Organic Pigments: Their Long-Term Fate

Fossil pigments provide evidence of modification of chemical structure under geological conditions.

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One of the first systematic studies in organic geochemistry (*1*) led to the surprising discovery that organic pigments from plants and animals are preserved in a wide range of geologic materials, often of great antiquity. This marked a decisive step in the development of our present concepts of the long-term preservation and transformation of organic compounds in nature. Much thought and effort has since been devoted to widening the scope of the original study and to interpreting the structural changes of the pigments in terms of the reactivity of the subsurface environment.

With the development of sensitive and selective analytical techniques like thin-layer chromatography, gas chromatography, and mass spectrometry, the study of fossil pigments has entered a new stage. We now have evidence that most sedimentary pigments occur as much more complex mixtures than had been anticipated. By studying the individual chemical structures we can often establish genetic relationships and can reconstruct reaction sequences. Most pigment molecules contain, attached to a central, unsaturated nucleus, a number of different functional groups. By determining how these change in the sediment we arrive at predictions about ways in which other molecules of related structure might react in the same environment.

Thus, the study of fossil pigments—and of other biochemical fossils—provides us with data on the modification of chemical structures under actual geological conditions. It avoids the extrapolation of laboratory results to a different time scale and to poorly known environmental conditions.

The Chemistry of a Fossil Crinoid

During fossilization, some biochemicals and their transformation products are imbedded at the source; compounds from other organisms and from the surrounding sediment remain excluded from many fossils. This explains why organic compounds in fossils occur at relatively high concentrations and only in moderately complex mixtures.

A Jurassic crinoid (*Apiocrinus* sp.) from northwestern Switzerland has been studied in detail (*2*). The intensely colored fossil contains an assemblage of numerous crystallized pigments (fringelites) and aromatic hydrocarbons, whose close chemical relationship suggests a common origin. Several considerations lead us to think that the hydrocarbons originated from the pigments, not vice versa. Hydroxyquinone pigments resembling the fringelites commonly occur in plants and animals and are found in many of the recent relatives of the fossil crinoid. The inorganic minerals of the fossil are strongly reduced. In such a reducing environment the pigments could well have been converted into hydrocarbons. The reverse reaction would lead from the stable hydrocarbons to the sterically strained pigments (Fig. 1), and it appears much less probable.

Several closely adjacent hydroxyl groups in fringelites D, E, and F of Fig. 1 considerably strain the aromatic ring, which would be most stable in planar configuration. This strain is most severe in fringelite D; it lowers the stability of this pigment. In the reducing sediments stabilization occurs through stepwise elimination of the sterically hindered groups. This process has been completed in fringelite H, where the only remaining hydroxyl groups are those stabilized by chelation to the quinone oxygens. The increase in stability from fringelite D to fringelite H is reflected in increasing concentrations of the pigments. The rates of these reactions decrease as the strain is lessened; the reactions proceed slowly enough to have preserved some fringelite D and larger amounts of the reduction intermediates E and F in this Jurassic fossil.

The further reduction of the very stable fringelite H proceeds very slowly; pigments still occur at 200 times the concentration of the hydrocarbons. Reaction intermediates which have lost some of their chelated groups are more readily reduced than fringelite H itself; this explains their absence in the fossil.

Reactions like those of the fringelites are geochemically irreversible. The reverse reactions are imperceptibly slow, even on a geological time scale. Given a favorable environment, such irreversible reactions proceed to completion. Reaction intermediates are unstable or "metastable." Typical examples of geochemically irreversible reactions are eliminations of oxygen functions (especially in sterically unfavorable positions), decarboxylations, some hydrogenations and isomerizations, cleavage reactions, and, in general, processes which destroy the ordered building pattern of biochemical products (Table 1).

Acid-base reactions and many reactions involving the exchange of hydrogen, on the other hand, may proceed sufficiently fast in either direction to lead to equilibrium in geological time spans. These we call geochemically reversible reactions. Here the environment determines the ratio of starting material to end product; if conditions in the environment change, this ratio adjusts to the change.

