Preface

The primary usefulness of this book is for organic geochemists, molecular palaeontologists, and molecular archeologists. Researchers interested in astrobiology from a paleontological perspective may also find this to be valuable. This provides an overview and an introduction to the science, including literature overview for non-geochemists. Analytical methods employed are included as a part of each chapter.

Kerogen and Sedimentary Organic Matter

Primary producers on Earth that contribute to large quantities of organic matter, of which about 0.1–1 % enters the geosphere as sedimentary organic matter. This sedimentary organic matter is broadly divided in two fractions: (1) bitumen (ca. 5 %) that is extractable using organic solvents and (2) kerogen that is insoluble in organic solvents.

Kerogen occurs in sedimentary rocks as finely dispersed organic macerals and is the most abundant form of organic carbon in the Earth’s crust. With increasing burial and heating, hydrocarbons are produced from kerogen, but kerogen itself is a three-dimensional polymeric macromolecule cross-linked by aliphatic chains (containing O or S functional groups) in which clusters of aromatic sheets form an important part (N, S and O functional groups). In typical kerogen, for every 1,000 C atoms there are c.500–1,800 H, c.25–300 O, c.5–30 S and c.10–35 N atoms. The oxygen-containing functional groups include carboxylic acids, alcohols, carbonyls, esters, ethers and amides.

Kerogen Types

Kerogen is classified as type I, II or III.
**Type I Kerogen**

Type I kerogen is relatively rare and is associated with lacustrine and open marine deposits. It has a high H/C ratio (about 1.5) and a low O/C ratio (<0.1) due to significant contributions from lipid material, especially long-chain aliphatic components. These aliphatic components, especially in lacustrine kerogens, are thought to predominantly derive from algaenan, with contributions from amorphous bacterial material. Compared with the other kerogen types it contains proportionally lower amounts of aromatic units and heteroatoms. Oxygen is present in the carbon skeleton mainly as ester and ether groups. The freshwater alga *Botryococcus braunii* is a major contributor to type I kerogens (e.g. torbanites of Scottish oil shales). The kerogen in the Eocene Green River oil shale of Colorado, Utah and Wyoming is another example of type I kerogen.

**Type II Kerogen**

Type II kerogen is more common than type I and can potentially be formed in a variety of environments. It has relatively high H/C and low O/C ratios. Aliphatic structures are important and comprise chains (chain length up to \( c. C_{25} \)) associated with polyaromatic units, which often form the nucleus of the kerogen structure. Ketone and carboxylic acid groups are more important than in type I kerogen and ester bonds are also abundant. In marine settings, a major source for Type II kerogen is the mixture of autochthonous organic matter from phytoplankton (and possibly also zooplankton and bacteria) and an allochthonous contribution of higher plant material.

**Type III Kerogen**

Type III kerogen is commonly found in terrestrial depositional environments and has low H/C (<1.0) and high O/C (up to 0.3) ratios, reflecting major contributions from vascular plant tissues. As with Type II kerogen, ketone and carboxylic acid groups are important with relatively minor contributions from ester groups. A significant proportion of oxygen in the carbon skeleton is in non-carbonyl groups (possibly heterocycles, ethers and methoxy groups). Aliphatic groups are present in relatively minor amounts, dominated by methyl and other short chain alkyl groups. Long chain alkyl compounds are also present in low amounts and probably originate from cuticular coatings (cutan and suberan) or from polymerisation of waxes.

**Type IV Kerogen**

Type IV kerogen primarily comprises black opaque debris which has no hydrocarbon generating potential. It is likely formed from higher plant matter that has been severely
oxidized or subjected to thermal stress (e.g. combustion) and then transported to its deposition site following reworking.

**Kerogen Formation**

The origin of kerogen has traditionally been attributed to random recombination reactions of biological components (neogenesis) or the survival of decay-resistant organic macromolecules (selective preservation). Earlier research has revealed an alternative process for kerogen formation by conversion of free aliphatic molecules into a resistant aliphatic macromolecule via *in situ* polymerization.

