CHEMICAL FOSSILS: A COMBINED ORGANIC GEOCHEMICAL AND ENVIRONMENTAL APPROACH

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ABSTRACT

The laboratory and field simulation of the geological processes believed to afford chemical fossils is under active study. The early stages of diagenesis are sometimes very rapid and even the slow process of maturation can be simulated in the laboratory.

INTRODUCTION

Several major texts document the subject of organic geochemistry[1–6]. Recent progress has been reviewed by Albrecht and Ourisson[7] and by Maxwell *et al.*[8] and in the published proceedings of several symposia[9–12]. The present paper is concerned mainly with current work on Recent sediments and will not review the literature.

Chemical fossils (*Table 1*) represent only a small part of the total abundance of carbon compounds in and on the earth. To understand their significance, one must examine present environmental conditions, that is, study

Table 1. Commonly studied classes* of chemical fossil

Alkanes†	Alcohols†	Quinones	Carbohydrates
Alkenes†	Ketones†	Porphyrins	Amino acids
Aromatics	Fatty acids†	Chlorins	Biopolymers
		Carotenoids	Kerogen

* Multifunctional compounds are encountered.
† n-, branched, acyclic and cyclic isoprenoid.

the organic geochemistry of the environment, since the principle of uniformitarianism employed by the geologists is just as relevant to geochemistry—'the present is key to the past'. This paper, therefore, is concerned with the study of contemporary sediments and the input and fate of organic compounds prior to consolidation. A variety of environments will need to be studied in order to find contemporary parallels for those which gave rise to ancient sediments and their content of chemical fossils. In examining a

particular aquatic environment, therefore, one must consider first, what enters the sediment as biological and other debris; second, what happens to this debris in the short term; and, third, what remains as the sediment becomes consolidated and biological action ceases. The final stage of maturation in the presumed conversion of biological compounds to chemical fossils (e.g. biolipids to geolipids) involves geothermal reactions which can also be studied in contemporary conditions and in the laboratory.

In this paper, I shall exemplify the current approach to the study of the geochemical fate of biolipids through two major biogenetic classes, polyacetate derived compounds, and isoprenoids. Polyacetate derived compounds are straight chain, branched chain and cyclic compounds formed by the acetate route. Typical examples include the straight chain fatty acids, ketones, alcohols and hydrocarbons and the corresponding iso- and anteiso-compounds. The patterns of abundances within the various homologous series are of especial significance. Isoprenoids, both acyclic and cyclic, include sesquiterpenes, diterpenes, triterpenes and tetraterpenes in the acyclic series, and steroids and triterpenoids in the cyclic series. The carbon skeletons and especially the stereochemistry of individual compounds are of prime importance.

Short-term changes in Recent sediments are undoubtedly an important part of the carbon cycle. Their study requires a combination of the approaches used in both organic geochemistry and environmental chemistry. Thus, the same analytical methods can be used for the characterisation of biolipids, geolipids and pollutants. Natural products vary greatly in their resistance to short-term degradation. Thus, oleic acid in Severn Estuary sediment is degraded mainly to CO_2 within a few days[13]. On the other hand, incubation of cholesterol in the same sediment for 89 days results in less than 30 per cent degradation[14]. The rapidity of degradation of an organic compound introduced into an environment may be dependent largely upon its suitability as a microbial nutrient. Microorganisms bring about degradation in the short term by two distinct ways: actual biodegradation within the microorganism and chemical degradation as a consequence of the immediate environment created by their activity. However, organisms may simply detoxify a compound rather than utilize it for food. The actual physical state of the compound is likely to be critical and here studies involving the interment of whole organisms into sediments will be important.

Partial analogies for the sediment situations exist in the utilization and transformation of biolipids by bacteria in pure or mixed culture. Thus, the sterol side chain can be partially or completely degraded by cultures of certain microorganisms[15,16] and it is reasonable to expect similar degradations in microbiologically active sediments. The symbiotic microbial populations within the gut of larger animals are also known to effect many conversions; for example, cholesterol is reduced to cholestanol within the intestines of the rat[17]. Natural aquatic environments are much more complex and only limited confidence can be placed in interpretations based on comparisons with microbial cultures or higher animal studies. However, such studies will provide useful analogies for those portions of the carbon cycle concerned with the short-term fate of biological products in sediments. Experimentally, the fate of a single radio-labelled compound can be followed

by observing the range of compounds actually formed. Estimates of the overall carbon budget can be made[18] and isotopic ratios used as guides to the origins of compounds found in specific environments (e.g. nitrogen from inorganic sources is poor in ^{15}N)[19].

At the heart of much of this work, however, is the need to adequately characterize and identify compounds. Fourier-transform nmr promises to give good ^{1}H and ^{13}C spectra with microgram and milligram sample sizes, respectively. Such equipment will be of value in environmental studies where the path of ^{13}C labelled compounds is to be followed.

Undoubtedly, the single most desirable facility for compound identification in environmental geochemical work is the combined capillary gas chromatograph–low resolution mass spectrometer–computer system[20,21]. Capillary GC–MS is used routinely in some laboratories[22], while MS–computer systems have been developed in others[23,24]. The more expensive high resolution mass spectrometer–computer system, gives detailed information about the composition of single compounds and complex mixtures[25].

Compound classification based on mass spectral ion series is useful for the preliminary characterization of complex mixtures analysed by GC–MS techniques. The small laboratory computer system used for the MS data reduction stage can be programmed to effect this preliminary treatment prior to the more demanding large computer treatment of unknown spectra by the 'artificial intelligence' and 'library matching' procedures[26–29]. Mass spectra are conveniently stored, distributed and exchanged in card image format on magnetic tape or disc as is done by the Mass Spectral Data Centre, Aldermaston, England.

The determination of relative and absolute stereochemistry is becoming more routine, suitable derivatization being followed by GLC at the microgram and nanogram level. Recent examples include studies of the rates and extent of epimerization of amino acids in sediments[30] and the stereochemistry of acyclic isoprenoid alkanes, alkanols, and alkanoic acids in the C_{10}–C_{25} range[31–33]. Separation methods now in common use are silver ion TLC, urea and thiourea adduction, and neutral–acid separation combined with derivatization (e.g. trimethylsilylation).

AQUATIC ENVIRONMENTS

Systematic studies of a variety of Recent (contemporary) sediments are required to provide the background information essential for the detailed understanding of the early stages in the history of chemical fossils. These studies should include measurement of total carbon, organic carbon, lipid content, the content of different classes of organic compounds and, in special cases, of individual compounds. Measurements of certain features such as salinity, mineral content and acidity, etc., should be made and the quantity and variety of microbiota defined. Classes of environment should include those variously combining marine and fresh water, eutrophic and oligotrophic, and arctic, subarctic, temperate, subtropical and tropical conditions. The measurements need to relate, wherever possible, to studies of the organic carbon budget of the ecosystem from which the carbon was contributed to the sediment. The data in *Table 2* indicate the very low amounts

of organic carbon in oceanic sediments and the high amounts in eutrophic fresh water lakes (Esthwaite) and anoxic marine basins (Black Sea) where the organic matter accumulates under the reducing conditions accompanying high productivity in the overlying waters.

