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The structure of kerogen and related materials. A review of recent progress and future trends

JÜRGEN RULLKÖTTER[1] and WALTER MICHAELIS[2]

[1]Institut für Erdöl und Organische Geochemie, Forschungszentrum Jülich GmbH (KFA), Postfach 19 13, D-5170 Jülich 1, F.R.G.

[2]Geologisch-Paläontologisches Institut und Museum, Universität Hamburg, Bundesstraße 55, D-2000 Hamburg 13, F.R.G.

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Abstract—Unravelling the chemical constitution of kerogen, asphaltenes, coal and humic substances is the most challenging objective in molecular organic geochemistry. Some compositional constraints are obtained from elemental analysis and from the determination of functional groups or the degree of aromaticity by IR or NMR spectroscopy. Pyrolysis of kerogen or related materials yields small structural units, some of which may be representative of moieties originally present in the macromolecules whereas others may have been formed by secondary reactions. In any case, there is no information on the mode of connection of the various structural units among each other.

Chemical degradation of kerogen and related materials so far commonly involved the application of strongly oxidizing reagents (e.g. $KMnO_4$), but also was done by reductive cleavage (hydrogenolysis). Although a variety of methods has been used over the years, much of the work lacked adequate detailed analysis of the reaction products and/or the reactions were not sufficiently specific to allow a reconstruction of larger structural entities. Nevertheless, a combination of elemental analysis, spectroscopic information and chemical data from pyrolysis, bitumen composition in natural rocks and sometimes chemical degradation were used to develop constitutional models of kerogens or asphaltenes of different origins and different levels of thermal evolution. The models at least were internally consistent with respect to the data set used, and although a fair amount of "chemophantasy" may have been incorporated, they certainly stimulated further work.

Recently, specific chemical degradation reactions like ether cleavage with boron trichloride followed by lithium aluminium deuteride reduction of the halides or oxidation with ruthenium tetroxide have shown how certain low-molecular-weight products are linked to kerogen or asphaltene macromolecules. Further reactions of this type will have to be looked for.

Any attempt to achieve substantial progress in kerogen structure elucidation will require the concerted efforts of (1) consecutive specific chemical degradation reactions, (2) the detailed quantitative molecular analysis of small degradation products, (3) a study of the degradation residues by spectroscopy and pyrolysis, and (4) the application of refined concepts of the preservation of biological macromolecules (e.g. aliphatic biopolymers).

Key words—aliphatic biopolymers, chemical degradation methods, humic substances, IR spectroscopy, kerogen, macromolecular organic matter, NMR spectroscopy

INTRODUCTION

With geological time being one of those key parameters in natural evolution on earth, which impose serious restrictions on the simulation of geological processes in the laboratory under controlled experimental conditions, research in geosciences over a long period of time has essentially been limited to descriptive and empirical approaches based on field observations. Only in the last few decades have there been strong tendencies to a more fundamental and quantitative assessment of geological facts, processes and materials.

Kerogen, although recognized as the quantitatively most important type of fossil organic matter a long time ago, has since been mostly dealt with in a descriptive or bulk geochemical manner by organic petrographers and petroleum geochemists. This was sufficient, however, to stimulate progress in the understanding of many of the gross factors influencing the accumulation and transformation of organic matter in the geosphere (Hunt, 1979; Tissot and Welte, 1984). In particular, it was helpful for the application of organic geochemical techniques in petroleum exploration. A true integration of organic geochemistry in modern petroleum exploration strategies aiming at an understanding of generation, migration and accumulation of hydrocarbons as interrelated processes on a geological time scale, however, requires a more fundamental knowledge and the development of quantitative methods (Tissot *et al.*, 1987).

Kerogen and related macromolecular fossil organic materials, despite having been a subject of investigation for a long time, have until today largely resisted a detailed elucidation of their chemical structures in molecular terms. The heterogeneous nature

of these materials, the lack of a detailed knowledge of the structures of biopolymers with a lipid-like backbone and their distribution in the biosphere are among the most important reasons which have impeded structure elucidation of kerogen and related materials.

The efforts in this direction until the end of the 1970s have been comprehensively compiled by Durand (1980a). It is the purpose of this review to summarize the subsequent developments and to describe the trends which may lead to further progress in the near future. A full coverage of all kerogen structure work, particularly that using the various pyrolysis techniques, is not intended. Emphasis is put rather on conceptual aspects of structure elucidation of macromolecular fossil organic matter.

DEFINITION

The most widely used definition of kerogen, according to Durand (1980b), is "the fraction of organic matter dispersed in sediments which is insoluble in organic solvents". Because in organic geochemistry a variety of solvents with a range of polarities is used which yield different amounts of extractable material (and thus leave behind different quantities of undissolved residue), this definition of kerogen—like all the others (Durand, 1980b)—is purely operational.

In Fig. 1 we have tried to use estimated variations in molecular size and increasing geochemical evolution, without giving numerical values, to illustrate the relationship between kerogen and other, mostly macromolecular types of fossil organic materials. The biomass of decayed organisms, after an initial stage of (partial) degradation during very early diagenesis, may form protokerogen or humic substances as intermediates on the way to kerogen and coal, dependent on the type of precursor material. Bitumen to a minor extent is inherited directly from the biomass, whereas the main portion is generated from kerogen during diagenesis under the influence of thermal stress. Asphaltenes are considered intermediates between kerogen and bitumen, i.e. they are entities soluble in polar organic solvents but structurally closely related to kerogen (Béhar and Pelet, 1985; Pelet *et al.*, 1986).

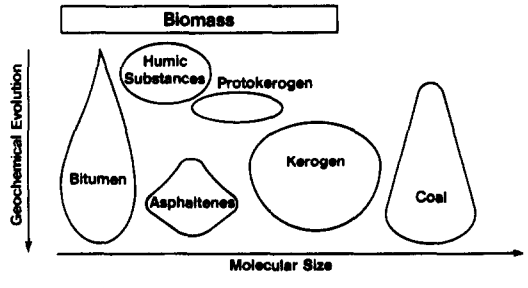


Fig. 1. Arbitrary relationships between biomass and various types of fossil organic matter as a function of molecular size and geochemical evolution.

In structural terms, kerogen until recently was commonly considered a heterogeneous random geopolymer (Fig. 2) which has been formed from highly organized biopolymers through enzymatic degradation by microorganisms and the formation of individual biomonomers, followed by a random polymerization and condensation of the preserved portion of these monomers (views of numerous authors summarized by Tissot and Welte, 1984). While this pathway mainly comprises the easily hydrolyzable biopolymers, e.g. proteins and polysaccharides, lipids are thought to be also incorporated into kerogen or its macromolecular precursors (humic substances, i.e. fulvic and humic acids, humin) at various stages of diagenesis. A significant proportion of the lipids, according to this view, survives as free bitumen constituents and gives rise to the group of compounds called geochemical fossils or biological markers (Fig. 2).

STRUCTURAL MODELS OF KEROGEN

Molecular organic geochemical studies commonly concentrate on the constituents of source rock extracts (bitumens) and crude oils with a bias to the hydrocarbon fractions of these soluble organic phases. Thus, it is not surprising that structural models of kerogens developed during the last twenty years are conceptually based on the results of these analyses (Vandenbroucke, 1980) and complement those of oxidative degradation of kerogen and of bulk physical and chemical analyses. They represent an attempt at randomly recombining the low-molecular-weight compounds to a macromolecular network with unidentified "residual kerogen" or polycyclic hydrocarbon moieties as central building blocks.

The Green River Oil Shale kerogen has been the target of most of these attempts. Older, very simplistic approaches, e.g. the models of Burlingame *et al.* (1969), Djuricic *et al.* (1971) and Schmidt-Collerus and Prien (1974) were jointly discussed by Yen (1976). Slightly more detailed is the model of Yen (1974) shown in Fig. 3. It is based on stepwise permanganate oxidation of a kerogen concentrate and spectroscopic analyses of the degradation products and of the precursor material. According to this model, Green River Oil Shale kerogen is essentually free of aromatic units. Major components are isoprenoids, steroids, terpenoids, carotenoids and other naphthenic clusters (mostly with 3–4 rings) held together by various kinds of bridges with the outer part of the kerogen containing more crosslinking than the central core. Long-chain alkane substituents (bridges) range from 17 to 31 carbon atoms in length, but free-end and flexible longer-chain polymethylene structures are said to be absent. Yen (1974) describes the kerogen matrix to be a multipolymer forming a heterogeneous, three-dimensional network. "Monomers" are difunctional bridges and multifunctional nuclei, which themselves may also be present

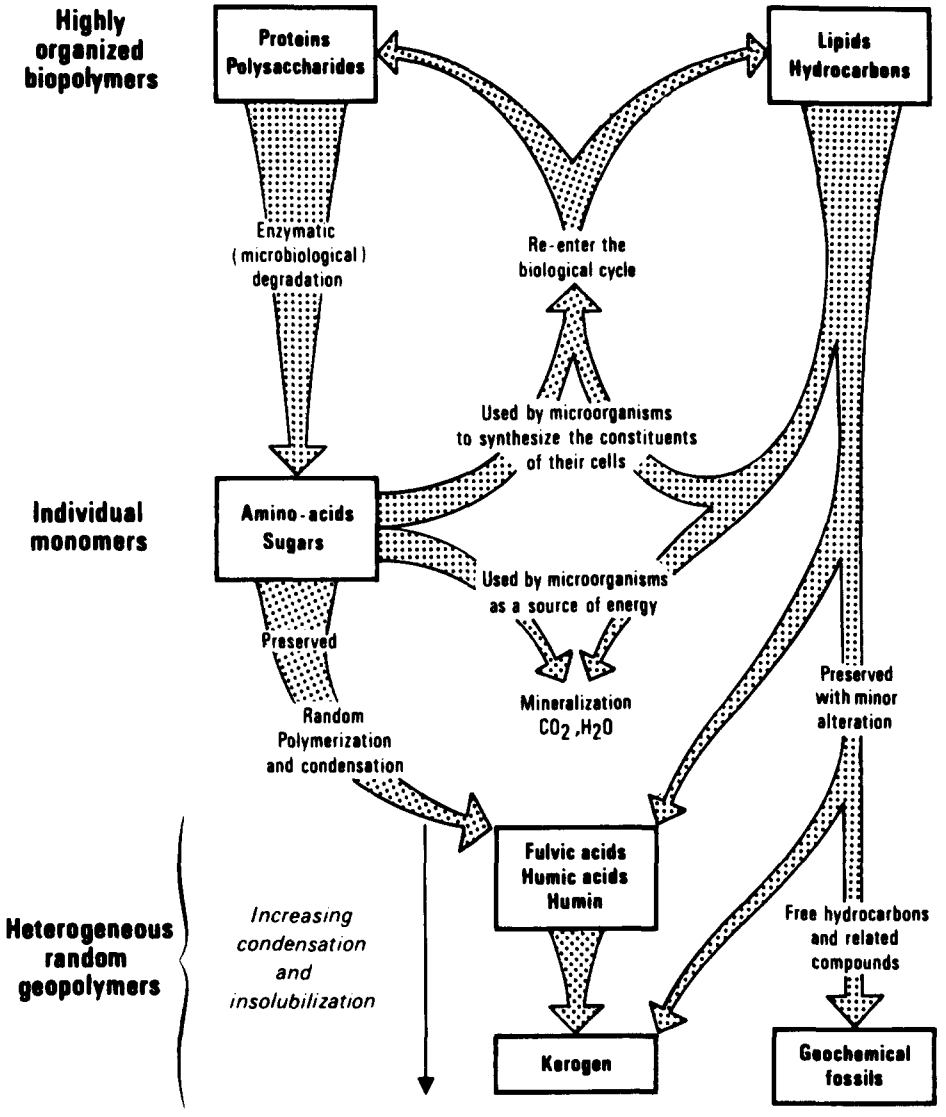


Fig. 2. From biomass to kerogen—a summary of the conventional view of processes involved in the transformation of biological organic matter to kerogen and geochemical fossils (from Tissot and Welte, 1984).