**Neogenesis**

In the classical theory of geopolymer formation, kerogen is believed to derive from humic substances similar to the humic and fulvic acids found in soils. During early diagenesis in aquatic environments, the organic material deposited from primary producers is broken down by microbial action into smaller constituents, which then undergo condensation reactions, giving rise to humic substances. Microbial degradation and condensation follow in immediate succession, leading to a zone in the top few metres of sediment (and possibly also in the water immediately overlying it) where both processes are active at the same time. With increasing time and burial depth, most of this humic material becomes progressively insoluble due to increasing polycondensation, associated with the loss of superficial hydrophilic functional groups (e.g. OH and COOH). Insolubilization can begin quite early and apparently continues to significant depths, as suggested by the occurrence of humic acids at several hundred metres depth in sediments that contain abundant terrestrial detrital material. The humin-like material that is formed continues to undergo condensation and defunctionalization, resulting in kerogen. With increasing time and burial depth, kerogen is formed, e.g. by incorporation of low molecular weight lipids via ether and ester bonds.

**Selective Preservation**

The selective preservation of resistant biomacromolecules (with the possibility of minor microbial alteration) is also thought to be an important process in the formation of kerogens, and particularly those formed at very early stages of diagenesis. Highly aliphatic, insoluble and non-hydrolysable biopolymers that resist biodegradation have been detected in the protective outer layers of some extant higher plants and algae as well as in the corresponding fossil remains. These materials are termed cutan and suberan in terrestrial plant cuticles, and are believed to make significant
contributions to kerogen. Equivalent materials, termed algaenans, are found in the cell walls of eustigmatophytes (an order of the Xanthophyceae) and many chlorophytes, but have yet to be detected in diatoms and haptophytes, and may be rare in dinoflagellates. Highly aliphatic macromolecules have also been identified in the cell walls of cyanobacteria and may make a contribution to amorphous kerogen. The algaenans from most algae contain high molecular weight, long \( n \)-alkyl chains (e.g. from the chlorophyte \textit{Tetraedron}, which is a major contributor to the Messel oil shale). Initial studies suggested that these algaenans were polyesters with \( n \)-alkyl chains of up to \( \text{C}_{35} \). Algae can also contribute aromatic material to kerogen in the form of polyalkylphenolic macromolecules.

These resistant biomacromolecular structures discussed above are likely to become concentrated as more abundant and readily hydrolysable biopolymers, such as proteins and carbohydrates, are degraded. There is also the possibility for less resistant material to be protected against biodegradation within coatings of resistant material (e.g. polysaccharides within higher plant cuticular membranes and lipids within microbial cell walls). Selective preservation is also consistent with the preservation of certain parts of organisms such as ultralaminar walls, cuticles, spores and pollen, often recognized in both coal and kerogen. Consequently, this model has gained increasing popularity over the last two decades.

Thus, classical and selective preservation models can be considered end-member models of the scale of alteration that may be undergone by the biomacromolecular precursors of kerogen.

**Sulphur Incorporation**

Conditions favoring the activity of sulphate-reducing bacteria result in the production of sulfide, which usually reacts with iron (II) ions especially in some types of clastic and argillaceous sediments. Where there is a limited supply of iron (II), free hydrogen sulphide and polysulphides are produced during early diagenesis, and these compounds can react with certain functional groups in organic compounds, fostering incorporation of both sulfur and organic compounds into kerogen. Additionally, reaction of organic matter with inorganic sulphur species may render OM resistant against bacterial degradation and remineralisation leading to formation of kerogen. In fact, normally labile compounds such as carbohydrates can be preserved in kerogen via early diagenetic reactions with sulphides.

**In Situ Polymerisation of Labile Components in Extant Organisms**

The cuticle of the great majority of pre-Tertiary arthropod and plant fossils comprises a macromolecule with a significant aliphatic component. The convergence in composition with type I and/or II kerogens suggests that polymerisation of free aliphatic
components may be important in the preservation of these fossils. Fossils provide a key to understanding the process because they allow the end product of diagenetic maturation to be related directly to starting composition.

