

Resistant biomacromolecules in the fossil record¹

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SUMMARY

This paper reviews the occurrence of biomacromolecules in fossil plants and animals. The range of techniques used in their release, preparation, microscopy and chemical analysis is described, as is the role of decay experiments in their investigation. The major plant parts that include resistant macromolecules are discussed: algal cell walls, cuticles, spore and pollen walls, propagules, periderm, secondary xylem, and secretions. The chemistry of animal cuticles preserved in the fossil record is briefly considered; they may incorporate macromolecules of plant origin during diagenesis. Some future directions for research are outlined: characterization of sporopollenin, characterization of extant and extinct tissues and organs, chemosystematic investigations, taphonomic processes, investigation of biomacromolecules in animal fossils.

Key-words: biomacromolecule, cuticle, fossil, organic geochemistry, plant, pyrolysis.

INTRODUCTION

The vast majority of plants lack biomineralized tissues, and their substantial fossil record therefore relies on the preservation of organic remains. Plants are comprised of a range of organic materials most of which are highly prone to decay. A variety of tissues, however, has evolved to provide structural strength and protection to the plant organs, and their chemical composition includes macromolecules. These tissues are consequently decay resistant and more likely than others to survive as fossils. In most cases the plant fossil record is, therefore, a result of selective preservation of organs and tissues which contain resistant biomacromolecules. These include algal cell walls (Fig. 1), leaf cuticles (Figs 2, 3c–e), pollen and spores (Fig. 4), seed coats (Figs 3a, b, 7), fruit walls (Fig. 11b–d), periderm (Fig. 11a), wood and resins (Fig 11b–c). The constituent macromolecules vary in their composition, susceptibility to decay, and behaviour during diagenesis. This produces potential biases in the fossil record both within and between organ/tissue categories (see Tegelaar *et al.* 1991; Van Bergen 1994).

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The most abundant biomacromolecules in extant vascular plants are lignins. They are the main contributor to lignites, brown coals and coals. Chemically modified versions of these aromatic biomacromolecules are preserved in fossil woods, seed coats and fruit walls (see De Leeuw & Largeau 1993; De Leeuw *et al.* 1995). Recent work on fossil seeds indicates a much greater variety of natural lignins than had hitherto been recognized (Van Bergen 1994). Macromolecular substances in plant secretions, especially resins, are also preserved in the fossil record, both as isolated amber pieces and *in situ* in plant fossils (Van Aarssen *et al.* 1990, 1994).

Recently, however, a series of resistant, aliphatic biomacromolecules has been documented in both modern and fossil algal cell walls, seed coats, periderm, spores and pollen, and cuticles (see review in De Leeuw & Largeau 1993). These macromolecules are also insoluble in organic solvents, and have a highly resistant nature which confers a high preservation potential. This provides the opportunity to investigate their origin, evolution and diversity through time using fossil material. Furthermore, factors which may control preservation potential can also be investigated both experimentally and by comparative surveys of different fossil preservation states and settings.

Fossilization segregates resistant tissues (which may be difficult or impossible to separate in modern material) thus making them easily accessible for study. Fossil tegmens (Fig. 3b), for example, are easily separated from the rest of the seed coat (Fig. 7) and leaves may consist only of a cuticle envelope (Figs 2b, 3c). For this reason studies of fossils are at the forefront of work on resistant biomacromolecules in plants. Even so, the investigation of the chemical composition of ancient plant material is still at a relatively preliminary stage, although activity in this field is gathering momentum (research on the composition of the organic remains of animals in the fossil record is even less advanced, and is discussed later in the paper). The approaches to such studies are inherently interdisciplinary, and the objectives are several.