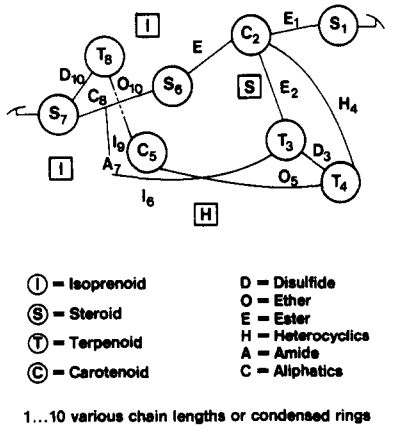


Fig. 3. Schematic structural model of Green River Oil Shale kerogen based on a series of nuclei (circles) crosslinked with bridges (lines) and trapped low-molecular-weight molecules (squares). Adopted from Yen (1974).

in the kerogen as trapped or 'loosely-held" components (Fig. 3). A more recent study claims to have confirmed this model with the only major difference that the presence of a significant proportion of aromatic material was indicated (McGowan *et al.*, 1985).

A more sophisticated chemical modelling of kerogen structure was performed by Béhar and Vandenbroucke (1986, 1987). In a statistical approach they have drawn models of different kerogen types (Tissot *et al.*, 1974) at different stages of their thermal evolution, i.e. beginning of diagenesis, beginning of catagenesis and end of catagenesis. The Green River Oil Shale (Type I), the Toarcian shales of the Paris Basin (Type II) and the deltaic mudstones of the Douala Basin and the Mahakam Delta (Type III) were chosen to be typical representatives of the kerogen types modelled. Basic parameters for the model design included elemental analysis (H/C and O/C values), aromaticity from ^{13}C nuclear magnetic

resonance (NMR) spectroscopy, and relative content of naphthenic and paraffinic carbon atoms from mass spectrometric ring number analysis of the extractable saturated hydrocarbons (Hood and O'Neal, 1959) because neither infrared (IR) nor NMR spectroscopy provide a measure of this ratio. The average size of polyaromatic nuclei was based on data obtained by electron microscopy of natural kerogens (Oberlin *et al.*, 1980). The distribution of functional groups was adopted from literature data on humic acids (Schnitzer and Khan, 1978), kerogens (Vitorovic, 1980) and coals (van Krevelen, 1961) and from a knowledge of the heteroatomic and molecular geochemical evolution of organic matter (summarized by Tissot and Welte, 1984, Chap. II-1–II-5). For the incorporation of biological marker units, the results of chemical and thermal degradation studies were used (see references in Béhar and Vandenbroucke, 1987). In order to be able to represent properly different types of structural units and to compare the different kerogen types with each other throughout their thermal evolution, the kerogen models were based on an initial carbon atom number of about 1500 in each case, giving rise to an initial molecular weight of the "representative unit" in excess of 20,000.

Figure 4 shows the result of this modelling approach for Type II kerogen at the stages of early diagenesis and late catagenesis. At the beginning of diagenesis and catagenesis, Type II kerogen is thought to be mainly formed from polycyclic structures with oxygenated functional groups (phenols, quinones, aromatic acids, etc.). Linear chains with about 30 carbon atoms represent former constituents of land plant cuticular waxes. Bulk changes during thermal evolution include the decrease in H/C and O/C atomic ratios as well as a reduction in molecular weight due to the loss of hydrocarbons and functionalized molecules (organic and inorganic). The product at the late stage of catagenesis is highly aromatic with the single aromatic units not exceeding 10 rings. This adheres to the results of Oberlin *et al.* (1980) who found this size of aromatic units in their electron microscopy studies using lattice fringe techniques. Such an approach has so far provided the only direct access to the chemical structure of kerogen, although applicable only at high degrees of coalification. Béhar and Vandenbroucke (1986, 1987) tried to incorporate some of the orientation of aromatic units found by Oberlin *et al.* (1980) into their late catagenetic stage model [Fig. 4(b)].

Béhar and Vandenbroucke (1986, 1987) pointed out that, although internally consistent with respect to bulk data, much of the chemical representation is only of statistical value. While the bonding sites of some of the small units, e.g. steroids, triterpenoids, acids, are fairly well known or can relatively safely be inferred, this is not true for the major portion of the structural moieties. This is particularly true for the distribution of functional groups on aromatic domains and the position of side chains. Also, bond types other than covalent were not taken into consideration. Finally, with the limitations imposed by the initial molecular weight, the possible incorporation of some specific high-molecular-weight biostructures into kerogen (cuticles, membranes, etc.) had to be disregarded, but was admitted to induce some additional heterogeneity in the actual kerogen structure.

Other than the more schematic kerogen model of Yen (1974) which illustrates lack of knowledge more than knowledge (Fig. 3), the very detailed and precise chemical representation of the kerogen modelling of Béhar and Vandenbroucke (1986, 1987) to those who are not aware of the assumptions and limitations suggest that the mystery of the chemical structure of kerogen has been solved. This has created a controversy from those who considered this chemical model (Fig. 4) pure "chemophantasy" and thus conceptually misleading, to others, including the authors of this review, who found this a stimulus for further in-depth studies of the chemical structure of kerogen.

THE IMPETUS FOR ADVANCED KEROGEN STUDIES

A petroleum geochemist involved in practical application of geochemical concepts to the exploration for oil and gas may argue that sophisticated chemical structural models of kerogen are far beyond the requirements for his daily work. Indeed, much simpler tools and concepts have been successfully applied to assess the hydrocarbon potential of organic matter in a source rock (e.g. Rock-Eval pyrolysis; Espitalié *et al.*, 1985) or to distinguish between different source rock types in terms of their hydrocarbon potential as a consequence of their depositional environments, i.e. their organofacies (Jones, 1987; Horsfield, 1989). Although this will hold true also in the future, there are a number of reasons which justify or even demand further advanced studies of the chemical structures of fossil macromolecules.

(1) While a geoscientist may consider the structural models of kerogen presented by Béhar and Vandenbroucke (1986, 1987) the unnecessarily sophisticated but logical result of a number of exact measurements, a chemist may be horrified by the recklessness of combining selected molecular and bulk information from various sources into a random molecular formula with an apparent accuracy, particularly of the cross-linking of the different small moieties, which is not at all supported by the analytical data. Thus, professional ambition as well as scientific curiosity is one of the obvious motivations for organic geochemists to face the challenge of unravelling the chemical structure of kerogen.

(2) In petroleum geochemistry applied to exploration, modelling of hydrocarbon generation in sedimentary basins has been developed as a tool to study the geological and chemical

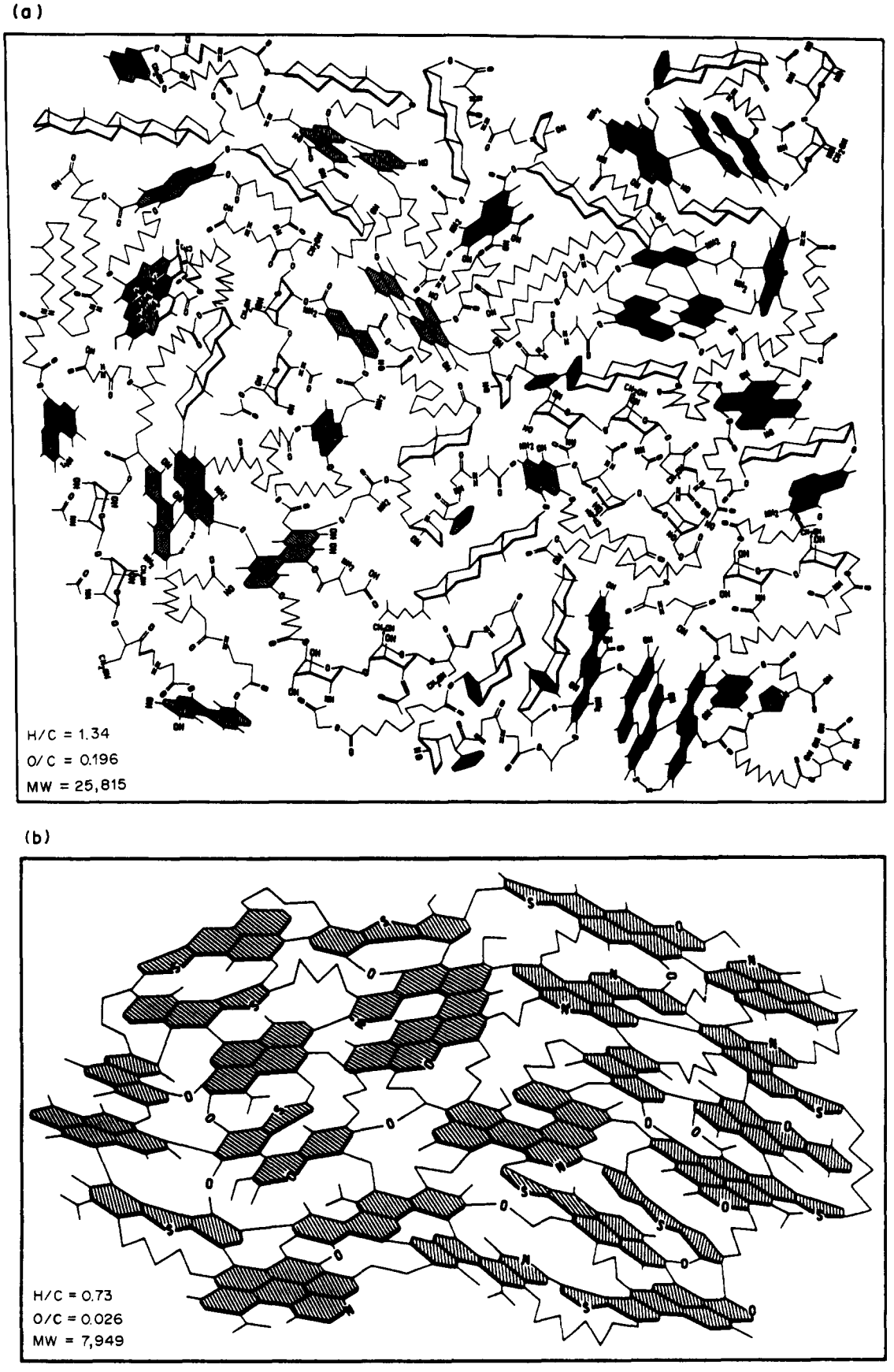


Fig. 4. Structural model of kerogen, based on a multitude of bulk and molecular organic geochemical analyses, for type-II organic matter at the stages of (a) early diagenesis and (b) late catagenesis. Adopted from Béhar and Vandenbroucke (1986).

processes involved, in a dynamic way, as a function of geological time (e.g. Welte and Yükler, 1981; Welte and Yalcin, 1988) in order to define more precisely future exploration targets. Besides modelling the geological system itself, as well as primary and secondary migration, calculation of the timing of hydrocarbon generation and of the quantities and composition of the products is one of the key steps in this integrated modelling approach. For this, the so-called "oil window" concept was insufficient but instead chemical reaction kinetics had to be used (Tissot and Espitalié, 1975). However, the validity of theoretically derived activation energy distributions, as well as experimentally determined "macrokinetic" activation energies and frequency factors for bulk oil generation and cracking to gas, is still highly debated (e.g. Burnham *et al.*, 1988; Espitalié *et al.*, 1988; Ungerer and Pelet, 1987; Ungerer *et al.*, 1987). A better knowledge of the chemical structure of kerogen would allow us to put constraints on the kinetic data for its conversion into petroleum hydrocarbons.