Evidence from Transformation of Arthropod Cuticle

Large numbers of invertebrates are protected by an outer layer known as the integument or cuticle. This provides protection against predators, a barrier to the environment and prevents desiccation. In arthropods, the cuticle consists of three major layers, the epicuticle, exocuticle and endocuticle. The epicuticle consists of waxes composed of straight-chain and branched hydrocarbons, wax esters, fatty acids, alcohols, ketones and sterols and prevents dehydration. The rest of the cuticle is composed of mainly two biopolymers, chitin and protein which form a complex structure cross-linked by catechol, aspartic and/or histidyl moieties. Chitin, a nitrogen containing polysaccharide provides the structural strength which is reinforced by the protein matrix. Additionally, CaCO$_3$ may provide biomineralisation and further strengthen the cuticle. The proportion of chitin to protein varies between taxa ranging from just traces in protozoa to up to 85% in some crustacean cuticles. The preservation potential of chitin is higher than that of associated proteins. However, almost all of the estimated (around $10^{11}$ t) of chitin produced annually in the biosphere is consumed by decay, and only mineralised or sclerotized cuticles are normally preserved. Organically preserved animal cuticles are known from rocks as old as Cambrian (in the Burgess shale, where they provide template for the precipitation of clay minerals) and are relatively common throughout the Palaeozoic (scorpions and eurypterids). Decay experiments have shown that the chitin component of the cuticle degrades even in anoxic conditions, but still at a slower rate than that of protein. Pliocene asphalt deposits (e.g. Rancho La Brea in California) and organic rich glacial deposits preserve abundant insect cuticles. The proportion of the chitin that survives reflects the nature of sediment. A lower proportion of chitin is preserved in peat deposits than in clastic sediments. The lithological control is also evident in the preservation of insect cuticles and shrimps from Pliocene sediments of Willershausen, Germany. Cuticles deposited in oxygen-depleted waters of lake bottom yield a higher proportion of chitin, than that near the lake margins. The oldest deposited chitin survives in Oligocene (24.7 Ma) beetle cuticles, which also preserve traces of protein from maar type lake deposits at Enspel, Germany. In each case where the fossil cuticles reveal little chemical alteration, their morphological details are also remarkably preserved. Cuticles preserved in Pleistocene amber do show good chemical preservation and those extracted from 25 Ma Dominican amber are chemically changed. Curiously, analyses of a diversity of cuticles from Tertiary marine crustaceans reveal no compelling evidence that the original chemistry is even partially preserved. Paleozoic and Mesozoic arthropod cuticles often show well-preserved surface detail but lack any chitin protein component of the original cuticle. The chemical composition of the
cuticles reveals the presence of an \(n\)-alkyl component. This aliphatic composition does not reflect the original chitin-protein composition. Thus, the fossilisation of arthropod cuticles cannot be attributed to selective preservation of decay resistant components, but this long-term preservation relies on the diagenetic polymerisation of lipid constituents of the cuticle to an aliphatic composition. Thus, cuticular analysis can provide an important key to understanding preservation of organic matter in ancient sediments.

**Evidence from Confined Pyrolysis Experiments/Experimental Heating**

Preservation of organic fossils and fossil cuticles cannot be explained fully by either the neogenesis or selective preservation models. Recent analyses reveal that, while younger fossil arthropods may preserve traces of the more resistant elements of the chitin-protein complex that constitutes the cuticle, older fossils are dramatically altered and often yield an aliphatic signature similar to that of many plant fossils and kerogen. Selective preservation does not provide an adequate explanation, as the aliphatic components occurring in the cuticle of modern arthropods are not decay-resistant and differ in structure from those present in fossils. Thus, the convergence in the macromolecular composition of plant cuticles, animal cuticles, and kerogen cannot be explained simply on the basis of the known biochemical compositions of the living organisms.