Table 2. Organic carbon in aquatic sediments

Sediment	% Organic carbon
Marine	
Oceanic[34]	0.1–0.3
Nearshore[35]	2.5
Anoxic basin	
permanent (Black Sea)[36, 37]	3–20
periodic (Saanich Inlet)[38]	7
Freshwater	
Lake (Esthwaite Water)[39]	10–18

Figure 1 depicts some of the environmental parameters involved in the deposition of aquatic sediments. The microorganisms, which include bacteria, fungi, protozoa and algae, play a large part in effecting changes brought about in organic matter contributed to the sediments. They consume and degrade it and on dying contribute their biomass which, of course,

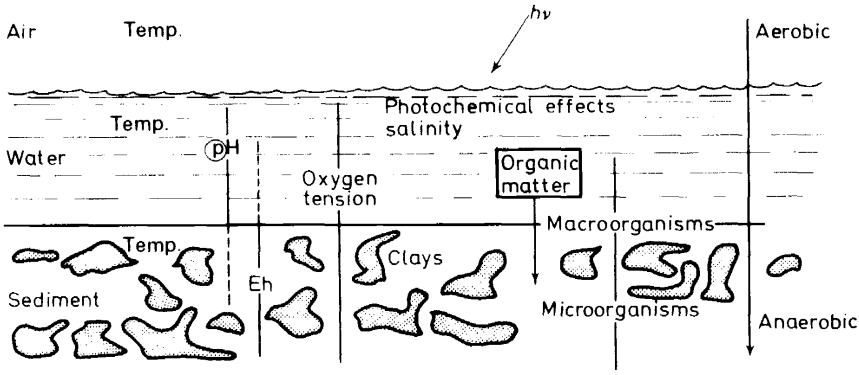
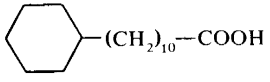


Figure 1. Aquatic environments. Some of the parameters involved in the deposition and early diagenesis of organic matter

may suffer further change. The reactions can be effected aerobically or anaerobically. It seems likely that some microorganisms may be very important in contributing and changing organic matter in sediments but, so far, have escaped detailed chemical attention. Thus, the sulphur bacterium, *Bacillus acidothermophillus*, which thrives in hot springs and hot dilute sulphuric acid, contains iso- and anteiso-fatty acids like other bacteria. However, it is markedly atypical in that it also contains fatty acids bearing a terminal cyclohexyl group; the C_{17} acid, (I) and the corresponding C_{19} acid together account for up to 65 per cent of the lipid, unsaturated and cyclopropane acids being absent. Only very small amounts of straight chain

acids are present. Furthermore, the triterpene hop-22(29)-ene(II, diploptene) and smaller amounts of other hopenes make up 0.3 per cent of the dry weight[40]. Again, *Methanococcus*, a bacterium which uses methane generated in sediments, contains hopene, 4,4-dimethyl sterols, 4-methyl sterols and 4-desmethyl



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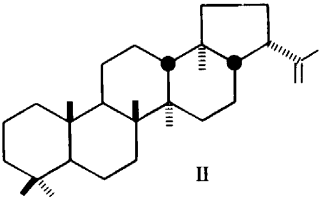


II

sterols. Contrary to previous suppositions, therefore, triterpenoids are not confined to higher plants[41,42]. Obviously it would be worthwhile examining the lipid chemistry of sediments in which particular bacteria, such as the above, are active. Indeed, some or all of the processes, such as reduction, hydrogen transfer, aromatization, chain shortening, chain lengthening, polymerization and depolymerization, may derive from the action of a wide variety of organisms.

Table 3 lists a few of the many transformations of biolipids which have been ascribed to the action of microorganisms. Three types of skeletons of geochemical significance are illustrated: polyacetate (exemplified by oleic acid),

Table 3. Microbial transformations of possible geochemical significance

Reaction	Precursor	Product	Microorganism
Reduction	Oleic acid[43]	Stearic acid	*Isotrichia intestinalis*
	Cholesterol[17]	Cholestanol	in rat intestine
	Phytol[44]	Dihydrophytol	in cow rumen
Hydroxylation	Oleic acid[45]	10-OH Acids 10-Keto acids	*Pseudomonas* spp
	Cholesterol[15,16]	Hydroxylated in various ring positions	many species
Chain degradation	Fatty acids[46] (C_2–C_{10})	CO_2, CH_4, H_2S	sediment microorganisms
	Cholesterol[47]	Androsta-1,4-dien-3,17-dione	*Arthrobacter simplex*
	Cholesterol[16]	20α-hydroxy-methyl pregna-1,4-dien-3-one	*Mycobacteria* sp.

the cyclic isoprenoid skeleton (exemplified by cholesterol), and the acyclic isoprenoid skeleton (exemplified by phytol). In most cases the studies were made without the use of radioactive labelled precursors. The conversions were demonstrated in pure or mixed cultures but not in sediments, though it is a reasonable inference that they are likely to occur there.

Some examples of claims for such changes taking place in soils and sediments are listed in *Table 4*. Again most of this work was carried out without benefit of radio labels. Nevertheless, it strongly supports the inferences made from studies with single cultures and mixed cultures of microorganisms, assuming the validity of the claims for the direct conversion, for example, of oleic acid into saturated acids and hydroxylated acids, of cholesterol into an oxidized form, and of a hydrocarbon and a sugar into carbon dioxide.