(3) Petroleum production is a domain of engineers and their mostly empirical methods. Reservoir organic geochemistry is a relatively young discipline and one of its objectives is to study the interaction between the mineral matrix of reservoir rocks and accumulated petroleum in order to understand better inhomogeneities in the distribution of the reservoir fluids and thus to improve possibly production strategies. A knowledge of the chemical structures of the high-molecular-weight (polar) constituents of petroleum (asphaltenes) may help to identify the surface-active components in petroleum or their relevant functional groups.

(4) It is well known that the use of pesticides in agriculture can cause environmental problems. Therefore, governmental authorities have become more restrictive in permitting new products to be introduced. Even for older products they now request additional experimental evidence that these can be used without the danger of intoxication of agricultural products, groundwater etc. A particular question in this context is that of the ultimate fate of pesticides and their metabolites. The application of radioactively marked pesticides has shown that in many cases a significant portion becomes "irreversibly" bound to soil organic matter (humic substances). The precise chemical structure of humic substances being unknown makes it difficult to say how the pesticide or its metabolite is bound to it and if and how they may eventually be remobilized. The same is true for the binding of toxic metals, both from anthropogenic sources and eroded natural rocks, to soil organic matter and their bioavailability.

METHODS OF KEROGEN STRUCTURE ELUCIDATION

Hierarchy of methods

Organic geochemistry uses a multitude of techniques to characterize kerogen and related materials and to derive structural information from these measurements. Unfortunately, a broad combination of these methods is scarcely used, an early and educative example being the study of Allan *et al.* (1980) on alginite from Permo-Carboniferous torbanites. Modern developments in instrumental analysis has revived the interest in kerogen structure elucidation from time to time. Each time, there was some hope that the new technique which became available would definitely solve the problem. With respect to the type of information, i.e. the extent of structural detail provided, the different methods can be grouped in a hierarchial order (Fig. 5).

Kerogen microscopy (Stach *et al.*, 1982; Teichmüller, 1986) describes morphological features of fossil remnants of biological organisms in transmitted and reflected light observations. In principle, conceptual chemical information can indirectly be derived from these observations by relating the information about individual species or classes of organisms morphologically preserved in sediments to what is known about the natural product chemistry of structural cell constituents of extant analogs (see later discussion of highly aliphatic biopolymers).

Elemental analysis provides a measure of the bulk atomic composition of the major elements present in macromolecular fossil organic matter (McIver, 1967; Durand and Monin, 1980). Besides carbon and hydrogen, the dominant elements, total nitrogen and sulfur concentrations can reliably be determined, whereas the direct measurement of oxygen contents is somewhat more difficult. Among the metals present, iron is often determined as one of the ways to assess the amount of inorganic sulfur in kerogen concentrates under the assumption that essentially all iron is bound in the form of pyrite (cf. Orr, 1986). Residual mineral contents in isolated kerogens often limit the accuracy of elemental analysis (Durand and Monin, 1980). Although the results of elemental analysis are widely used for kerogen typing (e.g. Tissot *et al.*,

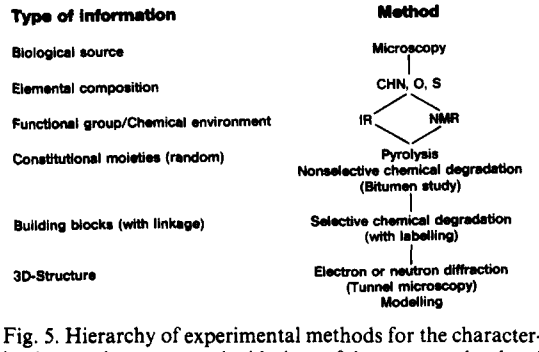


Fig. 5. Hierarchy of experimental methods for the characterization and structure elucidation of kerogen and related materials indicating the type of information obtained.

1974) they do not provide direct access to their chemical structures. Nevertheless, in combination with other data they are mandatory for any attempts of elemental mass balance, e.g. in the case of kerogen structure modelling.

Infrared and nuclear magnetic resonance spectroscopy, together with mass spectrometry, are the most common instrumental methods in chemical structure analysis. They provide information on the presence of functional groups (particularly IR spectroscopy) or on the chemical environment of carbon or hydrogen atoms (particularly NMR spectroscopy). This will be described in more detail in the following section. The data obtained are, of course, averaged over the entire material analysed, and the resolution is insufficient to separate all possible functional groups in their specific environments. In addition, it is difficult to quantitate the absorption or resonance signals, and so the data are often semiquantitative at best.

Microspectroscopy is a special case of the application of spectroscopic techniques to fossil organic material in that it aims at sufficient spatial resolution so that separate signals can be obtained for the different constituents of the maceral mixtures commonly present in kerogens. Spectral fluorescence microscopy after UV irradiation (van Gijzel, 1967; Teichmüller and Ottenjann, 1977), although in principle sensitive to the chemical nature of the material investigated, provides only very broad, unresolved signals due to the large number of chromophores in different chemical environments in kerogen. Only some empirical distinctions can be made for certain groups of macerals (e.g. Teichmüller and Ottenjann, 1977; Crelling, 1982; Senftle and Larter, 1988) without a chemical interpretation of the differences being possible. Thus, this technique today is mainly used to monitor bulk changes in fossil organic matter, e.g. as a function of thermal stress. As described in detail by Blob (1989), other microspectroscopy methods using Raman (Green *et al.*, 1983; Zerda *et al.*, 1981), infrared (Brenner, 1984) or near-infrared techniques (Fysh *et al.*, 1985; Blob *et al.*, 1988) also have strong technical difficulties and severe inherent limitations although some of them may have the potential to provide bulk chemical information like the proportion of aliphatic carbon atoms in single macerals (Blob *et al.*, 1988).

Pyrolysis and nonselective chemical degradation yield constitutional moieties of the macromolecular precursor material which to a certain extent will be representative of the initial chemical structure (see also later sections). The quality of the information depends on the type of pyrolysis or chemical degradation as well as the method of analysis of the products obtained. Important criteria are the extent of secondary reactions which may occur after pyrolysis or during chemical degradation and the proportion of detectable and identifiable products. In any case, the low-molecular-weight compounds produced are a random mixture and do not offer insight in the way these were crosslinked in the macromolecular organic matter. Bitumen studies for a long time were considered directly complementary to the methods described above, because it was assumed that the bitumen composition is a representative low-molecular-weight image of the corresponding macromolecular organic material in the same sediment. Apart from the fact that bitumen in a rock is mobile and thus is apt to depletion or enrichment (staining) effects, there meanwhile is solid evidence that diagenetic processes can be highly selective and lead to compositional fractionation between bitumen and kerogen. This has been clearly demonstrated for the quenching of labile functionalised lipids by inorganic sulfur species at the early stages of diagenesis (Sinninghe Damsté *et al.*, 1989b; de Leeuw and Sinninghe Damsté, 1990). It is also known that certain biological markers, e.g. 28,30-bisnorhopanes (Moldowan *et al.*, 1984; Noble *et al.*, 1985) and diasteranes (Seifert, 1978) are products of diagenetic reactions within the bitumen fraction and are not bound into kerogen. Furthermore, Eglinton and Douglas (1988) showed that the total amount of biomarkers in a sediment can be distributed between bitumen and kerogen in drastically different relative and absolute quantities.

Selective chemical degradation techniques deserve a higher rank in the methodological hierarchy of kerogen structure elucidation (Fig. 5). Liberation of building blocks of the macromolecular organic matter in this case is upgraded by information on the site(s) of their linkage. This is achieved either by the type of reaction itself or by the inherent possibility of performing labelling experiments. This will be discussed in more detail in a later section.

The three-dimensional structure determination may be considered the ultimate target of the investigation of kerogen and related materials. Direct access to this in principle is possible by electron, X-ray or neutron scattering and diffraction techniques. Apart from the electron diffraction work of Oberlin *et al.* (1980) showing the presence of clusters of aromatic units in high-rank fossil organic matter, there has been little practical application of these methods. If there is a good knowledge of the constitutional structure of kerogen, the 3D structure caused by noncovalent interaction may also be assessed by modelling programs such as those presently used to determine the 3D structures of enzymes and other polypeptides.

Spectroscopy

The use of a combination of spectroscopy techniques—in particular UV/VIS, IR, NMR and mass spectroscopy—today is the most common strategy for the identification of the chemical structures of organic compounds. Among these techniques, IR and NMR spectroscopy are those which can be most readily applied to kerogen analysis and at the same

time yield a broad range of information. For some time, there was the hope that it was just a matter of further instrument development and improvement of measurement techniques that the chemical structure of kerogen could be assessed by spectroscopy alone without the need of performing tedious chemical reactions of the study material. This was particularly true for solid-state ^{13}C-NMR spectroscopy with the potential application of highly sophisticated pulse techniques. There is a large number of publications on the use of ^{13}C-NMR spectroscopy for the analysis of chemical features of macromolecular fossil organic matter (a textbook summary of the technique and applications in geochemistry and soil chemistry has recently been published by Wilson, 1987). Admittedly, application of this technique has been beneficial in many cases, but realistically speaking, there has been deceivingly little progress with respect to structure elucidation considering the tremendous analytical effort. The main information still is that of gross molecular changes as a function of natural or artificial thermal stress.

IR spectra show a number of (relatively broad) absorption bands characteristic of different bond types and modes of vibration. Fossil organic solids all exhibit essentially the same bands with variations of their relative intensities being mainly due to differences in thermal history (e.g. Rouxhet *et al.*, 1980). The type of data obtained has virtually remained unchanged over the last fifteen years as shown in Figs 6(a) and 6(b). During a recent study of the artificial thermal alteration of a Japanese coal, Takeda and Asakawa (1988) recorded IR spectra at four different heating stages showing the removal or the change in relative abundance of different types of functional groups [Fig. 6(a)]. Essentially, aliphatic CH, CH_2 and CH_3 groups as well as carbonyl and hydroxyl groups are lost with increasing heating, whereas aromatic C=C and CH absorptions are less altered and preferentially survive at the highest temperature applied. Quite similar results had been obtained by Robin (1975) for kerogen samples which had experienced different extents of natural thermal stress with the difference (due to the different starting material) that the proportion of the organic CH band increased due to the neoformation of aromatic units during maturation. The data of fifteen years ago and today differ substantially neither in their quality nor in their type

