was therefore quite similar to that for biochemistry. Paper chromatography provided an analytical method which was capable of producing a qualitative and quantitative determination of individual organic compounds. This method was utilised in much of the amino acid geochemical analysis and to a lesser extent for the sugars and the nucleic acid bases. Thin layer chromatography provided an increase in sensitivity by a factor of 10-100 relative to paper chromatographic methods. This
technique has been used in the analysis of geological materials which are present only in trace amounts, such as the hydrocarbons, fatty acids and the pigments.

In the field of lipid analysis gas chromatographic methods have greatly enhanced our chemical knowledge of the individual constituents of petroleum. For the first time it was not only possible to separate organic mixtures into classes of organic compounds, but it was now possible to separate out the individual components from each of the classes. This was a particularly useful tool in the analysis of hydrocarbons. Improvements and additional refinements in the gas chromatographic techniques in recent years brought about improved separations. Separations of complex mixtures could best be effected on capillary columns.

The ability to separate out individual components from these organic mixtures enabled additional spectroscopic measurements to be carried out on each of these components. It was now possible, in theory at least, to separate out each of the hydrocarbon components from a petroleum fraction and characterise its structures unambiguously. The application of spectroscopic techniques lent themselves particularly to the subsequent structural characterisation. Among those receiving the widest application were:

1) Mass Spectroscopy (MS)
2) Infrared Spectroscopy (IR)
3) Nuclear Magnetic Resonance Spectroscopy (NMR)

In conjunction with gas liquid chromatography, mass spectrometry proved to be the most powerful of these spectroscopic methods. Its ability to provide structural information on samples as small as one microgram made it particularly useful to the organic geochemist. Moreover it was extremely versatile, and provided structural information on a wide variety of classes of organic compounds including the hydrocarbons, the fatty acids and their
esters, the porphyrins and chlorins, and the carotenoids. Among the hydrocarbons mass spectrometry was particularly valuable in the characterisation of the isoprenoid alkanes and the steranes and triterpanes, two hydrocarbon types which have been found in significant quantities in crude oils and sediments. The recent development of high resolution mass spectrometry in the identification of organic compounds has applications in structural problems outside of the organic compounds containing carbon and hydrogen only. From the point of view of the organic geochemist it promises to be most valuable in the characterisation of petroporphyrins, and of many oxygen containing compounds such as fatty acid esters. A most useful technique has just been developed which combines the gas chromatograph and the mass spectrometer. This technique avoids the necessity of having to isolate the individual components from the gas chromatograph and at the moment is the most powerful analytical technique available in organic geochemistry.

Both infrared spectroscopy, ultraviolet spectroscopy and nuclear magnetic resonance have all been utilised in organic geochemical analysis. The limiting factor in each of these methods has been the sample size. Infrared spectroscopy has found wide applicability in the structural characterisation of many isoprenoid hydrocarbons; reasonable spectra in the 1380 cm⁻¹ region could be obtained with about 20 μg. of sample contained in micro-cells and using a beam condenser. Ultraviolet spectra and visible spectra has proved most useful in structural identification of the porphyrins and the carotenoids. Nuclear magnetic resonance has some applications in the identification of hydrocarbons, but generally this technique is limited by the relatively large sample size that is required (1-20 mgs.). Various optical activity measurements have been carried out in certain cases and have provided valuable stereochemical information. In general, however, the stereochemical characterisation of individual components from organic mixtures has been carried out in only a very small number of cases.
The application of these spectroscopic methods in organic geochemistry is well illustrated by the work of Bendoraitis et al. (1963) and Hills and Whitehead (1966). Bendoraitis has isolated a series of isoprenoid alkanes from an east Texas crude oil by GLC and characterised their structures using mass spectrometry, IR, and NMR. Hills and Whitehead, in the characterisation of several pentacyclic triterpanes, including gammacerane, have carried out optical activity measurements, melting-point comparisons between geochemical samples and standards, high resolution mass spectrometric measurements, and IR and NMR measurements; X-ray crystallographic measurements have also been applied. This latter work illustrates the wide range of organic analytical methods that have been brought to bear in the rigorous structural characterisation of organic geochemical constituents.

In conclusion one might mention some of the many different applications of gas chromatography that have been found in the literature: see Meinschein (1961), Slowey et al. (1962), Hunt (1962), Hunt and Jamieson (1956), Orr and Callen (1959) and James and Martin (1952). This is but a selection of the wide applicability of gas liquid chromatography in organic geochemistry.

EXPERIMENTAL

It is not intended to dwell at great length in this thesis with the experimental procedure. This has already been reported in great detail in two other places (Eglinton et al., 1966; Belsky, 1966), and reference to these sources is suggested for specific details of any aspect of the experimental method. In addition, there are many publications in the literature of general experimental techniques in organic geochemistry, and Degens (1964) contains an excellent synopsis of all these methods.

A schematic summary of the experimental procedure is shown in Figure 3. This procedure was adopted for all the sediments that were analysed.
Figure 3. The General Outline of the Procedure
Employed in the Isolation of Hydrocarbons from Crude Oils and Shales.
Throughout this operation the prevention of contamination by materials which were used in the extraction method was carefully controlled. Solvents that had been redistilled and purified were checked for the presence of trace amounts of artifacts. No direct handling of the sample took place after the first ultra-sonication in 4:1 benzene:methanol solution. The neutral grade alumina and the silica gel G which were used for column chromatography and thin layer chromatography were found to contain significant quantities of impurities (1 μg. impurities per 100 grams alumina). A rigorous procedure was adopted, therefore. Before each column chromatography with neutral grade alumina, the column was washed with heptane, benzene and then heptane solvent again. The final heptane extract was evaporated and the residue analysed by GLC. At this stage the alumina was free of impurities. In preparative thin layer chromatography the plates were prewashed twice before each run. Generally this part of the procedure was the most likely to produce contamination, but with appropriate care this danger could easily be avoided. With the Precambrian samples which contained appreciably smaller amounts of total organic extract (0.1 to 0.1 ppm) than recent sediments and shales, it was always advisable to run a control, usually a rock-salt sample or granite. The analytical methods utilised were those that have been described previously (Belsky, 1966). In addition, a few other procedures have been developed and utilised much more widely than before; in particular, co-injection techniques with capillary gas liquid chromatography, and combined gas liquid chromatography-mass spectrometry. The analytical and spectroscopic techniques are now briefly outlined.

1. **Organic Synthesis of Hydrocarbon Standards**
   This will be described in detail in Chapter 4.

2. **Gas Liquid Chromatography (GLC)**
   i) **Preparative GLC.** The instruments used were Aerograph A90 P-2
equipped with a thermal conductivity detector and a 5 ft. by 1/4 in. stainless steel column. The solid supports were generally 60-80 mesh Chromosorb W, acid-washed (DMCS), 100-200 mesh gas chrom. Z, or 100-200 mesh gas Chrom. RA. Helium flow rates were 50-70 ml./min. at 50 p.s.i. Injector and detector temperatures of 200-300 degrees, and 185-280 degrees respectively, were used, depending on the boiling range of the material to be chromatographed. Phases generally used were silicone gum (3% SE-30), 5% TCEPE (tetra cyano-ethylated pentaerythritol), and 2.5% 7 m PPE (polyphenyl ether).

ii) Analytical GLC. An Aerograph 665-1 with 10 ft. by 1/16 in. packed columns was used to display the general patterns of hydrocarbon distribution. Later on, an Aerograph 204-Model B was acquired and used for similar purposes. Both instruments had hydrogen-flame detectors and helium as the carrier gas; flow rates were approximately 30 ml./min.

iii) Capillary GLC. All capillary gas liquid chromatography was carried out using a Perkin-Elmer instrument, Model 226. Capillary column 150 ft. by 0.01 in., 50 ml./min., helium; detector 185°C; injector 305°C; programmed generally 0.5 degrees/min.; Golay columns.

Phases:

a) OS-138 Polyphenyl Ether. Chemically it is an all metasubstituted six-ring polyphenyl ether (semi-polar liquid phase). Aromatic hydrocarbons tend to be retained longer than saturated hydrocarbons, and ketones are retained longer than alcohols with the same boiling points. Temperature range, R.T.-185°C. This phase was found to be ideal for the separation of complex hydrocarbon mixtures which contained no aromatic components. The only disadvantage was the temperature range, so that this phase could not be used for separation of high molecular weight hydrocarbons, for example, the steranes and triterpanes in the Green River Shale.
b) Castor Wax. Chemically it is a hydrogenated castor oil with the triglycerine of 12-hydroxy-stearic acid as the principle constituent. Used for the analysis of essential oils. Temperature range, 75-185°C.

c) Apiezon L. Non-polar hydrocarbon-liquid phase. Used for the general analysis of non-polar materials. Temperature range, 50-250°C. The upper limit depends on the previous conditioning. This phase was found to be suitable for the separation of saturated hydrocarbons. Usually it could separate hydrocarbons of higher molecular weight than the PPE phase. However it was still inadequate for analysis of the steranes and triterpanes of the Green River Shale.

d) SE-30 Silicone Gum Rubber. SE-30 is a methyl type silicone. It is used for high temperature work and can separate out the high molecular weight fraction of the Green River Shale. Temperature range 50-230°C. The SE-30 Golay column was found to bleed considerably at high temperatures, which resulted in the immediate loss of separating properties of the phase. Although great care was taken in the preconditioning procedure it was never possible to prevent this disadvantage, and the phase could only be used for a short period of time.

3. Thin-Layer Chromatography (TLC)

Thin-layer chromatography was used extensively in this treatise on both a preparative and analytical scale in the isolation and identification of pigments in the recent sediment from Mud Lake, Florida. In particular, it has been used in the characterisation of chlorins, such as pheophytin a, and carotenoids, such as β-carotene. An outline of the experimental procedures used is now given.

i) Silver-Nitrate Impregnated Silica Gel Plates. Silica Gel G plates impregnated with 12.5% silver nitrate (James and Morris, 1964)
were prepared as follows: 30 g. of Silica Gel G (Applied Sciences Lab., Inc.) and 7.5 g. of AgNO₃ in 60 ml. of distilled water were slurried, and the resulting mixture used to coat the TLC plates, using a Shandon spreader. The plates were dried in a current of air and then placed in an oven at 118°C for 30 min. Before use, the plates were prewashed with ethyl acetate. The plates were sprayed with a 0.001% solution of rhodamine 6 G and viewed under an ultraviolet lamp.

ii) Mannitol Plates. The mannitol used was Baker and Adamson reagent grade; 65 g. of mannitol in 100 ml. of acetone and 1 ml. of a solution of starch as a binder (5 g. of cornstarch in 10 ml. of distilled water) were mixed in a Waring blender for 45 sec. (Byrn, 1956). This slurry was used to coat the plates and the plates were subsequently air-dried.

iii) Polyethylene Plates. The polyethylene used for chromatography was a low melt-index (M.I.O. 444) from Dow Chemical Company (Byrn, 1966). Twenty five g. of powdered polyethylene in 125 ml. of acetone were slurried without a binder. The plates were coated with a 0.25 mm. thickness and were allowed to dry at room temperature.

4. Mass Spectrometry

Mass spectra were determined on a modified CEC 21-103 C mass spectrometer (for details of modifications and performance see Walls and Burlingame, 1964) equipped with a heated glass inlet system operated at 200°C. All spectra were determined at ionising voltage of 70 eV, ionising current of 10-50 μA and 160-180 V per stage on the multiplier. With more volatile compounds the probe used for introduction of the sample into the glass inlet system had to be pre-cooled with liquid nitrogen for successful operation.

In January, 1967, a mass spectrometer was purchased for the group from Associated Electrical Industries Limited, Manchester, England:
AEI MS 12. The low resolution MS 12 instrument was henceforth used in the analysis of all mass spectra. These spectra were also determined at ionising voltage 70 eV, and ionising current 100 μA; the heated glass inlet system was operated at 175°C.

5. **Combined Gas Chromatography-Mass Spectrometry (GC-MS)**

The Perkin-Elmer capillary gas chromatograph, Model 226 was combined with the mass spectrometer, MS 12, without the use of a molecular separator to diffuse out the helium carrier gas. This arrangement proved quite satisfactory. Flow rates were reduced to 30 ml/min. and the magnetic scan time over a decade was about 2 seconds.

6. **Other Physical Measurements**

   i) **IR Spectra.** IR spectra were recorded on a Perkin-Elmer infrared (Models 137, 237) using a thin film for the liquids. For high resolution infrared spectra of micro samples, the Beckman IR 7 was used with a Beckman beam condenser.

   ii) **NMR Spectra.** NMR spectra were recorded on a Varian A-60 spectrometer, at 60 Mc, with CCl₄ as solvent and tetramethyl silane as an internal standard; values on the tau scale (ppm) are reported with reference to TMS at a value of 10.

   iii) **UV Spectra.** Routine UV spectra were measured on a Beckman DK-2 ratio recording spectrometer with heptane as a solvent. All other spectra were recorded on a Cary-14 spectrometer (wavelengths are given in μm).

**DISCUSSION**

1. **The Problem of Contamination**

   In all organic geochemical analyses serious consideration must be given to the possibility that the sample under examination might be
contaminated either at some time in its previous history or during the laboratory handling procedure. This question becomes increasingly more important when one is dealing with samples which contain very small amounts of organic material such as Precambrian sediments and meteorites.

In the last decade considerable research effort has been devoted to the analysis of carbonaceous chondrites in the hope that this approach might shed some light on the question of extraterrestrial life. Nagy, Meinschein and Hennessy (1961) conducted a series of analyses on the organic extract from the Orgueil meteorite in which the hydrocarbon analysis from this meteorite suggested the presence of extraterrestrial life. Later, Claus and Nagy (1961) claimed that they had identified biogenic particles in the Orgueil meteorite whose morphology was entirely dissimilar to known terrestrial forms. Anders and Fitch (1962) criticised these findings and showed that such particles had a morphology very similar to a common air-borne pollen grain. Anders (1963) went on to criticise the evidence for extraterrestrial life formulated by Nagy et al. on the basis of the hydrocarbon analysis: This criticism has some relevance in the context of this discussion, and it is worth quoting from the text the comments of Anders:

"Carbonaceous chondrites are very porous, with a fractional pore volume of 15 to 20 per cent. They will therefore 'breathe' every time the barometric pressure changes, and since many of their mineral constituents, being of very small particle size, have large surface areas, considerable adsorption of atmospheric constituents will occur. Carbonaceous chondrites are known to adsorb even atmospheric argon and should be even better adsorbents for hydrocarbons. The Orgueil sample studies by Nagy et al. had been stored for a large part of its century-long terrestrial residence in the American Museum of Natural History, New York, N. Y. There are not many places on earth that burn and vaporize fossil fuels at a higher rate than New York City, and since fossil fuels, being of biogenic origin, display a mass spectrum similar to that sought by Nagy et al., only a few micrograms of contamination would be needed to produce a 'biogenic' mass spectrum in the meteorite extract. To be sure,
the bulk of the hydrocarbons in Orgueil, occurring at levels of a few parts per thousand, is of extraterrestrial origin; but in the mass range of interest, contamination with only a few parts per million of biogenic hydrocarbons would alter the mass spectrum appreciably."

Nagy, Meinschein and Hennessy added a rejoinder to this criticism:

"Total hydrocarbons in the range of 0.25 to 2.3 ppm have been reported for air near heavy traffic arteries. There have been no data available to us on high molecular-weight saturated hydrocarbons in city air. Making such reasonable estimates as a change of ± 3 per cent per week in atmospheric pressure, a conceded content of 0.5 ppm of higher saturated hydrocarbons in the air during the 97 years since the fall of the meteorite, the entry of these hydrocarbons into each gram of meteorite by 'breathing' can be calculated to be approximately 4 μg. This is more than 2 orders of 10 below the amount of these materials actually in the meteorite."

The above discussion underlines quite explicitly the problems involved in the analysis of meteorites. The uncertainty when it plunged through the earth's atmosphere and later when it was stored on the museum shelf, the meteorite's past history, where terrestrial contamination was not considered, threatens to undermine any conclusions drawn from these analyses. However, one can estimate the likelihood of contamination to a rough approximation by considering the level of the contamination in the surrounding environment. Blumer and Snyder (1965b) have shown that reagent-grade solvents such as pentane, iso-octane and methanol contain appreciable quantities of pristane and phytane (the concentration of pristane identified from a sample of commercial pentane was 3 × 10^{-6} g./litre) which can be reduced to a lower concentration on distillation. Nagy (1965) and Urey (1966) have presented data indicating optical activity in meteorites. This work was also criticised (Hayatsu, 1966) and the observed rotation was accounted for by the presence of colloidal sulphur particles which scatter and depolarise light. Meinschein et al. (1963b) draws attention to the
dangers of contamination in the handling procedure: from oily hands, wax pencils, pyrolysis particles in the atmosphere. Although all these handling contamination difficulties can probably be avoided with the adequate care, this author feels that the lack of knowledge concerning the meteorite's terrestrial history jeopardises any conclusions that one might draw.

With the Precambrian sediments one is still beset by the same problems because one is dealing with an organic extract that is of the same order of magnitude as that extracted from the meteorites. However, the information concerning the sediment's previous history is more definitive so that the question of contamination is not so critical. Other difficulties are encountered here, such as the problem of migration, which are discussed later. The recent sediments are considerably richer than the Precambrian sediments, and contamination should not be a hazard. The following table gives a representative summary of the total heptane extract from a series of different samples:

<table>
<thead>
<tr>
<th>Precambrian Samples (Years)</th>
<th>Pulverised Rock</th>
<th>Total Heptane Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonesuch Shale (1.0 x 10^9)</td>
<td>18 grams</td>
<td>3 mgs</td>
</tr>
<tr>
<td>Gunflint Chert (2.0 x 10^9)</td>
<td>1824 grams</td>
<td>2.1 mgs</td>
</tr>
<tr>
<td>Soudan Shale (2.7 x 10^9)</td>
<td>470 grams</td>
<td>5 mgs</td>
</tr>
<tr>
<td>Fig-tree (3.1 x 10^9)</td>
<td>1000 grams</td>
<td>20 µg</td>
</tr>
<tr>
<td><strong>Meteorite</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orgueil Meteorite</td>
<td>6 grams</td>
<td>100 µg</td>
</tr>
<tr>
<td>Devonian Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrim Shale (300 x 10^6)</td>
<td>480 grams</td>
<td>540 mgs</td>
</tr>
</tbody>
</table>
In conclusion, then, the problem of contamination is a serious consideration in any organic geochemical analysis and indicates that the most stringent precautions must be observed when analysis begins on the first samples returned from the moon.

2. Instrumentation

The value of combined gas chromatography-mass spectrometry has been shown in a recent paper by Oro (1967), in which he analyses the organic extract from the Fig-tree series whose age is considered to be $3.1 \times 10^9$ years. This Precambrian sediment contains a particularly small quantity of organic material ($\approx 0.1$ ppm). However, using the GC-MS instrument it is possible to obtain excellent mass spectra of the individual components which are present in very small quantities. The evidence indicates that both pristane and phytane are present. This analytical device obviates the necessity of having to isolate each component by GLC and reduces the contamination danger by minimising the handling operations. The combined GC-MS will prove invaluable in all organic geochemical analysis.

Mass spectrometric instrumentation techniques are presently being developed in organic geochemistry for the separation and structural characterisation of complex organic molecules. It is hoped to bring the most advanced mass spectrometric instrumentation, data processing, and interpretative expertise to bear upon the identification of organic molecular structures while expending the smallest amount of sample (microgram and submicrogram).
CHAPTER 3
THE CHRONOLOGICAL APPROACH TO ORGANIC GECHEMISTRY

GENERAL INTRODUCTION

In the last decade intensive research efforts have been devoted to the study of petroleum and oil shales of widely differing ages. The objectives of such a pursuit have not always been the same, nor has the chronological approach been adopted in a systematic manner. The petroleum industry has concentrated its attention on the structural determination of the individual components of petroleum. Detailed knowledge of composition is needed to fully characterise crude oils and to provide a foundation upon which theories of the origin and maturation of petroleum can stand. In this context the work of Martin, Winters and Williams (1963), Dean and Whitehead (1961), Bendoraitis et al. (1962), and Cummins and Robinson (1964) have shown the presence of a homologous series of normal alkanes and the presence of isoprenoid hydrocarbons in significant quantities in petroleum. The value of a chronological study lies in the attempt to establish a correlation between the morphological evidence and the chemical components of the organic matter in the rock. Furthermore, to understand the post-depositional transformations that take place in the rock it is necessary to study the chemical state of these components at different periods of geological time. Finally, a chronological study represents not only a reinforcement of the premise upon which organic geochemistry rests but it also offers the possibility of tracing on the molecular level the direction of biological evolution. It might be possible to arrange in an approximate order of evolutionary sequence the various classes of carbon compounds found in
sediments. Thus the gammacerane skeleton (see Chapter 1) might be considered more "primitive" than that of cholestane, since it is synthesised directly by enzymic cyclisation of squalene without any subsequent modification of the pentacyclic structure.

Chemotaxonomy attempts to classify living species on a chemical basis rather than on a morphological basis. Organisms differ quite markedly in the compounds they synthesise. In proposing theories for the origin and formation of petroleum it is essential to know the distribution of the various classes of organic compounds in living systems, and also in recent sediments.

Much research work has been directed towards chemotaxonomic studies. Alkanes have a wide distribution in both plants and animals. In plants they tend to be found in the cuticle waxes which act as protective coatings on leaves and stems. These waxes usually consist of a complex mixture of hydrocarbons that have very similar chemical and physical properties. Generally the concentrations of hydrocarbons do not exceed a few tenths ppm of the living matter. In a few cases, e.g. pristane, they can be highly concentrated in marine organisms. In general, alkanes of carbon number C_{25} - C_{35} are the dominant ones and there is a strong predominance of odd-carbon number alkanes over even-carbon number alkanes.

The most common lipids in plants and animals are fats which are esters of glycerol. On hydrolysis they yield a series of a long-chain fatty acids which may be saturated or unsaturated. The long-chain fatty acids are of even-carbon number. Among the saturated fatty acids palmitic acid, C_{16}, and stearic acid, C_{18}, are most abundant; among the unsaturated fatty acids palmitoleic acid and linoleic acid are most abundant. A great variety of other lipid-type compounds are
distributed in nature, including the terpenes, the steroids and the waxes. For a more detailed account of the chemical taxonomy of plants the reader is referred to publications by Swain (1963), and by Eglinton and Hamilton (1967).

In organic geochemical studies the isoprenoid hydrocarbons, and to a lesser extent the normal, iso- and anteiso-, homologous series, have been most studied. The saturated isoprenoid hydrocarbon, pristane, has been commonly isolated from animal and particularly marine sources (Sorensen and Mehlum, 1948; Bendoraitis et al., 1962; Blumer et al., 1963; Mold et al., 1963a). However, it is noticeably lacking in contemporary plants. Mono-olefins possessing the pristane skeleton have been isolated from zooplankton (Blumer and Thomas, 1965). An isoprenoid acid, from which pristane might be derived, has also been isolated from natural sources such as butterfat (Hansen and Shorland, 1953) and ox-blood (Lough, 1963). In addition, Ackman (1967) has recently isolated a series of isoprenoid acids from marine sources, the \( \text{C}_{16} \), \( \text{C}_{19} \), \( \text{C}_{20} \) isoprenoid acids. In contrast to pristane there are few reports of phytane occurring in living systems though it can be reasonably postulated to derive from phytol, which is part of the chlorophyll molecule. This aspect will be discussed at length in Chapter 6. For the present, this survey illustrates the ubiquitous presence of hydrocarbons in living organisms. Rough calculations indicate that although the level of hydrocarbon concentration is very small in living organisms, the total content is an order of magnitude greater than that necessary to account for the total hydrocarbon content of known petroleum deposits to date. This point is worth emphasising for despite the studies of Chibnall and Piper (1934) on the waxes in the 1930's, the proposal of Whitmore (1946-47) that hydrocarbons should also be found in sediments
was long in gaining acceptance and led to the mistaken belief that hydrocarbons were destroyed by bacterial action at the sediment surface.

Investigations by Smith (1952, 1954), Stevens et al. (1956), and Meinschein (1957) show that hydrocarbons are present in nearly all sediments. The level of concentration of hydrocarbons is quite low, and is generally of the order of a few tenths to a few hundredths ppm (Degens, 1965, p. 261). The hydrocarbons found in biogenic materials, soils, sediments and crude oils show considerable similarities. There is a preference of odd- over even-numbered normal alkanes in recent sediments that is not so marked in ancient sediments. Many of the compounds identified in crude oils and sediments can be related to biogenic precursors. However, there are differences also. Light hydrocarbons occur in ancient sediments and crude oils but are absent from living organisms and recent sediments. In addition, there is a greater abundance of hydrocarbons in ancient sediments. The isoprenoid hydrocarbons have been consistently found in crude oils and sediments of moderate ages (mesozoic and palaeozoic), in concentrations vastly greater than would be expected if they were derived on a thermodynamic basis. This is well illustrated by the work of Cummins and Robinson (1964) and by Eglinton et al. (1966) on the Green River Shale from Colorado. Fatty acids have also been isolated from recent sediments. In contrast to most biological systems, where fatty acids are even-numbered (Shorland, 1954), acids with odd numbers of carbon atoms are found along with those having even numbers of carbon atoms.

Recently several studies have been made on the hydrocarbon extracts from Precambrian sediments. These have attracted particular attention in the hope that the analysis of such extracts would provide evidence in establishing when life processes began on earth. Both Eglinton et al.,
(1964) and Meinschein et al. (1964), concurrently but independently, have provided evidence for the presence of the saturated isoprenoid hydrocarbons, pristane and phytane, in the Precambrian Nonesuch Formation at the White Pine, Michigan, and in the oil seeping from it \((1.0 \times 10^9 \text{ years})\). Additional evidence has been provided by these workers for the presence of porphyrins and steranes in the same formation. The same two isoprenoid hydrocarbons were characterised in a chert from the Gunflint Iron Formation \((1.9 \times 10^9 \text{ years})\) on the North Shore of Lake Superior (Oro et al., 1965b). These results agree with the micropaleontological observations made on the Gunflint chert by Barghoorn and Tyler (1963, 1965). Recently, evidence has been provided for the presence of isoprenoid hydrocarbons in the Soudan Shale Formation \((2.7 \times 10^9 \text{ years})\) from Minnesota (Meinschein, 1965; Belsky, 1966) and from the Precambrian of South Africa \((3.1 \times 10^9 \text{ years})\) in the Figtree series of the Swaziland system (Oro, 1967b). Barghoorn's extensive research efforts have complemented these results with the finding of micro-organisms in these Precambrian samples (Barghoorn et al., 1965c, 1966). This work provides strong evidence for Precambrian life existing at this period of geological time.

One final point should be emphasised. In carrying out the organic geochemical study one makes the assumption at the outset that the overall biochemistry of past organisms is similar to that of present day organisms. The isoprenoid hydrocarbon skeleton identified in Precambrian sediments is synthesised in biological systems by a very specific biosynthetic pathway. Whether such molecules could have been produced in Precambrian times without the assistance of those molecular systems, in particular the information-bearing system and the catalytic system, which form the basis of living organisms today, is the subject of Chapter 5.
Despite the interest that has been directed towards an organic geo-
chemical study of sediments of differing ages, this represents but a
small portion of the information that can be derived from such studies.
There is a need to increase that knowledge by further analysis of crude
oils and sediments. Part of this thesis has been oriented towards such
a chronological study, and the experimental work and results are now
described.

EXPERIMENTAL - RESULTS

PART I. The Organic Deposits from Mud Lake, Florida

1. Introduction

The richly organic algal ooze from Mud Lake, Florida has recently
been considered to represent a modern-day precursor of oil shales of
the type exemplified in the Green River Formation (Bradley, 1966).
Microscopic characteristics of the oil shale of the Green River Forma-
tion and its fossil micro-organisms indicate that the precursor of such
oil shales might indeed be such an algal ooze, accumulating gradually
in shallow lakes. The theory that algal oozes could give rise to oil
shales is not new (Zalessky, 1914, 1916; Thiessen, 1925; White, 1926;
Blackburn and Temperley, 1935-36). Algae have less cellulose and a
correspondingly greater proportion of fats and soaps. The contemporary

*Dr. W. H. Bradley writes: "This work of Temperley is definitive. Much
of the argument is negative and rests on the fact that by far the great-
est amount of organic matter in Temperate Zone lakes and ponds consists
of fragments of leaves of deciduous trees. This stuff and all higher
(cellulosic) plants from mosses up, decay anaerobically to produce hu-
mates and lignin, i.e., coaly material, whereas algae and bacteria,
waxy spore exines, etc., are not rich in aromatics but things may, or do,
convert somehow into hydrocarbons or potential hydrocarbons. Botryococcus
is actually the only fully reliable evidence for the 'oil shale or ker-
gen from algae theory'", (Personal Communication).
alga Botryococcus is present in microscopic remains in organic oozes. To complement this geological and paleobotanical evidence, the organic geochemist can isolate and identify the constituents of the genera of the blue-green algae known to be present in the Mud Lake, and compare them with the organic compounds isolated and identified in the Mud Lake, and at a later stage of "diagenetic" degradation in the Green River Formation. A comparison of the findings from these sources would represent, if the algal ooze precursor theory is justified, the detailed and precise analysis of the changes taking place at the molecular level from the contemporary genera of blue-green algae, through the post-Pleistocene deposits of the Mud Lake deposits, to the Eocene Period of the Green River Formation.

2. **The Environment of Mud Lake, Florida**

Mud Lake is about 18 miles northeast of Ocala, Florida, in Marion County. It is roughly circular in shape and about one mile in diameter. A narrow belt of tree jungle (usually shallowly flooded) forms an unbroken ring around the lake. Lakeward from the tree jungle belt is a mat of vegetation ranging in width from less than 10 feet to something like 200 feet. The outer part of this mat consists of floating plants. Beyond the edge of this mat no rooted aquatic plants grow, except for one very small patch of yellow water lilies. The lake bottom is bare and consists of greenish-grey flocculent ooze. At the edge of the mat, the water is about one foot deep and gradually deepens lakeward to about 2-1/2 feet during the dry season to about 3 or 3-1/2 feet during the rainy season. The entire lake bottom is made up of flocculent algal ooze; tiny fish are common all through the lake (see Figures 4 and 5). No

*Diagenesis = post-depositional transformations*
Figure 4. General view of Mud Lake, Florida.
Figure 5. Taking Eh measurements on Mud Lake, Florida with Dr. W. H. Bradley.
stream enters the lake; it is entirely rain and spring fed. In the
spring, clouds and patches of a green alga, *Spirogyra*, ranging from a
few inches across to 2 or 3 feet, are extremely abundant all through
the lake. Three species, *S. insignis*, *S. mirabilis*, and *S. triplicata*
were identified. For a detailed account of a microscopic examination
of the algal ooze, the reader is referred to the published work of Dr.

The algal ooze does not decay in wet, warm, oxidising environments.
Studies are in progress to determine why such a decay has not taken
place, but as yet no satisfactory theory has been proposed. Such a
theory would represent a breakthrough in our knowledge about the forma-
tion of oil shales.

3. Physical Measurements

The samples from the Mud Lake Deposits were kindly supplied by Dr.
addition, some of these physical measurements were carried out in Dr.
Bradley's laboratory and are included in this account to provide the
complete experimental data on the Mud Lake samples.

The samples for analysis will be designated as follows:

- **MW-0** = Mud Lake algal ooze at the mud water interface.
- **MW-1.6** = Mud Lake algal ooze at 1.6 feet, respectively,
  below the mud water interface.

1) The elemental analyses of the various levels are indicated in
the following table:
The $^{14}C$ ages of the sediments are:

<table>
<thead>
<tr>
<th></th>
<th>MW-1</th>
<th>MW-2</th>
<th>MW-3</th>
<th>MW-4</th>
<th>MW-6</th>
<th>Oil Shale</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>40.06</td>
<td>40.36</td>
<td>41.17</td>
<td>52.20</td>
<td>80.50</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>6.39</td>
<td>6.45</td>
<td>6.87</td>
<td>5.32</td>
<td>10.30</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.75</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>4.16</td>
<td>3.82</td>
<td>1.39</td>
<td>2.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1.13</td>
<td>2.62</td>
<td>2.59</td>
<td>1.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

The following data were obtained by Dr. W. H. Bradley with various sensing probes during a field trip to Mud Lake, Florida (February, 1967). The data are plotted in Figure 6.

\[
\text{Log } S = \text{Log (total free sulphur content)}
\]
\[
\text{Log } \Sigma \text{CO}_3 = \text{Log (sum of total carbonate concentration})
\]
\[
= \text{H}_2\text{CO}_3 + \text{HCO}_3^- + \text{CO}_3^-
\]

### Organic Geochemical Analysis of the Mud Lake Deposits

The isolation scheme shown in Figure 7 represents the scheme that we have utilised in the extraction of the organic components from Mud Lake, Florida (specifically MW-2).

Other extraction schemes have been utilised, and the efficiency of these procedures are reported:
Figure 6. A summary, in diagrammatic form, of several physical parameters at various depths of the Mud Lake.
Figure 7. The isolation of β-carotene from the Mud Lake - general outline.
2.17 g (MW-3) → (benzene:methanol [4:1 v/v], sonicate 45 min.) →
   120 mg total extract.
   17 mg heptane solubles.
8.28 g (MW-5) → (benzene:methanol [4:1 v/v], sonicate 30 min.) →
   98 mg total extract.