We believe that such reversible redox reactions have affected the composition of the aromatic hydrocarbons in *Apiocrinus*. It was surprising to discover that the major hydrocarbon of

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the fossil is a partially hydrogenated derivative (Fig. 1, II) of the parent compound of fringelite D. Though easily produced in the laboratory by reduction of the pigments, the parent hydrocarbon (I) itself is not represented in the fossil. In addition to fringelites D, E, F, and H, the fossil contains pigments which we consider precursors of hydrocarbons III and IV. Why, we wondered, are these represented at nearly equal concentrations if hydrocarbon I has been completely reduced to II?

The redox potential of these hydrocarbons cannot be measured in the laboratory. However, correlations between spectra and redox potentials of aromatic hydrocarbons suggest that hydrocarbon I is much more readily reduced than III. Thus, under appropriate reducing conditions, equilibrium would lead to complete reduction of I but to only partial reduction of III.

A similar situation may exist for the polycyclic aromatic and hydroaromatic hydrocarbons of ancient sediments and of crude petroleum. The concentration of oxidized and reduced partners of such redox couples reflects the reduction state of the environment. Because of the slowness of the reactions, the concentration may remain unchanged for a considerable time after changes in the environmental conditions occur—for instance, through uplifting and erosion.

Fossil Porphyrins

Tetrapyrrole pigments derived from chlorophylls and hemins occur in a great variety of sedimentary materials, including crude petroleum. Most abundant are the vanadium and nickel complexes. Frequently these are accompanied by small amounts of uncomplexed pigments. Complexes with gallium and iron as central atoms are thought to occur, though they have not yet been isolated in pure form. Chromatographic separation and structural identification of the porphyrins is simplified by "demetalation" of the complexes. Because of their great stability this requires severe treatment with concentrated acids. Often, artifacts are produced or the pigments are completely destroyed, especially when they are present in small amounts. Newer reagents (lithium or dithiooxamide in ethylene diamine, and butylphenyllithium in tetrahydrofurane) (*3*) promise to bring about "decomplexation" with better yield and without producing artifacts.

We have studied mainly the uncomplexed fossil porphyrins to avoid the uncertainty of demetalation (*4*). As a rule, free porphyrins are scarce in sediments, but the pigment-rich Triassic oil shale of the Grenzbitumen horizon of Serpiano, Switzerland, provides an excellent source. Treibs based much of his early work (*1*) on the analysis of this particular shale. He isolated what appeared to be four major pigments derived from chlorophyll and hemin, together with their metal complexes. When we applied thin-layer chromatography and mass spectrometry to the analysis of these pigments, each of them was resolved into a homologous series of six or more members. In addition, many minor pigment types were found to be equally complex mixtures of homologs.

Chlorophyll and hemin (Fig. 2) both have two acidic substituents; in chlorophyll these are esterified by methanol and the C_{20} isoprenoid alcohol phytol. The majority of the fossil porphyrins, on the other hand, are etioporphyrins —that is, they lack acidic substituents. Treibs thought that the etioporphyrins were derived from the acids by a simple decarboxylation; this would lower by two the number of carbon atoms in the molecule. The molecular-weight distribution obtained by mass spectrometry now suggests two or more additional processes in the transforma-