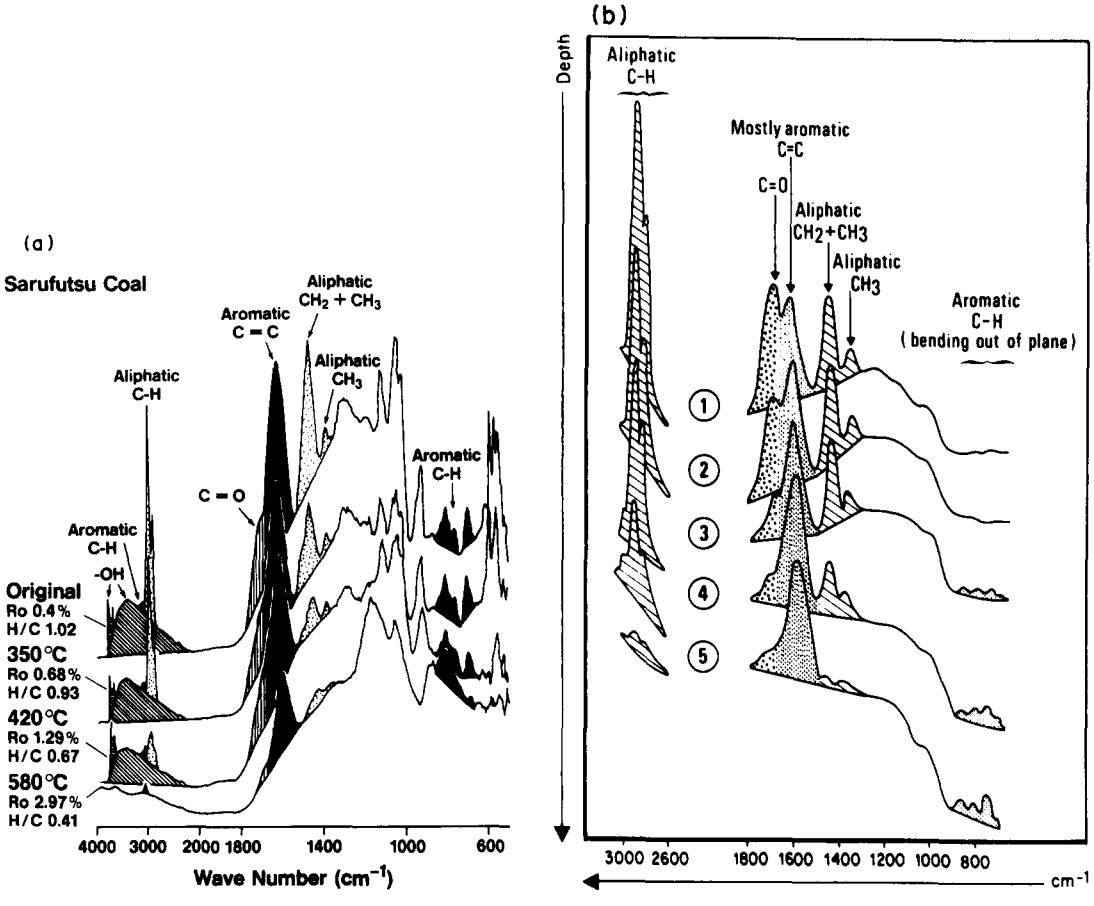


Fig. 6. Infrared spectroscopy in organic geochemistry. Examples show that the type and quality of information which can be obtained has virtually not changed over the last fifteen years. (a) Infrared spectra of Sarufutsu coal (Japan) before and after artificial maturation at different temperatures (after Takeda and Asakawa, 1988). (b) Infrared spectra of type-II kerogens at various levels of maturity (thermal stress increasing from 1 through 5) (from Tissot and Welte 1984; after Robin, 1975).

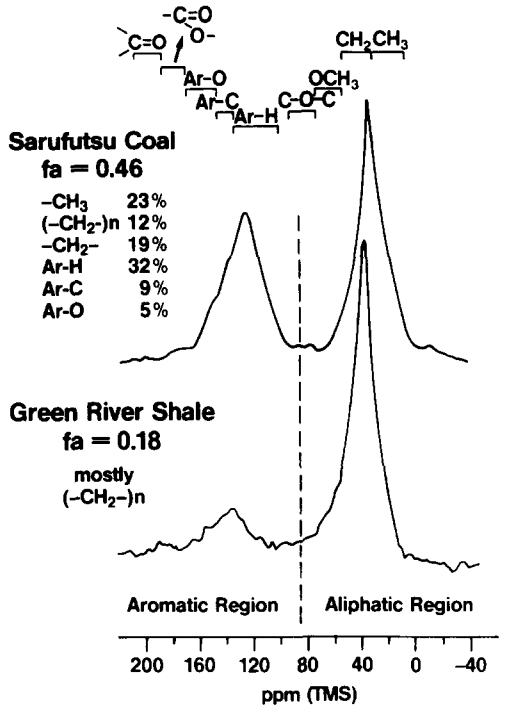


Fig. 7. Comparison of the solid-state ^{13}C-NMR spectra of Sarufutsu coal and Green River Shale (from Takeda and Asakawa, 1988).

of information. Modern infrared spectrometers linked to data systems allow, however, a somewhat better quantitation of the different absorption bands by deconvolution of partly overlapping bands (both from other organic matter absorptions and from those of residual carbonates or silicates) and by subtraction of the spectra of standards (Schenk *et al.*, 1986; Ganz and Kalkreuth, 1987, 1990). An example of the sophisticated use of modern Fourier transform IR spectroscopy for the rather explicit monitoring of chemical changes in (admittedly fairly homogeneous) fossil organic matter (Torbanites) as a function of thermal stress was given by Kister *et al.* (1990).

Typical solid-state ^{13}C-NMR spectra with cross polarization and magic angle spinning (CP/MAS) are shown in Fig. 7 which compares a coal sample to an oil shale (Takeda and Asakawa, 1988). Two broad signals in each spectrum are separated and said to represent an "aliphatic" and an "aromatic" region, respectively. Chemical shift ranges of more specific functional groups as derived from standard compounds of known structures are shown at the top of the figure. Commonly, the only information derived from such ^{13}C-NMR spectra is the aromaticity factor (f_a) from separate integration of the signals in the "aliphatic" and "aromatic" regions. As expected, this factor is higher in the coal than in the Green River Oil Shale. In this case (Fig. 7), Takeda and Asakawa (1988) even further subdivided the chemical shift ranges as indicated and calculated relative proportions of aliphatic CH_3, $(CH_2)_n$, and CH_2 moieties as well as various aromatic functional groups. This is highly artificial, however, because the chemical shift ranges of the functional group types indicated, in practice will overlap significantly and are not restricted to the adjacent ranges shown. Even the simple aromaticity (f_a) values may be erroneous particularly for organic matter of low maturity because the broad "aromatic" resonance signal may largely be caused by olefinic carbon atoms (Derenne *et al.*, 1987).

The chances of extracting more specific chemical information from ^{13}C-NMR spectra increase with increasing homogeneity of the organic solids (e.g. single source) and with decreasing extent of diagenetic evolution. In the course of an investigation of modern and fossil barks and plant residues, Wilson and Hatcher (1988) recorded the ^{13}C CP/MAS spectrum of Mangrove Lake buried wood from *Rhizophora mangle* (Fig. 8). Using the dipolar dephasing technique which is able to differentiate between carbon atoms bound to hydrogen atoms ("protonated carbon") and those not bound to hydrogen ("non-protonated carbon"), they detected a resonance signal among the "non-protonated" species (Fig. 8,

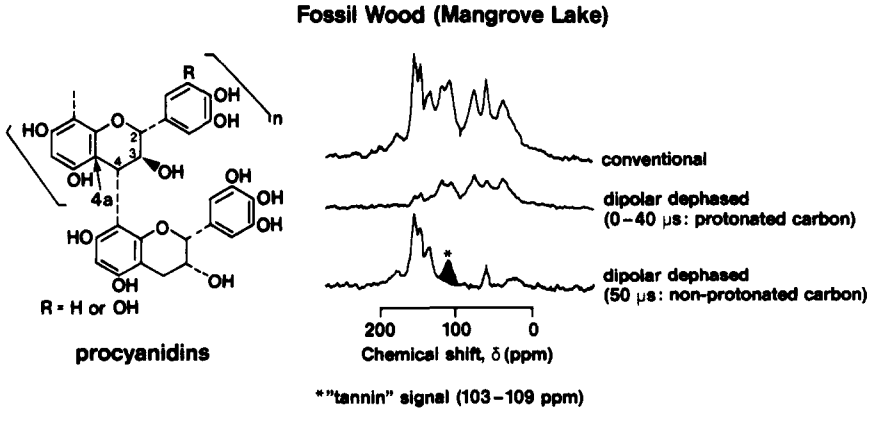


Fig. 8. Detection of a "tannin" signal in the dipolar dephased ^{13}C-NMR spectrum of fossil wood from Mangrove Lake (modified after Wilson and Hatcher, 1988).

lower trace) which is not that clearly visible in the conventional spectrum (Fig. 8, upper trace). Wilson and Hatcher (1988) assigned this signal to the non-protonated C-4a carbon atom in tannins (e.g. procyanidins) known to be present in recent barks. In this way, they claimed to have proven (a critical reader may prefer: have given some evidence) that tannins may survive in fossil wood to a certain extent. They have disproven, however, the former idea that the signal at 103–109 ppm often observed in soil samples is related to acetal groups of cellulose (e.g. Hatfield *et al.*, 1987) because these would show up in the "protonated-carbon" trace. In other ^{13}C-NMR studies, Hatcher (1987), Manders (1987) and Wilson (1987) have shown that this technique is useful for distinguishing guaiacyl and syringyl units in (fossil) woods.

Pyrolysis

Analytical pyrolysis is the simplest tool for the degradation of macromolecular fossil organic matter into smaller units and their subsequent on-line identification, commonly by gas chromatography, mass spectrometry or a combination of both. An up-to-date review of the use of analytical pyrolysis for the description of kerogen in terms of the quantitative distribution of various molecular subunits has been compiled by Larter and Horsfield (1990). Qualitative aspects have earlier been summarized by Meuzelaar *et al.* (1982), Philp (1982), Larter and Douglas (1982), Horsfield (1984) and Larter (1984).

Among the major identified compounds obtained by analytical pyrolysis of kerogen are saturated and unsaturated hydrocarbons (linear, branched and cyclic) with up to 35 carbon atoms, aromatic and naphthenoaromatic hydrocarbons with various sidechains and alkyl phenols (cf. Larter 1984; Horsfield, 1984; and references therein) as well as organosulfur compounds (Sinninghe Damsté *et al.*, 1989a). The formation of acyclic alkanes during pyrolysis requires hydrogen disproportionation between the products and the pyrolysis residue. The amount of this residue will vary, probably as a function of the hydrogen richness of the starting material. Also the residue will have constitutional features not present in the starting material. Likewise, the pyrolysis products to a certain extent may have undergone secondary reactions (e.g. rearrangements) which may lead to structural units not present in this form in the starting material. There are indications, however, that this effect is subordinate among the compounds registered in the detection unit coupled to the pyrolyzer, particularly if the pyrolysis products are removed relatively fast. This may not apply in the same way, however, to the polar high-molecular-weight material which often is the main pyrolysis product but has not been studied systematically. For a detailed discussion of the significance of the compound classes characterized so far in kerogen pyrolysates see Larter and Horsfield (1990).

Despite the fact that apart from the identified pyrolysis products (usually those clearly separated on the coupled GC column) there is a large amount of unidentified material consisting of chromatographically unresolved components, not GC-amenable tarry material and the pyrolysis residue, recent studies have shown that pyrolysis–GC may provide quantitatively relevant structural information at the molecular level for kerogens as a whole in certain cases (Horsfield, 1989). These also include immature humic kerogens which upon pyrolysis yield a variety of phenols substituted with oxygen-bearing functional groups related to diagenetically modified lignins (e.g. Saiz-Jimenez and de Leeuw, 1984; Hatcher *et al.*, 1988), typical Type-III kerogens whose pyrolysates are dominated by alkylphenols (Senftle *et al.*, 1986), and sulfur-rich kerogens yielding alkylthiophenes quantitatively related to the atomic S/C ratio of the starting material (Eglinton *et al.*, 1990).

Chemical degradation

Earlier attempts to degrade kerogen by chemical methods have been summarized by Vitorovic (1980). A compilation of reagents used to degrade humic substances, kerogen, coal, and asphaltenes in Fig. 9 includes more recent work, but most likely is not complete either. Oxidative, reductive and cleavage reactions can be differentiated with oxidation being the most widely applied but also most vigorous type of reaction.

Many of the reagents listed in Fig. 9 suffer from the restriction that they are not very specific and thus will neither provide an entirely correct representation of the structural units initially present in the kerogen (due to chemical modification) nor indicate the site and type of former bonding within the macromolecular network. Thus, increasing efforts have been made in recent years to find and apply specific chemical degradation and label the cleavage products in order to be able to reconstruct the type of bonding to the kerogen macromolecule. A number of such reactions are listed in Fig. 10.