Table 4. Degradations and transformations in soils and sediments

Precursor	Environment	Observations
Acetic acid	Lake sediment[46]	Palmitic acid, CO_2, CH_4, H_2S
Unsaturated fatty acids	Marine sediment[48]	Rapid degradation, partial conversion to saturated acids
Human fat	Soil[49]	Adipocere
Oleic acid	Soil[50]	10-Hydroxy stearic acid
Oleic acid	Sewage[51]	Stearic acid
Cholesterol	Soil[52]	60% Degradation in one year
Cholesterol	Soil[53]	Δ^4-Cholestenone and 5-oxo-3,5-seco-4-norcholestan-3-oic acid
n-Eicosane U-^{14}C	Soil[54]	$^{14}CO_2$
Glucose ^{14}C	Soil[55]	$^{14}CO_2$ by three metabolic pathways

Additional information is coming from the direct analysis of Recent aquatic sediments. Thus, Cranwell has found that the bottom sediments from Blelham Tarn and other lakes in the English Lake District contain pentacyclic triterpenoid cycloalkanes[56]. This finding and similar data for Rostherne Mere, Cheshire[14], suggest either that reduction of triterpenes occurs very rapidly or that these hydrocarbons are coming in from neighbouring peat deposits. Cranwell also reports that preliminary GC-MS analysis indicates

Table 5. Sterols and stanols of Mono Lake organisms and sediments[58, 59]

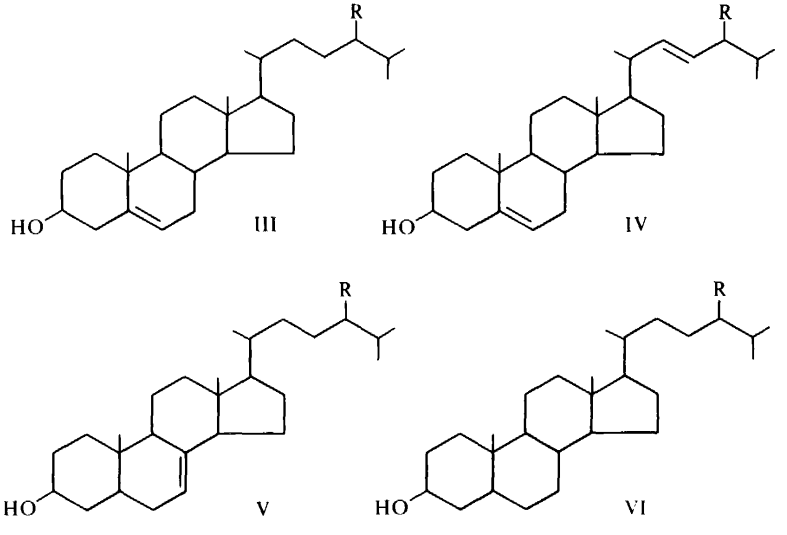
Organism or sediment	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	Ergost-7-en-3β-ol	24-Ethylcholest-7-en-3β-ol	β-Sitosterol	Cholestanol	Stigmastanol*	Ergostanol*
	III R = H	IV R = Me	III R = Me (24R)	IV R = Et	V R = Me (24 S)	V R = Et	III R = Et (24 R)	VI R = H	VI R = Et	VI R = Me
Algae	+	+	+	+	+	+				
Diatoms										
Shrimp	+									
Bottom mud	+	+	+	+			+			+
> 67 200 yr	+		+	+			+	+	+	
> 97 200 yr								+	+	+
> 120 000 yr	+	+	+	+	+		+			

* Also identified in Green River shale[60].

that the sterol fraction also contains stanols. For example, the upper 20 cm mud layer (which would be of age up to about 50 years) at Blelham contains cholestanol (campestanol) 24-methyl and (stigmastanol) 24-ethyl in addition to the corresponding Δ^5 unsaturated compounds. Presumably, reduction has occurred in the sediments, possibly as a result of bacterial action. This same indication of reduction processes is seen in the rise in the ratio of saturated to unsaturated fatty acids with depth of mud, as was reported by Parker and Leo[57] for the sediment column comprised of algal mats off the Texas coast.

Table 5 summarizes data obtained by Henderson *et al.*[58, 59] for the sterol and stanol contents of samples of Quaternary sediments from Mono Lake, California. There are some inconsistencies but the principal observation is that certain organisms abundant in the lake do not contain the stanols, whereas the bottom mud and the consolidated sediments do.

There is clearly much geochemical information to be obtained by analysis of these Recent sediments and from their intercomparison. The literature



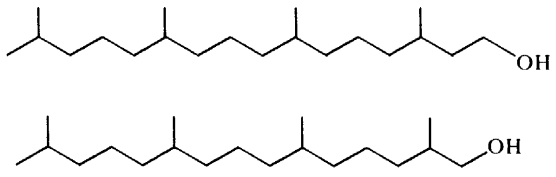
on the lipid constituents of organisms is increasing rapidly and it should be possible to infer the major precursors of compounds detected in the sediments.

LIPID ANALYSIS OF A CONTEMPORARY LACUSTRINE SEDIMENT

I have already stated the view that knowledge of the organic content of contemporary aquatic sediments is essential to an understanding of the origin of chemical fossils. Work designed to examine this premise is under way in

several laboratories; as an example I shall describe in some detail current exploratory research on the lipid content of bottom muds taken from Esthwaite Water in the English Lake District. These muds have been laid down during the 10 000 years which have elapsed since the last Ice Age. The lakes and their sediments are under continuous and wide-ranging study by the members of the Fresh Water Biological Association at Windermere. Esthwaite is eutrophic i.e. the sediment is anoxic for much of the year, with a large annual algal bloom.

This sediment has been extracted by Dr R. E. Cox of the Organic Geochemistry Unit, Bristol, who has shown that its content of certain compounds is similar to those described by Cranwell[56] for other sediments from the Lake District[61]. *Figure 2* outlines the separation scheme used and it can be seen that there are several fractions which await description. The separation procedures are based largely on TLC and on urea adduction and afford a number of fractions which are amenable to GC–MS study. Examination of the fractions is incomplete, but the alkanes are typically straight chain, with small amounts of isoprenoids and other branched-cyclics present in the branched-cyclic fraction. The alcohols include even-numbered, straight-chain alcohols and some isoprenoids, such as dihydrophytol(VII) and pristanol-(VIII).