1) **Heptane Extract.** The heptane soluble organic material was ob-
tained from the heptane extract in the partition between the solvents
heptane and methanol. Further separation of the organic constitu-
tants in this extract by silicic acid column chromatography has provided con-
vincing evidence for the presence of β-carotene in the Mud Lake deposit,
MW-2. The visible spectra of this compound is identical with a similar
compound isolated from the level MW-0. The spectroscopic evidence for
the presence of β-carotene is indicated below:

   a) **Mass Spectrometric Analysis.** All samples were determined
      on a modified C.E.C. mass spectrometer, Model 21-103 C, with ionising
      voltage 70 eV, and an inlet heated to about 200°C.

      **Standard:**
      m/e 536 (M); m/e 368 (M-168); m/e 236 (M-300).

      **Mud Lake Sample:**
      m/e 536 (M); m/e 420 (M-116); m/e 368 (M-168); m/e 236 (M-300).
      Also molecular ions present at m/e 548, 550, and 629.

   b) **Visible Spectra.** All spectra were recorded on a Cary-14
      spectrometer (values in μy).
<table>
<thead>
<tr>
<th>Solvent</th>
<th>MW-2</th>
<th>MW-0*</th>
<th>Standard β-Carotene</th>
<th>(Karrer)**</th>
<th>Lit.***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>max</td>
<td>474</td>
<td>476</td>
<td>Hep-</td>
<td>479</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>466</td>
<td>466</td>
<td>tane</td>
<td>.469</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>448</td>
<td>449</td>
<td>Sol-</td>
<td>.451</td>
</tr>
<tr>
<td></td>
<td>sh.</td>
<td>429</td>
<td>428-29</td>
<td>vent</td>
<td>.430</td>
</tr>
<tr>
<td>Benzene</td>
<td>max</td>
<td>489</td>
<td>490</td>
<td>.492</td>
<td>494</td>
</tr>
<tr>
<td></td>
<td>min</td>
<td>482</td>
<td>480</td>
<td>.484</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>463</td>
<td>462</td>
<td>.465</td>
<td>463</td>
</tr>
<tr>
<td></td>
<td>sh.</td>
<td>442</td>
<td>442</td>
<td>.441</td>
<td>440-44</td>
</tr>
<tr>
<td>CS₂</td>
<td>max</td>
<td>505</td>
<td>506</td>
<td>.506</td>
<td></td>
</tr>
<tr>
<td></td>
<td>max</td>
<td>480-81</td>
<td>481</td>
<td>.482</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sh.</td>
<td>456</td>
<td>457</td>
<td>.458</td>
<td></td>
</tr>
</tbody>
</table>

- max. = maximum, min. = minimum, sh. = shoulder

A comparison of the visible spectra of standard β-carotene and that isolated from the Florida Mud Lake is shown in Figure 8.

Structure of β-Carotene
Figure 8. Visible spectra of standard β-carotene compared with visible spectra of β-carotene isolated from Mud Lake.
c) Thin Layer Chromatography. The visible spectra recorded
above represent the spectral characteristics of the heptane eluate (see
Isolation Scheme, Figure 7) from silicic acid column chromatography.
Subsequent purification by thin layer chromatography using silica gel-G
plates, impregnated with 12% silver nitrate after the methods of De Vries
(1962) and Morris (1962) indicates that this fraction contains some
saturated alkanes and a small amount of unsaturated material, in addi-
tion to the \( \beta \)-carotene which remains at the origin.

<table>
<thead>
<tr>
<th>Eluate</th>
<th>Material</th>
<th>Rf. values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptane eluate</td>
<td>Saturated alkanes</td>
<td>0.9 → 0.95</td>
</tr>
<tr>
<td></td>
<td>Unsaturated alkanes</td>
<td>0.1 → 0.15</td>
</tr>
<tr>
<td></td>
<td>( \beta )-Carotene</td>
<td>0</td>
</tr>
</tbody>
</table>

**TLC Conditions**

a. The places were prewashed with ethyl acetate.
b. 118°/30 min.—activating conditions.
c. 0.01% Rhodamine B as detector.
d. 95% iso-octane—5% benzene as de-
veloper.

d) Gas-Liquid Chromatography. The saturated alkanes from MW-2
shows great similarity to those isolated from the MW-0 sample.* There
is a dominance of the \( n-C_{27} \), \( n-C_{29} \) and \( n-C_{31} \) alkanes, and an odd-even
normal hydrocarbon alternation is observed throughout. The distribution
of the saturated alkanes is shown in Figure 9.

**ii). Methanol Extract.** Evidence is given for the presence of
xanthophyll in the Mud Lake Sample, MW-2.

The outline on the following page represents the scheme employed
in the analysis of the methanol extract from the Mud Lake Sample, MW-2.

*W. V. Van Hoeven, Ph.D. Thesis, University of California, Berkeley
(to be published).
Figure 9. Gas chromatograph of total heptane solubles isolated from Mud Lake.
Crushed Rock (17.0 grams)

Yellow Brown Residue

Heptane Extract (30 mg) ← Methanol Extract (25 mg)

Silicic Acid Chromatography (325 mesh) Dim light.

Eluates

<table>
<thead>
<tr>
<th>Benzene</th>
<th>Benzene:Acetone</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pale Yellow</td>
<td>Dark Orange</td>
<td>Orange Band</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orange Band (2.5 mg)</td>
</tr>
</tbody>
</table>

The eluates could be divided into two distinct categories:

1) Pale-Yellow Green Bands -- Showing strong absorption at 408 μm and 667 μm regions, suggesting the presence of chlorophyll-like pigments.

2) Dark-Orange Bands -- Showing strong absorption in 440-490 μm region, suggesting the presence of carotenoid pigments.

The subsequent analysis of Eluate 10 was continued:

a) Visible Spectra. All spectra were recorded on a Cary-14 Spectrophotometer; wavelengths are given in μm.

The orange band shows an intense red coloration in carbon disulphide, a characteristic exhibited by the carotenoid xanthophyll.
This spectral evidence and the corresponding solvent shifts suggest the presence of xanthophyll. A comparison of the visible spectra of standard xanthophyll and that of the pigment extracted from the Mud Lake MW-2, are shown in Figures 10 and 11, respectively. The Mud Lake pigment is not completely pure, however. The visible spectrum shows a small absorption at 667 μm. In addition, the distortion of the 400 μm region

![Xanthophyll](image-url)
Figure 10. Visible spectra of yellow carotenoid pigment isolated from Florida Mud Lake (MW-2).
Figure 11. Visible spectra of standard xanthophyll.
compared to the standard xanthophyll may reasonably be attributed to small absorptions at 408 μ. Further chromatography on both silicic acid and polyethylene columns failed to remove this component.

b) **Thin Layer Chromatography.**

Silica Gel G: Activated 132°C/25 min.
Solvent: 70% Acetone/30% Benzene.
Thickness: 3 mm

The presence of one major component and three minor components is indicated. The major component was orange in color and had a retention time very similar to that of standard xanthophyll; the other components were pale yellow.

c) **Infrared Spectrum (Perkin-Elmer 137 Model)**

Absorption at 3300 cm⁻¹

No carbonyl absorption — argues for the presence of an hydroxy group.

Analysis of methanol extracts from MW-3 and MW-6 have provided tentative evidence for the presence of pheophytin a and rhodoxanthin, respectively:

1) From **MW-3 (see Figure 12)**

<table>
<thead>
<tr>
<th>Absorption</th>
<th>μ</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>666</td>
<td>666</td>
<td>Ether solvent</td>
</tr>
<tr>
<td>532</td>
<td>532</td>
<td></td>
</tr>
<tr>
<td>505</td>
<td>505</td>
<td></td>
</tr>
<tr>
<td>408</td>
<td>408</td>
<td></td>
</tr>
</tbody>
</table>

*"There is no uniformity in the literature regarding the designation of this compound. Some investigators employ the term "lutein" proposed by KUHN, and use the term xanthophyll only as a genetic term for hydroxyl-containing carotenoids, for which Karrer proposed the term phytoxanthins. In this monograph the name xanthophyll is retained for the yellow leaf pigment C₄₀H₆₀O₂."* — P. Karrer and E. Jucker, *Carotenoids*, Elsevier Press, New York, 1930, p. 197.
Figure 12. A comparison of the visible spectrum of standard pheophytin a with that of the sample from Florida Mud Lake (MW-3).
2) From NW-6 (Cary 11): NW-6 Sample

<table>
<thead>
<tr>
<th>Hexane</th>
<th>Benzene</th>
<th>MeOH</th>
<th>Cyclohexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>461</td>
<td>478</td>
<td>456</td>
<td>464</td>
</tr>
<tr>
<td>484</td>
<td>500</td>
<td>481</td>
<td>490</td>
</tr>
<tr>
<td>517</td>
<td>531</td>
<td>512</td>
<td>521</td>
</tr>
</tbody>
</table>

Standard Rhodoxanthin

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>458</td>
<td>474</td>
<td></td>
<td></td>
</tr>
<tr>
<td>489</td>
<td>504</td>
<td></td>
<td></td>
</tr>
<tr>
<td>524</td>
<td>542</td>
<td>(P. Karrer et al., ibid)</td>
<td></td>
</tr>
</tbody>
</table>

The spectroscopic evidence for xanthophyll and rhodoxanthin are summarized in Figure 13.

5. Microhydrogenation

To augment our physical measurements on the organic pigments isolated from the Mud Lake, Florida, we have hydrogenated these individual components and subjected the hydrogenated product to mass spectrometric analysis. This procedure would seem to be particularly suitable for members of the carotenoid series, where the low volatility of the carotenoid pigment aggravates the mass spectrometric examination. In addition, the fragmentation pattern of the perhydro-form will provide more definitive structural information than the corresponding mass spectrum of the unhydrogenated form which generally will only confirm the molecular weight of the compound.

The Brown micro hydro-analyser (Delmar Scientific Laboratories) is a specialised glassware unit for determination of unsaturation of micro and ultramicro samples by means of catalytic hydrogenation reactions.

---54---

**XANTHOPHYLL**

**VISUAL SPECTRA**

<table>
<thead>
<tr>
<th>IN BENZENE</th>
<th>MUD LAKE</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>482</td>
<td>487</td>
<td></td>
</tr>
<tr>
<td>473</td>
<td>482</td>
<td></td>
</tr>
<tr>
<td>455</td>
<td>461</td>
<td></td>
</tr>
<tr>
<td>431</td>
<td>437</td>
<td></td>
</tr>
</tbody>
</table>

**INTENSE RED COLORATION IN CS$_2$**

**INFRARED**

- OH GROUP; 3300 cm$^{-1}$

*The Xanthophyll structure is incorrect. See p.48 for correct structure.*

**RHODOXANTHIN**

**VISUAL SPECTRA**

<table>
<thead>
<tr>
<th>HEXANE</th>
<th>BENZENE</th>
<th>CYCLOHEXANE</th>
<th>METHANOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>461</td>
<td>478</td>
<td>464</td>
<td>456</td>
</tr>
<tr>
<td>484</td>
<td>500</td>
<td>490</td>
<td>481</td>
</tr>
<tr>
<td>517</td>
<td>531</td>
<td>512</td>
<td>512</td>
</tr>
</tbody>
</table>

---XBL 674 1046---

Figure 13. A summary of the spectral data for: a) xanthophyll, and
b) rhodoxanthin.
Using this technique it is possible to carry out hydrogenation reactions on $5 \times 10^{-5}$ mole of unsaturation with an accuracy of 0.5 to 1.0 percent. Sodium borohydride is used for both the generation of catalysts (platinum-charcoal catalysts) and for the source of hydrogen for the hydrogenation reaction, a procedure which eliminates the need for hydrogen cylinders and subsequent purification of the gas before use. A typical procedure is now outlined.

EXPERIMENTAL

A 2.0 cc solution of standard $\beta$-carotene in heptane (5 mg/cc) was hydrogenated in a Brown micro-hydroanalyser for nine hours using $\text{P} + \text{O}_2$ catalyst and a trace of acid. The hydrogenation was slow to start and was allowed to proceed for this length of time to ensure complete hydrogenation of the $\beta$-carotene. The product was isolated from the reaction mixture and dried.

- YIELD: 7 mg

- GLC analysis of the perhydro product at 300°C isothermally on 3% SE 30 column (25' x 1/4") indicated two peaks (25 min. retention time) which suggested that the starting material was not completely hydrogenated.

- MASS SPECTROMETRIC ANALYSIS of one of the peaks gave the following: m/e 558 (M), m/e 556, m/e 547, m/e 545, m/e 531 (M-29), m/e 492. There is almost certainly some partially hydrogenated material in this sample. Using this procedure and comparing the mass spectrum of the standard perhydro-form with that of the hydrogenated pigment isolated from the Mud Lake, it was possible to confirm the presence of $\beta$-carotene in the Mud Lake sample. Such a procedure can be used for other carotenoid pigments also.
PART II. San Joaquin Valley Oil

1. Introduction

A sample of a typical San Joaquin Valley oil was provided by the courtesy of Dr. L. Lindemann of the California Research Corporation, Richmond. The oil is considered to be about $30 \times 10^6$ years old, and the source rocks are thought to be of marine deposition.

The extraction procedure adopted is similar to that used by Eglinton et al. (1966) in the analysis of the Nonesuch Seep Oil and is outlined below:

```
San Joaquin Valley Oil (2.78 g)
   neutral alumina
       Total heptane eluate (1.75 g)
       neutral alumina
           Total benzene eluate (0.73 g)
           Total methanol eluate (~0.2 g)
           Residual (~0.1 g)
           U-V absorbing material (0.95 g)
               'Total' alkanes (0.80 g)
                   Branch-Cyclic Fraction (~50%)
                   Normals (~40%)
                       GLC
                       mass spectra
```
2. Gas Liquid Chromatography and Mass Spectrometry

The 'branch-cyclic' fraction derived from the 'total' alkane mixture by sieving was further analysed by preparative gas chromatography using a 3% SE 30 preparative column. The GLC pattern is shown in Figure 14. Individual peaks were collected and subjected to mass spectrometric analysis. The mass spectra of these fractions provided definitive evidence for the presence of the \( \text{C}_{16} \), \( \text{C}_{18} \), \( \text{C}_{19} \) and \( \text{C}_{20} \) saturated isoprenoid hydrocarbons. There is also good evidence for the presence of the \( \text{C}_{15} \) isoprenoid, farnesane, though the mass spectrum of this fraction is not as pure as those of the other fractions. The straight chain \( \text{C}_{15} \) alkane, \( \text{C}_{15} \)H\(_{32} \), has also been isolated from the 'normal' fraction and its structure has been confirmed by mass spectrometry. The mass spectra of the saturated isoprenoid hydrocarbons are shown in Figure 15. The gas chromatograms of the 'total', 'branch-cyclic', and 'normal' fractions, using an aerograph 665-1 instrument, 3% SE 30 phase, are shown in Figure 16. Rough calculations were made of the relative proportions of the saturated isoprenoid hydrocarbons present in the San Joaquin oil and these are shown in Table I. The normal hydrocarbons range in carbon number from \( \text{C}_{10} \) to \( \text{C}_{23} \), and maximize at \( \text{C}_{13} \).

<table>
<thead>
<tr>
<th>Isoprenoid carbon no.</th>
<th>Relative ratios</th>
<th>% Branch-cyclic fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{C}_{16} )</td>
<td>1.3</td>
<td>0.38</td>
</tr>
<tr>
<td>( \text{C}_{18} )</td>
<td>1.0</td>
<td>0.30</td>
</tr>
<tr>
<td>( \text{C}_{19} )</td>
<td>1.3</td>
<td>0.38</td>
</tr>
<tr>
<td>( \text{C}_{20} )</td>
<td>1.1</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Figure 14. 'Branch-cyclic' fraction of San Joaquin oil on gas chromatographic preparative column.
Figure 15. Mass spectra of the isoprenoid hydrocarbons isolated from the San Joaquin oil.
Figure 16. Gas chromatographs of 'total', 'branch-cyclic' and 'normal' fractions from the San Joaquin oil.
3. **Infrared**

The heptane eluate from the San Joaquin Valley Oil shows characteristic hydrocarbon absorption bands at \(2930 \text{ cm}^{-1}\), \(1480 \text{ cm}^{-1}\), \(1380 \text{ cm}^{-1}\) and \(1020 \text{ cm}^{-1}\).

4. **Electron Spin Resonance**

When the total benzene eluate is evaporated and an electron spin resonance spectrum obtained on this organic extract, the spectrum indicates that the transition metal vanadium is present. In view of the fact that absorption of the benzene eluate fluoresces this combined evidence suggests the presence of metal-porphyrin complexes in the San Joaquin Oil.

---

**PART III. Antrim Shale**

1. **Introduction**

A sample of the Antrim Shale from Midland County, Michigan, was provided by Mr. R. D. Matthews of Dow Chemical Company in the form of a core taken from a depth of 2608 feet. The Antrim Shale is the northern part of a large deposit which extends to the south and is referred to there as the Chattanooga Shale (Breger and Brown, 1962). The Antrim is considered to be Mississippian-Devonian in age from the spores in the shale, but there has been an academic argument about the placement of the geological time line for the Mississippian-Devonian boundary.

In a personal communication we received from Mr. R. D. Matthews, he drew our attention to the uncertainty concerning the age of the Antrim. He originally placed the Antrim at 265 million years:
"The Michigan Geological Survey has published a new stratigraphic succession in Michigan which places the Antrim in the Late Devonian (Chautauquan). A chronology published by Hough, which is based on work by Ladd and Ahrens, places the end of the Devonian at about 265 million years."

However, in a later communication he revises this estimate:

"The Antrim Shale of Michigan is lithologically similar to and occupies the same stratigraphic position as black shales in Ohio and the East Central United States. It is not continuous from Michigan to Ohio, and no paleontological evidence has been found which definitely establishes the age of the Michigan and Ohio units is the same...You should consider it Upper Devonian...

As to dating by geochronometry, Marshall Kay (1965) refers to Late Devonian as 350 million years ago. This is based on basal Middle Devonian rocks (by fossils) in Nova Scotia which have been intruded by granites having a calculated age of somewhat more than 350 million years."

In conclusion, then, the Antrim Shale from Michigan is one example of a Paleozoic sediment. It is a black shale (Upper Devonian, ca. 350 x 10^6 years old), correlating with other Chattanooga-type shales which extend under many square miles of the Central United States and have been much studied geologically. This latter age is the presently accepted one and differs from the one we have reported in earlier publications (Belsky et al., 1965; Johns et al., 1966; McCarthy and Calvin, 1967).

2. Analytical

The carbonaceous Antrim Shale is both rich in total carbon content and in extractable organic material. The percentage composition is shown in Table II.

<table>
<thead>
<tr>
<th>Table II. Antrim Shale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon content</td>
</tr>
<tr>
<td>Hydrogen content</td>
</tr>
<tr>
<td>Sulphur content</td>
</tr>
<tr>
<td>Total solvent extractables</td>
</tr>
</tbody>
</table>
The extraction procedure was similar to that used for the Soudan Shale and has been described in detail in an earlier report (Belsky, 1965). The core was crushed to a fine powder and the organic material isolated by sonication in 4:1 benzene-methanol solvent. The extraction scheme is outlined below:

- **Antrim Shale (479 g)**
  - Sonicate
  - 4:1 benzene-methanol
  - **Total Extractables (2.21 g)**
    - Neutral Al₂O₃
    - Heptane eluate
  - **'Total' Alkanes (0.75 g)**
    - 'Branch-cyclic' fraction (765%)
    - 'Normal' fraction (735%)

The individual components of the 'branch-cyclic' fraction were further analysed by gas liquid chromatography and mass spectrometry. Fractions were collected from the preparative column, 3% SE 30 phase (see Figure 17), and further purified on two additional phases: polyphenyl ether and tetracyanopentaerythritol. Members from four different homologous series were isolated and identified in the branch-cyclic fraction from the Antrim Shale: the saturated isoprenoid hydrocarbons, and the iso-, anteiso-, and cyclohexyl normal alkanes. The gas chromatograms of the 'total', 'branch-cyclic' and 'normal' alkanes using an Aerograph 665-1, and 3% SE 30 phase (column: 10' x 1/g"), are shown in Figure 18. The normal alkane distribution, which ranges from C₁₀-C₂₁.
Figure 17. 'Branch-cyclic' fraction of the Antrim Shale on gas chromatographic preparative column.
Figure 18. Gas chromatographs of 'total', 'branch-cyclic' and 'normal' fractions from the Antrim Shale.
Figure 19. Capillary gas chromatograph of normal alkanes from the Antrim Shale.
is shown in Figure 19, using a capillary gas chromatograph and Apiezon L phase.

A series of saturated isoprenoid alkanes, the C₁₆, C₁₈, C₁₉, C₂₀, and the C₂₁ isoprenoids, were characterised in the Antrim Shale. The chromatographic fractions corresponding to these particular compounds are indicated in Figure 17. The corresponding mass spectra of these compounds are shown in Figure 20. In addition, several other fractions were isolated from the 'branch-cyclic' fraction and their structures are indicated in Table III (refer to Figure 17):

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mass Spectrum</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No mass spectrum</td>
<td>C₁₅ isoprenoid, coinjection evidence</td>
</tr>
<tr>
<td>2(a)</td>
<td>Many peaks: m/e 226; m/e 238 prominent</td>
<td>mixture</td>
</tr>
<tr>
<td>2(b)</td>
<td>m/e 226(M) (Fig. 21)</td>
<td>C₁₆ iso-alkane</td>
</tr>
<tr>
<td>3</td>
<td>m/e 226(M) (Fig. 21)</td>
<td>C₁₆ anteiso-alkane</td>
</tr>
<tr>
<td>4</td>
<td>m/e 240(M) (Fig. 29)</td>
<td>C₁₇ isoprenoid (cf. Chap. 4)</td>
</tr>
<tr>
<td>5</td>
<td>Many peaks</td>
<td>mixture</td>
</tr>
<tr>
<td>6</td>
<td>m/e 254, not pure</td>
<td>C₁₈ iso-alkane</td>
</tr>
<tr>
<td>7</td>
<td>m/e 252; also m/e 264, 278 (Fig. 20)</td>
<td>C₁₈ cyclohexane, though not pure</td>
</tr>
</tbody>
</table>

The relative proportions of each of the isoprenoids present are listed in Table IV:
The characterisation of the C\textsubscript{17} saturated isoprenoid hydrocarbon represents the first report of this isoprenoid in the literature. The significance of such a finding and the detailed experimental work are described in Chapter 4.

A homologous series of iso-, anteiso-, and cyclohexyl alkanes have been isolated and identified in the Nonesuch Seep Oil (Johns et al., 1966). In the Antrim Shale two members of the iso- series have been identified: the C\textsubscript{16} iso-alkane, whose mass spectrum is shown in Figure 21 and the C\textsubscript{18} iso-alkane; the mass spectrum of the latter indicates that there is more than one component present even though prominent peaks are observed at m/e 254 (M) and m/e 211. Only one member of each of the anteiso- and cyclohexyl series has been identified: the C\textsubscript{16} anteiso-alkane (mass spectrum in Figure 20), and the C\textsubscript{18} cyclohexyl alkane. The mass spectrum of the C\textsubscript{18} cyclohexyl alkane (Figure 20) indicates that several components are present which are contributing to m/e 254 and m/e 278. This structural assignment is somewhat uncertain.

Many of the other fractions which were isolated remain unidentified. The mass spectra of these fractions indicates that a mixture of

---

**Table IV**

<table>
<thead>
<tr>
<th>Carbon number</th>
<th>Relative ratios</th>
<th>% Branch-cyclic fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{16} isoprenoid</td>
<td>9</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>C\textsubscript{18} isoprenoid</td>
<td>9</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>C\textsubscript{19} isoprenoid</td>
<td>5.5</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>C\textsubscript{20} isoprenoid</td>
<td>4.5</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>C\textsubscript{21} isoprenoid</td>
<td>4</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>
Figure 20. Mass spectra of the isoprenoid hydrocarbons, a cyclohexyl normal alkane and a C₁₆ iso-alkane, isolated from the Antrim Shale.
Figure 21. Mass spectrum of a) C\textsubscript{16} iso-alkane, and b) C\textsubscript{16} anteiso-alkane.
compounds is present. There are indications that both saturated and unsaturated components are present but no specific structure can be assigned to any one of these components. In this respect the Antrim Shale appears to be a more complex mixture of organic compounds than does the Nonesuch Seep Oil, where members of each of the homologous series were isolated in reasonably pure form.

PART IV: The Precambrian

The search for evidence of life processes in Precambrian sediments has been extended to the oldest carbonaceous sediments known on the Earth. Some of the work carried out by this group and by other workers has already been alluded to in the general introduction. In this work we directed our attention to three Precambrian sediments from three different continents:

A. The Soudan Shale from North East Minnesota in the Lake Superior region, $2.7 \times 10^9$ years old;

B. The Kalgoorlie Shale from the Australian Precambrian, whose age is considered to be $2.9 \times 10^9$ years old; and

C. The Fig-Tree Shale from South Africa, whose age is considered to be $3.1 \times 10^9$ years old.

A. The Soudan Shale

1. Introduction

The oldest carbonaceous rocks that are known in the North American continent are associated with the Soudan Iron Iron Formation of North East Minnesota in the Lake Superior region. The age of the Soudan Shale is believed to be greater than two billion years. Metamorphic minerals
in associated rocks give a potassium-argon age of \( 2.5 \times 10^9 \) years, and
an intruding granite has given uranium-lead and potassium-argon age
determinations which are in agreement at \( 2.7 \times 10^9 \) years old (Cloud
et al., 1965).

Samples were kindly supplied by Professor Preston Cloud, Jr. of
the University of California, Los Angeles. To avoid confusion it should
be stressed that two samples of the Soudan Shale have been examined:

Sample I: The Soudan Iron Formation cut from a surface exposure
north of Tower, Northeastern Minnesota--The Surface Sample.

Sample II: A sample taken from the 21st level of the Soudan mine,
Soudan, Minnesota, at a depth of 1800 feet below the ground--The Mine
Sample.

Cloud's description of the Soudan Iron Formation refers to the Mine
Sample, primarily. He first obtained samples from a known surface out-
crop north of Tower, "and then to minimise chances of recent contamina-
tion and to obtain material richer in carbon", he studied the mine
sample. A more detailed account of the geological features of the
Soudan Iron Formation is given by Goldich and others (1961).

2. Analytical

Sample I is stratigraphically related to Sample II, but differs
radically in its elemental composition, as the following table shows:

<table>
<thead>
<tr>
<th></th>
<th>% C</th>
<th>% H</th>
<th>% S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>3.05</td>
<td>--</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Sample II</td>
<td>5.16</td>
<td>0.40</td>
<td>25.28</td>
</tr>
</tbody>
</table>

Sample I has been previously analysed and has been reported by Belsky
(1966) and Johns et al. (1966). The analytical data on Sample II is now
reported. The elemental analysis shows a great difference in the sulphur content for the two samples. A striking difference also exists for the two samples in the following ratio:

\[
\frac{[\text{n-heptane soluble extract}]}{[\text{benzene-methanol (4:1) extract}]}
\]

as shown in Table V.

<table>
<thead>
<tr>
<th></th>
<th>Heptane soluble extract</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>280 \text{ mg}</td>
<td>.97</td>
</tr>
<tr>
<td>Sample II</td>
<td>5 \text{ mg}</td>
<td>.002</td>
</tr>
</tbody>
</table>

[The benzene-methanol (4:1) extract of Sample II includes considerable elemental sulphur. This was removed by the method of Blumer (1957)].

An exhaustive analysis of the heptane soluble extract, involving purification and isolation of individual components by GLC techniques and subsequent structural identification by mass spectrometry, was not carried out for Sample II. Such a comprehensive analysis on Sample II was beset with difficulties on account of the very small heptane soluble extract obtained from the rock, and the unavailability of the combined gas chromatograph-mass spectrometer. A comparison of the GLC distribution patterns of the total heptane soluble extract for the two samples is shown in Figure 22. The GLC patterns show some similarities despite the marked contrast between the samples' elemental composition. Isoprenoid hydrocarbons have not been unambiguously identified in Sample II, but coinjection evidence suggests that they are present in proportions similar to those found in Sample I. Variations are to be expected on the basis of the comparison between the elemental composition, which may
Figure 22. A comparison of GLC patterns of total heptane soluble extract from a) Soudan mine sample, and b) Soudan surface sample.
indicate a different ecological source for the hydrocarbons or alternatively a different environment under which the diagenetic processes took place.

B. The Kalgoorlie Sample

1. Introduction

Samples of Kalgoorlie Shale were kindly supplied to us by Mr. R. Woodall of the Western Mining Corporation Limited, Kalgoorlie, Western Australia. This sample was obtained from a diamond drill hole located 1-1/2 miles south of the Southern limit of mining on the main Kalgoorlie gold lodes. The age of the Kalgoorlie Shale which has been dated by the Sr-Rb method is considered to be 2890 \pm 560 million years, or about 2.9 \times 10^9 years old. The geology of the Kalgoorlie Shale has been described by Wilson et al. (1960).

2. Analytical Data

The elemental composition of the Kalgoorlie Shale is shown in Table VI.

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Depth (ft.)</th>
<th>% C</th>
<th>% H</th>
<th>% S</th>
</tr>
</thead>
<tbody>
<tr>
<td>3322</td>
<td>2661-2662</td>
<td>4.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3323</td>
<td>2889-2891</td>
<td>2.25</td>
<td>0.16</td>
<td>6.98</td>
</tr>
<tr>
<td>3225</td>
<td>5998-6000</td>
<td>1.51</td>
<td>0.24</td>
<td>0.45</td>
</tr>
</tbody>
</table>

The extraction of the organic material from the powdered shale was carried out according to the experimental procedure already described. The elemental sulphur was removed by the method of Blumer (1957) using a copper colloidal column. The scheme is outlined on the following page.
Very small quantities of heptane soluble material were obtained from the sample of Kalgoorlie Shale. The GLC distribution pattern shows a broad, unresolved hump of organic material, resembling the extract from the Soudan Shale (Sample II) but without the characteristic peaks that are shown in Figure 22. The approximate carbon number was in the C\textsubscript{15}-C\textsubscript{25} range. Broad cuts were collected and analysed by mass spectrometry. This served only to confirm the approximate carbon number range of the organic extract. No further analysis was carried out.

C. A Sample of Carbonaceous Shale, Fig-Tree Series, Swaziland System

1. Introduction

The Fig-Tree series of the Swaziland system is in Eastern Transvaal, South Africa. The carbonaceous silicate rocks from the Fig-Tree series were collected in the Sheba gold mine near Baberton, South Africa. The rock analysed was a black chert, and is considered to be $3.1 \times 10^9$ years old.

2. Analytical Data

The elemental composition of the Fig-Tree sample is shown on the following page:
The extraction procedure is similar to that outlined for the Soudan and Kalgoorlie samples. Again, only a small amount of total heptane soluble material was obtained from the crushed rock by sonication in 4:1 benzene-methanol. In a second extraction the crushed powder was digested in 48% HF for 36 hours. No additional organic material was obtained.

**Fig-Tree Sample (164 g)**

<table>
<thead>
<tr>
<th>Benzene-methanol (4:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.4 mg</td>
</tr>
<tr>
<td>Removal of elemental sulphur</td>
</tr>
<tr>
<td>Total Heptane Soluble (&lt; 1 mg)</td>
</tr>
</tbody>
</table>

The GLC pattern of the total heptane extract suggests the presence of normal alkanes in a homologous series. However, this evidence has only been provided by coinjection techniques and has not been confirmed by separating out the normals using molecular sieves and isolating each component for mass spectrometric analysis. In other respects the GLC pattern resembles quite closely that for the Soudan Shale (Sample II).

**DISCUSSION**

The analysis of a series of samples of different geological age has enabled one to i) make a comparative study of oils and sediments with the intention of identifying as many individual components as possible, and ii) carry out a systematic study of the components at selected carbon
numbers in an attempt to obtain some experimental indication of the course of diagenesis of the hydrocarbon materials with time.

The preliminary results from the Mud Lake Sample, Florida, indicate that this sample represents a typical example of a recent sediment. The normal hydrocarbon distribution in recent sediments has been investigated by several workers. Evans et al. (1957) and Kvenvolden (1962) have shown that the odd-carbon-number distribution of the normal alkanes predominates over the corresponding even-carbon-numbers in the C_{23}-C_{25} carbon range. Kvenvolden also comments on the conspicuous absence of lower molecular weight normal alkanes in recent sediments, particularly in the C_{6}-C_{13} region. Stevens et al. (1956) have observed the dominance of the n-C_{29} and n-C_{31} alkanes. These features are especially characteristic of the normal alkane distribution in recent sediments, and as Meinschein (1961) has noted, such a distribution is very different from that in crude oils. The total heptane extract from the Mud Lake exhibits a normal alkane distribution characteristic of a recent sediment. There is an odd- to even- predominance, and the n-C_{27}, n-C_{29}, and n-C_{31} alkanes predominate in the higher molecular weight region. One further aspect is worthy of comment. In a previous publication (Johns et al., 1966) we have identified the n-C_{17} alkane in the Nostoc variety of blue-green algae as the major hydrocarbon component. The ooze at the mud-water interface and below for about 6 inches consists wholly of minute fecal pellets, which are made up almost exclusively of blue-green algae. Although the Nostoc form of blue-green algae has not been characterised in these fecal pellets, the n-C_{17} alkane might be common to all forms of blue-green algae, and therefore would be expected to occur in the Mud Lake. Since this is not observed, it is just possible that the n-C_{17} alkane is singular to the Nostoc variety. A chemical analysis of the
predominant forms of blue-green algae in the Mud Lake -- Aphanathece, Oscillatoria limnetica, Microcystis incerta, and Spirulina, would confirm this conclusion.