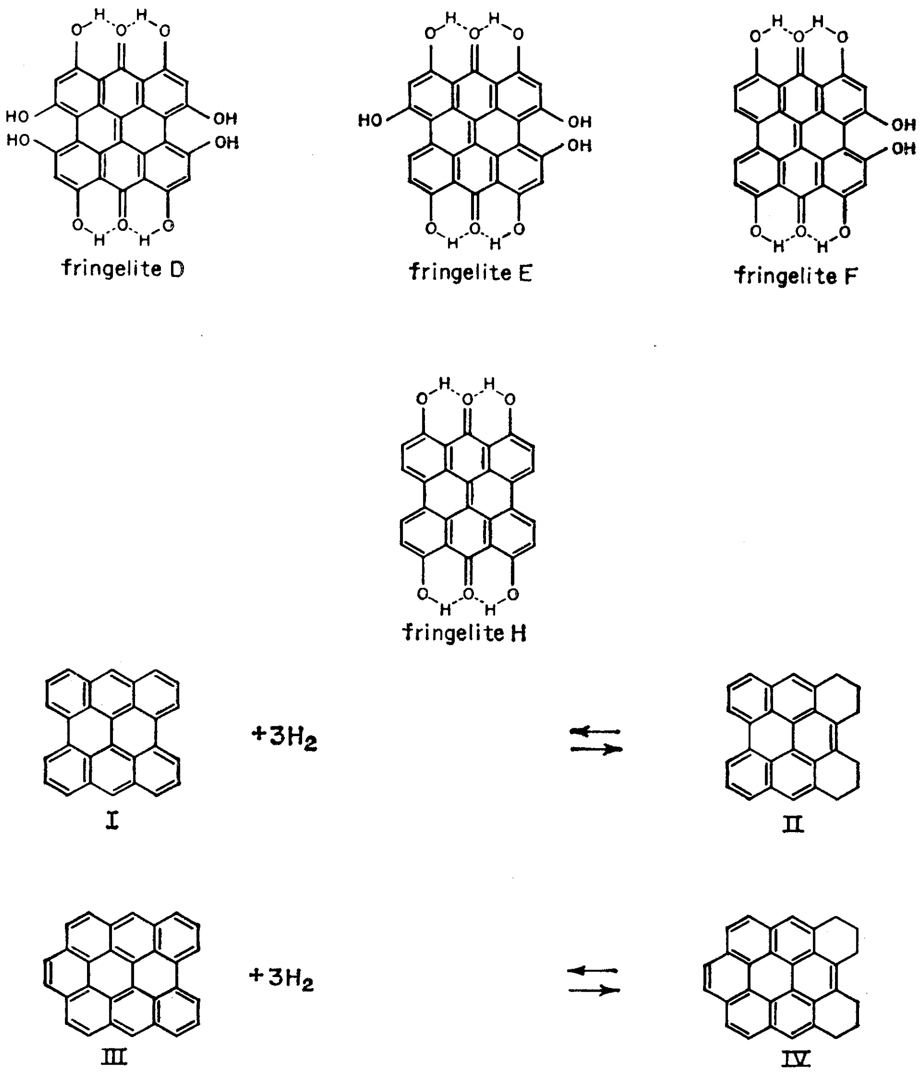
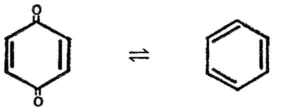
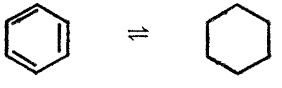
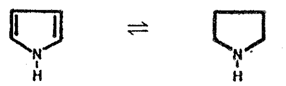


Fig. 1. Pigments and hydrocarbons of a Jurassic crinoid. The closely adjacent —OH groups of fringelite D exert a strain on the normally planar aromatic ring. Reduction in the sediments has lessened this strain in fringelites E and F and eliminated it in fringelite H. Hydrocarbons I–IV are derived from the fossil pigments. Their concentration is influenced by reversible redox equilibria.

tion of the acidic pigments to the etioporphyrins.

A few of the fossil etioporphyrins have the same number of carbon atoms as the original biosynthetic pigments. Others have lost a single carbon atom, but not two, as would be required in a complete decarboxylation. Still, as etioporphyrins these pigments have no carboxyl groups. This implies that one or both carbon atoms of the carboxyl groups have been retained by reduction to methyl groups (Fig. 2, A). This is the first geochemical evidence for the reduction of sedimentary acids. It implies such a low redox potential that it is not surprising that the carbonyl group of chlorophyll and double bonds of hemin and chlorophyll are completely reduced in the fossil porphyrins.

Table 1. Geochemically reversible and irreversible reactions. Only the reactions are listed for which we have evidence from the fringelites, the fossil porphyrins, and the phytol-derived hydrocarbons.