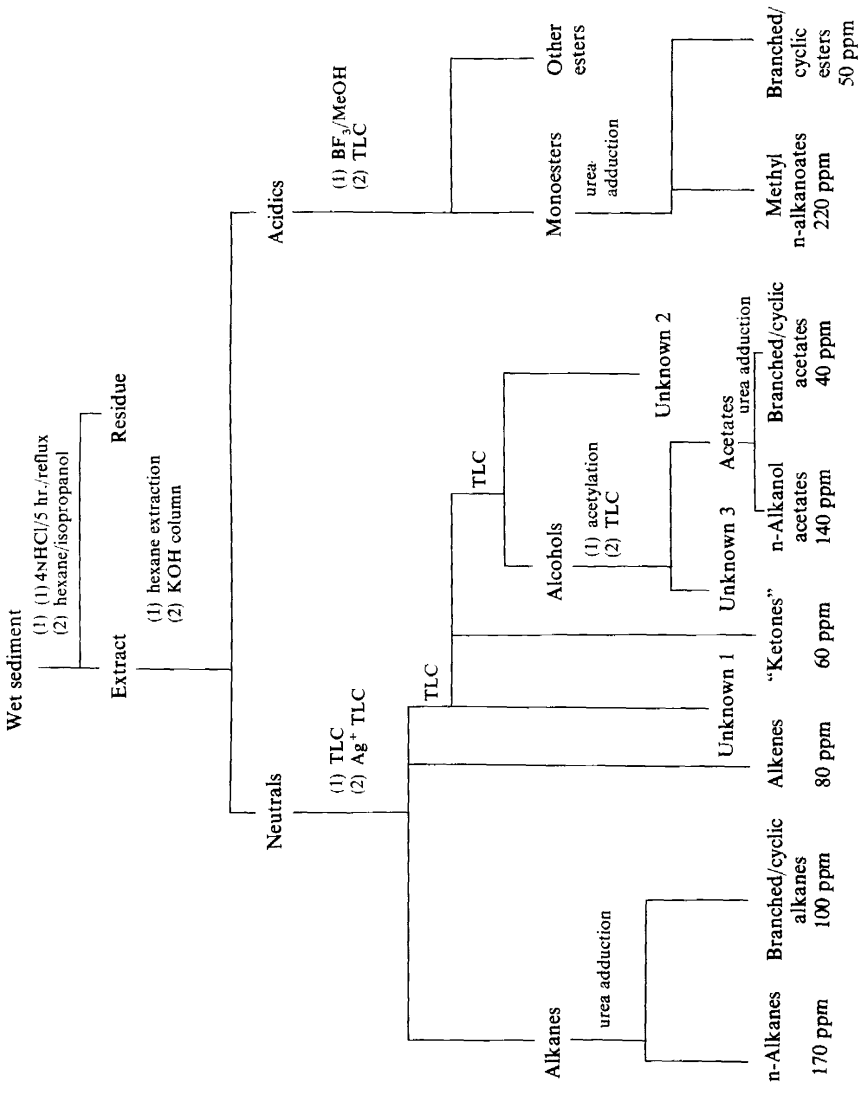
VII

VIII

The fatty acid fraction (*Figure 2*) has received the most study to date. Although incompletely resolved, capillary GLC produced a wealth of information (*Figure 3*). Small amounts of straight-chain acids (found as methyl esters) are still present after the urea adduction but the most significant features are the substantial amounts of the iso- and the anteiso-acids and the small amounts of the isoprenoid acids, such as phytanic (IX) and pristanic (X). *Table 6* lists the approximate percentage composition of this fraction.

Similar acid compositions have been found in other contemporary aquatic sediments, including those of nearby lakes (Blelham and Ennerdale)[56] and the Gulf of Mexico[63]. What is even more encouraging is that the Eocene Green River Shale also displays some similarities. These data are given in *Table 7*. Unfortunately the determinations have been made by different workers and by different methods. However, the patterns of relative abundance are impressively alike and similar to those found in certain bacteria. Further comparative work is desirable, especially employing a single method in a single laboratory. Even at this early stage, the normal fatty acids do show some interesting differences which may reflect the different conditions in the environment; thus the two eutrophic lakes appear to have double maxima at C_{16} and C_{22} while the single oligotrophic example (Ennerdale) has one

Figure 2. Neutral and acidic fractions from a wet lacustrine sediment. The sediment is from a core taken from Esthwaite Water in the English Lake District. This preliminary analysis was made by Dr R. E. Cox of the Organic Geochemistry Unit.[61,62] Quantities are expressed as p.p.m. of dry weight of sediment.



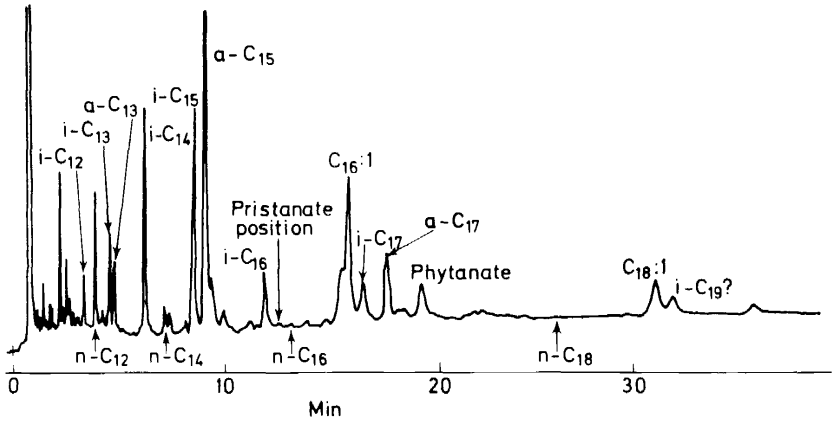


Figure 3. Gas chromatogram of urea non-adduct fraction of the total fatty acids from the sediment of Esthwaite Water.[61] Some straight chain acids are still present, especially the C_{16} and C_{18} monounsaturated homologues which are major constituents of the original lipid fraction. Conditions:—Acids as methyl esters; 50 m capillary column coated with butanediol succinate isothermal at 170 C, 40 p.s.i.

Table 6. Composition of urea non-adduct ester fraction from an Esthwaite core.[61]

Acid	Percentage composition*
i-C_{12}	1.0
i-C_{13}	3.0
a-C_{13}	1.5
i-C_{14}	6.5
i-C_{15}	8.0
a-C_{15}	24.0
i-C_{16}	5.0
C_{16}:1†	10.0
i-C_{17}	5.0
a-C_{17}	8.0
Phytanate	7.0
C_{18}:1†	8.0
i-C_{19}?	3.5
C_{19}:cy?‡	3.0

* Estimated from GLC peak areas (*Figure 3*).
† Position of double bond unknown.
‡ Possibly *cis* 9,10-methyleneoctadecanoic acid.

maximum at C_{26}. Bu'Lock has suggested[64] that the pattern of branched acids, e.g. the dominance of anteiso and iso- C_{15}, is more reminiscent of psychrophilic bacteria than of mesophilic or thermophilic species, which would seem reasonable in view of the low *in situ* temperatures (*ca* 5°C) of the bottom muds.