Carotenoids have been characterised in very recent sediments and in sediments as old as 100,000 years. They do not appear to survive into geological periods earlier than this. Within this time span the carotenoids seem to be remarkably preserved in the chemical state in which they have been found in living organisms; they are not much altered by geological conditions. Only Vallentyne (1957) has provided tentative evidence for diagenetic changes taking place where β-carotene has isomerised from the all-trans form to a cis-isomer. This, however, represents the only report of such a change. A summary of the carotenoid findings in recent sediments is given in Figure 23.

The identification of β-carotene, and the spectroscopic evidence which points to the presence of xanthophyll and rhodoxanthin, is in keeping with the findings of other workers. At the post-Pleistocene period it would seem that diagenetic changes have not taken place to any significant extent. This complements the findings of remarkably preserved fecal pellets, of which an estimated 10% are viable. However, its significance is diminished in view of the fact that β-carotene is also preserved in other recent sediments which do not have the preservation properties of the algal oozes. It would therefore be worth investigating algal ooze deposits which are much older in age than those from Mud Lake, Florida, or, alternatively, examine another class of organic compounds, such as the nucleic acids and the carbohydrates, to see if they have been affected by the geological environment in a period of 10,000 years.
Figure 23. A synopsis of the carotenoid findings in recent sediments.
The data plotted in Figure 6 are of some significance. At a depth of one foot below the mud-water interface, log S and log $ECO_3^-$ have a peak in abundance and, conversely, the $E_h$ curve has a peak showing reducing environment. These three points at a depth of one foot have been interpreted by Bradley as a site of important bacterial activity by facultative anaerobes. In this connection, Dr. A. J. Tousimis (personal communication) has just completed a biochemical and morphological characterisation of the 20 species of bacteria isolated from the Mud Lake in Florida. An examination of the bacteria might reveal whether there are compounds found in these bacteria and in the original samples.

The organic geochemical analysis of the Antrim Shale and the San Joaquin Oil provides a good example of Paleozoic sediment intermediate in time between the more recent sediments and the Precambrian, and of a typical crude oil. Both these samples show the prominence of the isoprenoid hydrocarbons in the 'branch-cyclic' fraction; this homologous series is the major constituent of the 'branch-cyclic' fraction. This aspect is consistently present in the 'branch-cyclic' fraction of a series of sediments and is well illustrated in Figure 24, where the 'branch-cyclic' fractions from the Antrim Shale, the Monesuch Shale, and the Soudan Shale are compared. A comparison of the percentage composition of the isoprenoid hydrocarbons present in different sediments is shown in Table VII.

The Antrim Shale is much more complex in character than the Soudan Shale. It was very difficult to isolate individual components in pure form and the mass spectra of the isoprenoid hydrocarbons from the Antrim Shale (see Figure 20) consistently show small amounts of additional compounds. This aspect will be discussed in detail in Chapter 4. The San Joaquin Oil shows a greater amount of hydrocarbon material in the lower
Figure 24. A Comparison of the ' Branched-Cyclic' Hydrocarbons from the Antrim Shale, the Nonesuch Shale and the Soudan Shale.
molecular weight region, an aspect which is also observed in the Moonie Oil (W. Van Hoeven et al., 1966) and other crude oils. In all respects the San Joaquin Oil is similar to a typical analysis of a crude oil.

Normal alkanes isolated from contemporary plant sources occur with carbon numbers greater than $C_{20}$ (Eglinton and Hamilton, 1963) and show an odd- over even-carbon dominance (Eglinton and Hamilton, 1963; Oro et al., 1965a). We have already mentioned that such distributions are also characteristic of recent sediments. Koons et al. (1965) have shown that normal alkanes from marine sources do not show this odd- to even- dominance. Except for the Green River Shale, such an odd- to even- preference has not been observed in any of the sediments and crude oils which we have analysed. Such a criterion has at times been invoked as evidence for
biogenicity, but it is considered unsatisfactory. The precursors of the normal alkanes, \textit{iso} and \textit{anteiso} series, and the cyclohexyl normal series will be discussed under "Diagenesis" in Chapter 6.

The analysis of the Precambrian samples has emphasised the need for analytical techniques capable of handling very small quantities of material. The continuous transfer operations involved in the extraction and analytical operations aggravate the difficulties. The combined gas chromatograph-mass spectrometer, which has just been acquired in this laboratory, will meet some of these difficulties and will greatly facilitate the analysis of Precambrian samples.

In all three samples analysed, no definitive identifications of individual components were achieved. The biogenic origin of the organic material in these rocks has not been ascertained. The analysis of the Soudan mine sample, however, provided valuable information in connection with the problem of migration; and for this reason was very significant in view of the finding of isoprenoid hydrocarbons in the surface sample. These findings have been challenged in view of the high temperature to which the Soudan Shale had been subjected. The whole problem of migration and the implied lack of homogeneity of the Soudan sample will be discussed in Chapter 5.

Much of the geochemical work on the Soudan mine sample has been carried out by Meinschein (1965) and he has claimed that pristane and phytane are present in the sample. Oro (1967a) has recently reported pristane and phytane in the Fig-Tree system, which represents the oldest findings of these isoprenoids in any sample. This complements the findings of microorganisms in the Fig-Tree by Barghoorn (1966) and, together, constitutes very good evidence, from two different approaches, for the presence of life forms at this period of geological time.
No additional work has been carried out on the Kalgoorlie Shale. This shale would be of great interest because it has been deposited in a different continent, and would give an indication of how widely distributed life-forms were in Precambrian times. The recent findings of microorganisms by Barghoorn and Schopf* from the Bitter Springs Formation in Central Australia seem to suggest that there were indeed life-forms existing in Precambrian times on the continent of Australia.

* Private communication, and subsequently published in Science, 15th October 1965, p.337.
CHAPTER 4

THE SYNTHESIS OF HYDROCARBON STANDARDS IN THE IDENTIFICATION OF

ISOPRENOID HYDROCARBONS IN GEOLOGICAL SAMPLES

GENERAL INTRODUCTION

With the development of new and powerful analytical techniques in the last ten years or so, organic chemistry has demanded a rigorous characterisation of the structures of individual compounds isolated from natural sources. This has been particularly true in the field of natural product chemistry, where the most recent spectroscopic and optical methods have been utilised in structural identification. In many cases total synthesis of the compound isolated from biological sources has constituted the final and absolute proof of the structure.

The field of organic geochemistry is confronted with many of the problems of natural product chemistry. Besides having to isolate individual compounds from complex organic mixtures, one is generally dealing with very small quantities of material. It is desirable, wherever feasible, to carry out as many physical measurements as possible on an individual compound. Thus ultraviolet, infrared and nuclear magnetic resonance spectroscopy, as well as gas-liquid chromatography and mass spectroscopy have all been used to characterise the components of complex mixtures. Organic geochemists have in the past tended to depend very much on mass spectroscopy as an analytical tool in structural identification. In the petroleum industry this has proved most useful. Most of the constituents are hydrocarbon in character, and yield a characteristic fragmentation pattern from which the structure can be
deduced. This has been the case in the identification of straight-chain hydrocarbons, branched hydrocarbons such as those that are isoprenoid in character, and cyclic hydrocarbons. Mass spectrometry has proved capable of assigning all aspects of the structure, except the stereochemistry, to an individual molecule.

Mass spectroscopy has been used extensively in organic geochemical studies. Only recently have some of the limitations of this analytical method been recognised. This is not so much a limitation of the method, but rather a limitation in the sensitivity of the fragmentation pattern to the presence of very small quantities of impurities. The inability of the organic geochemist to isolate pure components from a complex mixture of compounds has given rise to certain ambiguities in structural interpretation. We have already seen this difficulty in Chapter 3, in the interpretation of the structure of the C\textsubscript{18} cyclohexyl normal (Figure 20), where the presence of impurities alters the overall spectrum. As we shall see when we scrutinise the mass spectra of the isoprenoid hydrocarbons from the Antrim Shale more closely, these structural interpretations are also open to ambiguities. Since the designation of the exact positions of the methyl branch in the isoprenoid compounds must be achieved with absolute reliability if these compounds are to be used as "biological markers", it is important to obtain another physical measurement on the compound, other than its fragmentation pattern. Capillary gas chromatography has provided this measurement in the form of elution times of individual components. If standard compounds are available they can be coinjected into the mixture of organic compounds and used to identify specific compounds. If this procedure is repeated on different phases it can be used with a high degree of certainty. Coinjection techniques, in conjunction with mass spectroscopic measurements, have
enabled one to remove most, if not all, of the structural ambiguities. At the moment only the stereochemistry of an individual molecule remains undefined.

In most cases in organic geochemistry standard compounds are not available. Cason and Graham (1965) have shown the value of synthesising standard compounds in structural determination. Weedon et al. (1959) have used this approach in assigning stereochemical configurations to individual isoprenoid molecules. In the ensuing chapter, the synthesis of several standard isoprenoid hydrocarbons is described and their applications in organic geochemistry are discussed.

PART I. The Isolation and Identification of the C₁₇ Isoprenoid, 2,5,10-Trimethyltetradecane, in the Antrim Shale

In Chapter 3, the isolation and identification of a series of isoprenoid hydrocarbons from the Antrim Shale (ca. 300 x 10⁶ years old) was described and the procedure for the extraction of the total hydrocarbon content was outlined. The apparent absence of the C₁₇ saturated isoprenoid hydrocarbon from the Antrim Shale was commented upon by Johns et al. (1966) in a recent report, and this isoprenoid hydrocarbon has been found to be consistently absent from a series of shales and oils that these authors have examined. In the analysis of an east Texas crude oil, Bendoraitis et al. (1953) also failed to report the C₁₇ isoprenoid, which was conspicuously missing from a series of isoprenoid hydrocarbons that they characterised in the oil from C₁₀ to C₂₁. The only other member missing from this series was the C₁₂ isoprenoid, 2,6-dimethyldecane. Other authors (Eglinton et al., 1966; Cummins and Robinson, 1964) have failed to report this isoprenoid hydrocarbon in oils and shales. In view of this apparent absence of the C₁₇ isoprenoid hydrocarbon from
all samples analysed from a geochemical standpoint, it was decided to make a thorough examination of the 'branch-cyclic' fraction of the Antrim Shale in the region where the C₁₇ isoprenoid would be expected to be found.

Diagenetic considerations lead one to suppose that two C₁₇ isoprenoids might derive from a C₂₀ isoprenoid precursor if the assumption is made that phytol is the precursor of the isoprenoid hydrocarbons. These would be:

i) 3,7,11-trimethyltetradecane, produced by a single cleavage;

ii) 2,6,10-trimethyltetradecane, produced by two cleavages.

This is illustrated in the scheme below:

```
  
  3,7,11-trimethyltetradecane   2,6,10-trimethyltetradecane
```

Since a C₁₇ isoprenoid, if present at all in the Antrim Shale, seemed to be there in considerably smaller quantities than the other isoprenoid alkanes, it was decided to synthesise 2,6,10-trimethyltetradecane. Since a compound is inherently less likely to be produced by two cleavages rather than one, this compound would not be expected to be present in significant quantities if the scheme above is correct. Further, a preliminary analysis of the region of interest in the 'branch-cyclic' fraction of the Antrim Shale (that is, fractions 5 and 6 in Figure 17) indicated that at least two, and probably three, components were present which had molecular ions at 240. One of these fractions showed some resemblances to a C₁₇ isoprenoid, having prominent fragmentation peaks at m/e 183 and m/e 155. It was not possible to purify this fraction.
and it was decided that confirmation of the presence of a C_{17} isoprenoid could only be obtained if the standard was available for comparison.

The C_{17} saturated isoprenoid hydrocarbon was synthesised from farnesol using the scheme outlined in Figure 25 and described in detail in the experimental section. Farnesol was used as a starting material primarily because of its ready availability, but also because the valuable intermediate, hexahydrofarnesyl bromide, was produced in the synthetic scheme. This compound is a useful intermediate from which other saturated isoprenoid hydrocarbons can be obtained. Hydrogenation of farnesol generally produces a large proportion of hydrogenolysis, and to avoid this difficulty Cason and Graham (1965) hydrogenated farnesyl acetate and recovered the hexahydrofarnesol by hydrolysis of the hydrogenated acetate. The use of platinum oxide catalyst and a trace of acid produced 95% farnesane, but with a platinum-charcoal catalyst, generated in situ in the Brown hydrogenator, and by carefully controlled acid concentrations, the hydrogenolysis reaction can be kept to a minimum and an excellent yield of hexahydrofarnesol obtained. Two procedures were used for the synthesis of hexahydrofarnesyl bromide, one using anhydrous HBr gas, and the other using the method of Fischer (1928). In the latter case, where phosphorus tribromide and pyridine were employed, it was never possible to reproduce the excellent yield of Fischer and the former method was adopted. In the acetate cracking step three products were produced by pyrolysis of 2-acetoxy-5,9,13-trimethyltetradecane at 500°C. This is to be expected and they probably represent the cis- and trans-forms of 5,9,11-trimethyltetradec-2-ene, and 5,9,11-trimethyltetradec-1-ene. The mechanism of this acetate cracking step will be mentioned in Part II of this chapter in connection with some anomalies.
The synthesis of 2,6,10-trimethyltetradecane.
that seem to occur in the pyrolysis of 4-acetoxy-7,11,15-trimethylhexadecane. Hydrogenation of this mixture of tetradecenes (whose mass spectrum is shown in Figure 33) gave the desired product, 2,6,10-trimethyltetradecane, whose purity was demonstrated by capillary gas chromatography.

This hydrocarbon standard was now used in the structural characterisation of the C\textsubscript{17} isoprenoid isolated from the Antrim Shale. By coinjection techniques on a gas-liquid chromatograph capillary column and by comparing the mass spectrum of the standard with that of the compound isolated, convincing evidence has been provided for its presence in the shale. The gas chromatogram of the 'branch-cyclic' hydrocarbon fraction of the Antrim Shale is shown in Figure 26. The inset marked in Figure 26 is shown in detail in Figure 27, there it is compared with the gas chromatogram of the 'branch-cyclic' hydrocarbon fraction containing the coinjected C\textsubscript{17} saturated isoprenoid hydrocarbon. The gas chromatograms, programmed under identical conditions, are highly reproducible so that coinjection with known standards provides a very sensitive method of characterisation. The coinjection procedure has been repeated on two other phases, SE 30 silicone gum rubber and castor wax,\textsuperscript{*} and the corresponding coincidence again obtained. It should be emphasised that the small increase in relative peak height intensity of the C\textsubscript{17} saturated isoprenoid hydrocarbon shown in Figure 27 is reproducible in every instance under the stipulated conditions. Further, an estimation has been made of the relative proportion of the C\textsubscript{17} isoprenoid hydrocarbon found in the Antrim Shale and this is shown in Table VIII compared with the relative proportion of the other isoprenoid alkanes identified in the shale.

\textsuperscript{*}Standard Perkin-Elmer liquid phases: designation Z and C-H, respectively.
ANTRIM SHALE. BRANCH-CYCLIC. APIEZON L.

Figure 26. Capillary gas chromatogram of 'branch-cyclic' fraction from the Antrim Shale.
Figure 27. Inset from Figure 26. The identification of the C\textsubscript{17} isoprenoid hydrocarbon in the Antrim Shale by coinjection techniques.
Table VIII

Isoprenoid Content of 'Branch-Cyclic' Fraction of Antrim Shale

<table>
<thead>
<tr>
<th>Isoprenoid</th>
<th>% (by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{16} isoprenoid</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>C_{17} isoprenoid</td>
<td>0.05 ± 0.04</td>
</tr>
<tr>
<td>C_{18} isoprenoid</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>C_{19} isoprenoid</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>C_{20} isoprenoid</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>C_{21} isoprenoid</td>
<td>0.19 ± 0.02</td>
</tr>
</tbody>
</table>

(Identified isoprenoids represent approximately 1.6% of the 'branch-cyclic' alkanes of the Antrim Shale.)

Although an accurate estimation of the proportion of the C_{17} isoprenoid cannot be made, it is present in considerably smaller quantities than any of the other isoprenoids. This is in accord with a diagenetic scheme where phytol is considered the biological precursor of these isoprenoid hydrocarbons.

The upper part of Figure 28 shows a collection of the C_{17} isoprenoid region from a 10' x 1/4' preparative column, 3% SE 30 phase, before subsequent purification on two other phases (corresponding to fractions 5 and 6 in Figure 17). The peak labeled "C_{17} isoprenoid" was enhanced by coinjection of the standard. Further purification of the C_{17} isoprenoid was effected by reinjection on and collection from tetracyanoethylated pentaerythritol and seven-ring-meta-polyphenyl ether phases, respectively. The lower part of Figure 28 shows the relative retention times of the C_{17} isoprenoid and the C_{16} iso- and anteiso-alkanes, using 25' x 1/4' column with seven-ring-meta-polyphenyl ether as the phase.
Figure 28.

GLC of mass spectrometry sample collected from C_{17} isoprenoid region of Antrim Shale.

Capillary column: 150' x 0.1".
Split ratio: 100:1.
Program: 1/2° per min.
Apiezon L.

Isothermal: 138°C.
Dimensions: 25' x 1/4".
PRE phase.

C_{16} iso-alkane
C_{17} isoprenoid
C_{16} anteiso-alkane

Antrim Shale: C_{17} isoprenoid region; mass spectrometry collections.
The cut corresponding to the C_{17} isoprenoid was collected and analysed by mass spectrometry.

The mass spectrum of the standard C_{17} saturated isoprenoid hydrocarbon, and that of the sample collected from the Antrim Shale, are shown in Figure 29. The two mass spectra show considerable similarities to each other although certain discrepancies in the relative intensities of the peaks should be noted. This is due to the fact that a completely pure cut is difficult to obtain. The mass spectra were obtained from chromatographs in which the resolution was far inferior to that obtained on the capillary instrument.

The mass spectra of the C_{16} iso- and anteiso-alkanes isolated from the shale are shown in Figure 30 (refer to Figure 20). Consideration of these spectra should be made when comparing the mass spectrum of the C_{17} isoprenoid standard with that of the compound isolated from the shale. In the shale sample there would seem to be contributions from parent molecular ions at m/e 226 and m/e 238. The parent molecular ion at m/e 226, in part, might be reasonably attributed to the presence of the C_{16} iso- and anteiso-alkanes, which have retention times that are very similar to that of the C_{17} saturated isoprenoid hydrocarbon. The mass spectral peaks at m/e 211 and m/e 197, more intense than expected, could also arise from small amounts of the C_{16} iso- and anteiso-alkanes. Further, the C_{16} iso-alkane would contribute to the m/e 183 mass ion, which might account for a more intense m/e 183, relative to m/e 155, in the shale sample than is actually observed for the C_{17} isoprenoid standard. There is also mass spectrometric evidence for unsaturated components, including a mono-olefin of molecular weight 239. The remaining discrepancies might be better understood with a complete knowledge of the structure of the compounds in the cut taken from the C_{17} isoprenoid region of the gas chromatograph shown in Figure 28.
Figure 29. Mass spectrum of the C_{17} isoprenoid standard compared with that of the compound isolated from the Antrim Shale.
Figure 30. Mass spectrum of a) C\textsubscript{16} iso-alkane, and b) C\textsubscript{16} anteiso-alkane isolated from the Antrim Shale.
We have mentioned previously that from diagenetic considerations two C\textsubscript{17} isoprenoids might be expected to arise: i) 2,6,10-trimethyl-tetradecane, and ii) 3,7,11-trimethyltetradecane. Their mass spectra would be expected to have the intense fragmentation peaks shown below:

The two compounds with such basically different fragmentation patterns should be easily distinguished, and the latter structure cannot reasonably be postulated for the structure of the C\textsubscript{17} isoprenoid isolated from the Antrim Shale. In conclusion, we consider that the available evidence argues very strongly for the presence of 2,6,10-trimethyltetradecane as the major constituent of this region.
EXPERIMENTAL

SYNTHESIS OF THE C₁₇ SATURATED ISOPRENOID HYDROCARBON,
2,6,10-TRIMETHYLTETRADECANE.

FARNESOL, C₁₅H₂₆O [222]

The starting material, farnesol, was obtained from Givaudin.

NMR: Doublet at 4.05 δ (5.95 τ), R

Multiplet at 5.13 δ (4.87 τ), CH₂OH

and 4.29 δ (4.61 τ)

Broad singlet at 2.0 δ (8.0 τ), -CH₂-, hydrogens

Singlets at 1.58 δ (8.42 τ), methyl hydrogens

1.68 δ (8.32 τ)

IR: 3.0 μ; 5.99 μ; 10.0 μ.

GLC: indicates that it is probably a mixture of geometrical isomers.

MASS SPECTRUM: m/e 222 (M); m/e 224, 220; m/e 204 (M-18)

n-refractive index: n²².⁷ = 1.4877 [n²⁵ = 1.4855 (Fischer, 1928)]

ANALYTICAL: Found: C, 75.53; H, 10.60; Calculated for C₁₅H₂₆O:

C, 81.08; H, 11.71.

HEXAHYDROFARNESOL, C₁₅H₃₂O [228]
An 8.9 g sample of farnesol (1/25 M = 40 mM) in 25 ml of absolute alcohol was hydrogenated in a Brown\textsuperscript{2} hydrogenator [Delmar Scientific Laboratories; Brown, H. C., Brown, C. A. (1962)], using platinum charcoal catalyst (1.5 g of charcoal: [1/2 g C per 2/10 ml of metal]). The reaction was carried out at room temperature and followed quantitatively: 75\% reaction complete in 40 min. and 90\% reaction complete in 2 hr. After the reaction was 95\% completed, 0.1 g of platinum oxide was added. The reaction became very slow after 4 hr. A further 0.1 g of platinum oxide was added; there was no further reaction.

The crude reaction mixture was centrifuged and the ethanolic solution decanted off. The charcoal residue was heated to boiling in more ethanol to remove any product absorbed by the charcoal catalyst. Solutions were combined and 10 ml of distilled H\textsubscript{2}O added. This solution was extracted 3 times with 25 ml of n-heptane. Any borohydride salts remained in the aqueous layer. The heptane extract was dried overnight over anhydrous magnesium sulphate. The solvent was evaporated.

\textbf{Yield of dry reaction product = 9.1 g (100\%)}

GLC ANALYSIS later indicated that this consisted of:

- 94\% hexahydrofarnesol
- 5\% farnesane -- hydrogenolysis product
- 1-2\% mono, di, -- unhydrogenated starting product
- olefinic material

No attempt was made to remove the farnesane at this stage.

The use of PtO\textsubscript{2} instead of Pt/C gives a 95\% yield of the hydrogenolysis product, farnesane. The reaction is complete in 75 min. The theoretical amount of hydrogen uptake is exceeded. The nature of the catalyst is very critical. Therefore, a small amount of aldehyde (possibly from air oxidation of farnesol) will cause catalyst poisoning and
a slow reaction. This was observed in certain cases.

PHYSICAL CONSTANTS (REACTION PRODUCT):

Colourless, viscous liquid

REFRACTIVE INDEX: 22°C = 1.4392 [n18 = 1.4469; hexahydrofarnesol (Fischer, 1928)]; [n25 = 1.4303; farnesane (Fischer, 1928)]

ANALYTICAL: Found: C, 78.31; H, 13.79

Calculated: C, 78.86; H, 14.13

MASS SPECTRUM: m/e 228 (M), m/e 210 (M-18). Also peaks at m/e 226, 224, and m/e 208, 206.

INFRARED: 3.02 μ; 7.25 μ; 7.30 μ; (well resolved)

NMR: Triplet (2) 3.55 δ (6.95 τ); J = 7.5 cs (integrates to 2 hydrogens)  

R—CH₂—CH₂OH

No olefinic hydrogens

Singlets (2) 1.35 δ (8.65 τ), methylene

(-CH₂-) group

(a) 1.22 δ (8.78 τ), methylene

(-CH₂-) group

Singlets (2) 0.92 δ (9.09 τ), methyl hydrogens

(a) 0.89 δ (9.12 τ), methyl hydrogens

(a) 0.83 δ (9.18 τ), methyl hydrogens

Multiplet (a) 1.3-1.7 δ (8.7-8.3 τ), methine hydrogens

UV: (Cyclohexane solvent: good, down to 190 μ)

Absorption 220-192 μ (Cary Model 14)

indicates some unhydrogenated starting product

GLC: (10'x 1/16", SE 30°, isothermal 148°C;

Aerograph 665-1).
1 major peak (~95%)
5% farnesane
1-2% 2 smaller peaks (possibly unhydrogenated material)

**HEXAHYDROFARNESYL BROMIDE, C$_{15}$H$_{31}$Br [291]**

**HBr Synthesis:** An 8.0 g sample of hexahydrofarnesol (i.e., the reaction product containing 95% of the alcohol) in 35 ml of n-heptane was placed in a three-necked round bottom flask. Anhydrous HBr was passed into the solution. The reaction was stirred at room temperature for 2 hr. and the solution was then heated at 60°C for 10 hr. The reaction solution darkened during this 12 hr. period, suggesting that bromine dissolved in solution. The reaction solution was then cooled. The heptane solution was decanted from the HBr liquid at the bottom of the reaction flask. The reaction mixture was shaken with 50 ml of 5% NaHSO$_3$; a yellow heptane layer was produced. The heptane layer was separated. The NaHSO$_3$ solution was washed three times with 35 ml of heptane; the extracts were combined. The heptane extract was washed with 35 ml of water and then evaporated. The crude product was a yellow liquid. **Yield:** 8 g.

The crude product was purified on a neutral alumina column chromatograph (Merck reagent: 200 g of alumina; 20 cm x 3 cm) and eluted with n-heptane. The heptane eluate was evaporated and the pale yellow liquid was fractionally distilled through a 35 cm Podbielniak column at 1 mm pressure. Three fractions were collected: i) 89°C/1 mm; ii) 89-123°C/1 mm; iii) 123.5°C/1 mm. The first two fractions consisted of a C$_{15}$
hydrocarbon, mainly farnesane. Hexahydrofarnesyl bromide was isolated 
as a colourless liquid: 123.5°/1 mm. Yield: 5.02 g (48%).

**PHYSICAL CONSTANTS:** N, refractive index @ 22.7°C = 1.4605.

**ANALYTICAL:**
- **Found:** C, 61.76; H, 10.77; Br, 27.30.
- **Calculated:** C, 61.9; H, 11.05; Br, 27.45.

**GLC:**
- (using 10'x 1/16"; SE 30; isothermal at 144°C; Aero graph 665-1); indicates 99% purity.

**INFRARED:**
- No absorption at 3.0 μ; 7.25 μ; 7.30 μ; gem-dimethyl: well resolved. 7.94 μ, 8.24 μ, 8.56 μ [Bromine compounds show two bands at 600-500 cm⁻¹ (16.67 μ-20.0 μ)].

These may be the first overtones.

**NMR:**
- Triplet at 3.36 δ (5.6 μ), (2 hydrogens); J = 7 cps
- Broad singlet at 1.29 δ (8.71 μ), -CH₂- hydrogens
- Singlets at 0.91 δ (9.09 μ), methyl group
  - 0.88 δ (9.12 μ), methyl group
  - 0.81 δ (9.19 μ), methyl group
- Multiplet at 1.9 δ-1.4 δ (8.1 μ-8.6 μ), methine hydrogens

**MASS SPECTRUM:**
- m/e 292 (M⁻); m/e 290 (M⁻); (Br⁻⁷⁹, Br⁻⁸¹ in equal proportions).
- m/e 207 (M⁻⁻⁸⁻⁻⁵), m/e 205 (M⁻⁻⁷⁻⁻⁵).

**The Synthetic Procedure of Fischer:** An attempt was also made to 
synthesize hexahydrofarnesyl bromide following the method of Fischer 
(1928). To 8.0 g (3/100 M) of hexahydrofarnesol in 20 ml of redistilled 
n-heptane and 1 ml pyridine was added 8 g of PBr₃ in 10 ml of heptane 
with solutions being maintained between -10°C to -50°C. The temperature 
was not allowed to rise about 0°C during the addition. After standing 
for 12 hr. at room temperature the mixture was heated on a water bath for
4 hr. The reaction mixture was then cooled in ice and poured onto 10 ml H₂O/10 ml 10% NaHCO₃ with vigorous stirring. An emulsion formed which gradually separated on standing. The aqueous layer was extracted 3 times with heptane and the extracts combined. The heptane was evaporated.

The crude product was purified on an alumina chromatography column and fractionally distilled through a 35 cm Podbielniak column: Fraction (1) 98°C/3.5 mm; Fraction (2) 100-125°C/3.5 mm; Fraction (3) 141-142°C/3.5 mm. Fraction (3) represents pure hexahydrofarnesyl bromide.

Yield: 1.50 g (15%); n²³⁰°C = 1.4605.

PHYSICAL CONSTANTS: same as in the first synthesis.

5,9,13-TRIMETHYLTETRADECAN-2-OL, C₁₇H₃₇O [256]

Synthetic Procedure: Formation of Grignard Reagent: (General reference: Cason & Rapoport, "Laboratory Text in Organic Chemistry," p. 472). A 2.5 g sample (1/100 M) of hexahydrofarnesyl bromide in 5 ml of dry ether was slowly added to 0.25 g of magnesium in 5 ml of dry ether contained in a three-necked flask. The reaction was carried out under a nitrogen atmosphere. To initiate the reaction, the solution was gently heated and 3 drops of dibromoethane added. The addition took 1 hr and the green-black solution was heated under reflux for 15 min.

Acetaldehyde was obtained by depolymerising paraldehyde with dilute H₂SO₄. The acetaldehyde was distilled over (21°C) and dried. A solution of 0.35 g (<1/100 M) in 10 ml of dry ether was cooled to -5°C and added to Grignard reaction mixture (also cooled to -5°C). The addition took place with continuous stirring during 30 min. and the temperature
was not allowed to rise above 100°C. After all the acetaldehyde had been added, the reaction mixture was poured onto 10 g of crushed ice and stirred vigorously. This solution was then acidified with 1.5 ml of cold 15% H$_2$SO$_4$. The aqueous layer was separated in a separatory funnel and extracted 3 times with 25 ml of ordinary ether. The total ether extract was dried over MgSO$_4$.

The GLC analysis of the crude product indicated farnesane and the Wurtz-coupling product squalane had also been formed in the reaction together with the expected alcohol. To purify the reaction product further, it was eluted on an alumina column (Merck reagent: 30 g; 10 cm x 1 cm) with n-heptane (about 50 ml eluate) and then with ether. The eluates were evaporated down. Yield (from heptane eluate): 0.600 g (mainly saturated hydrocarbon from GLC, IR evidence. Yield (from ether eluate): 0.652 g (31%).

**PHYSICAL CONSTANTS:**

**INFRARED:**

2.98 μ; 7.25 μ, 7.30 μ.

**GLC:**

(10' x 1/16"; SE 30; 10°/min programm; Aerograph 665-1) indicates >98% purity.

No refractive index measured.

No mass spectrum obtained.

No analysis obtained.

2-ACETOXY-5,9,13-TRIMETHYLTETRADECANE, C$_{19}$H$_{38}$O$_2$ [298]

![Chemical Structure]

The procedure of Cason and Graham (1965) was followed. A 0.65 g sample of 5,9,13-trimethyltetradecan-2-ol, 1.1 ml of acetic anhydride,
4 ml of dry benzene were heated at 80°C for 4 hr. The reaction mixture was then cooled, poured onto 5 g of ice and stirred. The product was extracted with ether.

The crude product contained residual acetic anhydride. This was removed by extracting with NaHCO₃ (10%) and then distilling off any further residue also under vacuum (40°C/1 mm pressure). Yield (of product): 0.59 g (78%).

**PHYSICAL CONSTANTS:**

**GLC:** (10' x 1/16"; SE 30; 10°/min programm; Aerograph 665-1)
- >95% purity (also run isothermally at 150°C).

**INFRARED:** 5.70 μ, 8.02 μ; gem-dimethyl poorly resolved: 7.25 μ.

**NMR:** Quartet at 4.8 δ (5.2 τ); J{cps} = 6.0 cps
Sharp singlet at 1.9 δ (8.1 τ), methyl of acetate
Broad singlet at 1.22 δ (8.78 τ), -CH₂- methylene
Singlet at 1.1 δ (8.9 τ), methyl closest acetate (probably)
Singlets at .91 δ (9.09 τ), methyl hydrogen
.88 δ (9.12 τ), methyl hydrogen
.81 δ (9.19 τ), methyl hydrogen
Multiplet due to methine hydrogens

**MASS SPECTRUM:** No parent molecular ion; m/e 238 (M-60);
m/e 195; m/e 126.

5,9,13-TRIMETHYLTETRADECANES, C₁₇H₃₄ [238]

A 0.59 g sample of 2-acetoxy-5,9,13-trimethyltetradecane was pyro-
lysed at 515°C essentially according to Bailey and Golden (1952) in
1.0 cm x 20 cm pyrex tube filled to a depth of 16 cm with 3 mm pyrex helices. Dry \( \text{N}_2 \) was swept through the apparatus continuously and the acetate was allowed to drop onto the heated helices, drop by drop over 3 min. The pyrolysate was rinsed from the trap with 25 ml of normal heptane. The heptane solution was washed with \( \text{NaHCO}_3 \) (10%) solution, then with water, and dried. To remove the residual acetate, the crude product was chromatographed on an alumina column (Merck reagent grade; 1.0 cm x 8.0 cm) and eluted with 20 ml of n-heptane and then 20 ml of ether. The heptane eluate was dried and evaporated. Yield: 0.227 g (48%).