Polymerization may be responsible for the aliphatic character of organic fossil remains, i.e. the chitin/protein complex has been transformed into a predominantly aliphatic macromolecule. The process does not involve random incorporation of external components (neogenesis model), and presumably results from degradation and *in situ* polymerization of components in the cuticle. As discussed below, artificial maturation experiments using confined pyrolysis in gold tubes confirmed that the cuticles of living arthropods alone can source an aliphatic macromolecule similar to that observed in fossil arthropods, i.e. free cuticular lipids or hydrolysable lipids may be transformed *in situ*. Since such lipid components occur in all organisms it is reasonable to suggest that a comparable process may operate in both arthropod and plant cuticles. Moreover, a comparable process could occur during the diagenesis of phytoplankton and other microorganisms and thus account for the large quantities of organic material represented by some kerogens. Evidence for the *in situ* polymerization model was illustrated by confined pyrolysis of arthropod cuticles in gold tubes. Initial experiments performed on degraded scorpion cuticles (*Pandinus imperator*) showed a proportional increase in the aliphatic component of the cuticle with increasing temperature. During pyrolysis for 24 h at 700 bars and 260 °C, the macromolecular composition of the cuticle was significantly altered. Characteristic protein and chitin moieties were absent in the residue (as analysed by py-GC/MS) and both phenols and \(C_{5-20}\) \(n\)-alkanes and \(n\)-alk-1-enes were more abundant. At 350 °C, changes in the
cuticle composition were much more extensive: alkenes and alkanes were the predominant compounds released during pyrolysis, phenols were barely detected, and chitin and protein moieties were absent. Moreover, the pyrolysate of the cuticle that matured at 350 °C was very similar to that of a Carboniferous scorpion, suggesting that the experiments replicated at least aspects of geological. However, experiments were conducted only on modern scorpion cuticle.

Despite the limitations of artificial maturation experiments, these results showed that an aliphatic macromolecule was generated by thermal alteration of the original cuticular material. It was this that led to the formulation of the in situ polymerization model: that during thermal maturation, labile components such as chitin and protein are altered or lost while free and ester-bound aliphatic compounds are altered and recombine forming an aliphatic macromolecule. However, analyses were limited to the scorpion Pandinus and it is unclear how widely applicable these results were to cuticles with different chemical structures.

Contribution of Plants to Organic Matter

Terrestrial plants and marine phytoplankton in the oceans are primary photosynthesizers providing a source of food and energy for secondary organisms to utilise for the process of biosynthesising compounds needed for their own growth, survival and lifecycle. Ultimately, all living organisms, both primary and secondary photosynthesizers die and many are deposited in some kind of aquatic environment. The nature of the depositional environment plays a very important role in determining the amount of organic matter eventually preserved. Organic matter deposited in a highly anoxic environment, accompanied by rapid sedimentation/burial, is preserved in far greater concentration than in the oxic situation. Tissot and Weltehave estimated that around 0.1 % of the organic carbon from photosynthetic cycle escaped and was fixed in the geological record. Differences in preservation relate to the efficiency of microbial reworking of organic matter. In addition, environment with very high productivity is more likely to produce organic-rich sediment. Thus productivity and preservation are the two main driving forces dictating survival of organic matter over geologic timescales.

Study of Leaves and Cuticles

Leaves, whether broad leaf foliage or coniferous needles, are the most dominant aboveground inputs to most soils. Other aerial inputs such as branches, bark and fruits account for only 20 % in temperate deciduous forests and 20–40 % in coniferous forests of total litter fall. Coarse woody debris may contribute 40–60 % of the total detrital biomass.


### Preservation of Plant Fossils

Fossil plants are generally preserved as impressions, permineralisations and organically preserved structures. Most modern plants lack biomineralised tissues; hence their survival relies heavily on preservation of organic remains. These include wood remains, pollen, spores, algae, leaves and propagules. Except for wood, they are rarely preserved as complete plant organs but just as resistant outer coverings (i.e. pollen and spore walls, algal cell walls, cuticle, seed coats and fruit walls). The chemical and physical composition of the plant structures vary, resulting in differences in their susceptibility to decay. Thus, structures that are most resistant, either physically and/or chemically, have the highest organic fossilisation potential.