Table 7. Comparison of saturated fatty acid contributions in Recent and ancient sediments

Acid type		Recent								Ancient	
		Blelham		Esthwaite		Ennerdale		Gulf of Mexico		Green River Shale	
		Main[1]	% of total	Main[2]	% of total	Main[1]	% of total	Main[3]	% of total	Main[3,4]	% of total
Normal	$CH_3(CH_2)_nCO_2H$	**16**, *18*, *20*, **22**, 24	70	**16**, *18*, *20*, **22**, 24	86	24, **26**, 28	93	14, *16*, 18	94	16, 18, *28*, **30**	80
Iso	$CH_3CH(CH_2)_nCO_2H$ (CH₃ branch)	*14*, **15**, 16, 17	8	*14*, *15*, 16, 17	6	**15**, 16, *17*		14, 15, 16, 17	6	14, 15, 16, 18	8
Anteiso	$CH_3CH_2CH(CH_2)_nCO_2H$ (CH₃ branch)	**15**, *17*	12	**15**, *17*	6	*15*, **17**, 19		15, 17		15	
Branched	$CH_3(CH_2)_nCH(CH_2)_mCO_2H$ (CH₃ branch)	**17**, *19*	2	—	—	17, **19**	7	—	—	—	—
Cyclic	$CH_3(CH_2)_nCH-CH(CH_2)_mCO_2H$ (CH₂ bridge)	17, 19	2	—	—	17, *19*		—	—	—	—
Isoprenoid	$H-(CH_2-*CH(CH_3)-CH_2)_n-X$ (CH₃ branch); $X=CH_2-*CH(CH_3)-CH_2CO_2H$ etc)	20	4	20	1	19, **20**		—	—	19, 20	12

[1] Cranwell (1972)[56]; [2] Cox (1972)[61]; [3] Leo and Parker (1966)[63]; [4] Cox (1971)[62]

— Not reported. The figures in the columns refer to the total carbon number of the individual fatty acids. Relative abundance, **x** > *x* > x.

* Chiral centre.

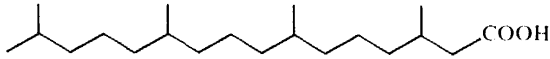
EXPERIMENTAL STUDY OF SHORT-TERM FATE OF BIOLIPIDS IN AQUATIC SEDIMENTS

The previous sections have outlined the reasons for thinking that the differences between the biolipid patterns of contemporary organisms so far examined and the geolipid patterns of Recent and ancient sediments may be explained by the (geologically speaking) rapid operation of processes such as reduction in the sediment. We are testing these beliefs experimentally at Bristol and have found that important changes do occur over periods of days and weeks. The techniques used are similar to those employed in biosynthetic studies within a single organism or enzyme preparation. In effect, the environment or small ecosystem is regarded as a single 'organism' into which one introduces radioactively labelled precursors. The label makes it possible to employ very small amounts of precursors and also to follow the formation of the various metabolites and degradation products.

Full *in situ* tests carried out by introducing the precursors directly into the environment are obviously the most convincing, but others conducted in the laboratory offer a useful guide. Model environments involving a number of organisms, water and sediment, etc., have also been employed by Metcalf and his colleagues[65–67]. We have used mud from the Severn Estuary, which is a major estuary where the water temperatures generally range from about 5 to 15°C. There is some contamination by sewage sludge[68] and other pollutants but comparisons made for different sites in the Estuary indicate no major differences in the chemical transformations brought about, in spite of considerable differences in the level of pollution. Present studies also include laboratory incubations, conducted in bottom muds from Esthwaite Water and Rostherne Mere (Cheshire) and sewage sludge (Avonmouth Foulwater Works, Bristol).

Work with four different classes of labelled precursors will be discussed to demonstrate this experimental approach to short-term diagenesis. These precursors and the rationale for their selection are as follows

(a) Oleic acid (XI) was chosen since it is a ubiquitous biolipid which occurs free or as glycerides and other esters in fatty and other components of most organisms, and is readily utilized[69–71].



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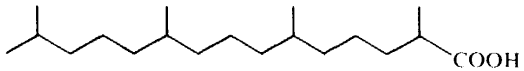
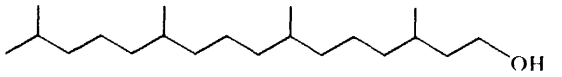


X

$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$ XI



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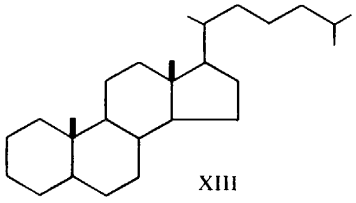

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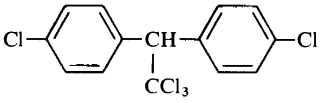


XIV

(b) Phytol (XII), the alcohol esterifying the chlorin ring system in chlorophyll which has contributed to most sediments in some quantity. The phytyl side chain is the presumed precursor of much of the C_{20} and lower acyclic isoprenoids (e.g. (VII) and (VIII)) found in sediments.
(c) Cholesterol (III, R = H) as an example of the sterols which are constituents of most organisms[72]. Sterols are the presumed precursors of the cycloalkanes, the steranes, e.g. cholestane (XIII) found in ancient sediments.
(d) DDT (XIV) as an example of persistent pollutants now entering sediments and slowly undergoing diagenesis in parallel with the biolipids.

Short term fate of oleic acid in Severn Estuary sediments[13, 69–71]

Figure 4 outlines the sequence of procedures used in the experiments. The oleic acid is dually (^3H, ^{14}C) labelled and is injected as the sodium salt into the sediment. Incubation is allowed to take place for a number of days and the CO_2 evolved trapped in aqueous KOH solution. A separate aliquot is acidified and the CO_2 flushed out to estimate the total conversion to CO_2. Acid hydrolysis and extraction with heptane affords an acid fraction which is then esterified. The methyl esters are separated by TLC into three main fractions—the polar products, the unsaturated fatty acid esters and the saturated fatty acid esters. These are characterized by radio GLC, chromatographic behaviour and scintillation counting.

Several of these experiments have now been conducted *in situ* and in the laboratory with incubation times of the order of 10 days. The results fall into a fairly consistent pattern with degradation in the laboratory proceeding faster than *in situ*. Nearly all the radioactivity is accounted for, the bulk (*ca* 80 per cent) of the ^{14}C and the ^3H being as $^{14}CO_2$ (or ^{14}C-carbonate) and ^3H_2O respectively, presumably as a direct result of bacterial metabolism. Most of the remaining ^{14}C (5–15 per cent) is in the lipid fractions which contain monohydroxy fatty acids and straight and branched-chain fatty acids. The dual labelling has permitted some conclusions to be drawn concerning the flow of products along certain metabolic pathways.