**PHYSICAL CONSTANTS:**

No refractive index measured.

No analytic composition was taken.

**GLC:**

1. \([10' \times 1/16''; \text{SE} 30; \text{isothermal @} 150^\circ C; \text{Aerograph} 665-1] \)

2 peaks, one with a shoulder, indicating 3 components; ratio: 45:45:10 (%)

2. \([\text{Perkin-Elmer} \text{ Model} 226 \text{ capillary column}; 150' \times .01''; \]

100:1 split ratio; attenuation 1 x 50; \( 2^\circ /\text{min.} \) from \( 140^\circ C \) onwards]. Again, 3 components; 2 of equal intensity, other 1/3 intensity of other two.

This suggests:

5,9,13-trimethyltetradec-2-ene, cis and trans forms

\[
\text{cis} \quad \text{and} \quad \text{trans}
\]

5,9,13-trimethyltetradec-1-ene.

This would account for the three peaks.
INFRARED:
6.07 μ (1645 cm\(^{-1}\)), weak; \(-\text{C} = \text{C}-\)
7.25 μ, 7.30 μ; gem-dimethyl
3.26 μ weak absorption, vinyl absorption
10.35 μ, trans olefinic
10.08 μ, 10.97 μ, vinyl absorption
7.92 μ (probably overtone from 15.8 μ region)

NMR:
5.32 δ (4.68 τ) Ethylenic protons; complex multiplet; coupling constants cannot be determined accurately.
4.72 δ (5.28 τ)
5.00 δ (5.00 τ)

Broad singlet 1.65 δ (8.35 τ) may be methyl adjacent to double bond.
Doublets 1.25 δ, 1.3 δ (8.75 τ) - methylene hydrogens
Normal methyl absorptions around 0.9 δ (9.1 τ)

MASS SPECTRUM: m/e 238 (M); m/e 196, m/e 182, m/e 168, m/e 140
(See Figure 33)

2,6,10-TRIMETHYL TETRADECANE, \(\text{C}_{17}\text{H}_{36}\) [240]

The mixture of olefins, 0.138 g in 10 ml of absolute alcohol, were hydrogenated in a Brown\(^2\) hydrogenator (Brown, H. C., Brown, C. A., 1962) using PtO\(_2\) as a catalyst (0.05 g of catalyst). The reaction was carried out at room temperature and was followed quantitatively. The total reaction time was 5 min. The crude reaction mixture was centrifuged and the ethanolic solution decanted off. The PtO\(_2\) residue was heated in more ethanol to remove any product absorbed by the catalyst. The
solutions were combined and extracted twice with 10 ml of n-heptane.
The heptane extract was evaporated and dried over MgSO₄. The solvent
was evaporated. Yield (of reaction product): 130 mg (94%). Any loss
of yield was probably due to mechanical transfer.

PHYSICAL CONSTANTS:

GLC: (Perkin-Elmer Model 226, gas-liquid chromatograph:
capillary column; 150' x .01'; apiezon L; 1 x 50;
20/min from 140°C; 100:1 split ratio). Indicates
1 component only (>99% purity).

INFRARED: 3.37 μ, 3.41 μ, 3.46 μ, alkane C-H vibrations;
7.25 μ; 7.30 μ, gem-dimethyl

REFRACTIVE INDEX: n₂³°C = 1.4291

MASS SPECTRUM: m/e 240 (M); m/e 183 (M-57), m/e 155 (M-85);
m/e 113 (m-127). See Figure 29.

PART II. THE SYNTHESIS OF TWO C₁₉ ISOPRENOID HYDROCARBONS, ISOMERIC WITH
PRIStANE, AND A COMPARISON OF THEIR MASS SPECTRA

The saturated isoprenoid hydrocarbons, pristane (C₁₉) and phytane
(C₂₀), have generally been characterised in crude oils and shales on the
basis of their mass spectral fragmentation patterns. In a few cases, in-
frared spectroscopy and nuclear magnetic resonance measurements (Eglinton
et al., 1966; Bendoraitis, et al., 1963) have confirmed these structural
assignments, where the amount of these compounds isolated is sufficient
to permit such measurements. However, the amount of isoprenoid compound
isolated is generally so small that it is not possible to carry out IR
or NMR measurements. In other cases, such as the Green River Shale, the isoprenoid hydrocarbons are the constituents of a relatively simple mixture and can be separated on a GLC preparative column into pure components. In most cases these isoprenoid hydrocarbons are usually the constituents of very complex mixtures from which it is extremely difficult to isolate them in pure form. As a result, the mass spectra of the individual isoprenoids usually contain a few impurities which in many cases can give rise to ambiguities in the structural interpretation. This is particularly the situation with the Antrim Shale, where the mass spectra of the C\textsubscript{19} isoprenoid and the C\textsubscript{20} isoprenoid isolated from the shale show considerable differences from the authentic pristane and phytane (see Figures 31 and 32). To resolve these ambiguities and to see what effect the shift of one methyl group (from the 14 position) to an adjacent carbon in the pristane molecule has on the overall mass spectrum of the molecule, it was decided to synthesise two C\textsubscript{19} saturated isoprenoid hydrocarbons, both of which are isomeric with pristane.

The two C\textsubscript{19} saturated isoprenoid hydrocarbons that were synthesised have the following structures:

1) \[
\begin{array}{c}
\text{2,6,10-trimethylhexadecane}
\end{array}
\]

2) \[
\begin{array}{c}
\text{2,6,10,13-tetramethylpentadecane}
\end{array}
\]

The former structure has already been postulated (Johns et al., 1966) as being consistent with the mass spectrum of the C\textsubscript{19} isoprenoid.
Figure 31. Comparison of the mass spectrum of authentic pristane with the mass spectra of a series of C_{19} isoprenoid hydrocarbons isolated from crude oils and shales.
Figure 32. Comparison of the mass spectrum of authentic phytane with the mass spectra of a series of C_{20} isoprenoid hydrocarbons isolated from crude oils and shales.
isolated from the Antrim Shale. From a diagenetic standpoint it could reasonably arise if squalane were a precursor (cf. Chapter 6). The latter structure, however, would not be expected to derive from biological precursors generally considered to give rise to isoprenoid hydrocarbons. One would expect that these two isomers, and pristane also, would have very similar mass spectra and that for a slightly impure sample of a C_{19} isoprenoid isomer isolated from a shale (which is the case for the Antrim Shale) assigning a specific structure to the isomer on the basis of mass spectrometry alone would be very difficult.

These two isomers were synthesised from the starting material hexahydrofarnesyl bromide, which had already been obtained as an intermediate in the synthesis of the C_{17} isoprenoid. The synthetic scheme, which in principle is similar to that for the C_{17} isoprenoid, is outlined below and described in detail in the experimental section.
The synthetic procedure is the same for each of the isomers except that in one case butyraldehyde is allowed to react with the Grignard reagent prepared from hexahydrofarnesyl bromide, and in the other case methyl ethyl ketone is allowed to react with the same Grignard reagent to produce a tertiary alcohol which is subsequently dehydrated with iodine. The purity of the two C₁₉ isoprenoids was demonstrated by capillary gas chromatography.

The pyrolysis of 4-acetoxy-7,11,15-trimethylhexadecane gave only two cracking products, which is somewhat curious in view of the fact that the pyrolysis of 2-acetoxy-5,9,13-trimethyleneoctadecane yields three. In the thermal cracking of esters three factors are considered to govern the nature of the product: i) the statistical supply of the hydrogens, which favours the less substituted alkene; ii) the steric effect; and iii) the relative stability of the olefin product (Berkner et al., 1959). In the cracking of a general ester of the type

![Chemical structure](image)

there are two possible transition states

![Transition states](image)

and

![Steric interference](image)

steric interference between the methyl and R groups.

Thus, the less substituted alkene is favoured both statistically (3:1) and sterically; it is not favoured on thermodynamic grounds, however.
De Puy has carried out extensive research on this thermal cracking reaction relating to the factors influencing the product distribution (1960). The pyrolysis involves cis elimination. The ease of pyrolysis depends on the ground state energy of the acetate. In the pyrolysis of cis- and trans-1-methyl-4-t-butyl cyclohexyl acetates the same ratio of exo/endo product was found as in the pyrolysis of 1-methyl cyclohexyl acetate, indicating that there are no conformational effects on the direction of elimination. The pyrolysis of sec-butyl acetates gives approximately a statistical olefin distribution. This no longer obtains as the chain length of the acetate increases. The departure from a statistical product distribution is accounted for by a consideration of the eclipsing effects in the transition state (De Puy et al., 1961a,b).

One would not expect any exclusive stereospecific preference for either the cis or the trans-alkene of the resulting product. Since steric effects, however, do play a significant role in determining the product distribution they will play some role in determining the geometric isomerism of the product. In the case of 4-acetoxy-5,9,13-trimethylhexadecane four products would be expected which should be separable by capillary gas chromatography. Only two products result, however, and the infrared spectrum of the product shows a strong absorption at 10.32 μ, which indicates an olefin with trans hydrogens is present; it does not absolutely exclude the possibility that a compound with cis hydrogens is present also. It is possible that the capillary gas chromatograph will not resolve a mixture of trans and cis components, but the evidence from the dehydration of 3,6,10,14-tetramethylpentadecan-3-ol, where the expected five components are all resolved, would seem to disfavour this explanation. This is not an exact analogy, but the evidence from the cracking products of 2-acetoxy-5,9,13-trimethyltetradecane,
where cis and trans components are apparently resolved, would seem to indicate that if four components are present in the cracking of 4-acetoxy-7,11,15-trimethylhexadecane they should all be resolved. To obtain a definite answer to this question the individual components should be separated and analysed further. For the present, however, the evidence would seem to point to steric control in the thermal cracking of 4-acetoxy-7,11,15-trimethylhexadecane, which gives rise only to the trans olefin.

The mass spectra of the mixture of the C\textsubscript{17} olefins and C\textsubscript{19} olefins, which both result from the thermal cracking of the corresponding acetates, are shown in Figure 33. These spectra show considerable similarities. They both have in common strong fragmentations at m/e 111, m/e 126, and m/e 196. The fragmentations at m/e 182 and m/e 196 indicate that allylic cleavage is a prominent fragmentation process. There is only slight evidence for loss of 28 m/u. from the C\textsubscript{17} isoprenoid alkene mixture, which contains some terminal olefin. These fragmentation patterns indicate that the mass spectra of such olefins could be very useful in structure identification.

A comparison of the mass spectra of the three C\textsubscript{19} hydrocarbon isomers, together with that of the C\textsubscript{19} isoprenoid isolated from the Antrim Shale, is shown in Figure 34. It is quite evident that the three C\textsubscript{19} isomers have very similar mass spectra, all having in common intense peaks at m/e 113 and m/e 183. Only in the region above m/e 200, where the fragmentation pattern is much weaker in intensity, do differences occur. The presence of a small impurity in a C\textsubscript{19} isoprenoid isolated from a geochemical sample would make the structural assignment of this isoprenoid very difficult. The mass spectrum of the Antrim C\textsubscript{19} isoprenoid, if anything, shows more resemblance to 2,6,10,13-tetramethylpent-
Figure 33. Mass spectra of a) a mixture of C_{17} isoprenoid alkenes; and b) a mixture of C_{19} isoprenoid alkenes.
Figure 34. Comparison of the mass spectra of three C$_{19}$ isomeric isoprenoid hydrocarbons with the mass spectrum of the C$_{19}$ isoprenoid isolated from the Antrim Shale.
decane than to pristane, although further evidence from GLC coinjection experiments indicated that this structure was not feasible.

The order of elution of the three C₁₉ isoprenoid hydrocarbons is shown in Figure 35. By coinjection of the three C₁₉ standards it was shown that the C₁₉ isoprenoid hydrocarbon isolated from the Antrim Shale had the pristane structure. This was also confirmed for the Soudan Shale and the Nonesuch Shale, where similar doubts existed.

The three C₁₉ isoprenoid isomers were also distinguished by a close analysis of the 7.25 µ (1380 cm⁻¹) region (the methyl symmetrical bending region) of their infrared spectra, obtained from a Beckman IR-7 instrument (Figure 36). Area analysis of the 7.25 µ (1380 cm⁻¹) region can be used in methyl group estimation (Brand and Eglinton, 1965). When two methyl groups are substituent on the same carbon atom the 7.25 µ (1380 cm⁻¹) peak splits into two components, one at ν7.22 µ (1385 cm⁻¹) and the other ν7.30 µ (1370 cm⁻¹). These C₁₉ isoprenoid isomers, therefore, in which the number of gem-dimethyl groups and single methyl groups is different for each isomer, can be identified on the basis of their infrared spectra. Nuclear magnetic resonance might also provide some information. However, the quantity of a pure compound isolated from a sediment is generally so small that this physical measurement is rarely possible. Coinjection of the C₁₉ isoprenoid 2,6,10-trimethylhexadecane standard into the 'branch-cyclic' hydrocarbon fraction of the Antrim Shale does not provide convincing evidence for its presence. Certainly, if this isoprenoid is present it is there in small quantities. On the basis of this evidence squalane would not appear to be a significant precursor of the saturated isoprenoid alkanes. This will be discussed again in Chapter 6.
ORDER OF ELUTION OF C₁₉ ISOPRENOID ISOMERS. APIEZON L. PHASE

CAPILLARY COLUMN: 150' X 0.1"
SPLIT RATIO: 100:1
PROGRAM: 1/2° PER MIN.
IR SPECTRA OF THE METHYL SYM. BENDING REGION
(BECKMAN IR-7 INSTRUMENT)

Figure 36.
In conclusion, then, this study emphasises the care necessary in assigning unambiguous structures to compounds isolated from complex mixtures of organic compounds where it is difficult to obtain pure samples of these compounds for spectroscopic analysis.

**EXPERIMENTAL**

**THE SYNTHESIS OF 2,6,10-TRIMETHYLBUNADECANE**

7,11,15-Trimethylhexadecane-10-ol, \( C_{19}H_{40}O \) [284]

Purification of Butyraldehyde. Butyraldehyde generally contained a small amount of butyric acid. This was removed by shaking with 10% \( \text{Na}_2\text{CO}_3 \) (\( \text{NaHCO}_3 \) reacts rather slowly with carboxylic acids and in that respect is not efficient.) washing with water, and combining ether extracts. However, this did not seem to effect complete removal of the butyric acid; distillation under vacuum seemed to enhance the oxidation of the aldehyde. The most suitable method, however, probably would involve fractional distillation under a nitrogen atmosphere. In this particular case this was not done. The extract after washing with 10% \( \text{Na}_2\text{CO}_3 \) was used.

**Synthesis of Grignard** (general reference: Cason & Rapoport, "Laboratory Text in Organic Chemistry", p. 472): A 1.0 g sample of (\( \approx 3 \text{ mM} \)) hexahydrofarnesyl bromide (synthesised in the preparation of 2,6,10-trimethyltetradecane) in 5 ml of dry ether was slowly added to 0.15 g of magnesium turnings in 5 ml of dry ether contained in a 3-necked
round bottom flask. The reaction was carried out under a N₂ atmosphere. To initiate the reaction, the solution was gently heated and 3 drops of dibromoethane added. The addition took 1 hr, and the green-black solution was heated under reflux for 15 min.

A solution of 0.25 g of dry butyraldehyde in 5 ml of dry ether was cooled to -5°C and added to the Grignard reaction mixture (also cooled to -5°C). The addition took place with continuous stirring during 30 min, and the temperature was not allowed to rise above 10°C. After all the butyraldehyde had been added, the reaction mixture was poured onto 10 g of crushed ice and stirred vigorously. This solution was then acidified with 1.0 ml of cold 15% H₂SO₄. The aqueous layer was separated in a separatory funnel and extracted 3 times with 25 ml of ordinary ether. The total ether extract was dried over MgSO₄.

The GLC analysis of the crude product indicated that some farnesane and squalane (Wurtz-coupling product) had also been formed in the reaction together with the expected alcohol. To purify the reaction product, it was eluted on an alumina column (Merck reagent; 30 g; 10 x 1 cm) with n-heptane (about 20 ml eluate) and then with ether. The eluates were evaporated down. Yield (from heptane eluate): 0.25 g; yield (from ether eluate): 0.47 g (47%).

**PHYSICAL CONSTANTS**

**REFRACTIVE INDEX:** n²⁰°C = 1.4518

**INFRARED:** 2.96 μ; 7.25 μ, 7.30 μ

**GLC:** (10' x 1/16"; SE 30; 8°/min; Aerograph 665-1)

Indicates >95% pure- a small residual farnesane

**MASS SPECTRUM:** m/e 284 (M), very weak; m/e 266 (M-18), intense; m/e 241

m/e 196; m/e 126
The procedure of Cason and Graham (1965) was followed. A 0.38 g sample of 7,11,15-trimethylhexadecan-4-ol, 0.75 ml of acetic anhydride, and 4 ml of dry benzene were heated at 80°C for 4 hr. The reaction mixture was then cooled, poured onto 5 g of ice and stirred. The product was extracted 3 times with 10 ml of ether.

The crude product contained residual acetic anhydride. This was removed by extracting with 10% NaHCO₃. Any further residual Ac₂O was removed by elution of the acetate from an alumina column (Merck reagent grade; 5 m x 1.0 cm; 8 g) with heptane (20 ml) and then ether.

Yield: 0.325 g (75%).

**PHYSICAL CONSTANTS**

**GLC:** (10' x 1/16"; SE 30; programm @8°/min, 1 x 10).

One component, 99% purity.

**INFRARED:** 5.71 μ; 8.01 μ; 7.25, 7.30 μ - gem-dimethyl; poorly resolved

**REFRACTIVE INDEX:** n²²°C = 1.4433

**MASS SPECTRUM:** No parent molecular ion; m/e 266 (M-60); m/e 196; m/e 182; m/e 140; m/e 126

**7,11,15-TRIMETHYLHEXADECENES, C₁₈H₃₈ [265]**

Synthetic Procedure. A 0.300 g sample of 4-acetoxy-7,11,15-trimethylhexadecane was pyrolysed at 515°C essentially according to
Bailey and Golden (1952). The procedure and apparatus is identical to that described in the cracking of 2-acetoxy-5,9,13-trimethyltetradecane mentioned beforehand. Yield (of crude product): 0.181 g.

To remove the residual acetate the crude product was chromatographed on an alumina column (Merck reagent grade; 1.0 x 5 cm) and eluted with 20 ml of n-heptane, followed by 20 ml ether. The heptane eluate was dried and evaporated. Yield: 0.141 g (48%).

**Physical Constants**

**Refractive Index:** Not taken

**GLC:** (Perkin-Elmer Model 226 gas-liquid capillary column: 150' x 0.1", 2°/min; 100:1 split ratio; Apiezon L; programme from 140°C) indicates 2 components; ratio 40:60% (approx.)

**Infrared:** 7.25, 7.30 μ - gem-dimethyl well resolved, Broad band at 5.98-6.20 μ; weak (-C=C- stretch) Sharp band at 10.32 μ \[ \text{H} \text{C=C} \text{H} \text{ absorption; trans} \]

**Mass Spectrum:** m/e 266 (M); m/e 196; m/e 182; m/e 140; m/e 126; m/e 111. See Figure 33.

**NMR:** 5.42 δ (4.58 τ), vinyl absorption 0.85-1.5 δ, methyl absorptions

2,6,10-Trimethylhexadecane, C₁₉H₄₀
Synthetic Procedure. The mixture of olefins, 90 mg, in 10 ml of absolute alcohol were hydrogenated in a Brown hydrogenator (Delmar Scientific Laboratories; Brown, H.C., Brown, C.A. 1962) using 12 mg of PtO₂ catalyst. The reaction was carried out in a similar manner as that described in the synthesis of 2,6,10-trimethyltetradecane.

Yield (of reaction product): 85 mg (94%).

PHYSICAL CONSTANTS:

GLC: (Perkin-Elmer Model 226, gas-liquid chromatograph capillary column: 150' x .01'; Apiezon L; 1 x 20; programm for 130°C @ 2°C/min; 100:1 split ratio)

Indicates one component (>99% purity).

n, REFRACTIVE INDEX: n = 1.4376

INFRARED: 3.34 μ, 3.88 μ; 3.47 μ alkane C-H vibrations

7.25 μ, 7.30 μ, gem-dimethyl (well resolved)

MASS SPECTRUM: m/e 268 (M); m/e 183; m/e 113. See Figure 34.

THE SYNTHESIS OF 2,6,10,13-TETRAMETHYLPENTADECANE

2,6,10,13-TETRAMETHYLPENTADECANE-3-OL, C₁₉H₄₀O [284]

Synthetic Procedure:

Purification of methyl ethyl ketone: Crude methyl ethyl ketone (CH₃COCH₂H₅) was purified by fractional distillation and the fraction boiling at 79°C collected and dried over MgSO₄ for 24 hr.
Synthesis of Grignard reagent (general reference: Cason and Rapoport, "Laboratory Text in Organic Chemistry", p. 472). A 0.38 g sample of (1.5 mM) hexahydrofarnesyl bromide (synthesised in the preparation of 2,6,10-trimethyltetradecane) in 3 ml of dry ether was slowly added to 0.06 g of magnesium turnings in 5 ml of dry ether contained in a 3-necked round bottom flask. The reaction was carried out under a nitrogen atmosphere. To initiate the reaction the solution was gently heated and 3 drops of dibromoethane added. The addition took 1 hr. and the green-black solution was heated under reflux for 15 min.

A solution of 0.1 g of dry methyl ethyl ketone in 3 ml of dry ether was cooled to -10°C and added to the Grignard reagent (also cooled to -5°C). The addition took place with continuous stirring during 30 min. and the temperature was not allowed to rise above 10°C. After all the methyl ethyl ketone had been added the reaction mixture was poured onto 5 g of crushed ice and stirred vigorously. This solution was then acidified with 1.0 ml of cold 15% H₂SO₄. The aqueous layer was separated in a separatory funnel and extracted 3 times with 25 ml of ordinary ether. The total ether extract was dried over MgSO₄.

The GLC analysis of the crude product indicated that some farnesane and squalane (Wurtz-coupling product) had also been formed in this reaction together with the expected alcohol. To purify the reaction product it was eluted on an alumina column (Merck reagent; 10 g) with n-heptane (about 20 ml) and then with ether. The two eluates were evaporated. Yield (from heptane eluate): 0.15 g. Yield (from ether eluate): 0.20 g (54%).
SYNTHESIS OF 2,6,10,13-TERAMETHYLPENTADECenes, C_{19}H_{38} [266]

Synthetic Procedure. 95 mg of 3,6,10,14-tetramethyldodeadecane-3-ol, and a few crystals of iodine, were refluxed in 4 ml of boiling toluene for 10 hr. The reaction mixture was cooled and washed with 5 ml of 5% NaHSO₃ and then with 5 ml of distilled water. The toluene extract was evaporated. The crude product was purified from any residual alcohol on an alumina column (Merck reagent; 5 g) and eluted with 15 ml of heptane. The heptane eluate was dried over MgSO₄ and evaporated. Yield: 45 mg (53%).

PHYSICAL CONSTANTS

REFRACTIVE INDEX: \( n^{20\text{oC}} = 1.4460 \)

INFRARED:
2.95 \( \mu \); 7.25 \( \mu \), 7.30 \( \mu \) - gem-dimethyl

MASS SPECTRUM:
No molecular ion; m/e 266 (M-18), intense;
m/e 255 (M-29)
m/e 196; m/e 126

GLC:
(Perkin-Elmer Model 226 gas-liquid capillary column: 1 x 20: 2°/min; 100:1 split ratio; Apiezon L; program for 130°C; also run on Castorwax -- similar results)

Apparantly 5 peaks

cis and trans

cis and trans
-130-

INFRARED: 3.35 μ, 3.39 μ; 3.45 μ; 7.25 μ; 7.30 μ - gem-dimethyl
Weak bands in 11.2 μ (–C=CH₂)
No band in 10 μ to 11 μ region

MASS SPECTRUM: Molecular ion at m/e 268

SYNTHESIS OF 2,6,10,13-TETRAMETHYLPENTADECANE, C₁₉H₃₀ [268]

Synthetic Procedure. The mixture of olefins, 33 mg, in 5 ml of
solvent (3:1, absolute alcohol:heptane) were hydrogenated in a Brown²
hydrogenator (Delmar Scientific Laboratories; Brown, H. C., Brown, C. A.,
1962) using 6 mg of PtO₂ catalyst and 3 drops of 10% concentrated HCl.
The reaction was slow and was followed to completion within an hour;
the presence of acid proved to be essential. The work-up procedure has
been previously described. Yield: 25-30 mg.

PHYSICAL CONSTANTS

GLC (Perkin-Elmer Model 226, gas-liquid chromatograph
capillary column: 150' x .01”; Apiezon L; 1 x 20;
programm from 130°C at 2°/min; 100:1 split ratio)
indicates one component, 94% purity

INFRARED
Weak spectrum, 7.25 μ; 7.30 μ

MASS SPECTRUM: m/e 268 (M); m/e 183 (M-85); m/e 113 (M-155)
See Figure 34.
A C_{20} isoprenoid has been isolated from many crude oils and shales and has been assigned the structure 2,6,10,14-tetramethylhexadecane (phytane), in all cases. Just as with the C_{19} isoprenoid, pristane, this structure has been based primarily on mass spectral evidence. The mass spectral interpretation has not always been unambiguous. The mass spectrum of the C_{20} isoprenoid isolated from the Antrim Shale and the Soudan Shale shows certain differences when compared with the spectrum of authentic phytane (cf. Figure 32). Other structures, such as 2,6,10-trimethylheptadecane, could reasonably be postulated which would equally well fit the mass spectral data. To confirm the structure of this isoprenoid hydrocarbon as phytane it was necessary to provide further evidence using capillary gas-liquid chromatography coinjection techniques.

During the investigation of the structure of the C_{20} isoprenoid, a curious feature was observed which has not been noticed hitherto. The C_{20} isoprenoid constituent in the 'branch-cyclic' fraction of the Antrim Shale appeared to have a shoulder when the capillary gas chromatogram was run on polyphenyl ether phase (PPE), suggesting the presence of two components. This was nothing more than the slightest indication. A much more pronounced shoulder, however, was observed when the 'branch-cyclic' fraction of the Soudan Shale was run under identical conditions, using the same phase, PPE. In many ways this was a much more remarkable observation, since the Soudan Shale is a relatively simple mixture of organic components compared with the Antrim Shale, the Monesuch Shale, and most crude oils. Three peaks are particularly prominent in the Soudan
Shale, and these had been identified as norpristane, pristane, and phytane. The capillary gas chromatograph of the 'branch-cyclic' fraction of the Soudan Shale, using Apiezon L phase, is shown in Figure 37. The three most prominent constituents appear as single, symmetrical peaks. When the same fraction from the Soudan Shale is injected into a capillary gas chromatograph, using PPE as the phase, the overall distribution remains the same, but the phytane peak, and to a lesser extent the pristane peak, shows the appearance of a shoulder (Figure 38). This is much more prominent when the inset of Figure 38 is expanded (Figure 39a). Here it can be seen quite plainly that the 'phytane' peak is composed of more than one component. A similar conclusion can be made for the 'pristane' peak. The 'norpristane' peak (the prominent peak which is not marked in the far left of Figure 39a) probably consists of one component. This observation is quite reproducible.

In the midst of these studies our attention had been drawn to the C_{20} isoprenoid, 2,6,11,15-tetramethyldodecane, isomeric with pristane, and having the following structure:

![Chemical Structure]

This compound had been previously studied by Sorensen et al. (1951), while investigating the structure of pristane. The aliphatic diterpenoid compound had been called "crocetane" because its structural skeleton was derived from the carotenoid pigment, crocetin. Crocetane had been synthesised by two different methods. Fischer and Löwenberg (1929)  

The identification of norpristane rests on mass spectral evidence only, but the fragmentation pattern can only reasonably be attributed to the norpristane structure.
Figure 37. Capillary gas chromatogram of the 'branch-cyclic' fraction from the Soudan Shale on Apiezon L phase.
Figure 38. Capillary gas chromatogram of the 'branch-cyclic' fraction from the Soudan Shale on PPE phase.
Figure 39a. Inset from Figure 38, showing the phytane peak with a shoulder.
Figure 39b. Inset from Figure 39, showing the same part of the capillary gas chromatogram as Figure 39a, but with 2,6,10,15-tetramethylhexadecane coinjected as a standard.
had employed the Wurtz-coupling of tetrahydrogeranyl bromide. Karrer and Golde (1930) had synthesised crocetane from crocetin by a series of reductive stages. This compound was now readily available, and our interest in this compound originated when considering the possibility of an abiogenic origin for the isoprenoid hydrocarbons. We considered isoprene to be a suitable precursor since this was a known product of an abiogenic synthetic process, employed in industry. A priori considerations suggest that an abiogenic polymerisation of isoprene could be distinguished from a biological polymerisation in that it would not be expected to proceed exclusively by head-to-tail linkages [but see the work of Natta (1958), described later in Chapter 5]; further, the tail-to-tail linkages should not occur exclusively with C₁₅ and C₂₀ polyisoprenoid compounds. Since tail-to-tail linkages do not occur apparently with the monoterpenoid compounds to give acyclic diterpenes in biosynthetic pathways, the compound crocetane, the result of a tail-to-tail linkage, might serve as a criterion of abiogenic origin for isoprenoid hydrocarbons. We proceeded to look for this compound in a series of shales, particularly those of Precambrian age, where we anticipated finding the demarcation line between the period of chemical evolution and the onset of biological evolution. It is always possible, of course, that crocetane could arise by diagenetic modification of a biological precursor. We shall return to this possibility later.

The two C₂₀ isoprenoids, phytane and crocetane, are not easily resolved by capillary gas chromatography. When the two compounds are run together on a capillary gas chromatograph, using apiezon L phase, no separation is observed (Figure 40). If the phase is changed to PPE the two compounds can be separated (Figure 41), a separation which could probably be enhanced even further if a column, greater in length than
Figure 40. Order of elution of phytane and 2,6,11,15-tetramethylhexadecane on Apiezon L phase.
Figure 41. Order of elution of phytane and 2,6,11,15-tetramethylhexadecane on PPE phase.
Figure 42. Mass spectra of a) 2,6,11,15-tetramethylhexadecane, and b) phytane.
150 ft., were used. The mass spectra of the two C₂₀ isoprenoids are compared in Figure 42. As would be expected, the two mass spectra have many features in common. The significant difference between the two mass spectra lies in the intense m/e 169 for crocetane, and a small m/e 183, and the intense m/e 183 for pristane on the other hand, with a relatively weaker m/e 169. This characteristic easily distinguishes the two compounds.

In contrast to the situation which exists for the three C₁₉ isoprenoid isomers which have just been considered, the mass spectral evidence clearly distinguishes the two C₂₀ isoprenoid isomers, whereas they are only separated on a capillary gas chromatograph with some difficulty.

The possibility that both these C₂₀ isoprenoid hydrocarbon isomers might be present in the 'branch-cyclic' fraction from the Soudan Shale was now investigated. That this possibility is reasonable should be considered in the light of the separation of the two C₂₀ isoprenoid isomers on Apiezon L phase and PPE phase respectively, compared with the shape of the C₂₀ isoprenoid peak in the 'branch-cyclic' fraction of the Soudan Shale on the same two phases. They show very similar properties.

Coinjection of the C₂₀ isoprenoid isomer, 2,6,11,15-tetramethylhexadecane, into the 'branch-cyclic' fraction of the Soudan Shale (see Figure 39b, which is to be compared with Figure 39a) provides very good evidence
for the presence of this isoprenoid in the 'branch-cyclic' fraction. 
The right hand side of the C₂₀ isoprenoid peak we have already shown to 
correspond to phytane. Although coinjection techniques provide convinc- 
ing evidence that both C₂₀ isoprenoid isomers are present in the Soudan 
Shale, the evidence is not unequivocal. A final proof might come using 
combined gas chromatography-mass spectrometry. Since the two components 
can be distinguished by the intensity of the m/e 169 mass spectral peak 
(or m/e 183), the C₂₀ isoprenoid fraction in the Soudan Shale could be 
scanned at different intervals and the changing intensity of these mass 
spectral peaks observed. The mass spectrum of the C₂₀ isoprenoid iso- 
lated from the Soudan Shale shows differences from that of authentic 
phytane, differences that might be interpreted in terms of a mixture of 
the two C₂₀ isoprenoid isomers. The GC-MS evidence should provide the 
answer to this problem.

The presence of the C₂₀ isoprenoid, 2,6,11,15-tetramethylhexadecane, 
in the 'branch-cyclic' fractions of other shales was investigated. The 
fraction marked 'phytane' in the 'branch-cyclic' fraction of the Nonesuch 
Seep Oil, thought to be of Precambrian age, also shows unmistakably the 
presence of a doublet (Figure 43). In the Antrim Shale the presence of 
two components in the C₂₀ isoprenoid peak of the 'branch-cyclic' fraction 
is not evident. Only the smallest hint of a shoulder is observed 
(Figure 44). When we come to the Green River Shale (Figure 45) the 
phytane peak in the 'branch-cyclic' fraction is perfectly symmetrical. 
There is no indication that this peak contains more than one component. 
This is a significant observation because it illustrates that the phytane 
from the Green River Shale, and possibly the Antrim Shale, does not 
occur with another constituent, as seems to be the case with the Precambrian
Figure 43. Capillary gas chromatogram of 'branch-cyclic' fraction from the Nonesuch Shale (PPE phase).
Figure 44. Capillary gas chromatogram of 'branch-cyclic' fraction from the Antrim Shale (PPE phase).
Figure 45. Capillary gas chromatogram of 'branch-cyclic' fraction (lower mol. wt. hydrocarbons) from the Green River Shale.
samples we have analysed, the Soudan Shale and the Nonesuch Seep Oil. It should be emphasised that these GLC patterns are strictly comparable, being run under identical or almost identical conditions.