The simplest and mildest reactions which can be applied to kerogen and related materials are base or acid hydrolysis of ester and amide bonds. The products will be free acids and alcohols in the first case and acids and amines in the second. These types of products at the same time represent an inherent labelling, i.e. it can be assumed with a high degree of confidence that former bonding was via acid, alcohol or amine groups, which ever part is released from the kerogen. The conjugate will remain bound to the kerogen residue unless it is released as a difunctional component after cleavage at a second site.

Treatment of kerogens with boron trichloride (BCl_3), boron tribromide (BBr_3) or trimethylsilyl iodide (TMSI) cleaves ether bonds (and esters if no prior hydrolysis was performed) and yields mono- or difunctionalized halogen derivatives in the case of mono- or diethers (Fig. 11). These are converted to

Method:	applied to:			
Oxidation	**Humic substances**	**Kerogen**	**Coal**	**Asphaltenes**
$KMnO_4/^-OH$	x	x	x	
HIO_4	x			
CuO	x	x		
NaOCl	x			
$C_6H_5NO_2$	x		x	
CH_3COOH/H_2O_2	x	x		
$K_2S_2O_8$		x		
$NaBO_2$		x		
RuO_4			x	
$Na_2Cr_2O_7/CH_3COOH$		x		
H_2O_2/CF_3COOH			x	
O_3		x		
$FeCl_3$-Ac_2O				x
NBS/O_2		x		
Reduction				
Zn	x			
Na amalgam	x			
H_2	x	x	x	x
$LiAlH_4$		x	x	
Na/NH_3			x	
$(C_6H_5)_3PBr_2$	x			
$Li/C_2H_5NH_2$				x
$K/C_{10}H_8$			x	x
Cleavage				
^-OH	x	x	x	x
H^+	x	x		
BX_3	x	x		
C_6H_5OH			x	
SMEAH		x		
TMSI	x	x	x	

Fig. 9. Summary of chemical degradation methods with indications to which types of fossil organic matter these have been applied (likely to be incomplete).

the corresponding hydrocarbons with lithium aluminium hydride ($LiAlH_4$), unless labelling is performed using the analogous deuteriated reagent ($LiAlD_4$). Two practical examples are illustrated in Fig. 12. Chappe *et al.* (1982) treated the polar lipids (resins and asphaltenes) of various sediments and petroleums with BCl_3 and obtained hydrocarbons obviously related to the glycerol tetraethers with head-to-head C_{40} isoprenoids characteristic of archaebacteria (methanogens). In the case of the Cariaco Trench deep sea sediment (Fig. 12, top) the acyclic C_{40} isoprenoid is the single most abundant component, but those with one or two cyclopentane rings (three isomers of the latter: 2,2′,2″) in the chain are prominent as well. That larger archaebacterial membrane units are preserved in kerogens had been shown earlier for the Messel oil shale by Michaelis and Albrecht (1979) and Chappe *et al.* (1980). Ethers in humic substances were cleaved with TMSI by Michaelis *et al.* (1989). From a lignite they obtained hopanes as major products, and among them 17β(H),21β(H)-pentakishomohopane (C_{35}) was the most abundant single compound (Fig. 12, bottom). Conversion of the primary iodides with $LiAlD_4$

Bond type	Method	Labelling
Ester	base hydrolysis: OH^-, crown ethers TMSI	-COOH, -OH
Amide	acid hydrolysis	-COOH, $-NH_2$
Ether	BCl_3, BBr_3, TMSI/$LiAlH_4$	$LiAlD_4$
Sulfur	Raney Ni/H_2	(D_2)
Ar-C-	RuO_4	-COOH
-C=C-	O_3	-COOH
Ar-O-C (Lignins)	hydrogenolysis (Rh-C/H_2), SMEAH	D_2

Fig. 10. Compilation of most important specific chemical degradation methods for kerogen and related fossil materials indicating the bond types attacked, the reagents used and the labelling options (inherent or optional) for the determination of bonding sites.

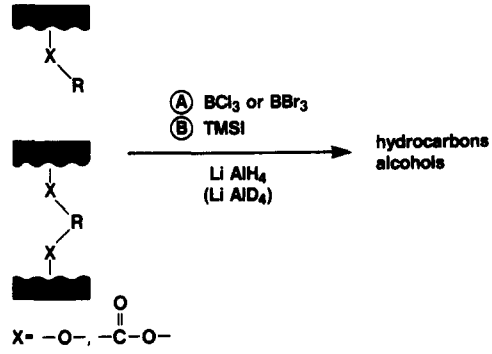


Fig. 11. Schematic representation of ether or ester cleavage by boron halides or trimethylsilyl iodide followed by reduction.

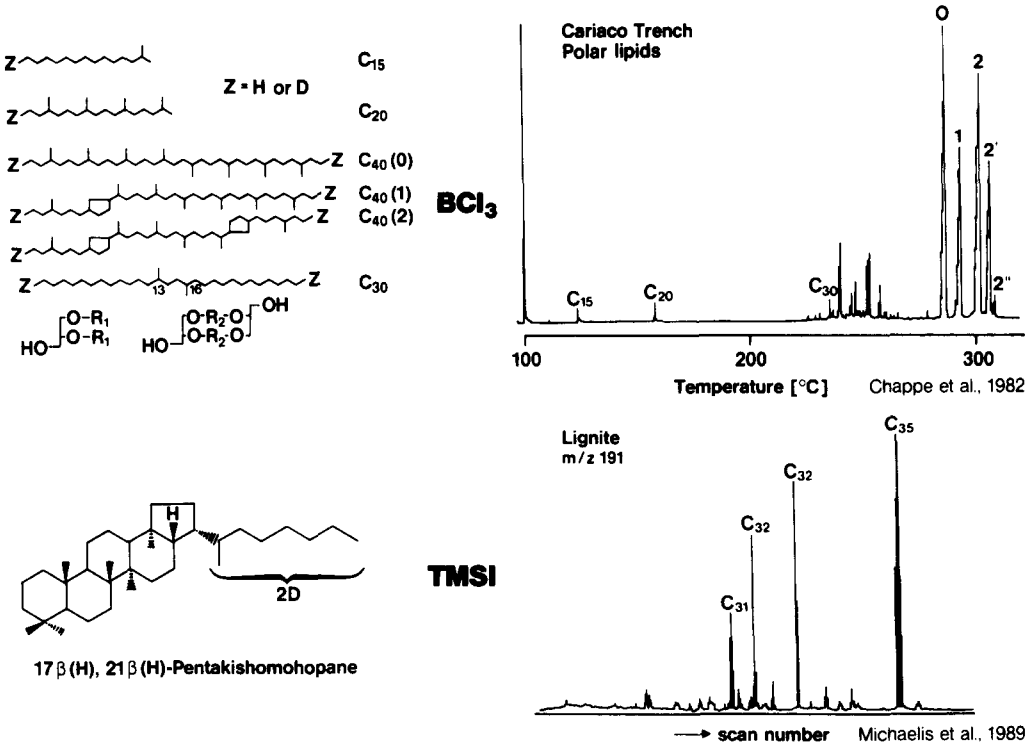


Fig. 12. Ether cleavage with BCl_3 or TMSI applied to polar lipids from a Cariaco Trench deep sea sediment yielding archaebacterial isoprenoid lipids (top: Chappe *et al.*, 1982; Copyright 1982 by the AAAS) and to lignite yielding bacterial hopanes (bottom: Michaelis *et al.*, 1989).

showed that the C_{35} hopane contained two deuterium atoms in the sidechain but none in the ring system. This clearly demonstrates the relationship between the hopanoids in fossil organic matter and bacteriohopanepolyols as their most likely precursors.

Of the two oxidative methods listed in Fig. 10, namely ozonolysis and ruthenium tetroxide oxidation, ozonolysis of carbon-carbon multiple bonds is the less specific one and has not been frequently used in recent years. Both oxidation methods yield carboxylic acids and thus provide a kind of inherent labelling. Ruthenium tetroxide (RuO_4) oxidation was introduced in organic geochemistry by Stock and Tse (1983) and Stock and Wang (1985, 1986) who adapted the method successfully for the degradation of coals. The effect of RuO_4 on the degradation of model compounds is shown in Fig. 13 (Trifilieff, 1987). Aromatic rings carrying alkyl substituents are oxidized in a way that carboxylic acids are formed with one carbon atom more than the former alkyl substituent, i.e. the carbon atom it was bound to in the aromatic ring now is the carboxyl carbon. The amounts of byproducts, i.e. not fully oxidized derivatives and carboxylic acids with one carbon atom less

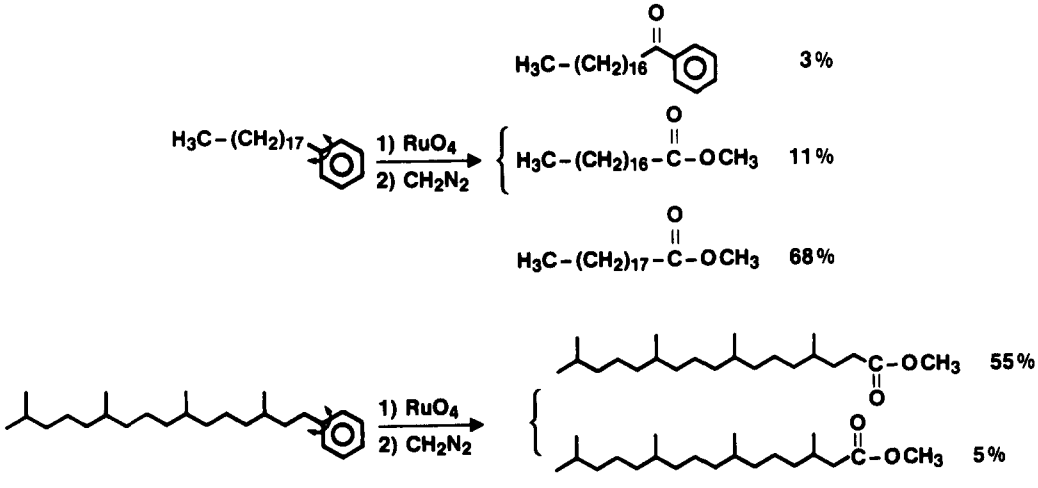


Fig. 13. Oxidation of model compounds with ruthenium tetroxide (after Trifilieff, 1987).

(i.e. without the ring carbon atom), are subordinate in the model reactions. Thus, labelling of the former bonding site is quite clear. Application of this reaction to crude oil asphaltenes (Fig. 14) yielded acyclic mono- and diacids as well as isoprenoid acids and carboxylic acids of the steroid and hopanoid series (Trifilieff, 1987). In these latter cases, carboxyl groups at C-3 in steroids and in the sidechain of hopanoids are fully consistent with the present understanding of the biological precursor molecules, i.e. 3β-hydroxysteroids and bacteriohopanepolyols hydroxylated in the sidechain. Highly aromatic organic macromolecules will yield benzene polycarboxylic acids, among others (Stock and Wang, 1986). Blanc (1989) showed that this type of product is very minor after RuO_4 oxidation of the highly aliphatic Messel oil shale kerogen but variably important if RuO_4 is applied to coals of different origin and rank.

Sulfur-rich macromolecules in geological samples have been at the centre of interest in the organic geochemistry groups in Strasbourg and Delft for some years (e.g. Schmid, 1986; Sinninghe Damsté *et al.*, 1988, 1989). An elegant method of analyzing these macromolecules is the removal of sulfur with Raney nickel and the identification of the resulting low-molecular-weight substances which often turn out to contain a significant proportion of (mostly saturated) hydrocarbons. Labelling of the bonding site by the use of deuterium instead of hydrogen in this reaction is tempting but usually leads to products with an excess number of deuterium atoms due to hydrogen exchange in the course of the reaction.