Two discrete pathways exist for the production of saturated fatty acids and are assumed to be operative in this system

—the degradative pathway: hydrogenation of the double bond preceding or following removal of C_2 units to give shorter chain-length fatty acids; and

—the resynthesis pathway: total breakdown of the carbon chain to C_2 units in the form of acetyl derivatives followed by resynthesis of the carbon

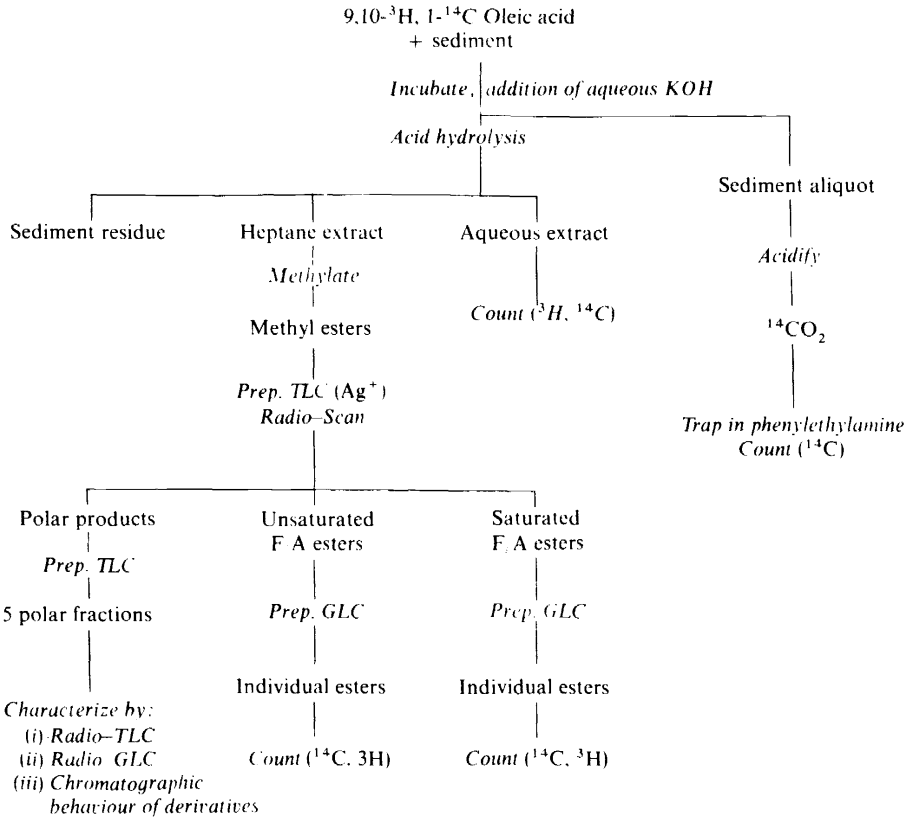


Figure 4. Short term fate of oleic acid (9,10-^{3}H, 1-^{14}C) in Severn Estuary sediment.[13,14] The experimental procedures are indicated diagrammatically. The hydrolysis step effects degradation of bacterial cell walls.[72]

chain producing, initially, palmitic acid and, subsequently, stearic acid or myristic and lauric acids.

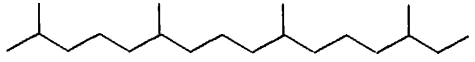
The findings to date, which include results for sediments from several sites in the estuary, are that: (a) the labelled oleic acid rapidly disappears with concomitant release of labelled CO_2 and H_2O; (b) labelled saturated branched and straight-chain fatty acids are formed in 0.1–2 per cent total yield by both the resynthesis and degradative pathways, the yields of straight-chain acids being $C_{16} \geqslant C_{14} > C_{12}$; (c) an additional pathway involving elongation and degradation interconverts C_{16} and C_{18}; (d) polar products, including monohydroxy and dihydroxy acids, are major products.

The inferences we have drawn from these experiments in estuarine tidal muds are that oleic acid (and presumably other unsaturated acids) contributed to the muds is rapidly utilized by microorganisms, most of the carbon

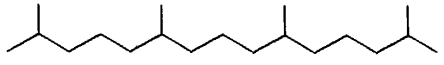
being metabolized to CO_2 but some being converted to straight-chain saturated fatty acids. The abundance pattern of the acids formed ($C_{16} \gg C_{14} > C_{12}$) matches that found by direct analysis of such sediments. Hence, it is probable that the fatty acid patterns of Recent and ancient sediments are partly explained by bacterial synthesis in the forming sediment.

Short term fate of phytol in a freshwater sediment[73]

The acyclic isoprenoid alkanes (C_{20} and lower) are ubiquitous constituents of ancient sediments, their most abundant precursor being presumed to be chlorophyll. Chlorophyll and its component alcohol phytol do not appear in general to survive long in Recent sediments, although there are some claims for chlorins in fossil material as old as Miocene[74, 75]. The hydrocarbons phytane (XV) and pristane (XVI) and the saturated alcohol dihydrophytol



XV



XVI

(VII) are found in Recent and ancient sediments[76] and are presumed to be degradation products of the phytol, arising by reduction and other processes taking place in the sediment. An experiment in progress involves injecting U-^{14}C labelled phytol into sediment from Esthwaite and allowing it to incubate in the laboratory at 18°C. *Figure 5* summarizes experimental findings.

The simulation was inadequate in that the core was only a portion of the environment and that the temperature was much higher than that (*ca* 5°C) at the bottom of the lake. Extraction gave 35 per cent of the label in the neutral fraction which was then separated into alcohol, ketone, ester and hydrocarbon fractions by silver-ion TLC. The alcohol fraction, which was the major one, was found to consist of phytol and labelled dihydrophytol. Conversion of the fraction to the corresponding acetate afforded sufficient mass for GC–MS studies; more material was present than in the 'cold' (unadulterated) sediment[61]. The stereochemistry of this dihydrophytol is not yet known. The ester fractions afforded labelled phytol and dihydrophytol on hydrolysis but the acid portions were unlabelled. Pristane and phytane did not appear to be labelled but this is not surprising in view of the short incubation time. Pristane and phytane are commonly thought of as *the* chemical fossils since they are prominent constituents of shales and petroleums. Pristane occurs in plants, animals and Recent sediments only in small amounts[77]. Of course, chlorophyll may not be the direct precursor; thus phytyl linoleate is found in autumn leaves and phytol itself is often found in yellow leaves[78].