### Transformation of Plant Cuticles

The cuticle is the external covering on leaf and green stem surfaces and serves to (1) reduce water loss, (2) control gaseous exchange and (3) provides a barrier to fungal pathogens. The cuticle in modern plants is composed of a solvent soluble wax fraction and insoluble matrix. This matrix makes up the framework of the cuticle and is composed of biopolyestercutin and/or an insoluble non-hydrolysable macromolecule cutan. The chemical structure of cutin is well understood; based primarily on C16 and C18 alkanoic acids. All three cuticular components (waxes, cutin, and cutan) are important to the decay-resistant nature of leaves and stems. In addition, the cuticle may also incorporate parts of the outer cell walls of the epidermis, which further contributes to its resistant nature.

The physical and chemical resistance of the cuticle helps preservation in the fossil record. However, in some cases, physically resistant outer coverings fail to survive, even though it is clear from other evidence that the species was present in the past, revealing that physical resistance does not necessarily guarantee fossilisation. Plant structures composed of both lignin and an aliphatic macromolecule are the most likely to be represented in the fossil record. However, the preservation potential of the aliphatic component itself is higher than that of lignin. In the case of the seed coat of *Typha*, cuticular layers containing the aliphatic macromolecule are selectively preserved, whereas thin walled lignified layers are lost upon fossilisation.
Conversely, plant remains lacking both lignin and an aliphatic macromolecule are unlikely to be preserved as organic fossils. Preservation of fossil leaves and cuticles is discussed at greater length later in the chapter as well.

The occurrence of plant cuticles in the fossil record has raised questions about the factors responsible for their preservation and their role in the formation of kerogen. Studies, investigating the chemical composition of cuticles, have concentrated on the resistant part of modern plant cuticles ‘cutan’, which yielded exclusively an aliphatic signature upon pyrolysis, thus being similar to that obtained by analyses of fossil specimens. More recent studies suggest the involvement of aromatic and/or fatty acid components in the overall chemical composition of the resistant part of the modern plant cuticle. Most modern plants whose cuticles have been shown to yield a residue of a non-saponifiable, chemically resistant macromolecule (e.g., *Agave americana* and *Clivia*) lack a fossil record, making investigation of the role of the resistant macromolecule in preservation processes especially difficult.

Fossil plant cuticles occur as minor constituents in coals and coaly shales and as major organic constituents in some organic-rich deposits of lacustrine or deltaic environments. Amongst other techniques, fossil cuticles have been studied using infrared (IR) spectroscopy in combination with pyrolysis–gas chromatography–mass spectrometry (Py–GC/MS). IR spectroscopy is a non-destructive technique for identifying functional groups in chemical structures, and pyrolysis yields chemical compounds formed by thermal degradation of the materials analysed. Both techniques enable analysis of very small samples such as fossil cuticles that are typically available only in milligram amounts. The combination of both methods allows detailed insight into the molecular structure of insoluble macromolecular material.

Mösle et al. (1998) and others at Royal Holloway England used recent and Mesozoic (Cretaceous) *Ginkgo* and conifer cuticles to study the chemical and structural changes during fossilisation and the significance of factors such as systematic affinity, original chemical composition and enclosing lithology that influence the preservation of the fossils, by using samples of similar thermal maturity and geological age. Samples of Recent *Ginkgo biloba*, Cretaceous *Ginkgo* and Cretaceous conifer cuticles (Frenelopsis and Abietites) from different enclosing lithologies and similar thermal maturity of the fossils were analysed by scanning and transmission electron microscopy, Fourier transform–infrared spectroscopy, and pyrolysis–gas chromatography/mass spectrometry. Recent and fossil *Ginkgo* cuticles under SEM revealed sheets, similar in appearance, varying in the abundance and texture of the cuticular papillae. TEM of the Recent *Ginkgo* showed an outer amorphous cuticle layer, a structured middle layer and an inner laminated layer of cell wall. The Cretaceous *Ginkgo* cuticles retained the amorphous layer and a modified structured layer. SEM of Cretaceous *Abietites* and *Frenelopsis* also show preservation of cuticle sheets, but each has distinctive morphology. These conifer cuticles are very thick (TEM), *Frenelopsis* cuticle has remarkable multi-laminar ultrastructure whilst *Abietites* is amorphous. *G. biloba* cuticle consists mainly of the natural polyester, cutin, as revealed by FT–IR and pyrolysis, indicated by an abundance of saturated, unsaturated and hydroxy fatty acids. IR spectra.
of fossil cuticles, like modern cuticles, showed aliphatic C–H, hydroxyl and carbonyl functions. However, in fossils, the carbonyl ester was transformed to carboxylic acid or ketone groups. Pyrolysates of fossils showed phenolic constituents like modern cuticles but loss of cutin fatty acid monomers and an increased prominence of a homologous series of \( n \)-alkene and \( n \)-alkane fragments up to \( n-C_{30} \). Since most recent cuticles, including those of conifers and *Ginkgo biloba*, do not yield a non-saponifiable highly resistant residue, they proposed that organic preservation of fossil species investigated involves the diagenetic stabilisation of chemically-labile aliphatic cutin constituents along with incorporation of waxes. These general chemical modifications characterise all fossil *Ginkgo* and conifer cuticles, irrespective of their enclosing lithology, systematic affinity, external morphology or internal ultrastructural preservation. However, there were clear chemical differences between the fossil samples that may be related to their systematic affinity.