1. To determine the degree to which ancient plant materials retain an original chemical signature which might be of chemosystematic significance.
2. To explore the range of biomacromolecules in plants which contribute to structural attributes and protection in life and to decay resistance, hence fossilization potential, after death.
3. To investigate the relationship between chemical and morphological preservation.
4. To understand the processes involved in the decay and diagenetic alteration of plants as they become fossilized.
5. To investigate the possibility that plant macromolecules may be diagenetically incorporated into organic fossils of animals.
6. To assess the nature and significance of the contribution of plant macromolecules to oil and gas generation.
7. To contribute to an understanding of the biosynthetic pathways and biological functions of resistant biomacromolecules in plants and the ways in which these influence (or are influenced by) issues such as plant success, survival, growth environment, pollution resistance, and palatability.

Here we describe the methods used to prepare plant material, both living and fossil, for analysis. We review the different plant organs and tissues that include biomacromolecules in their chemical composition (this approach contrasts with that usually adopted in the geochemical literature where the focus is on chemical compounds, e.g. De Leeuw & Largeau 1993). We consider the preservation of biomacromolecules in animal

cuticles, where diagenesis may lead to the incorporation of chemicals of plant origin. Finally, we consider where future research on biomacromolecules in fossils might lead.

METHODS

The methods used in molecular palaeobotany fall largely within the domain of organic geochemistry. The chemical composition of many modern plants is incompletely known, so the analysis of Recent examples is an important aspect of the interpretation of the fossils. At the least those techniques used to analyse the more resistant constituents of fossils have to be applied to their modern counterparts, so that the results can be directly compared.

Release and preparation

Target tissue must be separated from the plant material to allow analyses of morphologically well defined entities. Cuticles, for example, must be separated from the leaves or stems that they cover in modern plants. Only rarely can this be achieved physically; it is usually necessary to use ammonium oxalate (Nip *et al.* 1986; Tegelaar *et al.* 1991) or chromium trioxide (Kerp 1990). After separation the tissues are extracted sequentially, using ultrasound, with organic solvents. Purification of material for detailed structural elucidation of macromolecules may require vigorous extraction protocols such as those documented by Nip *et al.* (1986) and Tegelaar *et al.* (1989a, 1993).

Fossil plant materials can be released from some sedimentary rocks by simply washing with water or by physical/mechanical means. In other cases chemical treatments such as hydrogen peroxide and/or demineralization with HCl/HF are necessary. These routine palaeobotanical procedures have little or no effect on the chemical composition of the plant material (Van Bergen *et al.* 1994a,b,d; Collinson *et al.* 1994). Simple bleaching or chromium trioxide treatment may subsequently be required to facilitate separation of the different tissues in the fossil. After release fossil materials are subjected to organic extraction procedures comparable with those used to treat modern material.

Microscopy

It is instructive to subject materials that are to be analysed chemically to microscopic analysis (Figs 1–5, 7, 11). Light microscopy, scanning electron microscopy and transmission electron microscopy can be used to document gross morphology and ultrastructure so that resultant chemical interpretations can be related directly to morphology. Only in this way can it be established which material yielded which chemical signature. Microscopy can also aid in monitoring the effects of diagenesis (Fig. 7) and preparation procedures (Fig. 3). Indicators such as vitrinite reflectance (of associated material) and fluorescence can be used to assess the degree to which elevated pressures and temperatures have affected the fossils during their geological history.

Analytical techniques

Here our concern is with resistant biomacromolecules. A range of methods can be applied to their analysis: (i) elemental analysis (the ratio of carbon/hydrogen/nitrogen); (ii) pyrolysis, i.e. the breakdown of the biomacromolecule using heat in an inert atmosphere (Py), with or without gas chromatography (GC) and mass spectrometry (MS); (iii) spectroscopy, e.g. Fourier transform infra-red (FT-IR) spectroscopy or solid

state ^{13}C nuclear magnetic resonance (NMR); (iv) chemolysis, i.e. chemical degradation of the biomacromolecules using specific chemical reagents.