Hence, our preliminary conclusions from these initial studies are that phytol and probably any chlorophyll entering a sediment of this type becomes quite rapidly converted to dihydrophytol and other isoprenoid

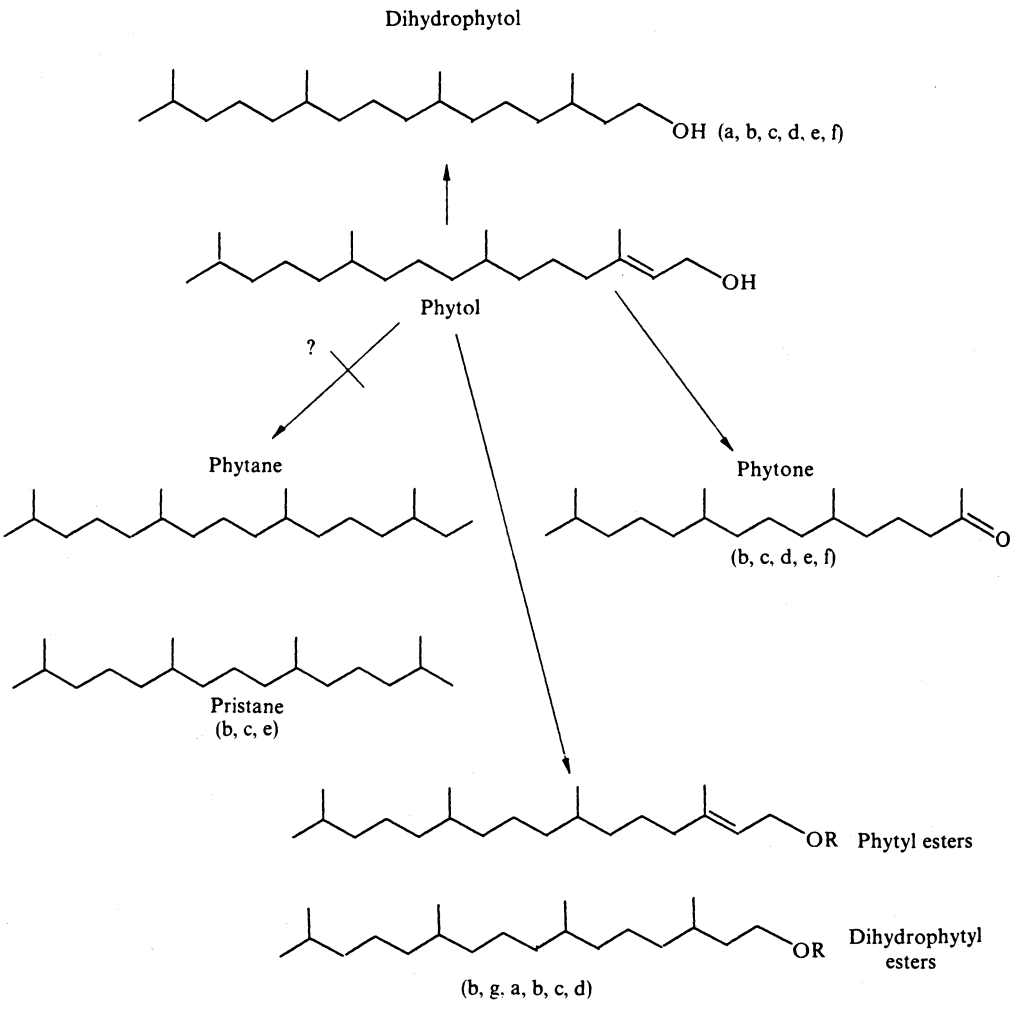


Figure 5. Short term fate of phytol (U-^{14}C) in a freshwater sediment.[73] Labelled (U-^{14}C) phytol (*ca.* 20 mg; 7R, 11R; from *Chlorella*) was dispersed in a solution of Tween detergent and injected into a sealed core (~30 cm) taken from Esthwaite. The solution was injected through a septum at about the 10 cm level and the core kept in the dark at ~18°C for 14 days. The products were isolated by extraction with heptane–isopropanol mixture. No activity was released from, or remained in, the sediment after subsequent acid hydrolysis. (yields based on ^{14}C).

Letters in parentheses indicate method of analysis: (a) acetylation; (b) Ag^+/SiO_2 (10%) T.L.C., plus radio-scan; (c) Capillary G.L.C. (B.D.S. column—30×10^3 theoretical plates) coinjection; (d) Liquid scintillation counting of an aliquot; (e) Radio GLC; (f) GC–MS; and (g) Hydrolysis.

compounds, which in turn will undergo further change until the sediment is compacted and biological action ceases. Beyond this point thermal alteration processes may result in the formation of pristane, phytane, etc., from

kerogen-bound isoprenoid side chains and other isoprenoid systems present in the sediment.

Short term fate of cholesterol in Severn Estuary sediment and sewage sludge[14, 69, 79]

In these laboratory experiments (*Table 8*), 4-^{14}C-cholesterol in Tween 80 suspension has been subjected to the action of Severn Estuary mud and of sewage sludge, both under anaerobic conditions. Cholesterol is only very slowly utilized by the microorganisms in the sediments as compared with the rapid rate of utilization of oleic acid and the intermediate rate of utilization

Table 8. Cholesterol—short term fate.[14, 69]

Incubation media	Temp, °C	Time days	Products			
			^{14}C lipid recovery* %	Sterols† %	Ketones† %	Polar products.† %
Severn sediment	20	90	70	90	5	5
Sewage sludge	38	30	15	90‡	10	n.d.

* As percentage of introduced radiolabel (no hydrolysis step in extraction): † as percentage of labelled lipid yield; ‡ of which 10% is *cholestanol*; n.d. not detected.

of phytol. Even sewage sludge at 38°C for 30 days affords as much as 15 per cent recovery of ^{14}C as sterol. The remainder presumably is degraded to CO_2. This work indicates two things: firstly, that unsaturated sterols are likely to be incorporated into forming sediments, and secondly, that the period of consolidation when microbiological activity is possible will result in substantial conversion into other products. These products appear to include ketones and more polar compounds which may be intermediates in the formation of the stanols. Some stanols were identified in the reaction mixture from the sewage sludge incubation. Diagenesis might be expected to follow such a pathway since it is known for certain biological systems. Thus, Bjorkhem and Gustafsson[17] showed that cholest-4-en-3-one is an intermediate in the conversion of cholesterol to cholestanol in rat intestine. Observations of saturated and unsaturated sterols in both Recent and consolidated ancient sediments are hence explicable in terms of differing extents of reduction in the sediment, reflecting the different conditions and length of time prior to consolidation.

Thermal alteration experiments have so far used cholesterol as precursor[69, 79–81]; these studies should now be applied to the degradation products discussed above. The thermal alteration studies that we have made at 200°C for various times (90 to 1000 hours) show that the products which appear to be intermediates between cholesterol and the cholestanes may be cholestenones, cholestenes and cholestadienes. Igneous intrusions in sedimentary formations are not rare; for example, they are widespread in certain areas of Australia. Such intrusions may have raised the sediments to

temperatures of 200°C and higher. However, some simulations of thermal alteration of deeply-buried sediments will need to be carried out at lower temperatures.

Short term fate of DDT in Severn Estuary sediment and sewage sludge[69, 82–84]

Persistent pollutants and their environmental transformation products now entering sediments are, in effect, incipient chemical fossils. What is incorporated into the sediments will depend on the particular environment, the compounds released into the environment and the pathways by which they reach the sediment. Pollution research should include studies of the fate of pollutants and their degradation products in sediments. Furthermore, the reactions revealed in such studies will be of general value in organic geochemistry. Recent developments have included laboratory studies of the fate of pesticides in self-contained model ecosystems[65–67]. DDT is now known to degrade differently under aerobic conditions (dehydrochlorination to DDE: a higher organism pathway) and anaerobic conditions (reductive dechlorination to give DDD: a microorganism pathway).