Reaction	Type	Reversibility
R–COOH ⟶ R–H	Decarboxylation	Irreversible
R–COOH ⟶ R–H + shorter-chain acids and hydrocarbons	Cooper-Bray (*5*) degradation	
$R-\overset{\overset{C}{\vert}}{C}-R \longrightarrow R-C-R$	Cleavage	Irreversible
R–C–C–R ⟶ R–C	Cleavage	Irreversible
$R_2C = CR_2 \longrightarrow R_2HC-CHR_2$	Reduction	Irreversible
$R-COH \longrightarrow RCH_3$	Reduction	Irreversible
$R-COOH \longrightarrow RCH_3$	Reduction	Irreversible
$R_2-C=O \longrightarrow R_2-CH_2$	Reduction	Irreversible
R–OH ⟶ R–H	Reduction	Irreversible
	Reduction	Reversible
	Reduction	Reversible
	Reduction	Reversible
R–H ⇌ R–Me	Complexation	Reversible?
R–COOR ⇌ R–COOH	Hydrolysis	Reversible

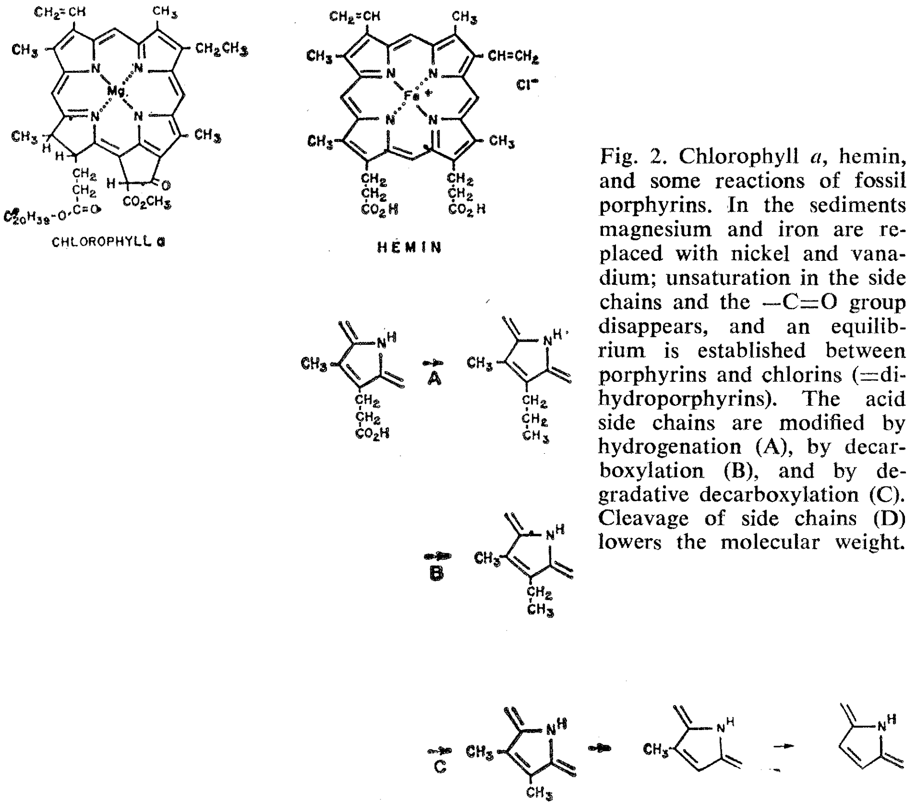


Fig. 2. Chlorophyll *a*, hemin, and some reactions of fossil porphyrins. In the sediments magnesium and iron are replaced with nickel and vanadium; unsaturation in the side chains and the —C=O group disappears, and an equilibrium is established between porphyrins and chlorins (=dihydroporphyrins). The acid side chains are modified by hydrogenation (A), by decarboxylation (B), and by degradative decarboxylation (C). Cleavage of side chains (D) lowers the molecular weight.