During liquid chromatography separation of Rozel Point oil and other sulfur-rich crude oils on silica, Schmid (1986) observed a narrow "red band" which was not directly analyzable by gas chromatography. Elemental analysis indicated a high sulfur content (>10%), and thus the isolated "red band" was reduced with Raney Ni/H_2 to remove the sulfur. About 30% of the starting material was recovered as saturated hydrocarbons of which *n*-alkanes with strong even-over-odd carbon number predominance and steranes represented the main compound classes (Fig. 15). Based on his results, Schmid (1986) drew a hypothetical partial model structure of the "red band" of Rozel Point oil (Fig. 16) with alkanes and steranes crosslinked with (poly)sulfide bridges which are thought to be responsible for the red colour.

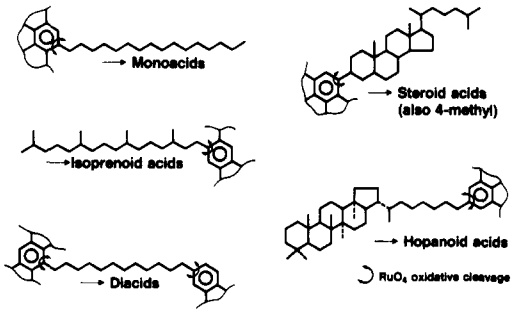


Fig. 14. Oxidation of crude oil asphaltenes with ruthenium tetroxide showing the type of products obtained (after Trifilieff, 1987).

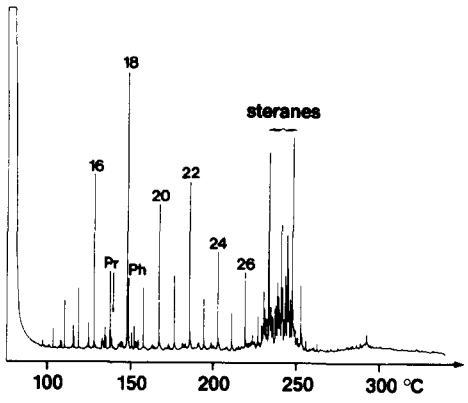


Fig. 15. Capillary column gas chromatogram of saturated hydrocarbons obtained from Raney nickel treatment of the "red band" of Rozel Point crude oil (after Schmid, 1986).

In a similar way, Rullkötter *et al.* (1991) treated the "aromatic hydrocarbon" fraction of a sulfur-rich Monterey crude oil from offshore California. The resulting saturated hydrocarbons (14%) showed an *n*-alkane distribution with strong even-over-odd carbon number predominance and a high abundance of *n*-alkanes with 37 and 38 carbon atoms [Fig. 17(a)]. They were tentatively assigned to be related to the well-known molecular markers of Prymnesiophytae algae (e.g. coccolithophores), viz. long-chain unsaturated alkenones (de Leeuw *et al.*, 1980; Volkman *et al.* 1980; Prahl *et al.*, 1988). In the saturated hydrocarbon fraction of the initial crude oil, the carbon number predominance is much less pronounced, and there is no enhanced concentration of the C_{37} and C_{38} *n*-alkanes [Fig. 17(b)]. Thus, high-molecular-weight material may contain palaeoenvironmental (and other) information which cannot be retrieved from the analysis of the low-molecular-weight constituents

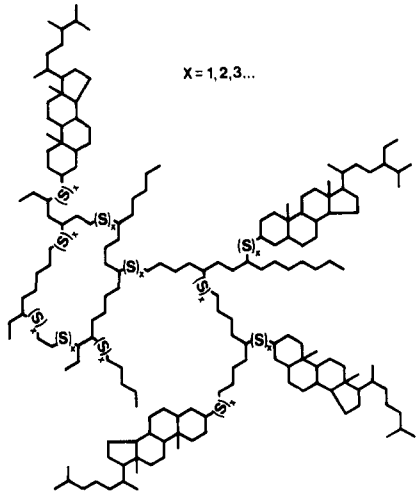


Fig. 16. Partial molecular structure (model) of the "red band" of Rozel Point crude oil (after Schmid, 1986).

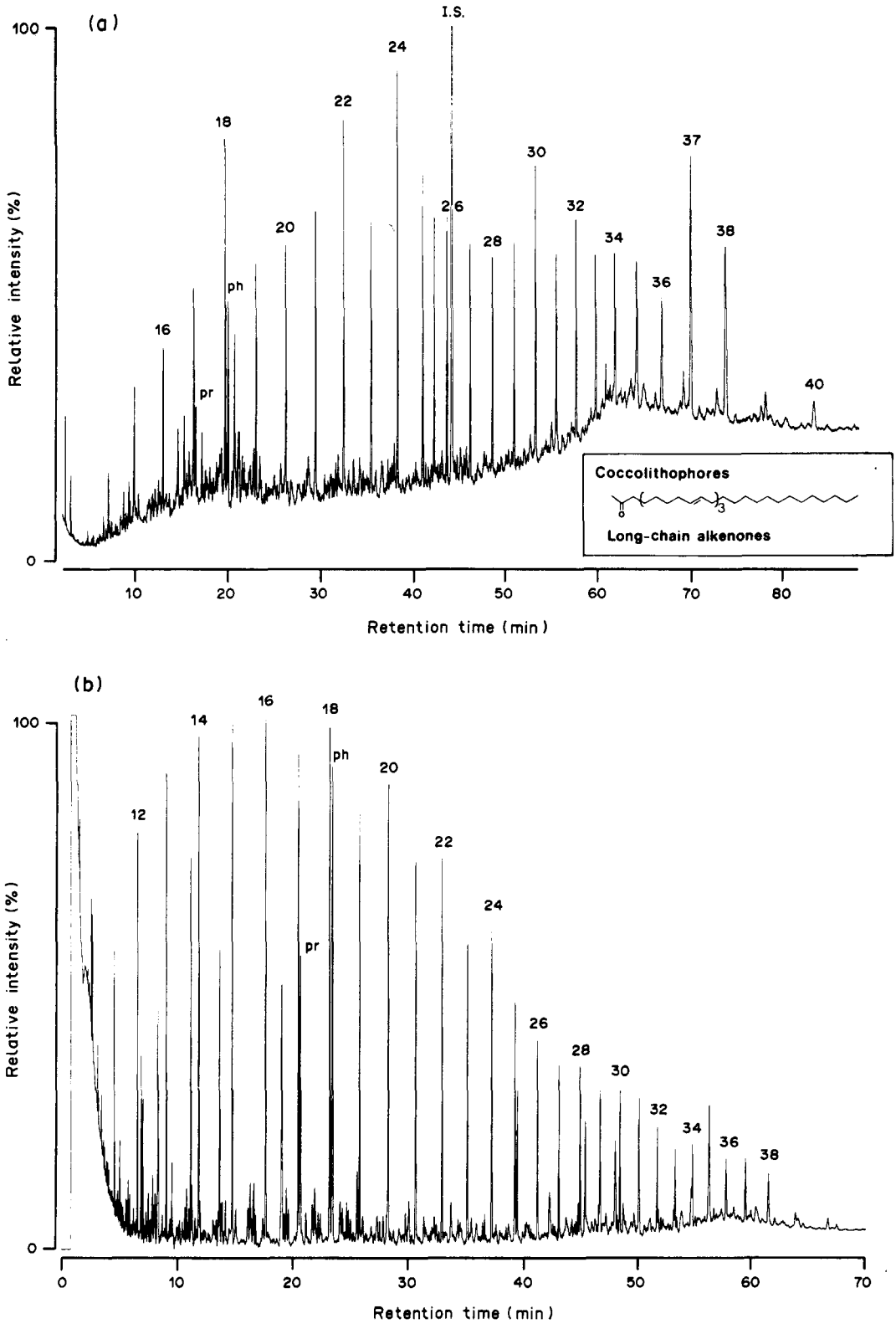


Fig. 17. Capillary column gas chromatograms of the saturated hydrocarbons of a Monterey crude oil (offshore California): (a) after desulfurization of the "aromatic hydrocarbon" fraction with Raney nickel; (b) original saturated hydrocarbon fraction (from Rullkötter *et al.*, 1991).

of geological samples. This is an additional reason to promote structure-specific analysis of kerogen and related materials.

Hydrogenolysis with a rhodium-on-charcoal catalyst (Fig. 18) leads to the cleavage of heteropolar bonds by the addition of hydrogen (or deuterium if

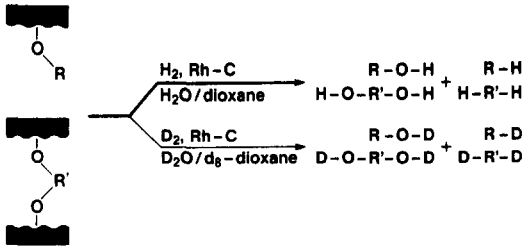


Fig. 18. Schematic representation of hydrogenolysis with H_2 and D_2 (after Mycke and Michaelis, 1986).

labelling if intended). Other common reducing agents are $LiAlH_4$, which will cleave esters (and at the same time may liberate trapped material as observed by Shaw and Eglinton, 1987; but this applies to many other reagents rendering the kerogen macromolecule less complex) and sodium bis-(2-methoxyethoxy)-aluminium dihydride (SMEAH). Hydrogenolysis yields are often remarkably high. For example, in a sequential degradation study of a bituminous coal Shaw and Eglinton (1987) applied hydrogenolysis after previous extraction, ester cleavage with $LiAlH_4$ and O-alkylation. In this way, they obtained as much as a total of 35% soluble material. Michaelis *et al.* (1989) used ether cleavage with BCl_3 and hydrogenolysis in parallel reactions to degrade humic material. The resulting hydrocarbon fractions had complementary compositions. The ether cleavage products were dominated by isoprenoid hydrocarbons from (archae)bacterial membranes, whereas hydrogenolysis mainly yielded triterpenoids of the hopane and friedelane type.

A particular problem with the application of mild selective degradation reactions to a macromolecular substrate like kerogen is the extent to which the reaction can be brought to completion. Not all functional groups the reagent potentially is able to attack are readily accessible, i.e. there may be a significant degree of sterical hindrance (protection) as has been shown, e.g. for fatty acids of which a minor part were released by hydrolysis but the main portion only during pyrolysis (Kawamura and Ishiwatari, 1985; Largeau *et al.*, 1986). Longer reaction times than common for low-molecular-weight substrates have to be taken into account, but this will only partly solve the problem. Alternatively, a reagent applied first may have to be used again at a later stage, when degradation with other types of reagents has provided access to sites which remained unchanged at the initial stage. Thus, the design of a consecutive chemical procedure for kerogen degradation should not necessarily be based on a strict hierarchy of increasingly more rigorous reactions but rather allow for some flexibility.

PRESERVED BIOPOLYMERS IN KEROGEN—A NEW CONCEPT

While there still is the prevailing opinion that kerogen is mainly a random condensation product of various biomonomers which were formed during diagenesis by hydrolysis of biopolymers (Fig. 2), lignin was considered one of the more resistant biopolymers which may enter the macromolecular fossil organic matter largely unchanged (Degens *et al.*, 1981; Tissot and Welte, 1984). Indeed, Mycke and Michaelis (1986) on mild hydrogenolysis (using H_2 as well as D_2) of humic material from river water samples obtained various mono- and dimeric lignin-derived phenols. In contrast to the products of more rigorous oxidation reactions (e.g. Ertel *et al.*, 1984), the hydrogenolysis products (Fig. 19) could readily be correlated to the characteristic subunits of intact recent lignin polymers from fresh spruce (Nimz, 1974).