Before discussing the significance of these results it should also be noted that the 'pristane' peak in the 'branch-cyclic' fraction of the Soudan Shale suggests the presence of more than one component though the shoulder is much less pronounced than for the 'phytane' peak. There is no evidence for the presence of more than one component in the 'pristane' peak of either the Nonesuch Seep Oil, the Antrim Shale, or the Green River Shale. The structural identity of this other component in the 'pristane' peak from the 'branch-cyclic' fraction of the Soudan Shale has not been pursued yet.

It is tempting to conclude from these results that we have come across an observation of great significance in an evolutionary sense --- that the finding of crocetane (which admittedly has yet to be confirmed unambiguously) in the Soudan Shale indicates that the biosynthetic pathway to the isoprenoid compounds as we know it today might not have been an important pathway at that period of geological time. The obvious process that comes to mind is that it be derived from an abiological origin. Since this question is of great concern to the organic geochemist, we have left this particular problem for further discussion in Chapter 5.

As a final comment, one should also emphasise that crocetane does not demand an abiogenic origin. There are several compounds which might reasonably be postulated as biological precursors. The most likely precursor is crocetin, whose structure was elucidated by Karrer and Salmon (1928-1933) in a series of papers, and is shown on the following page.
crocetin

By a series of diagenetic transformations, similar in kind to those employed by Karrér and Golde (1930) in the laboratory synthesis of crocetane, one can see how crocetane might arise under geological conditions. These carotenoids which have carbon skeletons fewer than 40 carbon atoms are probably natural degradation products of various C_{40} carotenoids (Weeden, 1965). Thus, both crocetin and bixin, whose structure is shown below, could give rise to crocetane.

bixin

If crocetane is indeed derived from a biological precursor, one thing is certain. Such compounds as crocetin and bixin are nowhere nearly so widely distributed in nature today as the chlorophyll molecule from which phytane is thought to be derived. This would necessitate postulating a very different ecology at this period of geological time than is known today.

EXPERIMENTAL

Phytane was synthesised from phytol and has been described previously (Eglinton et al., 1966). 2,6,11,15-tetramethylhexadecane was obtained from Chemical Samples Co., Columbus, Ohio.
PART IV. THE SYNTHESIS OF STANDARDS IN THE IDENTIFICATION OF A C21 SATURATED ISOPRENOID HYDROCARBON ISOLATED FROM OILS AND SEDIMENTS

As we have seen, the saturated isoprenoid alkanes, pristane (C19) and phytane (C20), have been isolated and identified in sediments dating from Precambrian times (Meinschein et al., 1964; Johns et al., 1966; Oro and Noonan, 1967). Other isoprenoid alkanes, in particular the C15 (farnesane), C16 (homofarnesane) and the C18 (norpristane) compounds have also been isolated and identified in oils and shales of differing ages (Eglinton et al., 1966; Johns et al., 1966; Van Hoeven et al., 1966). Bendoraitis et al. (1963) has characterised a C21 isoprenoid alkane, in an East Texas gas oil, to which he assigned the structure 2,6,10,14-tetramethylheptadecane. The mass spectrum of this C21 isoprenoid was plotted from a table containing the mass numbers, and the relative intensities of the ions (Bendoraitis et al., 1963) and is shown in Figure 46. This represents the only report of this isoprenoid alkane in oils and sediments. This structure has been tentatively assigned to C21 compounds isolated from the Soudan Shale (2.7 x 10⁹ years), the Nonesuch Seep Oil (1.0 x 10⁹ years) and other oils and sediments (Johns et al., 1966; Van Hoeven et al., 1966). However, impurities in the mass spectra of these compounds prevents one from assigning an unequivocal structure. This is well illustrated in Figure 47, where the mass spectra of a series of C21 isoprenoid alkanes is compared with the C21 isoprenoid standard, 2,6,10,14-tetramethylheptadecane. In view of the scarcity of reports of the C21 isoprenoid alkane, we have determined to characterise its structure unambiguously.
Figure 46. Mass spectrum of a $C_{21}$ isoprenoid hydrocarbon isolated from an East Texas Oil by Bendoraitis (1963).
Figure 47. Comparison of the mass spectra of a series of C₂₁ isoprenoid hydrocarbons, isolated from a series of crude oils and shales, compared with the mass spectrum of authentic C₂₁ isoprenoid, 2,6,10,14-tetramethylheptadecane.
Our interest was drawn to the characterisation of the $C_{21}$ isoprenoid alkane from another standpoint. We have considered the possibility that a $C_{30}$ isoprenoid, such as squalane, could be a precursor to the isoprenoid alkanes (McCarthy and Calvin, 1967; see also discussion in Chapter 6). The $C_{21}$ isoprenoid provides a more critical test of this hypothesis. The regular $C_{21}$ isoprenoid, $2,6,10,14$-tetramethylheptadecane, already reported by Bendoraitis (1962) and tentatively identified by Johns et al. (1966), could be derived from a $C_{40}$ isoprenoid precursor, or higher homologue, by analogy with our previous diagenetic schemes (see Figure 48). The $C_{21}$ isoprenoid $2,6,10,15$-tetramethylheptadecane could only reasonably arise if a $C_{30}$ isoprenoid such as squalane were a precursor. The mass spectra of these two isomers should exhibit only very minor differences. The close similarity of the mass spectra of three $C_{19}$ isomeric alkanes shown in Figure 34 would seem to justify such a prediction. An unequivocal identification of the $C_{21}$ isoprenoid present in the Antrim Shale and the Soudan Shale might provide evidence for the nature of the precursor.
Figure 48. The diagenetic pathways to the $C_{21}$ isoprenoid hydrocarbons

SYNTHETIC ROUTE TO THE $C_{21}$ ISOPRENOIDS

Figure 49. Synthetic route to the $C_{21}$ isoprenoids
In an attempt to resolve such ambiguities we have synthesised the two $C_{21}$ isoprenoid alkanes under consideration starting from the naturally available starting materials, farnesol and phytol. The synthetic scheme is shown in Figure 49 and is described in detail in the experimental section. The synthetic route to the $C_{21}$ isoprenoid alkane, 2,6,10,15-tetramethylheptadecane, again followed the basic outline that has been utilised in the previous syntheses. The intermediate hexahydrofarnesyl bromide was used as a starting material and was allowed to react with $\beta$-methyl valeryl chloride in a low temperature Grignard reaction with ferric chloride catalyst, according to the procedure described by Cason and Kraus (1961). Although these authors prescribe good yields (60-70%) for this reaction under carefully specified conditions, a low yield of the $C_{21}$ ketone, 3,8,12,16-tetramethylheptadecan-5-one, was obtained in several attempts. The $C_{21}$ ketone was separated from a large amount of farnesane and probably squalane, using a neutral alumina column, and was then reduced to the $C_{21}$ isoprenoid alkane by a Wolff-Kishner reaction. There was still some additional components in the reaction product of this reaction, and the $C_{21}$ isoprenoid alkane was separated from these compounds by preparative gas-liquid chromatography. The purity of the collected fraction was demonstrated by reinjection onto a capillary gas chromatograph. Only one component was observed.

These two standard compounds were for identification by capillary gas chromatography and mass spectrometry. They are readily separated from each other by capillary gas chromatography on both PPE phase (see Figure 50) and castorwax (see Figure 51). The mass spectra of the two

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The $C_{21}$ isoprenoid alkane, 2,6,10,15-tetramethylheptadecane, was synthesised by W. Van Hooven. For experimental details, refer to his Ph.D. Thesis, University of California, Berkeley (in preparation).
Figure 50. Order of elution of \( C_{21} \) isoprenoid isomers on PPE phase.
Order of elution of C_{21} isoprenoid isomers on castorwax phase.

Figure 51. Order of elution of C_{21} isoprenoid isomers on castorwax phase.
Figure 52. Mass spectra of C_{21} isoprenoid standards compared with the mass spectrum of the C_{21} isoprenoid isolated from the Soudan Shale.
compounds are compared in Figure 52, together with that of the C<sub>21</sub> isoprenoid isolated from the Soudan Shale. Again, they show considerable similarities to each other and are only distinguished in the region above m/e 225 where the intensity of the ions is very weak. It is quite evident that it is not possible to assign an unequivocal structure to the C<sub>21</sub> isoprenoid isolated from the Soudan Shale.

The coinjection of the standard C<sub>21</sub> isoprenoid alkane, 2,6,10,14-tetramethylheptadecane, into the 'branch-cyclic' fraction of the Soudan Shale indicates that the C<sub>21</sub> compound isolated from the Soudan Shale has the structure of this standard. This coinjection technique has been repeated on three different phases and an identical result was obtained in each case. Furthermore, coinjection of the alternative C<sub>21</sub> isoprenoid, 2,5,11,15-tetramethylheptadecane, indicates that if this compound is present at all it is present in very small quantities. The structural identification of this C<sub>21</sub> isoprenoid by capillary gas chromatography is shown in Figure 53. Using this procedure we have assigned the same structure to the C<sub>21</sub> isoprenoid compound isolated from the Monesuch Seep Oil and the Antrim Shale (300 x 10<sup>6</sup> years). The characterisation of the structure of the C<sub>21</sub> isoprenoid alkane, as 2,6,10,14-tetramethylheptadecane, suggests that its biological precursor is a C<sub>40</sub> compound, such as lycopene, or higher homologue, rather than a C<sub>30</sub> compound, such as squalene. A C<sub>25</sub> compound is not considered a likely precursor since, to our knowledge, no acyclic C<sub>25</sub> compound has been isolated from biological sources. The diagenetic processes giving rise to the C<sub>21</sub> isoprenoid hydrocarbon, and other isoprenoid hydrocarbons, are discussed at length in Chapter 6.
Figure 58. The identification of the C_{21} isoprenoid hydrocarbon, 2,6,10,14-tetramethylheptadecane, in the Soudan Shale by capillary gas chromatographic coinjection techniques.
EXPERIMENTAL

THE SYNTHESIS OF 2,6,10,15-TETRAMETHYLDEPTADECANE

**β-Methyl Valeric Acid**

This compound was obtained commercially from Baker Chemical Co.

**β-Methyl Valeryl Chloride, C₆H₁₁OCl [134.5]**

\[
\begin{align*}
\text{COCl} \\
\end{align*}
\]

A sample of 5 g of β-methyl valeric acid (M.W. 116) and 10 g of thionyl chloride (M.W. 119) were refluxed in round-bottomed flask on a sand bath for 2 hr. At the end of this period the reaction mixture was cooled and the excess thionyl chloride distilled off under vacuum for final purification. The acid chloride was distilled over and collected. **Yield: 5.2 g (90%).**

**PHYSICAL CONSTANTS**

**INFRARED:**

3.43 - 3.45 μ; -C-H stretch
5.55 μ; carbonyl absorption of acid chloride
6.9 μ; -C-H bending

**3,8,12,16-Tetramethylheptadecan-5-one, C₂₁H₄₂O [312]**

**Synthetic Procedure.** The procedure followed was essentially that described by Cason and Kraus (1961). A 0.95 g (~7 mM) sample of β-methyl valeryl chloride was dissolved in a mixture of dry ether and dry toluene and placed in a 50 ml 3-necked round-bottomed flask inside of which was a magnetic stirrer and which was fitted with a thermometer,
a dropping funnel and a condenser which was connected to a nitrogen line. A nitrogen atmosphere was maintained throughout the procedure. The solution was cooled to -60°C in a CO₂-isopropanol bath and anhydrous ferric chloride was added (22 mg). The ferric chloride was kept dry by weighing under anhydrous toluene and then dissolving by addition of ether. (For preparation of anhydrous ferric chloride solution, see later). The Grignard reagent, which had been prepared beforehand by the usual procedure, was added dropwise through the dropping funnel. The reaction mixture immediately became yellow in colouration. The rate of addition was adjusted so as to allow maintaining the temperature at -60°C (30-60 min. required), and then the mixture was stirred for an additional 60 min. after complete addition. The reaction mixture was decomposed in ice and water, and acidified to pH 3. The aqueous phase was separated and extracted with three 15 ml portions of ether. The total extracts were dried over MgSO₄.

The GLC and IR analysis of the crude product indicated that some hydrocarbon side product had been formed as well as some β-methyl valeric acid from the acid chloride. Column chromatography using neutral alumina further purified the product. After eluting with n-heptane this was then followed by ether and 20 ml of the ether eluate collected. This was found to contain all the ketone. The eluate was dried and the solvent evaporated. Yield: 300 mg (30%).

Preparation of Anhydrous Ferric Chloride Solution. A 55 mg sample of anhydrous ferric chloride was weighed under 3 ml of dry toluene and 20 ml of dry ether was added to dissolve the ferric chloride. This solution was centrifuged and then 0.9 ml (~22 mg) was pipetted from the supernatant. The clear solution was added to the acid chloride.
PHYSICAL CONSTANTS

GLC: (5' x 1/8'; SE 30; Aerograph 665-1) Contains only one component (2% hydrocarbon impurities).

INFRARED:
3.4 μ, 3.45 μ, 3.50 μ, -C-H stretch
5.80 μ, carbonyl absorption (ketone)
6.93 μ, -C-H bending
7.25, 7.30 μ, gem-dimethyl

MASS SPECTRUM: appears to be somewhat anomalous
m/e 310 (M), but also peaks at m/e 326, 332, and m/e 307, 305; m/e 282; m/e 226

2,6,10,15-Tetramethylheptadecane, C_{21}H_{44} [296]

Synthetic Procedure. A 130 mg (4 mM) sample of 3,6,12,15-tetramethylheptadecan-5-one, 1.5 cc of ethylene glycol and 75 mg of potassium hydroxide were placed in a pear-shaped flask. A 0.06 ml sample of hydrazine hydrate was pipetted into the reaction flask and the mixture was heated on a mantle to reflux temperature. This temperature was between 190-210°C. The reaction was refluxed for 2 hr. After this period of time the water formed in the reaction was distilled off. This distillation was continued for 1 hr. The entire mixture was heated at 250°C for 4 hr. to decompose the hydrazone formed. The reaction mixture darkened during this time to a dark brown tar-like residue. After 4 hr. the reaction was cooled and the product extracted with pentane. Yield (of crude product): 50 mg.
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The reaction product was further:purified by, .column chromatography"
The· heptane eluate,,Has,collected ,and ,found :to, contain
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tHO

principal

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components, -the desired, produCt andfarnesane,

These, Here easily.

separated by ,prepar,ative gas chromatography, (SE 30 phase? isothermal
, at 168°C; '25'x 1/4")"
PHYS rCAL

Yield:

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CONs~tAHTS

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(capillary gas chromat,ogr<aph, Perkin-'Elmer

GLC:

,',\!1odel 226; 150 i x'.Ol"; PPE; 2°/min. pro'. gramme)

INFRARED:

. -!.'

5 mg,

7.25~,

<\

BASS SPECTRUM'

.only one component

7.30 ~, gem-dimethyl absorption

• m/e 296 (M); m/e 267, m/e211, m/e 183;

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m/e 141, m/e 113 ••
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Figure 52.

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CHAPTER 5

ISOPRENOID HYDROCARBONS AS 'BIOLOGICAL MARKERS' -- A CRITICAL ASSESSMENT

INTRODUCTION

In Chapter 3 a chronological analysis of a series of oils and shales of different ages and from differing environments was described. In Chapter 4 ambiguities which can arise in the structural interpretation of the isoprenoid hydrocarbons were discussed in conjunction with a synthetic approach which was aimed at eliminating these uncertainties. Having established, therefore, the reliability of the experimental evidence in the characterisation of the structure of these 'biological markers' we now address ourselves to the most crucial problem of all: the validity of the conclusion that the isoprenoid hydrocarbons demand a biological origin and are indicative of life forms at the time of deposition of the rock. The premises upon which the organic geochemical approach rest have already been stated and it is to a critical assessment of these that we now turn.

The organic geochemical approach can be challenged from three standpoints. In the first place the sample analysed could be contaminated either in its past history or during laboratory handling. This is a subject we have discussed at some length in Chapter 2 in the context of the organic analysis of meteorites. Secondly, one has to consider the very real possibility that the organic material seeped into the shale at a period later than the formation of the shale. This is a question of great interest to petroleum geologists and one to which considerable research effort has been directed. The migration question cannot be answered unequivocally because of a lack of knowledge of the
factors involved, relating to oil seepage and the origin of oil in general. One approach has been to examine the geological environment of the sediment under consideration, to determine whether there are any seepage passages in the vicinity of the shale along which oil could have migrated. Another approach has been to examine the carbon isotope ratios of the organic extractable and the insoluble kerogen, to determine if they originated from the same source. Although this approach has not provided a definitive answer to the migration problem it has been possible to determine with some reliability whether migration has taken place or not. This approach is aggravated by uncertainties in our knowledge about kerogen formation. The third, and most crucial, objection to the organic geochemical approach, which strikes at the fundamental premise upon which this approach rests, is that the isoprenoid hydrocarbons could have originated from an abiogenic source and, therefore, are not necessarily indicative of life forms. Many attempts have been made to synthesise isoprenoid hydrocarbons abio-

genically and to characterise such compounds in mixtures such as the Fischer-Tropsch product (Studier, Hayatsu and Anders, 1965). There have been many attempts to find oil deposits which were not derived from biological detritus, and there have been several reports that such deposits have been found (Ponnamp eruma and P ering, 1966a, b; Sylvester-

Bradley and King, 1963). In none of the above cases is the evidence conclusive. However, Natta et al. (1959) have shown that isoprene can be polymerised to higher polymers exhibiting a regular head-to-tail arrangement. It therefore seems feasible that isoprenoid hydrocarbons could be derived from abiological sources by similar processes. This subject is so fundamental that it is worthy of some consideration, particularly in view of the claims that pristane and phytane are the
constituents of extraterrestrial meteorites (Oro et al., 1967a,b).
This, and the other criticisms of the isoprenoid hydrocarbons as 'biological markers', will now be discussed.

PART I. LABORATORY CONTAMINATION

The possibility of laboratory contamination during the handling operation in the extraction of organic materials from sediments has been emphasised by several authors (Blumer and Snyder, 1965; Eglinton et al., 1966; Oro and Noon, 1967a). Organic geochemists have sought to avoid these dangers by carrying out the extraction and subsequent analysis under aseptic conditions. Although laboratory contamination constitutes a constant danger it is the opinion of this author that it can be controlled if the proper preventions are taken. The greatest risk of contamination exists during the analysis of sediments, such as those of Precambrian age, which contain very small quantities of materials. In these cases it is always advisable to run a control to see if any stage of the extraction procedure introduces contamination. Isoprene rings and silicone O-rings are such a source, as well as reagent grade solvents. In most cases the quantity of material contained in these sources is far below that extracted from most sediments. Further, the impurities from alumina can easily be avoided with adequate precautions. Thus, with proper care, laboratory contamination should not be a difficulty. For details of the organic components from such sources as rubber stoppers, finger grease, etc., which are common sources of contamination, the reader is referred to Belsky (1966).
PART II. THE PROBLEM OF MIGRATION

The migration of petroleum fluids into a sedimentary rock formation, at a later period of time than when the sediment was deposited, is one form of contamination that is very hard to evaluate and to which organic geochemists have sought a definite answer. One approach to this problem has been to examine the geology of the adjacent rock formations from the standpoint of depth and relative impermeability. Another approach has been to analyse the organic extracts from various regions within the sample under consideration to see if the distribution of the organic material provides evidence of seepage from a different and younger source rock. Both these approaches, though valuable, do not provide objective answers to the question of migration. The most fruitful, and generally accepted, approach to migration has been to study the $^{13}\text{C}/^{12}\text{C}$ ratios in the organic extractable material and in the insoluble organic matter, kerogen. If both these derive from the same source they should have similar ratios. Before discussing this approach in the context of the Soudan Formation it is necessary to describe some of the research that has been carried in the study of carbon isotope ratios and also some of the work that relates to the origin of kerogen.

The $^{13}\text{C}/^{12}\text{C}$ ratio of a particular sample relative to a standard is defined as:

$$\delta \text{ in per MIL (‰)} = \frac{^{13}\text{C}/^{12}\text{C} \text{ sample} - ^{13}\text{C}/^{12}\text{C} \text{ standard}}{^{13}\text{C}/^{12}\text{C} \text{ standard}} \times 10^3$$

The standard that has commonly been chosen is CO$_2$ prepared from the fossil skeleton of Cretaceous belemnite, Belemnitella Americana, from the Peedee Formation of South Carolina. This scale is called the PDB scale. Thus a
positive δ value means that the $^{13}C/^{12}C$ ratio of the sample is greater than that of the standard; conversely, a negative δ value means that the $^{13}C/^{12}C$ ratio of the sample is less than that of the standard. The precision of this ratio is generally $± 0.1$ per MIL of the ratio.

Photosynthetic organisms are known to discriminate against carbon-13 in preference to carbon-12. Both terrestrial and marine plants have lower $^{13}C/^{12}C$ ratios than their respective carbon sources, atmospheric $CO_2$ and ocean carbonates. The exact mechanism of this fractionation is not known, but various proposals have been put forward which have experimental support. Park and Epstein (1960, 1961) proposed that two fractionation stages were involved, the first being a kinetic effect which occurred during the uptake of $CO_2$ from the atmosphere, the second fractionation step taking place during the fixation of dissolved $CO_2$ via the carboxydismutase enzyme into 3-phosphoglyceric acid.

Several workers have examined the $^{13}C/^{12}C$ ratios for various carbon reservoirs in nature (Craig, 1953, 1954; Keeling, 1958; Silverman and Epstein, 1958). The experimental data is taken from a similar display by Park and Epstein (1961) and is shown in the following chart:

<table>
<thead>
<tr>
<th>Marine limestone</th>
<th>Ocean bicarbonate</th>
<th>Marine algae</th>
<th>Marine petroleum</th>
<th>Atmospheric $CO_2$</th>
<th>Land plants</th>
<th>Terrestrial petroleum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$+5$ ≤ $δ$ ≤ $0$</td>
<td>$-10$ ≤ $δ$ ≤ $-5$</td>
<td>$-20$ ≤ $δ$ ≤ $-15$</td>
</tr>
</tbody>
</table>

$δ$ in %
Several features are evident from this chart. Both marine petroleum and terrestrial petroleum are enriched in C\textsuperscript{12} with respect to the present-day biological source from which they are derived. Since there is evidence which indicates that the C\textsuperscript{13}/C\textsuperscript{12} ratio of atmospheric CO\textsubscript{2} has been constant over large periods of geological time, the relative enrichment of the petroleum with respect to biological source has been explained by invoking a C\textsuperscript{12} enriched fraction in the plant which gives rise to petroleum. Park and Epstein (1961) showed that the lipid fraction, extracted from a series of plants, was enriched in C\textsuperscript{12} compared to the whole plant. The corresponding enrichment in petroleums can be explained if the lipid fraction is the primary precursor of petroleum.

Abelson and Hoering (1961) have investigated carbon isotope fractionation in a variety of photosynthetic and non-photosynthetic organisms. They confirmed the carbon isotope ratios of Park and Epstein on the lipid fraction. They showed that carbon isotope fractionation in the formation of amino acids by photosynthetic organisms also takes place but to a lesser extent. The carbons of the carboxyl group of aspartic and glutamic acids particularly, and of other amino acids, were heavier in C\textsuperscript{13} than the rest of the molecule. Silverman (1966) has also shown that the lipid fraction of organisms have consistently lower C\textsuperscript{13}/C\textsuperscript{12} ratios than do the whole organisms.

Many of the studies on the C\textsuperscript{13}/C\textsuperscript{12} ratios in the extractable and insoluble organic matter have been carried out by Dr. T. C. Hoering of the Geophysical Laboratory of the Carnegie Institution of Washington. The uncertainties arising from the conclusions that are drawn about migration originate in the lack of knowledge concerning the mechanism of formation of the insoluble organic matter known as kerogen. Kerogen...
accounts for more than 95% of the total organic matter that has been deposited in sediments. It has not been an easy task to determine the chemical composition of kerogen. With some difficulty kerogen is isolated from the sediment by HF dissolution. The C:H:N:S ratios are 81:7:10:2 (Degens, 1965, p. 253). Little chemical data is available about the structure of kerogen. It appears to be formed from humic acids, which are high molecular weight compounds of a complex nature, thought to be condensation products of phenols, quinones and amino compounds (ibid. Degens). The chemical nature of kerogen appears to depend on the following factors: (1) The chemical composition and molecular size of the original humic acid; (2) the redox potential in the early stages of diagenesis; (3) the thermal history of the sediment in which it is formed; and (4) the nature of the sediment.

Although considerable research efforts (Breger and Deuel, 1956; Breger, 1960; Breger and Brown, 1962) have been directed towards the understanding of kerogen formation, the details of the mechanism are not known with any certainty.

In his approach to the problem of migration, Hoering examined the \( \frac{^{13}C}{^{12}C} \) ratios of the organic extractables, and that of kerogen. Crude oils and their associated marine kerogens exhibit very similar \( \frac{^{13}C}{^{12}C} \) ratios. Eckelman et al. (1962) and Krejci-Graf and Wickman (1960) have shown that these differences never amount to more than 2 to 3 per MIL. This would seem to indicate that kerogen is probably derived from the same biological source as the crude oil, namely, the lipid fraction. However, it has been shown (Forsman and Hunt, 1958a, b) that kerogen cannot be the condensation product of the lipid fraction alone. To explain away this inconsistency Degens et al. (1963) have assumed that
other biological material must contribute to both kerogen and crude oil which still results in a sizeable C\textsuperscript{12} enrichment. Hoering (private communication) examined the C\textsuperscript{13}/C\textsuperscript{12} ratios for a series of sediments of differing ages and from different continents. His results are shown in Table IX.

Table IX

C\textsuperscript{13}/C\textsuperscript{12} Ratios in Coexisting Soluble and Insoluble Organic Matter

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Age</th>
<th>δ C\textsuperscript{13} (soluble)</th>
<th>δ C\textsuperscript{13} (kerogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent sediment</td>
<td>Aransas Bay, Texas</td>
<td>3 years</td>
<td>-22.01</td>
<td>-18.40</td>
</tr>
<tr>
<td>Green River Shale</td>
<td>Debeque, Col.</td>
<td>Eocene</td>
<td>-27.57</td>
<td>-28.80</td>
</tr>
<tr>
<td>Chattanooga Shale</td>
<td>Tennessee</td>
<td>Devonian</td>
<td>-27.48</td>
<td>-27.46</td>
</tr>
<tr>
<td>Woodford Shale</td>
<td>Oklahoma</td>
<td>Mississippian</td>
<td>-28.26</td>
<td>-27.82</td>
</tr>
<tr>
<td>Lignite Coal</td>
<td>North Dakota</td>
<td>?</td>
<td>-23.60</td>
<td>-22.76</td>
</tr>
<tr>
<td>Bituminous Coal</td>
<td>Pennsylvania</td>
<td>?</td>
<td>-22.84</td>
<td>-22.34</td>
</tr>
<tr>
<td>Kolm Shale</td>
<td>Sweden</td>
<td>Cambrian</td>
<td>-26.19</td>
<td>-27.43</td>
</tr>
</tbody>
</table>

The Precambrian

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location</th>
<th>Age</th>
<th>δ C\textsuperscript{13} (soluble)</th>
<th>δ C\textsuperscript{13} (kerogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>McMinn Shale</td>
<td>Australia</td>
<td>Precambrian</td>
<td>-30.59</td>
<td>-30.71</td>
</tr>
<tr>
<td>Soudan Shale</td>
<td>Minnesota</td>
<td>Precambrian</td>
<td>-25.00</td>
<td>-34.81</td>
</tr>
<tr>
<td>Dark Limestone</td>
<td>Transvaal, South Africa</td>
<td>Precambrian</td>
<td>-25.01</td>
<td>-38.21</td>
</tr>
<tr>
<td>Wintersdorp Shale</td>
<td>&quot; &quot;</td>
<td>Precambrian</td>
<td>-25.78</td>
<td>-36.86</td>
</tr>
<tr>
<td>Fig-Tree Shale</td>
<td>Swaziland, South Africa</td>
<td>Precambrian</td>
<td>-27.55</td>
<td>-26.94</td>
</tr>
</tbody>
</table>

Attention should be drawn to two aspects of this table. In the first place, the C\textsuperscript{13}/C\textsuperscript{12} isotope ratios of the organic extractables and the
kerogen are very similar for the members of the first part of the table. These are generally of much more recent age and are consistent with the findings of Eckelmann et al. (1962). Secondly, when the sediments of Precambrian age are examined, discrepancies become more numerous. Although some Precambrian samples show consistent $^{13}C/^{12}C$ ratios, there are enough discrepancies to cast doubt on the hypothesis that any disagreement in the $^{13}C/^{12}C$ ratios for the organic extractables and the kerogen is indicative of migration. This could, of course, still be the case, but until we know what the precursors of kerogen are and the exact mechanism of its formation, any table, such as Table IX, represents only an empirical correlation.

Hoering has interpreted the results on the Soudan Formation indicate that the organic matter has migrated in from somewhere and is not part of the Soudan system. He also provided strong evidence that the observed differences in carbon isotope ratios for the organic extractables and the kerogen do not arise from partial fractionation by physicochemical processes, of various classes of compounds having widely different carbon isotope ratios. This can be seen from the results of Hoering in Table X, where the various classes of organic compounds which comprise the heptane, carbon tetrachloride, benzene and methanol extracts show similar $^{13}C/^{12}C$ ratios (T. C. Hoering, personal communication).

Evidence from other sources relating to the possibility of migration into the Soudan Shale is inconsistent with this interpretation of Hoering's. However, Weinschein (1965) has carried out a thorough analysis of various samples from the Soudan Formation in which he examines the extracts from the surface of the rock, from the drushed sample, and from the silicate phases of the sample. If migration has taken place at a later date, one would expect to find some evidence of fractionation in
Table X

<table>
<thead>
<tr>
<th></th>
<th>McMinn Shale</th>
<th>Woodford</th>
<th>Nonesuch</th>
<th>Green River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kerogen</td>
<td>-30.71</td>
<td>-27.82</td>
<td>-28.15</td>
<td>-28.80</td>
</tr>
<tr>
<td>CCl₄ Eluate</td>
<td>-30.67</td>
<td>-27.76</td>
<td>-30.30</td>
<td>-29.40</td>
</tr>
<tr>
<td>Benzene Eluate</td>
<td>-30.67</td>
<td>-28.28</td>
<td>-29.81</td>
<td>-27.59</td>
</tr>
<tr>
<td>Methanol Eluate</td>
<td>-30.70</td>
<td>-28.01</td>
<td>-27.71</td>
<td>-27.64</td>
</tr>
</tbody>
</table>

the distribution of organic material in these various samples. No such evidence was observed. Furthermore, our own work on the two samples from the Soudan formation, the surface sample and the mine sample, has shown an overall comparison of the GLC patterns of the total heptane extract (see Figure 22 in Chapter 3) do not show noticeable differences. Digestion of the surface sample in HF and subsequent analysis of the hydrocarbons released gave results which were in accord with those of Meinschein (Johns et al., 1966).

The Soudan Shale is metamorphosed and may have been subjected to temperatures as high as 350°C or 400°C (Cloud, Gruner and Hagen, 1965). It has been argued that isoprenoid hydrocarbons and steranes, which have also been identified in the Soudan (Burlingame et al., 1965) could not have survived such temperatures for any significant length of time without undergoing thermal cracking. The isoprenoid hydrocarbons and the steranes, therefore, must have migrated into the rock at a later period of geological time. Whereas this argument seems quite valid,
uncertainty does exist concerning the geothermal history of the sediment, and it is difficult to determine with any certainty the maximum temperature to which the shale has been subjected. The $\delta^{13}C$ value for the extractable material from this Soudan Shale is comparable with that found in other ancient sediment, whereas that for the kerogen of the Soudan Shale is abnormally low. The large negative $\delta^{13}C$ value for the Soudan and most other Precambrian sediments can be rationalised if we assume that the kerogen derives from specific source materials within the sediment. From the evidence available on the nature of kerogen (Degens, 1965, p. 256) it seems clear that a difference does exist between Precambrian and recent kerogen. The results of Sackett et al. (1965) serve to emphasise that $\delta^{13}C$ values are very sensitive to environmental changes ($\delta^{13}C$ values of -20 to -31 percent corresponding to a temperature range of 26°C to -2°C, are reported for contemporary phyto- and zooplankton as a reflection of temperature variation in the Atlantic Ocean), a factor which must be taken into consideration in assigning significance to $\delta^{13}C$ values.

Whereas $\delta^{13}C$ values represent a most useful and reliable guide as a criterion of biological origin of organic extracts, such values do not as yet provide a definitive answer to the problem of migration. The problem of migration into the Soudan Formation, therefore, remains inconclusive, and further work aimed at a greater understanding of the uncertainties that have been alluded to in this discussion, and particularly concerning the formation of kerogen, may resolve the conflicting evidence.
PART III. THE ISOPRENOID HYDROCARBONS -- ABIGENIC CONSIDERATIONS

ABIGENIC THEORIES OF HYDROCARBON FORMATION

Most of the fundamental building units of the living system, the amino acids, the sugars, the purines and pyrimidines, and others, have all been synthesised in varying amounts by the 'primordial atmosphere' experiments in which energy, in the form of electron irradiation, acts on a gaseous mixture of methane, ammonia and water. This kind of mixture probably comprised the atmosphere of the prebiotic earth. In addition to the synthesis of these fundamental building units, polymerisation and dehydration reactions have been effected which bring about the formation of short-chain polymers such as peptides and nucleotides.