Decarboxylation of the acid groups (Fig. 2, B) competes with their reduction; it produces a sizable, but not the principal, fraction of the fossil etiopigments. The majority of the etioporphyrins contain less carbon than the products of reaction B. It has been suggested that fatty acids in sediments are degraded by a low-temperature decarboxylation involving free-radical intermediates and yielding acids and alkanes of lower molecular weight (*5*). Some of the fossil etioporphyrins may well have been produced by this or a similar mechanism (Fig. 2, C).

A few of the fossil etioporphyrins have still lower molecular weights. In these, cleavage of alkyl groups (Fig. 2, D) must have accompanied the complete degradation of the propionic acid side chains.

Sediments and crude petroleum often contain traces of reduced porphyrins. In these chlorins, one of the four pyrrole rings has been saturated by the addition of hydrogen. Chlorophyll itself is a chlorin; thus, we might consider the fossil chlorins to be direct descendants of chlorophyll. On the other hand, they could be reduction products of fossil prophyrins. That we have isolated chlorins structurally related to the fully aromatic pigment hemin suggests the latter. This interpretation is supported by mass spectral analysis of the fossil chlorins derived both from chlorophyll and from hemin. Except for the mass difference caused by the two extra hydrogen atoms, the fossil chlorins and porphyrins have the same molecular-weight distribution. This suggests a reversible redox system at equilibrium. Chlorins probably were formed only after the porphyrins had already undergone their major structural changes.

The fossil chlorins are among the most strongly reduced compounds recovered from sediments. In pure form, they react with the oxygen of the air. In solution, chlorins reduce vanadyl ions to trivalent vanadium. That chlorins survived during uplifting and erosion must be attributed to the very considerable buffering action of many other strongly reduced compounds in this organic-rich shale.

Isoprenoid Hydrocarbons from Phytol

Phytol, a C_{20} isoprenoid alcohol (Fig. 3, I) is a constituent of chlorophyll (Fig. 2). Above, I discussed the geochemical fate of the tetrapyrrole ring fragment of this pigment; now, we shall consider the reactions which phytol undergoes in organisms and in sediments.

Phytol is synthesized by all green photosynthetic plants. An estimated 90 percent of the total production occurs in the sea, mostly in planktonic algae. The small crustaceans, which are among the most important intermediates in the food chain between algae and higher organisms, liberate phytol from chlorophyll by hydrolysis. The phytol is then converted into a wide range of saturated and olefinic hydrocarbons (*6*) (Fig. 3, II–X).

Several isomeric phytadienes are formed (Fig. 3, II–IV), presumably by acid-catalyzed dehydration of phytol in the digestive tract of the animals. Pristane (V) and three isomeric olefins (VI–VIII) with the same carbon skeleton are formed by an alternate degradation mechanism, which causes the loss of one carbon atom. Pristane is stored in amounts up to 5 percent of total body lipids by several species of *Calanus*. It has an unusually low density and melting point and may therefore serve as a buoyancy regulator and may aid in keeping the stored lipids of these species in the liquid state.

In cold water, planktonic crustaceans increase the unsaturation of the fatty acids ingested with their food (*7*); this lowers the melting point of their body fat. A similar process may be responsible for the formation of several C_{19} di- and triolefins (Fig. 3, IX and X) from pristane.

The phytol-derived hydrocarbons are less easily metabolized than other lipids; therefore they are often retained by organisms which feed on zooplankton. Pristane and the monoolefins can be traced throughout the marine food chain; they occur in the fats of large marine fishes and mammals.

Saturated hydrocarbons related to phytol occur in crude oil and in ancient sediments. Their presence in the billion-year-old Nonesuch shale of Michigan has been interpreted as evidence of life in Precambrian times (*8, 9*). No olefins have been reported; their reduction should proceed rapidly in most sediments. Phytane (Fig. 3, XI), the parent hydrocarbon of phytol, is surprisingly abundant; it accounts for 0.2 percent of East Texas crude oil and 2.2 percent of Colorado oil-shale bitumen. It may have been formed by hydrogenation of phytadienes, either those derived directly from organisms or those generated *in situ* by catalytic dehydration of phytol adsorbed on clay minerals. Conversion of phytol to phytane by hydrogenation and reductive elimination of the hydroxyl group also appears possible, though less probable.