Philp and Calvin (1976) proposed that kerogen-like material is inherited virtually unchanged from living algae. They tested this hypothesis by oxidative chemical degradation of recently-deposited algal oozes from Laguna Mormona (Baja California). Based on the products they had obtained, they came to the conclusion that "the basic nucleus of the material consists of cross-linked polymethylene chains with increasing amounts of normal and branched acids attached to the periphery with increasing sample depth". In general, however, relatively little attention has been payed to the common observation of organic petrographers for many years that biological cell structures are preserved morphologically intact in sediments to an extent allowing taxonomic assignments in great detail (cf. Stach *et al.*, 1982). While this is not overly surprising for woody material largely consisting of lignin (considered fairly resistant), this does not apply in the same way to the different types of liptinites. Strangely enough, this has not stimulated a systematic search for resistant biological substances in algae, spores, cuticles and other precursors of oil-prone macerals.

Thus, the discovery of previously unknown insoluble, non-hydrolyzable highly aliphatic biopolymers in extant organisms (Fig. 20) as well as in fossil organic matter in recent years was surprising as well as revolutionary for the concept of kerogen formation. Berkaloff *et al.* (1983) and Largeau *et al.* (1984, 1986) found a resistant polymer (PRB *A*) based upon unbranched, saturated, cross-linked hydrocarbon chains (up to C_{31}) in the extant alga *Botryococcus braunii* and in immature Torbanite and Coorongite (Dubreuil *et al.*, 1989). In addition to the bridged structures, the polymer appears to contain a substantial part of singly bound alkyl chains as esters of unbranched, saturated or *cis*-unsaturated fatty acids which are sterically protected against chemical degradation. Structural and morphological similarities between PRB *A* and immature Torbanite demonstrated that the resistant polymer (*c.* 10% of the total mass in extant *Botryococcus braunii* colonies) was selectively preserved during diagenesis and thus provided a major contribution to Torbanite formation. In race *A* of *Botryococcus braunii* the aliphatic portion in the

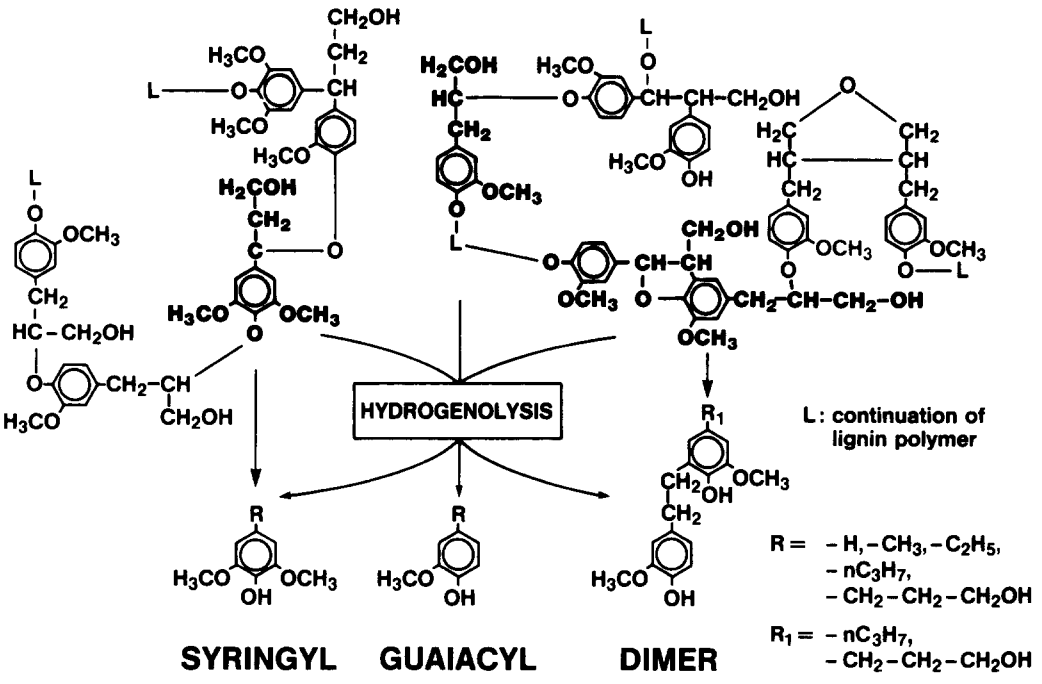


Fig. 19. Schematic representation of lignin hydrogenolysis indicating formation of mono- and dimeric alkylphenols (after Mycke and Michaelis, 1986).

polymer matches reasonably well the composition of the major lipids (Fig. 20), whereas race *B* mainly contains botryococcenes in the extractable lipids despite possessing the same aliphatic biopolymer as race *A* (Kadouri *et al.*, 1988). In contrast to this, the major extactable lipid of the third race of the same alga (race *L*) is lycopadiene, with the cell wall biopolymer being "polylycopadiene" (Derenne *et al.*, 1988, 1989, 1990).

Similar insoluble, non-hydrolyzable aliphatic biopolymers were detected in the protective layers of terrestrial higher plants. They occur in the cuticular membranes and barks together with the hydrolyzable polyesters cutin and suberin in relative amounts varying widely from one plant to another. Nip *et al.* (1986a) isolated such highly aliphatic biopolymers from the cuticles of *Agave americana* and two other plants. After exhaustive extraction on base and acid hydrolysis to remove cutin and polysaccharides, respectively, the residual aliphatic biopolymer, as characterized by analytical pyrolysis and solid-state ^{13}C-NMR spectroscopy, was found to contain polymethylenic chains (at least up to C_{35}), but also a substantial proportion of polysaccharide moieties

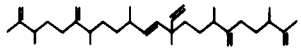
	Major Lipids	Cell wall Biopolymer
Botryococcus braunii (race *A*)	$CH_3-(CH_2)_7-CH=CH-(CH_2)_n-CH=CH_2$ $CH_3-(CH_2)_5(-CH=CH)_2-(CH_2)_n-CH=CH_2$ n=13,15,17,19 / $C_{25}-C_{31}$	"PRB *A*" $-O-(CH_2)_n-O-(CH_2)_m-O-$? (cycloalkyl units)
B braunii (race *B*)	e.g.  "botryococcenes"	"PRB *B*" identical to "PRB *A*"
B. braunii (race *L*)	 "lycopadiene"	"PRB *L*" "polylycopadiene"
Plant Cuticles	Waxes	$-(CH_2)_n$-Polysaccharide moieties/ Cutins
Plant Periderms	Waxes	$-(CH_2)_n$-moieties/ Suberins

Fig. 20. Different insoluble, non-hydrolyzable highly aliphatic biopolymers in extant organisms and comparison with their major soluble lipids (after Tegelaar *et al.*, 1989b).

probably covalently bound to the polymethylene units and sterically protected from hydrolysis (Nip *et al.*, 1987; Tegelaar *et al.*, 1989b). A similar type of biopolymer was isolated from barks of several angiosperms (Tegelaar *et al.*, 1991). The investigation of humic and fulvic acids (Saiz-Jimenz and de Leeuw, 1987) and of fossil cutinite (Nip *et al.*, 1989), mainly by analytical pyrolysis, revealed hydrocarbon distributions strongly resembling those of the biopolymers of extant higher plants. This confirms earlier studies of Allan and Larter (1981) who had found evidence for long-chain aliphatic structures in sporinite (outer walls of spores and pollens) by oxidative chemical degradation and agrees with the discovery of resistant materials in some modern exines (references cited in de Leeuw and Largeau, 1990).

Two examples will further illustrate the wide distribution of highly aliphatic biopolymers in extant plants and fossil organic matter. The kerogen of the Messel oil shale has been inferred from optical microscopy to be a mixture of remains of small algae, bacterial biomass and subordinate terrigenous organic matter (e.g. Jankowski and Littke, 1986). Supporting evidence has been gained from the analysis of the extractable bitumen which showed bacterial components (e.g. hopanoids) and 4-methylsteroids (believed to be chemotaxonomically related to dinoflagellates) as major biological markers (e.g. Ensminger, 1974; Rullkötter *et al.*, 1988; Robinson *et al.*, 1989) and from chemical degradation of the kerogen which yielded long-chain isoprenoids from archaebacterial membranes (Chappe *et al.*, 1982). The nature of the small coccoid algae in the Messel oil shale was further studied by Püttmann and Goth (1988) using scanning electron microscopy. In contrast to the previous inference from extract data, these were not dinoflagellates but appeared to be *Tetraedron* species (green algae) which are not expected to contain 4-methylsteroids. In a subsequent study, Goth *et al.* (1988) compared the pyrolysis products of Messel oil shale kerogen rich in algal bodies to those of algaenan (insoluble, non-hydrolyzable highly aliphatic biopolymer) isolated from cultured *Tetraedron minimum* algae (Fig. 21). The fingerprint patterns show striking similarities except for the relative concentration of pristene which is believed to originate from bound tocopherol in organic constituents other than *Tetraedron* algae in the Messel shale. Goth *et al.* (1988) concluded that the highly aliphatic biopolymer abundantly present in Messel shale kerogen was selectively enriched during diagenesis as a consequence of degradation of the more labile components (e.g. polysaccharides) of the *Tetraedron* cell where, in the case of the extant species, the highly aliphatic cell-wall biopolymer accounts only for about 5% of the total biomass.

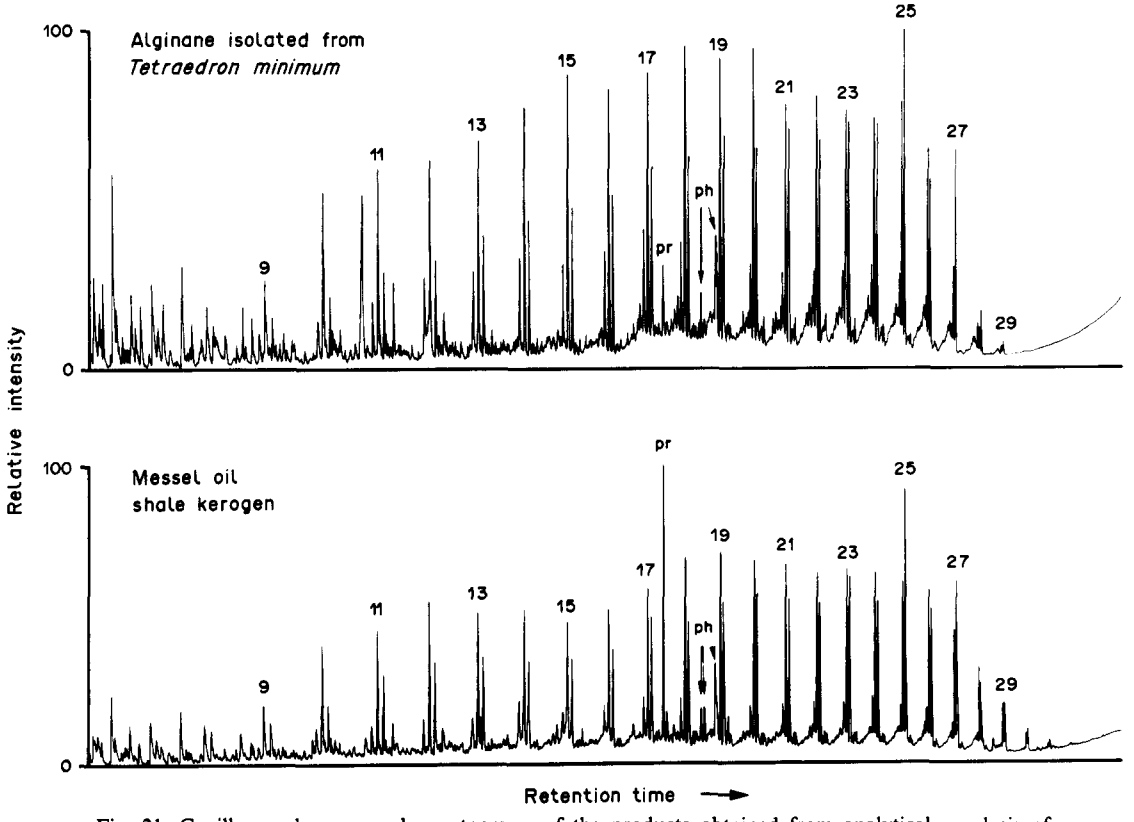


Fig. 21. Capillary column gas chromatograms of the products obtained from analytical pyrolysis of algaenan isolated from *Tetraedron minimum* algae (top) and of Messel oil shale kerogen rich in *Tetraedron* species (bottom) (after Goth *et al.*, 1988). Reprinted by permission from *Nature*, **336**, 759–761. Copyright © 1988 Macmillan Magazines Ltd.