Table 9 summarizes our results employing radiolabelled DDT in Severn Estuary mud and in sewage sludge, incubated in the laboratory. It is not yet clear whether these reactions are the direct result of metabolism by the

Table 9. Short term fate of *p,p'*-DDT (U-^{14}C phenyl) in Severn Estuary sediment and sewage sludge.[82, 84]

Conditions of incubation	^{14}C recovered	Components of extracted material (as % of total)				
	In extract %	DDT	TDE(DDD)	DDCN	DDMS	DBP
Sewage (anaerobic), laboratory, 37°C, 80 days	32	tr	29	70	tr	tr
Severn sediment, laboratory, 20°C, 21 days	88	48	52	nd	nd	nd
Severn sediment, *in situ*, 5–20°C, 46 days	51	93	7	nd	nd	nd

Extraction with boiling acetone and analysis by EC-GLC: tr Trace (< 1.0 %); nd not detected. Compounds: *p,p'*-DDT, $R_2CH.CCl_3$ (XIV); TDE, $R_2CH.CHCl_2$; DDE, $R_2C{:}CCl_2$; DDCN, $R_2CH.CN$; DDMS, $R_2CH.CH_2Cl$; DBP, $R_2C{:}O$; where $R = ClC_6H_4$.

microorganisms, enzyme reactions, or whether they are chemical transformations brought about under the reducing conditions resulting from the actions of the microorganisms. The new degradation product, DDCN, was discovered in the course of this work: the same compound has also been identified by Dr Sören Jensen and his collaborators at Stockholm[85]. The sewage sludge again appears to be more active than the Severn Estuary sediment, although the conditions employed are not directly comparable. Muds can be recycled by organisms and by normal weathering processes. Hence, they may act as both pollutant banks and pollutant sinks, continually or intermittently incorporating and releasing pollutants and their metabolites and degradation products.

CONCLUSIONS

The work described herein indicates that the origin of chemical fossils may be complex, with several pathways leading to any single compound. Some of the points which have emerged may be listed as follows

(a) Contemporary aquatic environments can vary widely in regard to the balance of the organic matter contributed to the forming sediment. Paleo-ecological factors must have been similarly important in determining the composition of ancient sediments.

(b) Algae, especially blue-green algae such as *Lyngbya* and *Nostoc* and phytoplankton in general, often contribute extensively to lake sediments as indicated by visual observations and by their content of n-C_{17} and other alkanes.

(c) Bacteria and other microorganisms may greatly affect the organic content of sediments and probably give rise to much of the geolipid fraction extracted from Recent sediments. Thus, sterols and triterpenoids, once thought characteristic of animals and higher plants, are present in certain bacteria. Abnormal rather smooth distributions (Carbon Preference Index

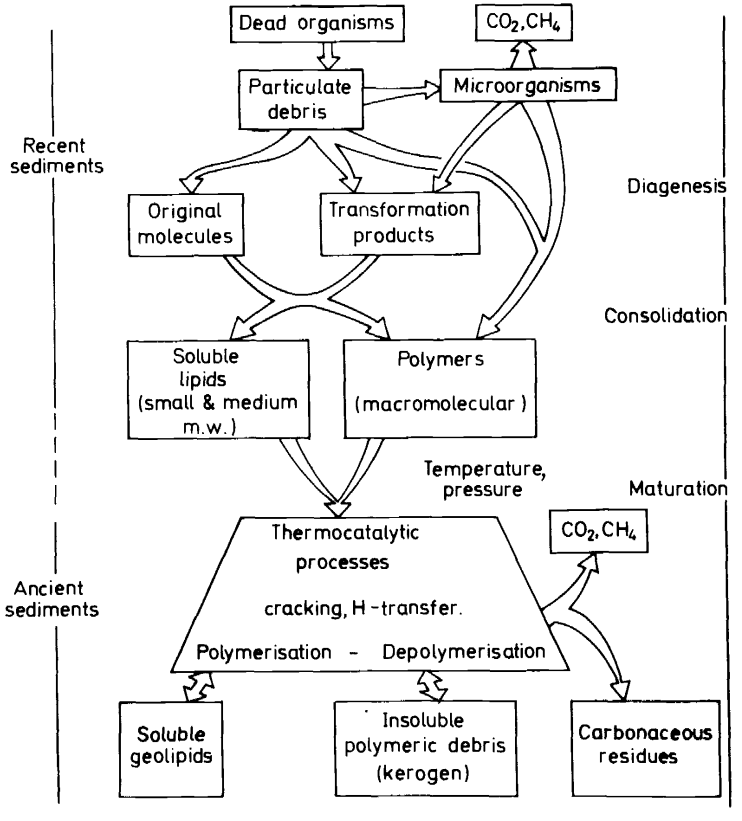


Figure 6. Outline scheme for the fate of carbon compounds in the geosphere.

of near unity) of n-alkanes have been tentatively ascribed to bacterial metabolism.

(d) Reduction, such as the reduction of sterols to stanols, occurs rapidly in Recent sediments but is usually incomplete prior to consolidation. Microorganisms are very likely important in effecting this and other reactions.

(e) Oxygenated and unsaturated compounds can survive long periods of geological time as components of sediments and fossils. Numerous 'finds' include steroids, triterpenoids and related compounds, saturated fatty acids and alcohols. Sometimes geolipid fractions contain a series of structurally related compounds such as the 4-methyl stanols, -stanones, and -steranes of the Messel oil shale (Eocene)[86, 87]. Such series are particularly informative in being very probably related biogenetically and geogenetically. Laboratory thermal alteration, including reduction and H-transfer, does occur but is slow.

Figure 6 links some of the points into an overall framework. The formation of chemical fossils is presented in a dualistic fashion: major changes can be effected at low temperatures in the young sediment, at elevated temperatures in deeply-buried ancient sediments or in both ways. Thus, dead organisms contribute debris which may contain original compounds, transformation products resulting from the action of microorganisms in the particular chemical environment, or both. Unchanged or changed compounds or both are contributed to the soluble lipid fraction and to the polymeric debris within the sediment. The thermal maturation processes, which would be undergone by a deeply-buried sediment or one subject to temperature-increases resulting from igneous intrusions, can in turn generate soluble geolipids and also insoluble debris. The debris becomes increasingly carbonaceous as the process is continued. Hence, the soluble geolipids (chemical fossils) may represent unchanged compounds or compounds which are the result of one or more of the various steps indicated in the diagram.

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