Like phytane, pristane (Fig. 3, V) occurs at high concentrations: 0.5 percent in East Texas crude oil and 1.0 percent in Colorado oil-shale bitumen. The derivation of pristane from phytol by oxidation to an acid, followed by decarboxylation and hydrogenation, has been suggested. It could also be formed from norphytene (VII), a C_{19} monoolefin which occurs in organisms and is a by-product of the catalytic dehydration of phytol.

We should not exclude the possibility that some of the ancient pristane was formed by organisms at the time of deposition of the sediments. This is almost certainly the case for the pristane which we have isolated from the topmost sediment layer of a Norwegian fjord (*10*). At the sediment surface, the reductions which could generate pristane from phytol without biochemical mediation proceed too slowly. This is evident from the excellent state of preservation of the highly unsaturated carotenoids in surface sediments.

Several C_{14} to C_{18} isoprenoid hydrocarbons (Fig. 3, XII–XV) have been isolated from petroleum and oil shales. These may be cleavage products of phytol, phytane, or pristane. In addition to these fragments, cleavage reactions also produce low-molecular-weight compounds, with boiling points in the gasoline-boiling-point range. Because of their structural simplicity their biochemical ancestry is not easily recognized.

The fossil tetramethylheptadecane (Fig. 3, XVI) is thought to originate from carotenoids or other isoprenoids with more than 20 carbon atoms. By fragmentation these may also have produced some of the sedimentary phytane, pristane, and lower-carbon-number hydrocarbons.

Isoprenoid acids with the carbon skeleton of phytane, pristane, and farnesane have been isolated from a California petroleum (*11*). Through simple and stepwise decarboxylation with alkyl radical intermediates these acids might produce isoprenoid paraffins of lower molecular weight than pristane.

Phytadienes dimerize to substituted cycloalkenes at moderate temperatures, even without a catalyst. Through cleavage and disproportionation these might give rise to some of the substituted dialkylbenzenes and dialkylcyclohexanes of crude petroleum.

From a structural analysis of fossil pigments we have arrived at tentative reconstructions of the events in their transformations. At present we are re-

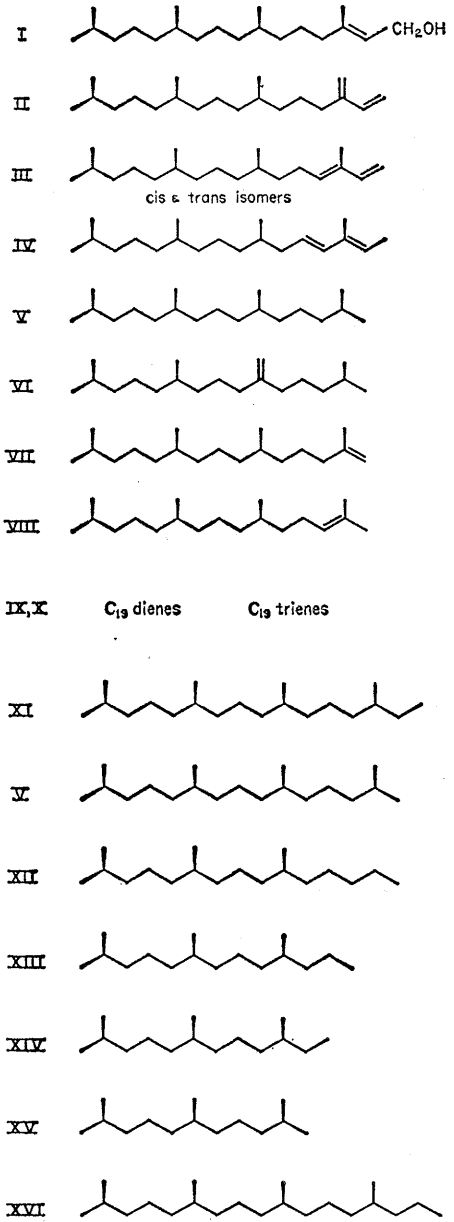


Fig. 3. Phytol (I) and phytol-derived hydrocarbons from organisms, sediments, and petroleum. Compounds II–X have been isolated from marine zooplankton, compounds XI–XVI occur in ancient sediments and petroleum. Pristane (V) is common to the two groups and has been found in a recent marine sediment.