Unless 4-methylsteroids will some time also be found in *Tetraedron* species, this interpretation requires, however, that in the soluble lipid fraction of the Messel shale 4-methylsteroids from dinoflagellates were selectively preserved and *Tetraedron* steroids as well as dinoflagellate cell structures preferentially destroyed. This could be an extreme example of selective preservation during deposition and early diagenesis which will cause false palaeoenvironmental interpretations if organic geochemical data of either bitumen or kerogen are used on their own.

A similar misinterpretation may have persisted until recently in the literature with respect to the origin of long-chain (wax) alkanes in crude oils which were generated from source rocks containing terrigenous organic matter. These the long-chain *n*-alkanes often exhibit a rather pronounced preference of the odd-carbon-number homologs and were believed to originate from the defunctionalization of the major components of higher-plant cuticular waxes (Tissot and Welte, 1984, and references therein). Recently, the insoluble, non-hydrolyzable highly aliphatic biopolymer found in cuticles of extant plants was suggested as an alternative precursor of the long-chain *n*-alkanes in crude oils (Nip *et al.*, 1986b). To demonstrate this possibility, Tegelaar *et al.* (1989c) artificially matured cutinan, a highly aliphatic biopolymer from the cuticle of *Agave americana*, by heating it for four weeks at 325°C in a closed tube and compared the resulting *n*-alkane distribution in the bitumen-like material formed to that of an Indonesian crude oil rich in wax alkanes (Fig. 22). The hydrocarbon generation potential of cutinan is clearly evident, and there is a striking similarity between the *n*-alkane distributions as well as that of methyl-branched alkanes (marked with dots in the chromatogram of the oil sample in Fig. 22).

Many kerogens which appear to have formed preferentially via selective preservation of aliphatic biopolymers do not exhibit a shortening of *n*-alkane chain length in their pyrolysates or pronounced aromatization with increasing level of induced thermal stress, as revealed by two-step pyrolysis–gas chromatography and microscale sealed vessel pyrolysis (Horsfield, 1989; Horsfield *et al.*, 1989). This is in contrast to kerogens in which neoformation of macromolecular moieties during diagenesis is supposed to have played a more important role. The pyrolysates of such kerogens show significant compositional changes under the same heating conditions.

The discovery of the insoluble, non-hydrolyzable highly aliphatic biopolymers in extant organisms and geological samples has led to a reappraisal of the processes involved in kerogen formation (de Leeuw and Largeau, 1990; Tegelaar *et al.*, 1989d). In the modified scheme (Fig. 23; cf. to Fig. 2), more emphasis is placed on the selective preservation of biopolymers. This means, that the role of consecutive and random polymerization and polycondensation reactions of biomonomers formed by hydrolysis or other degradative pathways during deposition and early diagenesis in kerogen formation may be less important than previously thought. Tegelaar *et al.* (1989d) assigned different (relative) "preservation potentials" to various types of biomacromolecules and concluded that kerogen may be a physical mixture mainly of selectively preserved and sometimes partly altered, resistant biomacromolecules (Fig. 23). Further application of transmission electron microscopy may show that much of the previously termed unstructured ("amorphous") kerogen particles in sediments will disclose many more biological structures of "membranes" and cell walls directly inherited from phytoplankton and microorganisms (Raynaud *et al.*, 1988, 1989). A further indication is the observed close morphological relationship between fossil "ultralaminae" and the thin resistant outer walls of green microalgae (Largeau *et al.*, 1990).

The new concept of kerogen structure may have a correspondence to the recent debate on the molecular

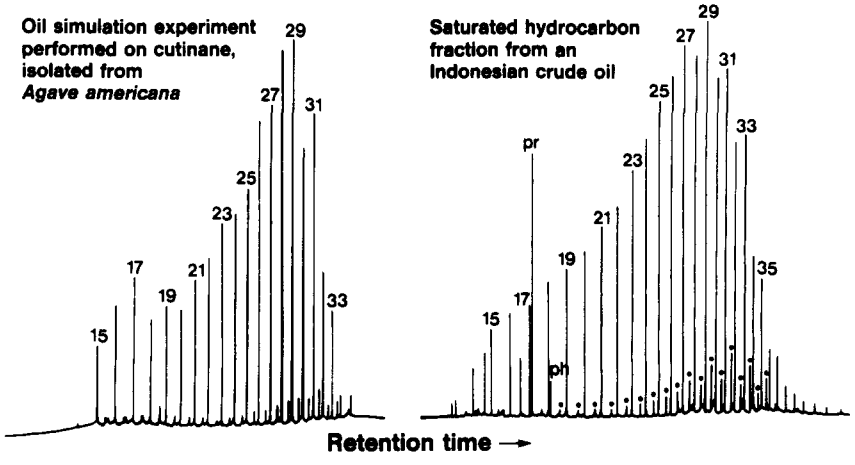


Fig. 22. Capillary column gas chromatograms of the saturated hydrocarbons from an artificial maturation experiment using cutinan from *Agave americana* (left) and from an Indonesian crude oil (right) (after Tegelaar *et al.*, 1989c). Reprinted by permission from *Nature*, **342**, 529–531. Copyright © 1989 Macmillan Magazines Ltd.

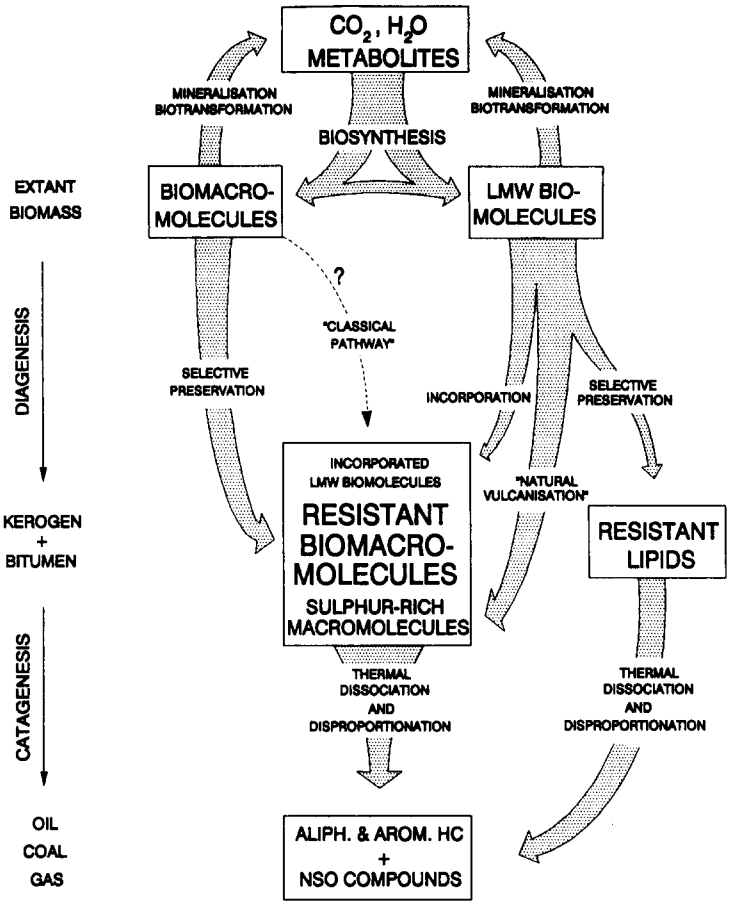


Fig. 23. Revised mechanism for kerogen formation based on the concept of selected preservation of (highly aliphatic) resistant biopolymers (from Tegelaar *et al.*, 1989d).

structure of coals, i.e. the two-phase model of coals (Given *et al.*, 1986; Derbyshire *et al.*, 1989). The proposed dualisms of macromolecular framework and a second phase of relatively small molecules, if real, may also be a consequence of selective preservation or reaction processes during early diagenesis. This means that the mobile phase may not structurally be representative in any way of the macromolecular coal component.

CONCLUSIONS

The elucidation of the chemical constitution (structure) of kerogen and related fossil macromolecules is still a challenging task. Despite the tremendous efforts, little progress has been achieved by applying instrumental analysis alone to this problem. The increase in knowledge that was reached in recent years is mainly due to the application of specific chemical degradation reactions and to the developments of a new concept about the interrelationship between the composition of extant biological organisms and fossil macromolecular organic matter.

Thus, in summarizing the previous discussion, kerogen structure elucidation requires the coordination of:

—Consecutive specific chemical degradation reactions. In addition to those reactions known and already applied, there should be a continuous search for other, complementary reactions. Each degradation reaction should be carefully tested on simple and on more complex model compounds. Much of the success in the interpretation of the degradation results may depend on the intelligent selection of these model compounds.

—A detailed quantitative molecular analysis of small degradation products. Kerogen structure work suffered from restrictions in this respect several years ago, but due to the development of more sophisticated analytical techniques it is less of a problem nowadays. Difficulties are encountered in the interpretation of mass spectral data because many of the compounds produced by chemical degradation of fossil organic matter will not be found in mass spectral libraries. Also, quantitation of products in the reaction mixtures is still not routinely applied.

—Studies of the degradation residues using spectroscopic methods (particularly NMR) and analytical pyrolysis. Although mostly bulk average data are generated this way, monitoring the changes during consecutive chemical degradation will be helpful,

and there is the expectation that spectra and pyrograms become simpler and easier to be interpreted in the course of the degradation scheme.

—Application of the new and refined concepts of selective preservation of biological material during deposition and early diagenesis. This requires an intensified cooperation with microbiologists, molecular biologists and biochemists and a consideration of the developments in natural product chemistry.

It should be clearly manifested by now, that there is not necessarily a direct relation between bitumen composition and kerogen structure. The concept of selective preservation rather requires that there is a discrepancy between these two fractions of fossil organic matter. This has become most obvious in the recent studies of organic sulfur compounds in sediments and crude oils. This may also be an argument in favour of the validity of the recently discussed two-phase model of coal. Bearing the concept of selective preservation and the discovery of the insoluble, non-hydrolyzable highly aliphatic biopolymers in mind, it becomes evident that simulation of kerogen formation in the laboratory by heating, e.g. amino acids and sugars (melanoidin formation) may not be the optimal approach.

The elucidation of the structure of kerogen and related fossil macromolecules is not a scientific playground but will have an impact on:

—Petroleum exploration by improving the understanding of chemical reaction kinetics and thus the advanced integrated basin modelling tools as well as the palaeoenvironmental assessment of the hydrocarbon generation potential of source rocks.

—Petroleum production by providing a basis for the understanding of organic/inorganic interactions in a reservoir and thus by allowing the development of better non-empirical exploitation strategies.

—The solution of environmental problems, e.g. by giving a clue to the determination of the type of bonding of pesticides or their metabolites or of heavy metals to organic matter in soils.

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