Some naturally occurring hydrocarbons have been considered to have an abiogenic origin. Among the protagonists of this theory, Wilson (1962) has taken the extreme view that all hydrocarbons, and particularly those of petroleum deposits, have an abiogenic origin. He suggested that a small percentage of high molecular weight hydrocarbons, which are contained in some of the meteorites reaching the earth, have been subjected to high enough temperatures to bring about thermal cracking and produce hydrocarbon products of lower molecular weight. Wilson proposed that the maria on the surface of the moon are the residue of this material after the lighter material had been removed by evaporation. He demonstrated experimentally how straight-chain hydrocarbons, which are present in petroleum in significant quantities, might be formed by nonbiological processes. Methyl radicals, produced by the interaction of solar photons on methane, could react with the terminal end of molecules bound to inorganic materials to produce
straight-chain hydrocarbons of gradually increasing molecular weight. While admitting that some of the minor constituents are of biological origin, Wilson maintains that this represents contamination by terrestrial detritus.

An asphalt lake, in Trinidad, dating from Miocene times, has attracted considerable attention for some centuries now and has aroused much speculation concerning its origin. Dauvillier (1965) has proposed an abiogenic origin for the asphalt material of the lake, a proposal which Wilson (ibid. 1962) had suggested in considering hydrocarbon deposits on the earth that might show some resemblance to the maria of the moon. A recent geochemical analysis (Ponnampерuma and Pering, 1966) has characterised the overall hydrocarbon content of this deposit. The hydrocarbon extract is an extremely complex mixture, the aliphatic hydrocarbons being predominantly cyclic in character. No individual compounds were identified, but the overall GLC pattern does show some similarity to the overall hydrocarbon distribution synthesised as the discharge product in the sparking of methane gas. The origin of the hydrocarbons in the asphalt deposit is aggravated by the finding of porphyrins in the organic extract, probably derived from the vegetation which is on the shore of the lake. The possibility that the hydrocarbon deposit could be derived, at least in part, from a similar source, must also be considered. The complexity of the hydrocarbon extract differs quite markedly from the extract of a typical biological specimen, and no satisfactory theory has been proposed to account for this distribution.

Sylvester-Bradley and King (1963) have provided evidence for abiogenic hydrocarbons from hydrothermal deposits in Mountsorrel, Leicestershire, where the bitumen occurs in mineral veins. Although it is
possible that these hydrocarbons could have migrated from another source. The authors consider that this theory is unlikely. The oil deposits are found underlying sedimentary rock, and because oil floats on water, the oil would not have migrated downwards into the igneous rock. Ponnam-peruma and Pering (1966) have analysed the aliphatic hydrocarbon fraction from the Mountsorrel deposit and, as with the deposit from the asphalt lake in Trinidad, this fraction was found to be very complex in character, showing very little resemblance to the aliphatic hydrocarbons in a typical biological shale. However, no individual compounds were identified in the deposit and the abiogenic origin of these hydrocarbons, proposed tentatively by the author, awaits adequate organic geochemical evidence. An anomaly in this connection are the $^{13}C/^{12}C$ isotope ratios which have a large negative value, indicating that the hydrocarbon material is derived from biological sources (Silverman, private communication).

The abiogenic theory for the origin of petroleum has been given serious consideration by Sylvester-Bradley. Such a theory has to explain how inorganic hydrocarbon gases are polymerised. Sylvester-Bradley notes (ibid. 1963) that heavy hydrocarbons which 'show some evidence' of abiogenic origin are found in three common sources: (1) in thucholites with pitchblende; (2) in hydrothermal deposits; and (3) in igneous hydrocarbon mixtures which are in association with alkaline or basic intrusions.

Mueller (cf. Sylvester-Bradley, 1963) comments that the arguments advanced for the abiogenic origin of thucholite hydrocarbons are very strong indeed. Their high-oxygen-hydrogen ratios are in sharp contrast with the lower values that are generally associated with biogenic substances. However, for the hydrothermal deposits and the igneous
hydrocarbon mixtures Mueller prefers a biogenic origin, since "the composition, structure and physical properties are well within the range of distillates, etc., of biogenic residues."

Many other processes have been postulated and justified on an experimental basis for the abiogenic theory of hydrocarbons. Davis and Libby (1964) have shown that high yields of heavy hydrocarbons result from the polymerisation of solid methane by cobalt-60 gamma rays. The Fischer-Tropsch product, obtained by the reaction of carbon monoxide with hydrogen in the presence of catalyst, at about 300°C and under pressure, has been analysed in this laboratory (Belsky, 1966). The GLC hydrocarbon distribution consists of regularly spaced peaks, with a maximum at n-C<sub>23</sub>, determined by coinjection methods. The collection of those peaks with a retention time in the proximity of the isoprenoid alkanes, pristane and phytane, followed by gas chromatographic purification and subsequent mass spectrometric analysis does not give any indication of the presence of isoprenoid alkanes in this product. The compounds that have been identified in this region are the normal hydrocarbons, n-C<sub>17</sub>, n-C<sub>18</sub> and n-C<sub>19</sub>, as well as the straight-chain monolefins, C<sub>18</sub> and C<sub>19</sub>. The GLC distribution of the total alkanes from the Fischer-Tropsch product is shown in Figure 54 compared with the hydrocarbons of a methane spark discharge product. Both hydrocarbon products derive from an abiogenic synthesis. The overall hydrocarbon distributions are quite different, however, and it can be seen that the existence of a complex mixture of unresolved hydrocarbons, characteristic of the GLC hydrocarbon patterns from the asphalt lake deposit in Trinidad and the hydrothermal deposit from Mountsorrel, is not a reliable criterion of abiogenic origin, a criterion that has been tentatively proposed by Ponnampерuma and Pering. Our work on the Abbott Seep Oil
Figure 54. Gas chromatogram of the 'total' alkane fractions from
i) the Fischer-Tropsch product, and ii) methane spark discharge product.
(Johns et al., 1966) where another complex mixture of hydrocarbons is characteristic of the 'branch-cyclic' fraction (see Figure 55) would also seem to invalidate such a criterion, for the evidence here strongly indicates that the hydrocarbons of the Abbott Seep Oil are of biogenic origin. The origin and mechanism of formation of these hydrothermal deposits is of great interest, but it would seem a little premature to invoke an abiogenic origin without a knowledge of the structures of the individual compounds in these deposits. A detailed organic geochemical analysis of the hydrocarbon components should provide a greater understanding of their origin.

Isoprenoid hydrocarbons have been sought, but their presence has not been conclusively proved in hydrocarbon mixtures of known abiological origin. Anders et al. (1965) have claimed that they have identified pristane in the Fischer-Tropsch product but this identification awaits confirmation. Marx (1964) has proposed that hydrogenation of graphite could provide the major hydrocarbon constituents of petroleum. Marx and Breisacher (1963) showed that higher paraffins could be formed in the thermal reaction of graphite and hydrogen. Hydrogenolysis will take place at the exposed surfaces of the graphite hexagonal lattice; isomers involving least internal carbon-carbon breaking will be preferred. Thus this theory would predict that normal hydrocarbons should be formed more readily than branched hydrocarbons, and mono-branched hydrocarbons should be present in greater quantities than the multi-branched hydrocarbons. This prediction is borne out experimentally when petroleum deposits are examined. The formation of the monoterpenes, 2,6-dimethyl-octane is shown at the top of page 181.
Figure 55. Gas chromatograms of the 'total, 'branch-cyclic' and 'normal' fractions from the Abbott Seep Oil.
Despite this ingenious theory the experimental demonstration of it has yet to be achieved. Thus, isoprenoid hydrocarbons have not been synthesised by any of the abiogenic processes mentioned in the preceding paragraphs, and the most reasonable assumption on the available evidence has been that they are derived from biological precursors. The significance of the stereospecific polymerisation of isoprene, by Natta et al. (1959), will be discussed later.

THE ORIGIN OF THE HYDROCARBONS IN A CANADIAN THUCHOLITE

The hydrocarbon material of thucholite has been the subject of much controversy, for the theory has developed that these hydrocarbons could have an abiogenic origin. Both Sylvester-Bradley and Mueller (1953) have favoured the abiogenic theory, and considerable geological evidence has accumulated which supported such a hypothesis. Conflicting evidence has come from a study of the carbon isotope ratios, by Hoefs and Schidlowski (1967), who examined the hydrocarbons occurring in the gold-uranium conglomerates of the Witwatersrand system, South Africa. The δ C\textsuperscript{13} values ranged from -22.4 per MIL to -32.8 per MIL, indicating
that the organic material had probably a biogenic origin. In view of the interest that has been aroused by these Thucholite formations, we decided to analyse the hydrocarbon extract from an organic geochemical standpoint.

The Thucholite sample, which was supplied by Professor Clifford Frondel from Harvard University, is from the Thucholite of Conger, Parry Sound District, Ontario, Canada. Thucholite is supposed, from its manner of occurrence in a graphite pegmatite, to be of nonbiological origin. Much of the geological research on this sample was carried out by Spence (1930), who concluded from his study that the hydrocarbon material had indeed a nonbiological origin. Frondel comments:

"Many similar occurrences have been described over the world, but considering the ease with which organic material can be distilled out of sedimentary or metasedimentary metamorphic rocks, and caught up therein, one always has some doubts about the supposed nonbiological origin. Spence's occurrence is one of the more convincing." (personal communication)

An elemental analysis of the powdered shale gave the following results:

<table>
<thead>
<tr>
<th>Element</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>64.49</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>3.34</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Sulphur</td>
<td>0.40</td>
</tr>
<tr>
<td>Ash</td>
<td>15.2</td>
</tr>
</tbody>
</table>

The sample of Thucholite was crushed by a Wig-L-Bug to a black powder. The hard metal spheroids, presumably uranium ore, which remained after the crushing operation were removed from the black powder. The rest of the extraction procedure followed the outline we have already described, and is shown on the following page.
Small quantities of total heptane soluble material were obtained from the extraction of 20 g of powdered Thucholite. The total heptane extract was not sieved to remove the normal hydrocarbons. The capillary gas chromatograph of the total heptane soluble material is shown in Figure 56, compared with the total heptane solubles from the Soudan Shale, considered to be of biogenic origin. The normal hydrocarbons in the Thucholite extract, which range from n-C\textsubscript{16} to n-C\textsubscript{27}, and maximise at n-C\textsubscript{22}, have been characterised by coinjection evidence and then subsequently confirmed by mass spectrometry. The two small peaks which can be observed close to the n-C\textsubscript{17} and n-C\textsubscript{18} components are of interest since this is the region in which one would expect to find pristane and phytane, if they are present at all. Coinjection of standard pristane and phytane indicates that these two components do indeed correspond
Figure 56. Capillary gas chromatogram of of total heptane solubles from a sample of Thucholite compared with that of the Soudan Shale.

THUCHOLITE EXTRACT. TOTAL HEPTANE SOLUBLES. APIEZON L.

Soudan Shale. Total fraction. Apiezon L.
to pristane and phytane. This identification rests on coinjection evidence alone and has not been confirmed by mass spectrometry. However, the evidence does indicate that isoprenoid hydrocarbons are present in the total heptane soluble material from the Thucholite sample.

The GLC of the total heptane solubles on an Aerograph 665-1 instrument and 3% SE 30 phase (10' x 1/16") is shown in Figure 57, and is to be compared with the total heptane fraction extracted from the other samples -- the San Joaquin Oil, the Antrim Shale, the Soudan Shale and the Nonesuch Shale in Figure 16 and Figure 24, respectively, in Chapter 3. The capillary gas chromatograph of the total heptane soluble material, shown in Figure 56 compared with that from the Soudan Shale, should also be compared with (i) the capillary gas chromatograph of the Fischer-Tropsch product and the methane spark discharge product (Figure 54), two abiogenic hydrocarbon distributions of widely differing character, and (ii) that of the Green River Shale, a typical biogenic sample (Figure 58).

Two features are immediately evident from total heptane solubles of the Thucholite extract. The maximisation of the normal hydrocarbons is of higher carbon number than that of other shales and crude oils that have been examined, which tend to maximise at n-C\textsubscript{17}. In this respect the total heptane solubles shows some resemblance to the normal hydrocarbon distribution in the Fischer-Tropsch product, where the maximum occurs at n-C\textsubscript{23}. Further, the absence of any significant amounts of hydrocarbon material below n-C\textsubscript{17} is another feature which differentiates the Thucholite sample from the GLC distributions of other oils and shales. The total hydrocarbon distribution is concentrated in the range from n-C\textsubscript{17} to n-C\textsubscript{24}. 


Figure 57. GLC of the total heptane solubles from a sample of Thucholite.
Figure 58. Capillary gas chromatogram of 'total' alkanes from the Green River Shale.
In other respects the Thucholite sample shows features which are characteristic of the hydrocarbon extract isolated from any sediment. A series of normal hydrocarbons have been characterised in the hydrocarbon extract and the presence of the isoprenoid alkanes, pristane and phytane, is strongly suggested. When compared with the Soudan Shale 'total' fraction these similarities are quite evident. The carbon isotope ratios, on similar Thucholite-type samples, suggest that the hydrocarbon material has a biogenic origin also.

In conclusion, then, the hydrocarbons isolated from the Thucholite sample show many of the characteristics of a biogenic sample. Differences do exist which should be clarified when a detailed understanding of all the mechanisms giving rise to hydrocarbons in a geological environment are elucidated. At the present time there is no convincing evidence available to suggest that these hydrocarbons have other than a biogenic origin.

**THERMODYNAMIC CONSIDERATIONS**

Two conflicting theories have arisen which endeavour to explain how organic compounds might have been formed in the primordial atmosphere. These two theories involve nonequilibrium processes, on the one hand, and equilibrium processes, on the other. The nonequilibrium processes are Miller-Urey type reactions which utilise as an external energy source an electric discharge to bring about the chemical reactions. These reactions have been considered to take place in a reducing atmosphere where the elements C, O, N are in their reduced form as methane, ammonia and water. This atmosphere has been proposed for that of the primitive earth by analogy with the atmosphere of other planets which spectroscopic evidence indicates are reducing in
character. Reactions of the Miller-Urey type have been very successful in synthesising the fundamental building units of the living system. The equilibrium processes have been in relative obscurity until just recently because of their inability to produce significant quantities of complex organic compounds. The biologically important compounds are predicted to occur only in trace quantities on the basis of equilibrium calculations.

Suess (1962) first drew attention to the equilibrium theory again when he showed that complex organic compounds could be formed in a 'fractionated' gas phase, where H/C ratio had fallen to about 1/1000 of its cosmic value. Most recently, Dayhoff, Lippincott and Eck (1964) calculated the concentrations of a large number of biologically significant molecules which would be present in an atmosphere if thermodynamic equilibrium obtained under different conditions of temperature, pressure and elemental composition. To include many regions of interest to models of the primitive atmosphere, temperatures between 300°K and 1000°K, and between 10^{-6} and 300 atmospheres were considered. Under a variety of conditions it was found that many of the compounds of biological interest such as ribose, adenine, serine and others, were found in negligible concentrations (less than 10^{-35} M) at all these compositions. Although these concentrations were much smaller than those produced in Miller-Urey type reactions, these research workers emphasised the importance of not only the concentrations of the biological molecules but also the relative concentrations of other compounds, which are related, and which may compete with them in the special reactions postulated for the origin of life.

Studier, Hayatsu and Anders (1965) utilised these calculations to explain the distribution of organic compounds in carbonaceous chondrites.
These workers considered that neither Miller-Urey reactions nor biological processes were capable of accounting for this distribution, particularly the observation that ethane and its homologues are present at less than $10^{-3}$ (the abundance of methane) and also in explaining the preponderance of aromatic compounds. They showed that the observed distribution agrees with a distribution calculated by Dayhoff et al. (1964) for conditions of thermodynamic equilibrium in a C-H-O-N mixture of 500°K, and they went on to suggest that the organic constituents of meteorites may all have originated by equilibrium processes. Urey and Lewis (1966), and Burlingame and Schnoes (1966) have criticised this theory on various accounts. The ad hoc assumption that graphite is absent from the equilibrium mixture has been regarded by Urey as arbitrary and untenable. In addition, it still does not provide the concentrations of nitrogen compounds capable of undergoing further stages in chemical evolutionary processes. Some of the experimental evidence has also been questioned. The origin of the organic compounds of meteorites is still in doubt. Such compounds could have been produced by either high energy radiations or by living organisms of terrestrial origin contaminating the meteorite, or alternatively by the equilibrium processes postulated by Dayhoff et al. (1964).

The equilibrium theories have focused attention on the origin of the hydrocarbons. Isoprene, the hydrocarbon whose polymer constitutes natural rubber, would be the product of the hypothetical reactions postulated by Dayhoff et al. (1964). A saturated isoprenoid hydrocarbon such as phytane containing 20-carbon atoms has more than one million isomers. Dayhoff's calculations indicate that the concentration of any one of these isomers is so small that even a million of them would have a negligible total concentration.
To examine further the thermodynamic stability of certain of these hydrocarbons we tabulated the free energy of formation of these compounds, particularly those that might be considered to be precursors of the isoprenoid hydrocarbons in an abiological synthesis under equilibrium conditions. The free energy of formation, ΔF°, at different temperatures between 0°K and 1500°K, for a series of C₅H₈ hydrocarbons including isoprene, are shown in Figure 59. Among the C₅H₈ isomers the conjugated compounds are always more stable than the other isomers, which is to be attributed to the extra resonance stabilising energy of the conjugated system. Isoprene is the most stable of all the C₅H₈ isomers up to 1000°K, where the entropy factor due to the methyl branch destabilises the compound with respect to cis and trans 1,3-pentadienes. On a thermodynamic basis the isoprene molecule would be expected to be most readily formed, therefore.

The relative stability of the saturated C₅H₁₂ isomers can be seen in Figure 60, where ΔF° is plotted against temperature in the range 0°K to 1500°K. At the lower temperatures, the more highly branched isomer, neopentane, is the more stable compound, whereas at the higher temperatures this becomes the least stable compound and n-pentane is more stable. The 'cross-over' point is approximately 475°K. This same entropy effect, which destabilises the more branched compounds at the higher temperatures, can be seen conspicuously for the C₆H₁₄ isomers, listed in Table XI.

The free energy of formation (ΔF°) for the normal alkanes at various temperatures between 0°K and 1500°K is shown in Table XII. From this table it is evident that methane is the thermodynamically most stable of the normal alkanes at all temperatures. This indicates that the reaction C₂₀H₄₂ → 20 CH₄ + 19 H₂ proceeds with the liberation
Figure 59. The change in the free energy of formation, $\Delta F^\circ$, with temperature for a series of C5 hydrocarbons.

- Isoprene
- 1,4-pentadiene
- 1,2-pentadiene
- 2,3-pentadiene
- 3-methyl-1,2-butadiene
- 1, cis-3-pentadiene
- 1, trans-3-pentadiene

Temperature, $T$, °K
Figure 60. The change in the free energy of formation, $\Delta F^\circ$, with temperature for a series of C$_5$H$_{12}$ isomeric hydrocarbons.
of free energy at, say, 500°K of 20 (-7.84) -124.6 = -281.4 Kcals of energy, since \( \Delta F^\circ \) for \( \text{H}_2 \) is zero at all temperatures. The normal alkanes which we find in ancient sediments represent a situation which is not an equilibrium process. However, the greater proportion of methane to ethane in carbonaceous chondrites suggests that this could represent a situation where the equilibrium reaction has gone to completion.
Another interesting result arises if we consider the equilibrium reaction:

\[ \text{C}_5\text{H}_{12} \rightleftharpoons \text{C}_5\text{H}_{10} \rightleftharpoons \text{C}_5\text{H}_8 \]

Isopentane \textit{\textsuperscript{\textbullet}} Isoprene

The free energy of formation of these compounds plotted against temperature is shown in Figure 61. At temperatures below 800°K the order of stability is \( \text{C}_5\text{H}_8 < \text{C}_5\text{H}_{10} < \text{C}_5\text{H}_{12} \). At approximately 970°K isoprene becomes the most stable of these compounds and thermodynamics would predict, therefore, that the reaction shown above should go to completion above these temperatures.

When considering the likelihood of an abiological route to the isoprenoid hydrocarbons via isoprene such thermodynamic considerations are of some significance. It can be seen that isoprene is not only the most stable of the \( \text{C}_5\text{H}_8 \) isomers but also could be derived from saturated analogues of the same carbon numbers above certain temperatures. Thus, there is built into the isoprene molecule a thermodynamic stability. This is analogous with the generation of higher degrees of molecular order in the polypeptides. When a polypeptide becomes eight or ten units long, there is built into this molecule factors which may give rise to secondary structure. The secondary structure is an intrinsic part of the overall linear array of consecutively linked amino acids, and no additional external influences are needed to produce the helical coiled geometry. The next development in the structural assembly is the characteristic of auto-catalysis, namely, that certain kinds of structures in some way control or induce the subsequent structural character of the molecule. It is this kind of an intrinsic quality which may be contained in the thermodynamic stability of the isoprene molecules. Analogies here can also be found in present
-1960-

day organic chemistry. Considerable research efforts are being directed towards finding the exact conditions under which the squalene molecule is cyclised to a tetracyclic molecule in stereospecific manner in the laboratory, a reaction that would be analogous with the enzymic cyclisation. Attempts to achieve this have been partially successful. Here, again, it is thought that squalene can be induced into a specific conformation from which the cyclisation reaction can be initiated. Such thermodynamic considerations give credence to the idea that the isoprene molecule is a stable intermediate in the formation of the higher homologues in this series. In the subsequent polymerisation step, we shall now compare the stereospecific mechanism by which the biological system brings about this reaction, with the mechanism of the non-biological polymerisations.

BIOSYNTHETIC CONSIDERATIONS

The biosynthetic pathway of the isoprenoid hydrocarbons has been fully elucidated (for a general review, see R. B. Clayton, 1965). It has been established that in the biological system isopentenyl pyrophosphate plays an important role. This five-carbon fragment whose structure is shown below is the precursor to all the intermediates

\[
\text{CH}_3\text{-C-CH}_2\text{-CH}_2\text{C}(\text{PP})
\]

involved in the isoprenoid pathway. Polymerisation of this five-carbon unit takes place by nucleophilic attack at the 5-position of isopentenyl pyrophosphate and subsequent displacement of the pyrophosphate group to give a 10-carbon fragment, or monoterprenoid compound. This displacement involves a head-to-tail linkage and has been shown to take place in a
sterespecific manner (Cornforth et al., 1966). This biological mechanism is repeated in further polymerisation reactions. However, the head-to-tail mechanism is replaced by a tail-to-tail one at two places:

1) The $C_{15}$ compound, farnesyl pyrophosphate, reacts with another molecule of farnesyl pyrophosphate to give the hydrocarbon, squalene:

![Squalene structure]

2) In the biosynthetic pathway to the carotenoids, $C_{40}$ terpenoid compounds, an analogous tail-to-tail linkage is formed between two $C_{20}$ compounds.

These general features characterise the isoprenoid pathway in biological systems.

Certain other aspects of the stereospecific character of the biosynthetic pathway should be mentioned here. The work of Lindgren (1965) has shown that homologous alipathic $C_{30}-C_{45}$ terpenols are found in the wood extractives of birch (Betula verrucosa Erh.). The alcohols have the general formula:

$$H-(CH_2-C(CH_3) = CH-CH_2)_n-OH$$

where $n$ is 6, 7, 8, 9.

About 60% of their double bonds have the cis configuration. This finding shows that $C_{30}$ terpenoid and $C_{40}$ terpenoid structures have been isolated from living organisms containing both the tail-to-tail linkage and the head-to-tail linkage. Higher terpenoid compounds have been isolated from pig liver, which is a rich source of dolichol. The alcohol has fifteen or sixteen of its eighteen internal isoprene units in the
cis configuration (Butterworth et al., 1966):

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_3 \text{-C}=\text{CH-CH}_2 \text{-}(\text{CH}_2 \text{-C}=\text{CH-CH}_2)^{18} \text{-CH}_2 \text{-CH-CH}_2 \text{-CH}_2 \text{OH}
\end{align*}
\]

Dolichol

The biosynthetic pathway to rubber and gutta, which is also a polyisoprenoid and is produced by a small number of tropical species, has been discussed by Bonner (1963). Both rubber and gutta are polymeric substances derived from isoprene in which the isoprene units are linked together through 1,4 linkages. Rubber contains from 500 to 5000 isoprene units, while gutta contains about 1000 units. It is of interest to note the double bonds of the individual isoprene molecules are in the cis configuration for rubber and in the trans configuration for gutta. No polymers have been found which contain both the cis and trans geometry.

In recent years, studies on the non-biological polymerisation of small organic molecules such as propylene and butadiene have indicated that these reactions proceed with considerable stereospecificity. It had been hitherto thought that stereospecificity in polymerisation of the isoprene molecule was characteristic of the biological system. The work of Tobolsky and Rogers (1959) seemed to confirm this. Dispersions of metallic lithium, sodium and potassium were used to initiate the polymerisation of isoprene. Depending on the nature of the metal and the solvent (e.g. diethyl ether, tetrahydrofuran) the polymerisation proceeded by 1,2 linkages, 3,4, linkages and 1,4 linkages, in varying amounts. With lithium and diethyl ether, for instance, the product contained 93% 1,4 cis configuration and 7% 3,4 configuration.
Natta was the first to show that the polymerisation of conjugated di-olefins can proceed in a stereospecific manner. He showed that butadiene, isoprene and 1,3 pentadiene were converted to polymers containing 99% of linear 1,4 trans structures by means of a highly stereospecific catalyst Al(Et)$_3$-VCl$_3$ (molar ratio 2:1) in heptane (Natta et al., 1958). The presence of even small amounts of impurities impairs the stereospecificity of the reaction. The infrared spectrum of the polymer resembles that of natural rubber. X-ray diffraction studies show the linearity of the polymers and a periodicity of 4.82 Å along the main axis. The use of TiCl$_3$ produces a stereospecific 1,4 cis linked polymer. Natta and his co-workers then showed that by changing the catalyst to a lithium alkyl derivative, the same cis 1,4 rubber resulted.

The exact mimicking of the stereospecific features of the biosynthetic pathway in the terpenoid series would seem to dispel the notion that the head-to-tail linkage is unique to biological systems. Natta's work has shown that not only the 1,4 trans configuration of the biopolymer, gutta, but also the 1,4 cis configuration of the natural rubber can be reproduced by non-biological methods. These findings call into serious question the validity of the isoprenoid compounds as 'biological markers.' Certainly the structure of the isoprenoid hydrocarbons taken in isolation can no longer be considered to be unambiguously derived from a biological precursor. The presence of the isoprenoid hydrocarbons in crude oils and sediments must be viewed against the background of the other components present.

By emphasising these stereospecific non-biological polymerisation processes it is not intended to nullify the value of isoprenoid compounds as 'biological markers.' The characteristic tail-to-tail linkage seems to take place exclusively at C$_{15}$ to give C$_{30}$ compounds such
as squalene, the precursors of the steranes and triterpanes, and at 
C₂₀ to give C₄₀ compounds such as lycopene. It may be that the non-
branched 4-carbon unit in this tail-to-tail linkage is the criterion 
for which we are looking in assigning a biological origin to compounds 
isolated from crude oils and sediments. For the present, the findings 
of Natta and his co-workers support the hypothesis that such polymeri-
sation processes could have proceeded stereospecifically in the primor-
dial atmosphere during the early history of the earth.

Let us consider a possible reaction where isoprene is polymerised 
abiogenically. One would predict, a priori, that three compounds 
should be formed, the head-to-tail linkage (h-t), the tail-to-tail 
linkage (t-t), and the head-to-head linkage (h-h). This is illustrated 
below:

```
     (h-t)       (t-t)       (h-h)
     isoprene    
```

(The saturated compounds are drawn as products, though there is no 
reason to expect that this would necessarily be the case, unsaturated 
compounds probably resulting.) There are some analogies for this 
type of mechanism. Ramsden, Engelhart and Naegele (1967) have recently 
shown that conjugated olefins react readily but slowly with metallic 
magnesium to form organomagnesium derivatives which have the reactiv-
ity of Grignard compounds. When isoprene is the conjugated di-olefin, 
two moles of isoprene react with one gram atom of magnesium. Hydroly-
sis of the organomagnesium derivative yielded monoterpenoid hydrocarbons 
which all have the tail-to-tail linkage. The structures of the primary 
products are given on the following page;
The structure of the organomagnesium derivative has been postulated as a diallylic derivative of the following type:

\[
\text{CH}_3 \quad \frac{\text{C}}{\text{CHCH}_2\text{CH}_2\text{CH}} \quad \frac{\text{C}}{\text{CHCH}_2\text{CH}_2\text{CH}} \quad \text{Mg}
\]

The same workers have also shown that an organomagnesium compound forms with myrcene, a C\textsubscript{10} monoterpen:

The subsequent hydrolysis products have not been structurally identified. The demonstration of a synthetic procedure whose mechanism involves a tail-to-tail linkage illustrated that this type of polymerisation of isoprene by a nonenzymic method is possible. If dimerisation were to occur by a radical ion addition, a head-to-head, or a tail-to-tail, product would be expected.

When we consider the addition of another molecule of isoprene to produce a C\textsubscript{15} compound, and a further molecule to give a C\textsubscript{20} compound, then several products should result whose structural skeletons are shown in Figure 62. These compounds might be expected to be present in
Figure 62. Hypothetical scheme in a non-biogenic polymerisation of isoprene.
hydrocarbon mixtures if they were derived by an abiogenic process
which did not proceed stereospecifically. Thus, the presence of such
structures in organic extracts might be used as a criterion for abiogenic origin.

The C_{10} isoprenoid compounds have not been extensively analysed
in crude oils and sediments. Bendoraitis et al. (1962) have reported
the presence of 2,6-dimethyloctane in an East Texas crude oil, and
Mair et al. (1966) have also reported this compound, and 2-methyl-3-
ethyl heptane, which they consider to be derived from limonene by thermal cracking. The same authors have also reported the presence of
2,7-dimethyloctane in a crude oil (Mair et al., 1962). Other reports
are limited.

Since by far the vast majority of the constituents of organic ex-
tracts from crude oils and sediments have not been identified, it would
be worthwhile carrying out an extensive search for the type of compounds
that would be predicted to form in a postulated abiological polymeri-
sation of isoprene. There is a practical problem in that it would be
necessary to have synthetic standards available for structural identi-
fication of these compounds. The synthesis of many of these compounds,
almost all of which are not readily available, would be extremely com-
licated. Only in the case of the C_{10} compounds is the synthetic route
straightforward. However, the C_{10} compounds seem to be present in
significant quantities only in the crude oils, such as the San Joaquin
Valley Oil. The 'branch-cyclic' fraction from the Soudan Shale and
the Antrim Shale are almost devoid of lower molecular weight compounds
in the C_{10} regions. It would seem, therefore, that the C_{15} compounds
and the C_{20} compounds might be more profitably sought. It was with
this objective in mind that we looked for crocetane, 2,6,10,15-tetramethylhexadecane, in a series of oils and sediments. These results are reported in an earlier part of the thesis.

Isoprene has been synthesised in extremely large quantities on a commercial basis by Dow Chemical Company. Many of the side products of this industrial synthesis, and particularly those present in small quantities, have not been analysed. In the hope that some polymerisation products of isoprene might be present in this higher molecular fraction, it was decided to carry out a careful analysis of this sample. The results are now described.

A SEARCH FOR THE POLYMERISATION PRODUCTS OF ISOPRENE

Our interest was directed towards an investigation of the higher molecular weight material produced in the ethylene plant of the Dow Chemical Company, Freeport, Texas, when it was learned that isoprene was produced as a side product in sizeable quantities. The production of ethylene involves feeding in various amounts of propane and ethane into a pyrolysis furnace where these compounds undergo thermal cracking at temperatures approaching 1500°K. It was thought that at these temperatures small amounts of products resulting from a thermal polymerisation of isoprene might be present. Accordingly, we analysed the heavy residue (designated T-10 bottoms) produced in this commercial process.

The ethylene plant recycles all of the propane and lighter constituents internally. The C_40 compounds and heavier constituents are separated in a series of distillation towers. An analysis of the T-10 bottoms indicates that this mixture comprises a large proportion of aromatics and a very small quantity of non-UV absorbing compounds.
Dicyclopentadiene is the major constituent of this latter category, and its presence was confirmed by us in approximately the proportion as above. It is interesting to note the relative proportions of the following compounds:

1) **T-7 overhead**
   - Pentanes: 8.3%
   - Pentenes: 14.2%
   - Isoprene: 18.8%
   - 1,3-Pentadiene: 14.2%

2) **T-7 overhead**
   - Cyclopentadiene: 11.4%
   - Cyclopentene: 10.9%
   - Cyclopentane: 1.2%

3) **T-9 overhead**
   - Butadiene: 62.7%
   - Butenes: 22.2%
   - Butanes: 7.1%

Throughout, there is a tendency for the more unsaturated compound to predominate. This is in keeping with the thermodynamic stabilities of the compounds at elevated temperatures. It suggests that the direction of the reaction is so as to produce the most thermodynamically stable product at the temperature of the reaction. It is not surprising, therefore, that isoprene is produced in such large quantities, since this is the most stable of the C₅ compounds above 975⁰K of Figure 61.