Hydrogenation of the carboxyl group:

$$C_nH_{2n+1}COOH \rightarrow C_nH_{2n+1}CH_3$$

Simple decarboxylation:

$$C_nH_{2n+1}COOH \rightarrow C_nH_{2n+2} + CO_2$$

Stepwise decarboxylation with alkyl radical intermediates (Cooper and Bray):

$$\begin{aligned} C_nH_{2n+1}COOH \rightarrow {} & C_nH_{2n+2} + C_{n-1}H_{2(n-1)+1}COOH + \\ & C_{n-1}H_{2(n-1)+2} + C_{n-2}H_{2(n-2)+1}COOH + \\ & C_{n-2}H_{2(n-2)+2} + C_{n-3}H_{2(n-3)+1}COOH \; . \; . \; . \end{aligned}$$

Cleavage:

$$C_nH_{2n+1}COOH \rightarrow C_{n-x}H_{2(n-x)+2} + C_xH_{2x+2}$$

Fig. 4. Transformation of a saturated straight-chain fatty acid in the sediments. A wide-range mixture of fatty acids and paraffin hydrocarbons is produced. The average molecular weight is lower than that of the starting material.

luctant to interpret these changes in terms of reaction mechanisms, though some convincing steps in this direction have already been taken (*5*). Eventually, we hope, a broader knowledge of sedimentary reactions and their mechanisms will enable us to predict the fate of most organic substances in the sedimentary environment.

Even now we may try to apply the information derived from the pigments to a wider range of compounds. Thus, carboxylic acids in a sediment should undergo transformations resembling those of the propionic acid side chain of the porphyrins. The possible reactions of a saturated, straight-chain fatty acid $C_nH_{2n+1}COOH$ are summarized in Fig. 4. The overall result is the formation of a mixture of fatty acids and paraffins of wide boiling-point range and lower molecular weight than the starting compound, showing a smooth statistical distribution of carbon numbers. This agrees well with the finding that petroleum paraffins have a lower average molecular weight than the biogenic fatty acids of recent source sediments. Also, their carbon-number distribution is smooth, while chains of odd carbon number predominate in the paraffins, and chains of even carbon number predominate in the fatty acids, of recent sediments.

Hydrogenations account for many of the reactions of ancient pigments (Table 1). They also play a dominant role in the processes which are believed to form crude petroleum. What is the source of the hydrogen and what are the driving forces for these reactions?

At the time of deposition the sedimentary organic matter includes a wide range of oxidation states and is far from redox equilibrium. After burial, the most highly oxidized materials are reduced by reaction with compounds of lower reduction state. As geologic time passes, reactions which are too slow to proceed in the laboratory eliminate the least stable compounds and bring the sedimentary organic material closer to equilibrium.

Pigments and petroleum precursors are only a small fraction of the sedimentary organic material. Their hydrogenation is coupled with the dehydrogenation of other compounds. Straight- and branched-chain compounds may lose hydrogen in condensations, cyclizations, and aromatizations. Hydrogen is made available also in the formation of those large aromatic entities which are an important part of the petroleum asphaltenes and of kerogen. These reactions amount to an overall stabilization of the sedimentary organic material.

We have studied sediments of intermediate age. In these, most of the unstable compounds have already disappeared; fringelite D, and to a lesser degree fringelites E and F, are exceptions. Sufficient time has lapsed since the Jurassic period for some reactants to have been brought into equilibrium with the environment. With time, a larger fraction of the sedimentary organic matter will undergo such stabilization and equilibration. The most stable organic compounds survive for extremely long periods, if the temperature of the sediment remains sufficiently low to exclude slow pyrolysis. The discovery of fossil porphyrins in a Precambrian sediment has recently been reported (*9*). It will be most interesting to learn how their structure compares to that of our Triassic pigments.

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