Möslle et al. (1998) investigated material from four North American Carboniferous localities, which have yielded conifer, cordaite and pteridosperm cuticles in co-occurrence. The emphasis was to establish if chemosystematic signatures survive in spite of chemical alteration and, if so, if these are indicative of evolutionary relationships. The cuticles of different Late Carboniferous plants—Cordaitales from Lone Star Lake, Kansas, USA; Cordaitales, and pteridosperms (*Neuralethopteris* and *Eusphenopteris*) from Joggins, NS, Canada; and conifers of the genus *Walchia* from Garnett and Hamilton, both in Kansas, USA. Cuticles were preserved as sheets from larger leaves or as entire cuticle envelopes from smaller, scale-like leaves. Each cuticle has morphology diagnostic of the parent plant group reflected in external appendages and/or external/internal patterns indicative of the arrangement of underlying epidermal cells. Cuticle pyrolysates revealed a highly aliphatic character; however there were differences in the distribution and relative abundance of aliphatic hydrocarbons between the different genera. The pyrograms of the two *Cordaites* and the two *Walchia* were similar to one another but distinct from pyrograms of other seed plant cuticles, including those from the same localities. Hence, cuticles retain some chemosystematic signature.

Comparison amongst the pyrolysis profiles fossil samples showed that *Walchia* and *Cordaites*, both of the conifer/cordaite clade, are as different from each other as either is from *Eusphenopteris* or *Neuralethopteris* (pteridosperms). Indeed the *Cordaites* were more similar to *Neuralethopteris* whilst the *Walchia* are more similar to the *Eusphenopteris*. Thus, although the samples retained a genus-specific chemical signature it did not provide evidence of chemosystematic relationships at higher rank and does not reflect a sister group relationship between cordaites and conifers.

The model of selective preservation of plant cuticles stresses the contribution of the chemically resistant material termed cutan, which was proposed to produce the aliphatic homologous series observed in pyrograms of fossil specimens. These studies of cuticles from Recent conifers and *Ginkgo* showed the rarity of cutan as a significant cuticular constituent as determined by pyrolysis and showed the absence of cutan as chemically resistant material in the tested cuticles. Therefore, additional processes need to be invoked to account for the observed “aliphatic” signatures of
fossil plant cuticles. Possible explanations include: (1) the incorporation of cutin monomers via linkages more stable than carboxylic acid ester and (2) the formation of ether linkages by intermolecular reaction of alcohol and epoxy groups was proposed by Schmidt and Schönherr (1982) and elaborated by Tegelaar et al. (1991). This would certainly engender cutin monomers resistant to acid- or base-catalysed degradation, and capable of yielding hydrocarbon fragments of chain length less than the original (typically 16 or 18 C atom) upon pyrolysis. The generation of fragments of chain length greater than \( C_{30} \) could be explained as a result of the di- or polymerisation of cutin constituents via carbon–carbon linkages along with the chemically stable incorporation of pre-existing chains of extra-cuticular material (in waxes up to 37 C atoms).

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