The analysis of the T-10 bottoms was similar to that described for the San Joaquin Oil in Chapter 3, and is outlined on the following page.
T-10 Bottoms (2.2 g)

Neutral alumina column chromatography
Pentane solvent

Total Pentane Eluate (10 g)

Alumina chromatography

Non-UV Absorbing Material (\(\sim\)225 mg; 530 mg)

The preparative GLC pattern of the non-UV absorbing material is shown in Figure 63. Fractions were collected and analysed by mass spectrometry. The results are listed in Table XIII.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Mass Spectrum</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>m/e 120 (M); m/e 66</td>
<td>Figure 64</td>
</tr>
<tr>
<td>2</td>
<td>m/e 132 (M); m/e 66</td>
<td>Figure 65</td>
</tr>
<tr>
<td>3</td>
<td>m/e 146 (M); m/e 80, 67</td>
<td>Methyl cyclopentadiene cyclopentadiene dimer.</td>
</tr>
<tr>
<td>A</td>
<td>m/e 146 (M)</td>
<td>Structures unknown,</td>
</tr>
<tr>
<td>B</td>
<td>m/e 146 (M); m/e 148 (M)</td>
<td>but show similarities to fractions 1, 2, 3.</td>
</tr>
<tr>
<td>C</td>
<td>m/e 160 (M)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>m/e 268 (M); m/e 183</td>
<td>Pristane</td>
</tr>
<tr>
<td>5</td>
<td>m/e 254 (M)</td>
<td>(n-C_{18})</td>
</tr>
<tr>
<td>6</td>
<td>m/e 282 (M)</td>
<td>(n-C_{20})</td>
</tr>
</tbody>
</table>
DOW SAMPLE. T-10 BOTTOMS

PHASE: 3% SE 30
ATT‘N: 1 x 2

Gas chromatogram of the non-UV absorbing material from the T-10 bottoms, run on a preparative column.

Program

Isothermal

250°C

550°C
The mass spectrum of Fraction 1 is shown in Figure 64, and the structure of this compound is probably butadiene-cyclopentadiene dimer:

\[ \text{\includegraphics[width=0.2\textwidth]{butadiene-cyclopentadiene-dimer.png}} \]

This compound has a molecular weight of 120 and would be expected to dissociate very readily to give the cyclopentadiene molecule, m/e 66. The mass spectrum of Fraction 2 is shown in Figure 65 compared with a standard mass spectrum (API #1616) of dicyclopentadiene. This spectrum has a characteristic m/e 66 which confirms the identity of Fraction 2 as dicyclopentadiene:

\[ \text{\includegraphics[width=0.2\textwidth]{dicyclopentadiene.png}} \]

In addition, the ultraviolet spectrum of Fraction 2 indicates that there is no absorption above 225 μm, which is also in accord with the dicyclopentadiene structure. Fractions 3, A, B, C have not been identified unambiguously but their structures are probably dimers of the same type as Fractions 1 and 2. Fraction 3 shows considerable resemblance to the mass spectrum one would expect from the methyl cyclopentadiene-cyclopentadiene dimer:

\[ \text{\includegraphics[width=0.2\textwidth]{methyl-cyclopentadiene-dimer.png}} \]

The higher molecular weight regions aroused considerable interest because Fractions 4, 5, 6 exhibit mass spectra which show similarities to
Figure 64. Mass spectrum of Fraction 1.
Figure 65.
typical compounds isolated from crude oils and shales. Fraction 4 was identified as pristane, and Fractions 5, and 6 as n-C\textsubscript{18} and n-C\textsubscript{20} compounds, respectively. The normal alkanes were removed by occlusion in molecular sieves and showed a distribution in carbon number from C\textsubscript{16} to C\textsubscript{21}, maximising at n-C\textsubscript{17}. This again shows resemblance to the normal alkane distribution in several crude oils that we have analysed. The normal alkanes constituted <.001% of the total pentane eluate. Because of the similarity of the higher molecular region of the T-10 bottoms to that of a typical crude oil, we decided to analyse a sample of compressor injector oil to see if any of this had been mixed in with the product from the pyrolysis furnace. A 1.3 g sample of compressor injector oil was chromatographed on neutral alumina column, and gave 0.94 g of non-UV absorbing material. The GLC pattern of the non-UV absorbing material of the compressor injector oil and that of the T-10 bottoms are compared in Figure 66. A comparison of the higher molecular weight region of both samples shows considerable similarities. Many of the characteristics of the overall pattern are identical for both samples. Only a few minor differences exist in this region. It would therefore seem that the T-10 bottoms are contaminated with hydrocarbons from the injector oil.

This finding would seem to undermine the reliability of any other results which might be obtained on further analysis of this sample. Further work was not continued. One other result should be noted. When standard 2,6-dimethyl octane was coinjected with a sample of the T-10 bottoms into a capillary gas chromatograph, no evidence was obtained for its presence in this sample. This might be expected to be present in the pyrolysis furnace as a dimerisation product of isoprene,
I. DOW SAMPLE, T-10 BOTTOMS, TOTALS, (NON-AROMATIC)

II. COMPRESSOR INJECTION OIL

Figure 66.
either in its saturated, or partially saturated, form. Since compressor injector oil has found its way into the higher molecular weight fraction it is not possible to make definitive conclusions about the origin of the components of the T-10 bottoms. Polymerisation products of isoprene may indeed be present in this fraction in small amounts, but it would be difficult to prove with any degree of probability that they were not components of the compressor injector oil.

The production of isoprene in significant quantities as a side product in the formation of ethylene from ethane by thermal cracking, is the first demonstration of a process that could be reasonably invoked to explain how isoprene could be synthesised in the primitive atmosphere. Ethane itself, although thermodynamically less stable, could be formed from methane which has generally been considered as a component of this atmosphere. Natta’s studies on the polymerisation of isoprene have shown how isoprenoid compounds containing exclusively head-to-tail linkage might be formed by non-biological methods. We therefore have a speculative mechanism which could account for the formation of isoprenoid hydrocarbons from one of the components of the primitive atmosphere.

CONCLUSIONS -- THE CRITERIA FOR ABIGENIC ORIGIN

In the preceding pages of this chapter we have described the various approaches, both theoretical and experimental, that have been adopted in trying to establish whether the components of the organic extracts isolated from crude oils and shales have a biogenic origin or an abiogenic origin. It would be true to say that for some organic extracts this question has not been answered with any reliability and criteria have yet to be found which will provide an unequivocal answer.
to this problem. At the present time the evidence points overwhelmingly to a biogenic origin for almost all the organic extracts of crude oils and shales. It is when we come to analyse such extracts from meteorites, from Thucholite samples and from hydrothermal deposits that the origin of the organic extract is much less clear cut. In view of the recent polymerisation studies which have brought into question the validity of isoprenoid hydrocarbons as 'biological markers' it is important to search for criteria which will provide an unambiguous answer to these uncertainties and which will reconfirm the fundamental premise upon which the organic geochemical approach rests. This is more than just an academic exercise, for it is these same uncertainties about the origin of the organic extracts that are likely to arise when the lunar samples are returned to earth for analysis. It is therefore imperative that these uncertainties be resolved.

Among the many approaches that have been adopted in endeavouring to find criteria which will determine whether organic material has an abiogenic or biogenic origin, one particular approach, which has not been utilised to any significant extent, appears to be most promising. This approach involves the determination of the precise stereochemistry, and particularly the absolute configuration of the optical centres, of the individual constituents of these organic extracts. In Chapter 4 we emphasised the importance of being able to characterise the structure of the individual constituents with utmost certainty. In the experimental approach it is necessary to carry out as many physical measurements as possible on an individual compound to designate an unambiguous structure. No attempt was made, however, to determine the stereochemistry of the isoprenoid hydrocarbons isolated from the crude oils.
and shales, nor, in general, have such determinations been made by other research workers in organic geochemistry. There are very good reasons for this. Until just recently it has been felt generally that the very specific structural architecture of the 'biological markers' was in itself adequate evidence for a biological origin. With the isoprenoid hydrocarbons, for instance, the characteristic methyl branch at every fourth carbon atom is so specific that it has been generally accepted to be indicative of a biogenic origin. Further, it has been very difficult to isolate the individual components in sufficient quantity and purity from the very complex organic mixtures to obtain a reliable optical measurement. Finally, with the isoprenoid hydrocarbons in particular, the optical rotations are so small that much larger quantities of the compound than normal (20-50 mg), as opposed to the optical measurements carried out by Hills and Whitehead (1966) on milligram amounts, are required. Such quantities of individual compounds are impossible to obtain from most organic geochemical samples. For these reasons, reports of optical measurements having been carried out in organic geochemistry are scarce.

To overcome this practical problem, another approach has to be adopted to determine the optical configurations of these 'biological markers', and specifically those of the isoprenoid hydrocarbons. This approach involved the separation of the diastereoisomers of the isoprenoid hydrocarbons. This approach involves the separation of the isoprenoid hydrocarbons by capillary gas-liquid chromatography. Both Weedon et al. (1959) and Djerassi et al. (1959) have independently determined the absolute configuration of phytol. It has been shown that phytol is 3, D-7, D-11, 15-tetramethylhexadec-trans-2-ene-1-ol, as shown on the following page.
The absolute configuration at carbons 7 and 11, therefore, have been shown to be the same as that of the D-configuration. This was confirmed synthetically by Weedon et al. (1959). Starting from citronellal, having an optical purity of 80%, C18 ketones were synthesised whose optical rotations were compared with another C18 ketone obtained from the ozonolysis of phytol ($\alpha_D^{21} + 0.03^\circ$). In the course of this synthesis, a C18 ketone was converted into the fully saturated hydrocarbon, pristane, by a Wittig reaction. Starting from C18 ketones having different optical configurations, two 'pristanes' were obtained, the meso-form, and a mixture of d,l forms of the other diastereoisomer. The optical rotations of both these forms were shown to be zero and $0.18^\circ$ ($\alpha_D^{26}$) respectively. The availability of these standards provides the opportunity of correlating the absolute configuration of pristane isolated from biological sources with pristane isolated from geological samples. Pristane isolated from biological sources is thought to have the meso-configuration but, to our knowledge, this has not been correlated with pristane isolated from any geological samples. The two forms of pristane are shown on the following page.
The separation of the diastereoisomers of pristane in very small amounts by capillary gas-liquid chromatography would provide a major breakthrough in experimental techniques in organic geochemistry. More significantly, it would enable one to establish a criterion whereby one could recognise organic compounds derived from abiological sources from those derived from biological sources, since, a priori, one would not expect a C19 saturated isoprenoid hydrocarbon, having exclusively the meso- configuration, to be produced in an abiogenic synthesis.

Attempts have been made in this laboratory to overcome this practical problem and have so far met without success. One would
expect that the ideal phase should be of high molecular weight, not necessarily being optically active, but certainly having a predominance of one diastereoisomeric configuration. In this connection, dihydrophytyl adipate was used as a GLC phase. Dihydrophytol is formed by hydrogenation of phytol (Cason and Graham, 1965) and has two centres, at 7 and 11, which have the D,D configuration of phytol. The other centre, at C_{21}, produced on hydrogenation is presumably racemic. The ester was formed by reaction of dihydrophytol with adipic acid under normal esterification conditions. The purified ester was then used to coat a Perkin-Elmer capillary column, 150' x .01' in dimensions. Various practical problems arose at this stage. The coating of such columns is a delicate procedure which has not been mastered by this group. Thus, when it was attempted to separate the diastereoisomers of farnesane, severe tailing of the farnesane peak was observed. In spite of this, there was no evidence that any separation had taken place. Other phases have been tried, utilising the wax in the free flowing preen gland secretion from living geese (Odham, 1963), which consists of C_{14} and C_{15} branched fatty acids. Similar negative results were obtained by Belsky (1965). One of the difficulties in the phases so far used has been the relatively high bleed rate at temperatures above 150°C. This has aggravated the separation of these diastereoisomers, and future attempts should probably seek phases of higher molecular weight. One such phase might be the ester formed from dihydrophytol and pristanic acid, which, besides producing an ester that should not bleed readily, will also possess a specific diastereoisomeric configuration at six different carbon atoms. Further efforts need to be continued to bring about this separation.
Although this practical problem still remains an obstacle, the stereochemical approach will provide the only reliable criterion in determining the biogenic origin of an organic extract. For the present, the most reliable approach still lies through the carbon isotope ratios. Although not definitive in some cases, such measurements have already discredited certain proposals for an abiogenic origin of the hydrocarbons from hydrothermal deposits (Ponnamperuma et al., 1966).

Further insights into this problem might also be obtained from a development of the carbon isotope approach. Nobody has studied the carbon isotope ratios of an individual molecule, although work is presently in progress on the gammacerane molecule, which can be crystallised from the total heptane fraction of the Green River Shale, (Robinson, private communication). There are practical problems involved in this approach. The isolation procedures involving crystallisation and chromatographic techniques themselves bring about isotope fractionation of individual molecules, so that it would be necessary to isolate the total amount of each component from the hydrocarbon mixture, or at least obtain an accurate estimate of the extent of the isotope fractionation at each stage of the isolation procedure. Since each of the biological molecules have a different biosynthetic pathway, the carbon isotope ratios should be different for each of these molecules. Diagenetic processes should affect the carbon isotope ratios, but these changes should be smaller than the fractionation brought about by the biological system. Using high resolution mass spectrometry, it should also be possible to determine the carbon isotope distribution at each of the individual carbon atoms of, say, the pristane molecule. This particular approach would seem to be a most
useful one to adopt in ascertaining the origin of the isoprenoid hydrocarbons found in geological deposits.

Other criteria, such as the distribution of normal alkanes in sediment extracts are only reliable for the most recent sediments. The identification of other 'biological markers' than the saturated hydrocarbons is most useful in complementing the evidence for biological origin. Except for the porphyrins, however, and the steranes and triterpanes, other classes of 'biological markers' do not survive into the most critical region of interest, the Precambrian, which imposes a severe limitation on this approach. The finding of iso-, anteiso- and cyclohexyl homologous series in crude oils and sediments, while not in themselves necessarily indicative of biological origin, do augment the evidence from the isoprenoid hydrocarbons, when identified in the same extracts. However, these are nowhere nearly as ubiquitous in organic geochemical samples as the isoprenoid hydrocarbons.

The problem of the origin of the isoprenoid hydrocarbons remains inconclusive. The evidence still indicates that isoprenoid hydrocarbons are in most cases derived from biological sources, even in the Precambrian samples. It is in this geological time period, however, that we are looking with renewed interest for the transition between chemical evolution and the advent of biological systems. Natta has already demonstrated an experimental, non-biogenic route to the isoprenoid compounds. The possibility of non-biogenic isoprenoid hydrocarbons is a very real one, and criteria need to be established which will distinguish between those derived from an abiogenic origin and those derived from biological systems.
CHAPTER 6

DIAGENESIS

INTRODUCTION

Diagenetic studies are concerned with understanding the transformations that take place in the organic matter from living organisms after it has been deposited in the geological environment. The general factors which influence these changes can be divided roughly into three broad categories:

1) The chemical changes due to microorganisms, which involve hydrolysis of the large macromolecules from living systems.

2) The subsequent formation of 'humic acids' or 'heteropolycondensates' by condensation of the hydrolysis products.

3) Inorganic maturation of the organic matter over long periods of geological time under the primary influence of temperature, (Degens, 1965).

An understanding of the mechanism of these diagenetic changes has been the objective of organic geochemists for some time for several reasons. Diagenetic studies shed light on both the course of biochemical evolution and the origin of petroleum. In addition, they provide information on the thermal stability of biochemical constituents in the geological environment.

The primary factor influencing the changes of the organic matter deposited from living organisms is the presence of microorganisms in the upper portion of a recent sediment. The destruction of the organic matter, its alteration and subsequent reformation have all been attributed to the presence of microorganisms. Estimates concerning the population of microbes
in soils and sediments indicates that the population decreases quite markedly with depth of burial. In the surface layers populations can amount to 100 million bacteria per gram. Although it has been shown that there is an inverse relationship between the microorganism population and the depth of burial, it is not known whether they become extinct at some depth of burial corresponding to some period of geological time. Claims have been made that viable bacteria have been isolated from ancient sediments. Most of these reports have not been confirmed and the experimental difficulties of ensuring aseptic conditions cast much doubt on the validity of these findings.

Microbial activities virtually cease a few feet below the surface of the sediment. Degens (1965) suggests that the subsequent diagenetic changes involve i) inorganic maturation, consisting principally of polymerisation and condensation of the deposited organic matter with the associated minerals; ii) a redistribution of the pre-existing organic molecules, for example, in the formation of petroleum deposits; iii) alterations due to temperature, and possibly pressure also, which may be catalysed by clay minerals. A knowledge of the thermal stability of classes of organic compounds is of value in elucidating the mechanism of degradation. Complementing this information is the presence, or absence, of such classes of compounds at various periods of time. The studies of Conway and Libby (1958) measured the rates of very slow reactions under various conditions. The reaction studied was the decarboxylation of alanine, and the rates were measured using radioactive labelling and low level counting techniques. At room temperature, the half-life for decarboxylation was found
to be about $10^9$ years; at higher temperatures (420-430°K) the half-life was only 100 years. Such data indicates that the amino acids, which may be derived from the microbial hydrolysis of proteins, could be stable over millions of years under low temperature conditions.

The stability of the biogenic macromolecules, such as the proteins, the nucleic acids and the carbohydrates, is extremely low. Usually these molecules undergo hydrolysis by microorganisms or the aqueous environment during the early stages of diagenesis. Such changes are of interest because they provide an insight into the mechanisms that operate on the organic molecules during the first period of deposition.

Shales contain most of the organic matter derived from living organisms. To a lesser extent organic material is also found in limestones and hardly at all in sandstones. Total organic matter is about the same for recent shell carbonates, limestones and clay muds.

It is not intended to discuss in detail the diagenesis of all classes of organic compounds that are of interest to the organic geochemist. An excellent review of the stability of organic matter in diagenetic environments has been provided by Degens (1965, Chapter 5). In this he discusses the amino acids, the porphyrins, the lipids and the macromolecules. For a more detailed discussion of the diagenetic changes affecting these classes of compounds the reader is referred to this review. Since the hydrocarbons have been the subject of major study in this thesis, and particularly the isoprenoid hydrocarbons, the discussion will concern itself with the biological precursors of these hydrocarbons and the diagenetic processes giving rise to them.
DISCUSSION

BIOLOGICAL PRECURSORS OF PRISTANE AND PHYTANE

The biological precursors of the C₁₉ and C₂₀ isoprenoid hydrocarbons, pristane and phytane, have been the subject of much interest. Phytane is almost universally considered to be derived from the phytol side chain of the chlorophyll molecule, a precursor originally proposed by Béndoraitis et al. (1962). Phytol (Figure 67, 1) is synthesised by all green photosynthetic plants. Apparently 90% of the total production occurs in the sea, mostly in planktonic algae. Phytol is hydrolysed from the chlorophyll molecule by small crustaceans, which are important intermediates in the food chain between algae and higher organisms (Blumer, 1965b). Curphey (1952) has considered the probable early distribution of chlorophyll to illustrate how the physical changes of such a distribution can affect the process of decomposition of this substance. He considered algae as the source of chlorophyll. In Paleozoic times the algae were attached to the sea bed. The presence of fossil calcareous algae such as *Epiphyton* would tend to prevent the aerobic decomposition of the hydrolysed chlorophyll molecules. Organisms that are not protected by calcareous growths would tend to undergo early aerobic decompositions which would be accelerated by air and light.

Blumer (1965b) has carried out studies on recent marine sediments in which he finds no detectable trace of phytane. Phytane is not a known constituent of living organisms, there being but one report of this isoprenoid hydrocarbon in these sources (Ciereszko et al. 1963). Pristane, on the other hand, has been isolated in large quantities from marine sources (Soren-
son and Mehlum, 1948; Blumer, et al. 1963); it is also present in some terrestrial plants and animals (Brieskorn and Zimmerman, 1965; Mold, et al. 1963a). Pristane is especially abundant in marine crustaceans such as copepods. These copepods derive pristane from phytane in their food. Blumer was able to show that pristane, unlike phytane, was present in the recent marine sediments which he examined, indicating that some of the pristane found in crude oils and shale extracts is derived from marine sources. He concluded that phytane and isoprenoids of lower molecular weight, including some pristane, appear to be postdepositional products.

DIAGENETIC THEORIES

Various theories have been proposed to explain how phytol is converted into phytane and lower molecular weight hydrocarbons. Blumer (1965 a,b) has suggested that phytol is first dehydrated to give a series of phytadienes which can then undergo hydrogenation to give phytane or oxidative cleavage to the isoprenoid hydrocarbons of lower molecular weight. In support of this mechanism, Blumer has isolated several isomeric phytadienes (see Figure 67, 2,3,4) from the digestive tract of animals. Three isomeric olefins, whose structures are shown below and in Figure 67 (6,7,8), have been isolated from similar sources. These molecules have one less carbon than the phytadienes and are obvious precursors of the pristane molecule (Figure 67,5).
The isoprenoid acid, phytanic acid, has been isolated from natural sources such as butter fat (Hansen and Shorland, 1953) and ox blood (Lough, 1963), as well as from petroleum (Cason and Graham, 1965).

Since decarboxylation of this acid would yield pristane, this is also a likely precursor of pristane in crude oils and sediments. Thus, there are several diagenetic routes which could give rise to pristane. Certainly some of the pristane is derived directly from living organisms, for the finding of pristane in recent marine sediments, which we have already mentioned, and the concurrent finding of highly unsaturated carotenoids, such as \( \beta \)-carotene, preserved in their biological state in recent sediments, indicates that diagenetic changes proceed too slowly to have produced pristane from phytol. Further, Blumer has suggested that the lower molecular weight hydrocarbons, \( C_{14} \) to \( C_{18} \), may be cleavage products of phytol, pristane or phytane. Whether this process involves thermal cleavage or oxidative cleavage is not specified.
ISOPRENOID HYDROCARBONS FROM MARINE PLANKTON (After Blumer)

Figure 67.
The information that can be obtained from a study of the chemical state of the porphyrins found in the geological environment has justified certain reaction mechanisms that have been proposed for these transformations. The propionic side chain of the tetrapyrrole nucleus undergoes decarboxylation to give the hydrocarbon side chains of etioporphyrin. A molecular weight distribution of the etioporphyrins in crude oils and sediments, obtained by mass spectrometric analysis, indicates that a homologous series of etioporphyrins is present, differing in molecular weight by 14 in the side chain. It has been shown, moreover, that some of the fossil porphyrins have the same number of carbon atoms as the original biosynthetic pigments; others have lost one carbon atom, but none have lost two. This implies that one, or both, of the carbonyl groups has been reduced to methyl groups. Blumer (ibid. 1965c) points out that this is the first geochemical evidence for the reduction of sedimentary acids. As a result, this enables one to postulate the reaction mechanisms that fatty acids might also undergo. Such reactions are outlined below:

\[
\begin{align*}
C_nH_{2n+2} & \overset{-CO_2}{\longrightarrow} C_nH_{2n+1}CO_2H & \overset{\text{Reduction}}{\longrightarrow} C_nH_{2n+1}CH_3 \\
\text{Decarboxylation} & \quad \text{via alkyl radical} & \quad \text{Cleavage} \\
& \quad \text{intermediates} & \\
& \text{Fatty acids of lower} & \text{Lower molecular} \\
& \text{molecular weight} & \text{weight hydrocarbons}
\end{align*}
\]
These general types of reaction would be expected to play an influential role in diagenetic modifications in sediments. Such a general theory agrees with the observation that petroleum paraffins have a lower average molecular weight than the biogenic fatty acids of recent sediments. In addition, the marked predominance of even-numbered fatty acids in living systems and recent sediments has disappeared, a characteristic which would be predicted by the above scheme.

The source of hydrogen is a matter of some speculation. Hydrogenation reactions may be coupled with dehydrogenation reactions, such as those that give rise to aromatic compounds. The dehydrogenation processes that give rise to asphaltenes and kerogen are probably a primary source of hydrogen, therefore.

Other theories have been postulated to explain the occurrence of isoprenoid hydrocarbons in geological samples. Those described by Curphey (1952), Bendoraitis et al. (1963) and Johns et al. (1966) are deserving of some attention. Curphey has suggested that phytol is converted into a C\(_{18}\) ketone by epoxidation followed by cleavage. The ketone is then hydrogenated to form the saturated alkane.
To account for the formation of lower molecular weight saturated isoprenoids he suggested that thermal cracking of the $C_{18}$ hydrocarbon might give rise to these compounds. He did not propose a diagenetic scheme which would account for the presence of pristane.

Bendoraitis and his co-workers suggested a more general scheme in which he attempted to account for the presence of a series of saturated isoprenoid hydrocarbons ranging from $C_{10}$ to $C_{21}$ in carbon number. He suggested that carotenoids might be precursors to these isoprenoid hydrocarbons. Carotenoid pigments, such as $\beta$-carotene and lycopene, are conjugated polyenes which are extremely susceptible to chemical attack, particularly to oxidation and to cleavage reactions to yield aldehydes, ketones and acids, which can then be further modified.

Two major reactions were postulated which accounted for a whole series of hydrocarbons: an oxidative cleavage followed by either decarboxylation of the acid or, alternatively, reduction to the corresponding hydrocarbon. This scheme is outlined:
2,6,10,14-tetramethylheptadecane

2,6,10,14-tetramethylpentadecane

2,5,10-trimethylundecane

2,6-dimethylheptane

DECARBOXYLATION

LYCOPENE

REDUCTION

2-methylheptane

2,6-dimethyloctane

2,6-dimethylundecane

2,6,10-trimethyldodecane

2,6,10-trimethylpentadecane

2,6,10,14-tetramethyloctadecane

2,6,10,14-tetramethyloctadecane
This scheme accounts for all the isoprenoid hydrocarbons that have
been so far found in crude oils and sediments. (These are denoted with
an asterisk (*).) The limitation of this scheme is that it does not
predict the occurrence of the C\textsubscript{16} isoprenoid hydrocarbon, 2,6,10-
trimethyltridecane. He managed to get around this difficulty by
postulating that it could be derived from the biological precursor,
squalene, by oxidative cleavage and subsequent decarboxylation.

\begin{center}
\includegraphics[width=0.5\textwidth]{2,6,10-trimethyltridecane.png}
\end{center}

2,6,10-trimethyltridecane

In keeping with the overall mechanism, one would predict that the C\textsubscript{17} iso-
prenoid hydrocarbon, 2,6,10-trimethyltetradecane, should also be pro-
duced from squalene if reduction of the acid, instead of decarboxylation,
takes place. However, until our report of the C\textsubscript{17} isoprenoid hydrocarbon
in the Antrim Shale, this isoprenoid had not been identified in any
crude oils or sediments. Also, the C\textsubscript{12} isoprenoid hydrocarbon, 2,6-di-
methyldecane, could be derived from squalene but there are no reports
of this isoprenoid either in organic geochemical sediments. The C\textsubscript{16}
isoprenoid, on the other hand, has been identified by us in several
samples. In the Antrim Shale it represents the highest proportion of
any isoprenoid present in that sample. The inherent weakness of this
scheme is its inability to predict the relative proportions of the
isoprenoid hydrocarbons present led us to consider another mechanism to account for the presence of the lower molecular weight isoprenoid hydrocarbons.

Bendoraitis had already recognised that phytol was the most probable precursor of both pristane and phytane. We now considered that phytol might give rise not only to the $C_{19}$ and $C_{20}$ isoprenoid hydrocarbons but also to a whole series of isoprenoid hydrocarbons that have been so far identified in sediments. This scheme is outlined in Figure 66 (upper portion). The conversion of phytol to phytane could occur through several pathways. Saturation of the allylic double bond in phytol, followed by dehydration reactions, would lead to phytane. Oxidation of the carboxyl group, followed by decarboxylation and saturation, would give rise to phytane. Alternatively, phytanes of the type shown could give rise to phytane by hydrogenation of the double bond.

Oxidative cleavage of the double bond would give rise to the $C_{19}$, $C_{18}$ and $C_{16}$ acids/could be reduced to the saturated hydrocarbon. Another
scheme which is preferred by us involves prior saturation of the biological precursor, followed by thermal cracking of this hydrocarbon molecule. An interesting analytical technique recently developed by Holman, Deubig and Hayes (1966) gives credence to this theory. These workers analysed the pyrolysis products of phytane; a series of monolefins of carbon number C\textsubscript{15} (M.W. 210), C\textsubscript{16} (M.W. 224), C\textsubscript{18} (M.W. 252) and C\textsubscript{19} (M.W. 266) resulted. Conspicuously absent was the C\textsubscript{17} olefin whose molecular weight is 238. This is exactly analogous with the diagenetic scheme we have postulated. The formation of the C\textsubscript{17} isoprenoid hydrocarbon, as can be seen from Figure 66, requires two cleavage points, an inherently less likely process. This scheme predicts, therefore, that the C\textsubscript{17} isoprenoid hydrocarbon, if present at all, would be present in relatively smaller proportions than any of the other isoprenoid hydrocarbons which should be formed by this scheme. This is in keeping with present findings since the only report of the C\textsubscript{17} isoprenoid hydrocarbon is in the Antrim Shale and the relative proportion of this isoprenoid is considerably less than that of any of the other isoprenoids.

The absence of the C\textsubscript{17} saturated isoprenoid hydrocarbon finds some analogy with the work of Cason and Graham (1965) on a series of isoprenoid acids they isolated from a California petroleum. They identified the C\textsubscript{11}, C\textsubscript{14}, C\textsubscript{15}, C\textsubscript{19} and C\textsubscript{20} isoprenoid acids, but found no evidence for the presence of the C\textsubscript{17} or C\textsubscript{18} isoprenoid acids. If one considers that these acids are derived from C\textsubscript{20} monolefins by oxidative cleavage one can rationalise the absence of the C\textsubscript{17} and C\textsubscript{18} isoprenoid acids.
The formation of the \( C_{17} \) isoprenoid acid would require oxidation of a \( C_{20} \) monolefin to yield a \( C_{18} \) methyl ketone, followed by oxidation of the methyl ketone, and the \( C_{18} \) isoprenoid acid would require reduction of the carboxyl group of the methyl ketone followed by oxidation of the methyl group itself. Both these processes are not considered likely to occur.

\[
\begin{align*}
\text{C}_{19} \text{ acid} \\
\text{C}_{18} \text{ ketone}
\end{align*}
\]

Similar reasoning will account for the absence of the \( C_{12} \) and \( C_{13} \) isoprenoid acids, though the absence of the \( C_{16} \) isoprenoid acid remains anomalous.

The concentrations of the normal and isoprenoid hydrocarbons at various depths in the Green River Shale has been studied by Robinson et al. (1965). Both the normal hydrocarbons and the isoprenoid hydrocarbons decrease in relative concentration (weight percent of bitumen) with the depth of burial. The \( n-C_{17} \) is the most abundant of all the normal alkanes at all depths. The odd-carbon numbered normal hydrocarbons are present in higher concentrations than the adjacent even-
numbered normal hydrocarbons, except for \( n-C_{20} \) and \( n-C_{22} \). This predominance of odd-numbered hydrocarbons over even-numbered hydrocarbons decreases with depth. The relationship between the isoprenoid hydrocarbons is most significant. The \( C_{20} \) isoprenoid decreases in relative concentration with depth, while that of the \( C_{15}, C_{16}, C_{18} \) and \( C_{19} \) isoprenoids tend to increase. This suggests quite strongly that the \( C_{20} \) isoprenoid is a precursor to the lower molecular weight isoprenoid hydrocarbons. The variations in the amount of \( n-C_{17} \) with depth are more difficult to rationalise. Since this is somewhat irregular one can only attribute such variations to the amount of available precursor, possibly stearic and unsaturated \( C_{18} \) fatty acids. The precursors of the \( n-C_{17} \) hydrocarbons are not known with any certainty, however, and since we have already noted that the blue-green algae of the Nostoc variety contain the \( n-C_{17} \) as the major hydrocarbon constituent, this, too, could give rise to the \( n-C_{17} \) hydrocarbon of the geological samples. These studies of the variation in the concentration of the isoprenoid hydrocarbons with depth are a strong vindication of the hypothesis that phytol is the precursor of the lower molecular weight isoprenoid hydrocarbons.

Both Bendoraitis et al. (1963) and Robinson (1965) invoke oxidation and reduction mechanisms in their diagenetic degradation schemes of phytol. Robinson et al. consider both oxidation of the double bond of phytol to provide a \( C_{18} \) ketone and subsequently a \( C_{17} \) acid and reduction of the double bond to produce dihydrophytol, as intermediary pro-
cesses in the formation of lower molecular weight hydrocarbons. One
mechanism which has not been greatly developed is the thermal cracking
of a fully saturated C_{20} isoprenoid. It has been argued that thermal
cracking temperatures are far in excess of those to which petroleum
shales have been subject in their thermal history, and that such a
mechanism is therefore untenable. Welte (1965) has recently given
serious attention to this mechanism. He suggests that the simple
destruction of carbon-carbon bonds is to be accounted for by thermal
cracking mechanisms rather than by oxidative mechanisms. To support
this hypothesis he uses the experimental evidence of both Hunt (1962)
and Abelson (1964). Hunt subjected kerogen to high temperatures over
short periods of time (e.g. 400°C for 10 min) with the exclusion of
oxygen, and found that low molecular weight hydrocarbons, ranging in
carbon number from C_{1} to C_{8} were generated; these consisted of both
saturated and aromatic components. Abelson has considered the ther-
mal cracking processes as first-order reactions and has used the
Arrhenius equation to calculate the time it would take to generate
low molecular weight hydrocarbons. He concludes from the calculated
temperature-time curve that this process would take 100 million years,
at temperature of 100°C to obtain a significant production of hydro-
carbons. The decarbonylation reaction which has a lower activation
energy would require a correspondingly shorter period of time, of
the order of one million years, for the reaction to have proceeded
to the same extent. These calculations indicate that the objection that
too high a temperature was required for thermal cracking to take place is no longer valid, and that the temperature factor is more than compensated for by the time factor. It should be noted that such calculations do not take into account any catalytic effects of clay minerals which may play a significant part in petroleum generation. At the present time, these effects are little understood, and it is difficult to estimate how significant a role they might play.

Although phytol is considered to be the major precursor of the isoprenoid hydrocarbons, two other recent findings are of interest in this connection. Kates (1966) has shown that a phytol-containing lipid (diphytyl phospholipid) is common to certain salt bacteria. It is possible that both pristane and phytane could be derived from the isoprenoid side chains of the phosphate-containing lipid:

\[
\text{CH}_2-\text{O-CH}_2
\]
\[
\text{CH}_2\text{O-CH}
\]
\[
\text{CH}_2\text{-O-P-O-CH}_2\text{-CHOH-CH}_2\text{-O-P-}
\]

Sen Gupta et al. (1966) have isolated a homologous series of isoprenoid acids from sea fish oil. Ackman (1967) has isolated the same homologous series of isoprenoid acids from marine lipids, in addition to the iso and anteiso fatty acids which they reported previously (Ackman and Sipos, 1965). They isolated phytanic acid (C\text{20}) and pristanic acid (C\text{19}), both
of which had been reported by Cason and Graham (1965) in their studies on a California petroleum. In addition, they isolated the C_{16} isoprenoid acid, 4,8,12-trimethyltetradecanoic acid, from the same source, an isoprenoid acid which Cason and Graham had failed to report. These fatty acids are potential precursors of the hydrocarbons in petroleum. When more is known about how widespread a distribution these acids have in biological systems it will be possible to assess how significant a role they play as precursors of the isoprenoid hydrocarbons.

THE ROLE OF SQUALENE AS PRECURSOR OF THE ISOPRENOID ALKANES

During our studies on the C_{17} isoprenoid hydrocarbon we considered the possibility that a C_{30} isoprenoid compound could be a precursor to this C_{17} hydrocarbon rather than a C_{20} isoprenoid compound such as phytol. Squalene was an obvious choice. The squalene molecule could be reduced to the fully saturated analogue, squalane, which could then undergo thermal cracking to produce the C_{17} isoprenoid. This diagenetic scheme is illustrated in the lower portion of Figure 66. If squalene is a major precursor one might expect to find other hydrocarbon types present also, in particular the C_{19} isoprenoid, 2,6,10-trimethylhexadecane, a compound which we synthesised from farnesol in Chapter 4. The coinjection of this C_{19} isoprenoid into the 'branch-cyclic' fraction of the Antrim Shale indicates that if this isoprenoid hydrocarbon is present at all it occurs in very small quantities.

The situation which arises for the C_{21} isoprenoid provides a more critical test of the hypothesis that squalene is a significant precursor
to the isoprenoid hydrocarbons. The regular C_{21} isoprenoid, 2,6,10,14-tetramethylheptadecane, could be derived from a C_{40} isoprenoid precursor, such as lycopene, by analogy with the diagenetic schemes we have suggested previously, when phytol and squalene were considered as biological precursors. The same C_{21} isoprenoid could also be derived from head to tail oligomers of isoprene of carbon number greater than C_{20}. There are many such available precursors in living systems. Isoprenyl alcohols whose structures have been characterised as undeca-isoprenol-1 and dodeca-isoprenol-2, have been isolated from silkworm feces (Fukawa et al., 1966). They also confirmed the presence of solanesol which has the following structure:

\[
\begin{align*}
\text{CH}_{3} & \\
\text{H-} & \text{(CH}_{2 \cdots \text{C}=\text{CH-CH}_{2 \cdots} \text{)}}_{n-\text{OH}} \\
n = 9 & \text{ solanesol}
\end{align*}
\]

This compound had been characterised earlier by other workers (Rowlands et al., 1956) who had isolated this compound from flue-cured tobacco. Its structure was confirmed by Folker and his co-workers (1959) as a C_{45} isoprenoid compound. Lindgren (1955) has isolated a series of C_{30}-C_{45} terpenols from birch wood, and Morton et al. (1963) characterised dolichol (C_{100}) in pig liver. The name 'bactoprenol' has been given to the most abundant lipid formed by three species of Lactobacilli from mevalonic acid. The structure of this compound was confirmed as a C_{55} isoprenoid alcohol.
The side chains of the ubiquinone, Vitamin K$_1$ and K$_2$ and Chlorobium chlorophylls also contain long-chain isoprenoid alcohols. Any of these compounds is a likely source of the C$_{21}$ isoprenoid, 2,6,10,14-tetramethylheptadecane. If a hydrocarbon derived from solanesol were a precursor to the C$_{21}$ isoprenoid, a C$_{24}$ compound should also result (see Figure 69). If this compound is present at all it is there in small amounts. The C$_{21}$ isoprenoid, 2,6,10,15-tetramethylheptadecane, could only reasonably arise if a C$_{30}$ isoprenoid such as squalene were a precursor. The structures of these two C$_{21}$ isoprenoids are given below and the possible diagenetic mechanisms which could give rise to these compounds are shown in Figure 69.

\[
\begin{align*}
\text{2,6,10,14-tetramethylheptadecane} & \\
\text{2,6,10,15-tetramethylheptadecane} & 
\end{align*}
\]

Our studies in Chapter 4 indicate that the C$_{21}$ isoprenoids identified in the Antrim Shale, the Nonesuch Shale and the Soudan Shale all have the structure of the regular isoprenoid, i.e., 2,6,10,14-tetramethyl-
heptadecane. Coinjection of the C21 isoprenoid, 2,6,10,15-tetramethylheptadecane, into the 'branch-cyclic' fraction from these samples indicates that it is not present in any significant amounts. The concomitant absence of the C19 isoprenoid, 2,6,10-trimethylhexadecane, in addition to the C21 isoprenoid, 2,6,10,15-tetramethylheptadecane, indicates that squalene is not an important precursor of the isoprenoid hydrocarbons. Further, the diagenetic mechanism of Figure 68, where squalene is postulated as a precursor to the C17 isoprenoid hydrocarbon would not predict that this isoprenoid hydrocarbon should be present in smaller amounts relative to the other isoprenoids, as does the diagenetic scheme where phytol is a precursor. The fact that in the single instance where the C17 isoprenoid has been isolated and identified it is present in significantly smaller concentrations than any of the other isoprenoids identified is an inherent weakness of the squalene precursor hypothesis. This is somewhat analogous to the limitations of Bendoraitis' scheme, to which we have already alluded, where he postulates squalene as a precursor to the C16 isoprenoid, 2,6,10-trimethyltridecane.

No single diagenetic mechanism is available which will account for all the organic geochemical results that have been obtained. It is possible that several precursors may give rise to the same isoprenoid hydrocarbon, a situation which almost certainly exists with the C19 isoprenoid hydrocarbon, pristane, which is derived from naturally
Figure 68. Diagenetic pathways to the C\textsubscript{17} isoprenoid hydrocarbon.
Figure 69. Diagenetic pathways to the C$_{21}$ isoprenoid hydrocarbons
occurring sources and as a degradation product of phytane. The thermal cracking theory, which we have postulated with phytol as a biological precursor, seems to account for all isoprenoid hydrocarbons that have been identified in crude oils and sediments. In addition, it explains why the C_{12} isoprenoid hydrocarbon and the C_{17} isoprenoid hydrocarbon have not been characterised in the homologous series. Finally, one can make predictions with this scheme which can be directly tested on an experimental basis. Such predictions, if borne out, could provide unequivocal evidence for the thermal cracking theory as the major process in the formation of isoprenoid hydrocarbons in petroleum. Thus, we can make the following predictions, illustrated in the scheme below:

\[\text{phytol} \rightarrow \text{phytane} \rightarrow \text{C}_{16} \text{ isoprenoid} \rightarrow \text{C}_{17} \text{ isoprenoid} \rightarrow \text{C}_{18} \text{ isoprenoid}\]
The three isoprenoid hydrocarbons are all isomers of the corresponding hydrocarbons that have the same carbon number as those identified in the crude oils and shales which we have analysed, namely, norpristane \( (C_{18}) \), homofarnesane \( (C_{16}) \) and \( 2,6,10 \)-trimethyltetradecane. The corresponding isomers would have the following structures:

1) \( C_{16} \) isoprenoid: \( 3,7,11 \)-trimethyltridecane, which should be present in similar amounts as homofarnesane.

2) \( C_{17} \) isoprenoid: \( 3,7,11 \)-trimethyltetradecane, which should be present significantly larger amounts than the \( C_{17} \) isoprenoid already characterised in the Antrim Shale, since its formation requires only a single carbon-carbon cleavage.

3) \( C_{18} \) isoprenoid: \( 3,7,11 \)-trimethylpentadecane which should not be present in large amounts, if at all, since its presence requires two carbon-carbon cleavages, a less likely process.

Here we have a very special prediction which can be tested experimentally. If these isoprenoids are identified in crude oils and shales it would provide a final vindication of the thermal cracking theory. If, on the other hand, their presence is shown, this would seem to suggest that the initial degradation process takes place by attack at the functional end of the phytol molecule which could then be followed by thermal cracking mechanisms. Alternatively, but less
likely in view of the ubiquitous presence of chlorophyll and, therefore, phytol in living systems, the isoprenoid hydrocarbons could be derived from isoprenoid acids of the type identified by Ackman (1967). The experiment outlined above should provide an unequivocal method of distinguishing between these various mechanisms.

THE ORIGIN OF THE NORMAL, ISO-, ANTEISO- AND CYCLOHEXYL NORMAL ALKANES

The derivation of the normal alkanes from biological precursors has been a subject of considerable speculation. In an earlier chapter we have already mentioned that normal alkanes isolated from contemporary plant sources generally have carbon numbers greater than C20 (Eglinton and Hamilton, 1963) and show an odd over even predominance in carbon number (Eglinton and Hamilton, 1963; Oro et al., 1965c). Normal alkanes from marine sources do not generally show this special odd to even preference (Koons et al., 1965). It was commonly suggested that the alkanes are formed from fatty acids by diagenetic modification. Fatty acids isolated from terrestrial sources range in carbon number from C14 to C20 and in marine sources from C20 to C26. An analysis of the fatty acids in blue-green algae, whose microscopic remains have been identified in Precambrian sediments (Barghoorn et al., 1965), might be expected to reflect more accurately the fatty acid precursors that are likely to give rise to the normal alkanes in these sediments. Parker and Leo (1965) have identified fatty acids ranging in carbon number from C12 to C18 in blue-green algal mats, and our own finding of the n-C17 hydrocarbon in the Nostoc variety of blue-green algae is
in keeping with our observation that normal hydrocarbon distribution from several shales have their maximum at n-C₁₇ (see Figure 70).

Robinson et al. (1965) have offered an explanation which will account for the high C.P.I. values for n-alkanes at relatively high depths of burial in the Green River Shale, and the low C.P.I. value for the n-alkanes at much lower depths. The carbon preference index (C.P.I.) for the n-alkanes in the C₂₅ to C₃₃ range was calculated as the ratio of the sum of concentrations of odd-carbon number n-alkanes to the sum of the concentrations of even-carbon number n-alkanes (Bray and Evans, 1963; Cooper and Bray, 1963). The high C.P.I. values can be accounted for invoking the decarboxylation mechanisms of the even-numbered fatty acids which would produce odd-number n-alkanes. The lower C.P.I. values at greater depths can be explained if it is assumed that a degradation mechanism is operating which causes loss of one carbon atom from each of the n-alkanes. Since the odd-carbon number alkanes are present in proportionately greater concentrations than the even-carbon number n-alkanes, this mechanism will result in a gradual equalisation of the distribution of n-alkanes, with greater depth of burial, and, therefore, age, a feature which has been observed in the crude oils and shales which we have analysed.

The simple decarboxylation mechanism which has been suggested to explain how n-alkanes could be derived from fatty acids has been given further consideration by Breger (1965), who points out that no simple bacteriological mechanism is known that will directly decarboxylate a fatty acid. He proposes that this decarboxylation mechanism
Figure 70. Relative distribution of n-alkanes in a series of oils and sediments
may not be as simple as once was thought, and that it may well involve prior conversion into more reactive compounds, such as \(\alpha,\beta\)-unsaturated fatty acids or \(\beta\)-hydroxy acids, which will then undergo decarboxylation reactions more readily. This hypothetical process would depend on a combination of chemical and bacteriological mechanisms. This theory is in contrast to that of Jurg and Eisma (1964) who showed that fatty acids could be converted into hydrocarbons of various chain lengths when such acids were heated in the presence of clay minerals, which had a catalytic effect on the decarboxylation reaction.

The branch chain fatty acids derived from lipid sources are possible precursors of the iso- and anteiso-alkane homologous series. Deuel (1951-57) has shown that the C\(_{10}\) to C\(_{26}\) iso-branched acid and the C\(_{9}\) to C\(_{31}\) anteiso-branched fatty acids are the constituents of hair, while in bacteria the C\(_{15}\) and C\(_{17}\) iso-acids are the major constituents of the lipid fraction isolated from Bacillus subtilis. Ackman (1967) has identified iso and anteiso fatty acids in marine lipids which he considers to be precursors of the iso and anteiso alkanes in petroleum deposits. Analogous to the n-alkanes there are a few reports of the occurrences of iso- and anteiso-alkanes in natural waxes (Carruthers and Johnson, 1959; Mold et al., 1963b, 1964; Dunning et al., 1960).

The diagenetic processes which give rise to the cyclohexyl normal alkanes are not known. A cyclic naphthenic acid resembling the terminal structure of \(\beta\)-carotene has been reported (Lochte and Littman, 1955) which lends credence to Bendoraitis' hypothesis that carotenoids may be significant precursors of petroleum hydrocarbons.
There are no obvious precursors of the cyclohexyl normal homologous series. However, it is possible that this homology is derived from unsaturated fatty acid derivatives which become saturated by intramolecular cyclisation, or, alternatively, by a mechanism proposed by Breger (1965) involving the Diels-Alder reaction. He suggests that $\alpha,\beta$-unsaturated acids might readily undergo a Diels-Alder reaction at room temperature with conjugated dienes to produce a variety of partially unsaturated cyclic compounds of the type shown:

\[
\begin{align*}
\text{CH}_2\text{(CH}_2\text{)}_5\text{CO}_2\text{H} & + \text{CH-CH}_3 \\
\text{CH}_2\text{(CH}_2\text{)}_3\text{CH}_3 & \rightarrow \\
\text{CH}_2\text{(CH}_2\text{)}_5\text{CO}_2\text{H} \\
\end{align*}
\]

Eleostearic acid

This mechanism is a matter of some speculation but could conceivably give rise to a cyclohexyl normal homologous series.
EXPERIMENTAL

The synthesis of the following standard hydrocarbons discussed in this chapter is described in detail in Chapter 4:

1) 2,6,10-trimethyltetradecane
2) 2,6,10-trimethylhexadecane
3) 2,6,10,14-tetramethylheptadecane
4) 2,6,10,15-tetramethylheptadecane
PART I. GENERAL SYNOPIS AND SIGNIFICANCE OF RESULTS

The evidence for life-forms at periods of geological time earlier than 600 million years ago has been clearly established. Both the organic geochemical approach and the micropaleontological approach have identified remnants of living organisms, either in the form of 'biological markers' or microfossils that have been embedded in the sedimentary rock at the time of its deposition. Most of the oldest sedimentary rocks known on the surface of the Earth have been analysed and in almost all cases the 'biological markers', pristane and phytane, have been identified. It is therefore worthwhile reconsidering what is now known concerning the early history of the Earth, the development of the living system, and the geological transformations that lead to petroleum deposits. Most important, it is necessary to reassess the criteria of the organic geochemical approach for the existence of life-forms at a given time in the light of what is known about the nature of the organic compounds identified in carbonaceous chondrites, and with a view to an examination of the lunar samples which will be available to us in the very near future.

Many theories have been advanced to explain how the Earth was formed. The presently accepted theory invokes the cold aggregation of discrete particles. During the degassing, the 'primitive atmosphere'
gases would be produced, \( H_2, CH_4, H_2O, N_2, NH_3 \). Oxygen is not considered to be a component of this early atmosphere and is thought to have arisen at a much later stage. There was probably always a small concentration of oxygen as a result of photolytic dissociations in the outer atmosphere. Anaerobic life could have survived in the absence of oxygen. This evolution of living systems under anaerobic conditions provides the foundation from which organisms capable of photosynthetic release of oxygen arose. Photosynthesis eventually produced an oxygen-containing atmosphere building up to present levels. Berkner and Marshall (1965) have postulated that the Earth accumulated without an external primordial atmosphere, considering the present atmosphere of the Earth to be of secondary origin. Volcanic eruption was thought to be the source of all the early gases, with the exception of oxygen. These authors calculated that oxygen generated by photodissociation of water vapour would be self-regulated, at less than 0.1 percent of the present atmospheric level, since the oxygen so produced by this mechanism would protect the underlying water vapour from further dissociation. Such a level would not be capable of supporting oxidative metabolism. Atomic oxygen and ozone generated in small quantities from molecular oxygen would form a thin layer near the Earth's surface since atomic oxygen and ozone will be produced at much lower altitudes than they are today; this could be responsible for a higher degree of oxidation of the Earth's crust in these early stages than might be expected. The self-regulated equilibrium is upset only when the rate of production of oxygen exceeds the balance of the limiting concentration pro-
duced by photodissociation. The still intense UV radiation limits the location of biological development to the lower regions of ponds and similar habitats. With the production of photosynthetic oxygen it became possible for oxidative metabolism to evolve and for multicellular Metazoa to arise and diversify. This period was thought to be close to 600 million years ago when we have the first clear evidence of such species from the fossil record. From that time onwards the level of oxygen in the atmosphere continued to increase.

This model of Berkner and Marshall illustrates in outline what is presently considered to represent the early evolutionary history of the Earth's atmosphere. Although the advent of an oxidative metabolism does not occur until the start of the Precambrian this is in keeping with the previously mentioned micropaleontological findings of Barghoorn and Tyler and Barghoorn and Schopf, who have correlated the morphological remnants of microfossils in Precambrian sediments with the morphology of blue-green algae. The occurrence of these microfossils indicates that the waters were sufficiently shallow to allow light to penetrate to the water-surface sediment. Conclusions about the Earth's atmosphere during Precambrian times cannot be drawn with any certainty on the basis of paleontological evidence. If one assumes that photosynthetic systems were reasonably widespread during Precambrian times, and most recent evidence would seem to indicate this, one can say that this period may represent an intermediate stage in the evolution of the highly oxygenic atmosphere which prevailed at the beginning of the Cambrian. The diversity of form that is evident from all of Barghoorn's
microfossil remnants indicates that at this stage of evolutionary development considerable variation in the morphology of these most ancient organisms had already arisen.

The organic geochemical results in Precambrian samples complements these microfossil findings. These samples seem to have been taken from such a wide-spread geological environment, which has included the analysis of Precambrian sediments from different continents, that for all of them to contain the same hydrocarbon contaminants would seem to be a remote possibility. The consistent finding of pristane and phytane in all the sediments analysed has reinforced the organic geochemical position that the isoprenoid hydrocarbons are reliable 'biological markers'. These two compounds have been found in samples as widely different as the Green River Shale of Wyoming, where an abundance of other classes of biologically derived compounds such as the steranes and triterpenes have been found, and the Precambrian samples, where the organic extractable material is very small and in most cases the conclusions made have rested on the identification of these isoprenoid hydrocarbons.

It is quite apparent from an overall comparison of the GLC distributions of the hydrocarbon material from a series of crude oils and shales that, although pristane and phytane have been isolated and identified in all these samples, the occurrence of these hydrocarbons in relation to the other hydrocarbons present is different in each case. It is this rather obvious/significant observation that has been little investigated by organic geochemists. Thus, the simplicity of the GLC
distribution of the 'branch-cyclic' fraction of the Green River Shale (Figure 5) suggests that this sample is quite unique amongst all the samples that we have examined. The isoprenoid hydrocarbons are present in the highest concentrations and no other compound has been identified in significant quantities in the \( C_{15} \) to \( C_{20} \) region. The homologous series of \( \textit{iso}, \textit{anteiso} \) and cyclohexynormal alkanes seem to be absent from the Green River Shale. The 'branch-cyclic' fractions of the Soudan Shale, the Nonesuch Shale and the Antrim Shale (Figure 24) all contain the isoprenoid hydrocarbons, pristane and phytane, and yet the overall GLC distributions seem to exhibit a unique patterns, which is different in each case. The Soudan Shale is remarkable in that the isoprenoid hydrocarbons are by far the most prominent of any other compound present (see Figure 37). In this respect it is quite similar to the Green River Shale, and very different from the GLC distributions of other Precambrian samples that have been examined, which are generally much more complex in character. The Antrim Shale and the Nonesuch Shale (Figures 44 and 43) are far more complex, particularly the Antrim Shale. The isoprenoid hydrocarbons, although present, no longer occur as the most prominent constituents. The presence of other homologous series has also been shown, and there is a preponderance of lower molecular weight material which has yet to be characterised. This is even more pronounced in the San Joaquin Oil and in crude oils which other workers have identified. In all these, however, it is emphasised that there is unambiguous evidence for the presence of isoprenoid hydrocarbons. The
question which is asked, therefore, is whether the differences in the character of the hydrocarbon distribution can all be attributed to ecological variations or variations in the thermal history of the sediment, which has been the rationale that organic geochemists have traditionally provided for these differences, or do they represent something of greater significance, as yet not understood. What is the significance, for instance, of the GLC distribution of the hydrocarbons extracted from meteorites which are complex in character and in which evidence has been provided for the occurrence of the isoprenoid alkanes, pristane and phytane? This is the vital question to which there is no unequivocal answer at the moment, and which represents one of the major problems in organic geochemistry today.

There is no doubt that at some time considerable effort is going to have to be spent in the careful and thorough analysis of the individual constituents of petroleum and shale extracts. This presents itself as a monumental and tedious task. At the moment only the smallest proportion of these constituents has been identified. However, the clue to understanding the processes that are involved in petroleum formation lies in a detailed knowledge of the chemical nature of its constituents. Such a knowledge will provide insights into not only the diagenetic mechanisms involved but also into the most fundamental question of all, the origin of these hydrocarbons.

The comprehensive analysis of petroleum constituents is an essential part of future organic geochemical research efforts. However, there are other approaches to answering these basic questions which have
been discussed in some detail in the chapters of this thesis. In the first place, the identification of the individual components isolated from organic geochemical extracts must be carried out with a reliability that does not merely assign this or that particular component into a broad class of organic compounds. For the development of ideas relating to biochemical evolution it is essential, wherever possible, to characterise the specific structure of each component, including its geometrical isomerism and the stereochemistry. Too often in the past, organic geochemists have developed theories with inadequate experimental identification. With the availability of analytical techniques that are capable of isolating and characterising the structure of very small amounts of individual components it is now possible to obtain several physical measurements on each compound. This eliminates many of the ambiguities that can arise in structural identification. Secondly, organic geochemistry must direct its attention towards finding criteria which will ascertain the origin of the organic constituents of petroleum and sediments. Two of the approaches discussed in Chapter 5 appear to be the most hopeful. The stereochemistry of individual compounds isolated from geological sources must be correlated with the stereochemical configuration of the molecules from which they are thought to derive. Up to now the inadequacy of experimental techniques and the small quantities of the geological sample available have prevented extensive stereochemical studies being
carried out. If isoprenoid hydrocarbons, and steranes and triterpanes, derived from biological precursors are to be distinguished from the same compounds synthesised by abiogenic processes it will be necessary to have a detailed knowledge of the stereochemistry of these molecules. Likewise the $^{13}C/^{12}C$ measurements, which have hitherto been taken on mixtures of many constituents such as the hydrocarbons of petroleum, should now be performed on the individual constituents of these mixtures to see if any reliable correlations can be established between the hydrocarbons isolated directly from biological systems and those derived from biological systems and subsequently deposited in a geological environment. This approach should be able to distinguish between individual compounds derived from biological sources and those synthesised by abiogenic methods. Utilising these methods, namely, the stereochemical characterisation and the carbon isotope measurements, it should be possible to determine the origin of the hydrocarbons found in thucholites and hydrothermal deposits and in the extracts of extraterrestrial objects such as meteorites. When the lunar samples are returned to this planet for analysis it is to the origin of the organic material and the mechanism of derivation that interest will be directed. Organic geochemistry must endeavour to establish criteria which will provide an answer to these fundamental questions.
PART II. EXTRATERRESTRIAL LIFE

In 1961 a study of the Orgueil meteorite revealed evidence that testified to former extraterrestrial life. Although this evidence has been severely criticised and additional evidence has been provided which suggests that the probable origin of the organic material is a contaminating source, the search for extraterrestrial life has been continued with renewed vigour. Such a finding would be as important as the finding of actual living organisms and would represent one of the most significant scientific discoveries of all time. The establishment of life-forms on other planets within the relatively small confines of the solar system would suggest its occurrence also in the vast expanse of the universe.

The search for extraterrestrial life has been approached from both the morphological standpoint and from the organic geochemical standpoint, in exact analogy to the approach adopted in the search for Precambrian life. The objects that have been studied have been the meteorites, and particularly the carbonaceous chondrites, which from time to time have plunged through the Earth's atmosphere to be subsequently found, embedded in the Earth's crust. The origin of the carbonaceous chondrites is a subject of much controversy and no theory has been proposed which has gained universal acceptance. The studies on meteorites have been viewed essentially as preparatory studies in the search for extraterrestrial life on other planets when mankind's technological progress will allow him to land on the surface of the Moon, on Mars and on Venus, and study directly the chemical constituents of extra-
terrestrial bodies, free of contamination dangers. At the moment, the uncertain history of the carbonaceous chondrites has prevented any reliable conclusions being drawn from the analysis of their organic content, and it appears unlikely that they will provide a definitive answer to the question of cosmic biology.

In the experimental approach to the detection of extraterrestrial life two fundamental aspects of the problem should be recognised. In the first place, one has to define in physical terms what is meant by life and then design an experiment in which established criteria are to be utilised in its detection. Secondly, one must have some knowledge of the environment of the planet to be sampled. There is no simple definition which will adequately describe in physical terms what constitutes life. Even though there is no formal definition of the phenomenon available it is not difficult to recognise its existence and distinguish between living and inorganic matter by the judicious choice of suitable biochemical experiments. The phenomenological approach is generally the basis of all life detection experiments so far proposed. The weakness of this approach lies not so much in the inability of formulating a definition of life whereby it can be recognised but rather in the fundamental assumption which relates all life to a common biochemical ancestry (Lovelock and Lipsky, 1967). Both Schrodinger (1944) and Bernal (1961) have discussed the physical basis of life and have formulated the following description: "Life is one member of the class of phenomena which are open or continuous reaction
systems able to decrease their entropy at the expense of substances or energy taken in from the environment and subsequently rejected in a degraded form". The spontaneous generation of life, according to recent calculations from quantum mechanics, is an extremely improbable event (Wigner, 1961; Lansberg, 1964). On the basis of the above description life is recognised from nonlife by the following phenomenology:

1) the unique presence of structure and order in the living systems which have been brought about by utterly improbable events, in any thermodynamic sense;

2) the existence of a self-replicating system that is far removed from a state of equilibrium.

These two phenomena should represent the basic experiments designed to distinguish planets bearing life from those on which life does not exist. Accordingly, experiments have been formulated which attempt to achieve this objective. These have been outlined by Lovelock and Lipsky (1967). Order in the living system is identified by the specific chemical structures and sequence of structures of the components of living systems. The analytical techniques used in the identification procedure are those that are commonly used in organic chemistry today. Combined gas chromatography and mass spectrometry has proved the most versatile of these analytical techniques in the characterisation of chemical structures. Polymeric compounds of biological origin have sharply defined molecular weights, whereas those produced by inorganic polymerisation do not. Thus, the manifestation of order in molecular weight distribution could be detected by electrophoresis or similar such polymer
separating techniques.

The search for evidence of nonequilibrium processes is more difficult to carry out. Chemical processes which are not in equilibrium can be detected by differential thermal analysis in which a sample of the planet's surface is treated first in the planet's atmosphere and then in an inert atmosphere, the differential signal between the two experiments indicating a reaction between the surface and the atmosphere if life processes were present. A comparison of the hydrocarbon distribution from abiogenic synthesis, for example in the Fischer-Tropsch process, and hydrocarbons of biological origin indicates the striking contrast between the products of equilibrium processes, on the one hand, and nonequilibrium processes, on the other. The hydrocarbons from the abiogenic synthesis fit very closely to the expected position distribution, indicating a state of chemical equilibrium. The hydrocarbons from many biological sources show an odd to even distribution and are far removed from a state of equilibrium. The example cited is the extreme case rather than the typical one for diagenetic modification of the biological material can drastically alter the original distribution so that it becomes unrecognizable.

The second consideration which must be taken into account in the search for extraterrestrial life is the physical environment of the planet. One of the implications of the general theory of chemical evolution is that this process is not restricted to explaining the origin of life on this planet but can be invoked as a plausible process in any other part of the universe, where favorable conditions of temperature and atmos-
pheric composition exist. Oparin (1938), in his treatise on the origin of life, has concluded: "all these difficulties disappear if we ... take the standpoint that the simplest living organisms originated gradually by a long evolutionary process of organic substance and that they represent merely definite mileposts along the general historic road of evolution of matter". Oparin takes the position, therefore, that life will arise spontaneously in the universe wherever a chemical environment resembling that of the primitive Earth is found. Another viewpoint considers life as a highly improbable event, so improbable that it cannot be regarded as the outcome of random chemical evolution (Horowitz, 1962).

At the present time, this questions remains one of speculation to which no definitive answer can be given.

Within the near future it will be possible to investigate these speculations on an experimental basis. It is intended to explore not only the lunar surface but also that of Mars and Venus. Horowitz (1966) has given some consideration to the possibility of life on Mars. Although recognising that the physical conditions on Mars are not conducive to life as we know it on that planet, he does not permit the conclusion if life ever existed on Mars it is now extinct. The average temperature on Mars is about -55°C. The atmosphere contains carbon dioxide and a small amount of water vapour but no oxygen has been detected. The rarefied atmosphere and absence of a magnetic field are responsible for the constant bombardment of the surface by cosmic rays and solar radiation. The recent Mariner photographs suggest that Mars is a dead planet which does not contain the variety of ecological habitats which characterise the Earth. All these factors suggest a very severe environment which
is not at all likely to support life.

A more optimistic interpretation of these conditions is provided by Horowitz (ibid.). He claims that none of the conditions that are known to prevail on the surface of Mars excludes it as a possible abode of life. He points out that the Martian temperatures are similar to those encountered in Antartica where microbial life has been detected. Again, although the intensity of the cosmic radiation is far greater than the amount reaching Earth, it is still not at a level which would destroy any vestige of life. The scarcity of water on the planet is the most serious objection to the existence of life. The amount of water vapour which has been detected in the Martian atmosphere is less than that contained in the Earth's atmosphere by a factor of a thousand. No evidence has been found either from astronomical observations or from Mariner photographs for the existence of river valleys or ocean beds on the surface. Despite these physical conditions which would suggest that no life could exist, other observations, such as the seasonal change of colour in the maria, remain unexplained and leave the question of life on Mars unsolved.

The physical conditions on Venus are such that there is no possibility of life existing on that planet. The atmosphere contains a large amount of CO₂ and possibly some water. Direct visual observation of the planet is difficult, for when Venus is closest to the Earth the planet is covered by very dense clouds so that its surface has never been seen. The surface temperature of Venus has been estimated from the radio emission
of the planet in the microwave region. A temperature between 300–400°C is indicated, which would exclude all possibility of life on this planet.

Experiments are being designed with a view to acquiring more information relating to the possibility of life on these planets. The "fly-by" experiments will enable the analytical composition of the atmosphere to be determined by UV and IR spectroscopy. In addition, more reliable information about the surface temperatures of these planets can be obtained. The landing of spacecraft on these planets will allow a much more detailed study of these planets to be carried out. GLC determinations of the atmospheric composition and an organic geochemical analysis of the planetary soil will now be feasible. Devices for detecting microbial life are also being developed. These experiments will be carried out with the utmost precautions having been taken to avoid contamination risks from terrestrial sources, the exhaust from the rockets on landing, for example, or the handling during the sample collection.

At the moment all interest is being directed towards the Apollo landing on the lunar surface in the very near future. It is true that conditions on the Moon are not conducive to the presence of life as it occurs today. As with the case of Mars, however, the high radiation flux, the extreme cycling temperature and high vacuum does not exclude any possibility of life existing, or having existed. Certainly it constitutes a fruitful area for organic geochemical exploration. The information gained from these analytical measurements on extraterrestrial samples
may provide insights into the fundamental questions relating to the origin of life and its relation to the cosmos. The discovery of life on another planet, both in a scientific and a philosophic sense, would be one of the most monumental events in the entire history of mankind.
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