Elemental analysis provides a simple indicator of compositional and therefore structural differences. Pyrolysis techniques are well-established for detailed examination and characterization of the molecular composition of small samples of organic material, particularly those not susceptible to dissolution (Nip *et al.* 1986; Tegelaar *et al.* 1991, 1993; De Leeuw & Largeau 1993; Collinson *et al.* 1994; Van Bergen *et al.* 1994a–d). Pyrolysis techniques provide a ‘fingerprint’ which can be identified by comparison with the results of previous investigations and model compound studies. Some quantitation of the result can be achieved by incorporating a known mass proportion of a standard such as polystyrene into the sample prior to analysis (e.g. Eglinton *et al.* 1991). Alternative methods of quantitation using a low molecular weight compound are under investigation.

Samples may be further analysed using solid state ^{13}C nuclear magnetic resonance (NMR) and chemical degradation. ^{13}C NMR spectra reveal the types and relative proportions of different carbon ‘environments’ present, e.g. aliphatic, carboxyl, carbonyl, aromatic (Hemsley *et al.* 1994). Comparison of single pulse and cross-polarization magic angle spinning (CPMAS) data can determine whether these occur in ‘mobile’ or ‘rigid’ environments (e.g. Gidley & Robinson 1990). FT-IR spectroscopy provides an additional source of structural information by revealing the presence of particular chemical bonds (e.g. C-H, O-H, C-O, C=O) through spectra indicating bond stretching under infra-red irradiation. Chemical degradation techniques may reveal how the products released by pyrolysis are bound within the macromolecular matrix. They include RuO_4 oxidation, which causes lysis of olefinic, ether (after subsequent saponification) and aromatic moieties, among many other methods (e.g. CuO , HI , BCl_3 , BBr_3 , Et_3Si , $\text{Pb}(\text{OAc})_4$). The stable carbon isotope ratios of individual components present in pyrolysates or chemolysates can be further characterized using irm-GC-MS (isotope ratio monitoring-gas chromatography-mass spectrometry).

Decay experiments

The fossilization of plant materials involved at least some decay of the original organism. Chemical analysis of plants at different stages of decay, and under different conditions, can therefore throw important light on the transformations that occur following the death of the plant. Experiments in which plant materials are exposed to microbiological degradation under controlled conditions allow the labile and resistant chemical constituents to be identified. This clearly aids the interpretation of results from fossil specimens and helps to determine the effect of different depositional settings.

PLANT PARTS INCLUDING RESISTANT MACROMOLECULES

There is a wide range of biomacromolecules in modern organisms, many of them in plants. Here our concern is only with those that are particularly decay resistant and therefore have a high preservation potential (Tegelaar *et al.* 1989a), i.e. lignins, tannins, sporopollenins, algaenans, cutans and suberans. A review of the chemistry of these biomacromolecules, which is beyond our remit, can be found in De Leeuw & Largeau (1993). Here we consider the different plant organs and tissues that include biomacromolecules in their chemical composition.

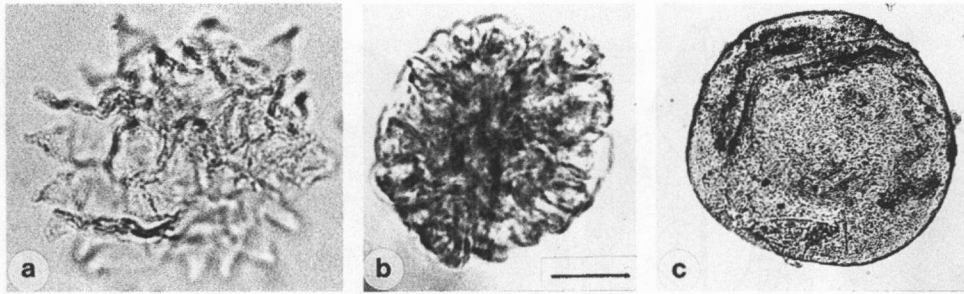


Fig. 1. Fossil algal cell walls composed of the highly aliphatic biomacromolecules named algaenans. (a) *Pediastrum*, (b) *Botryococcus*, (c) *Tasmanites*; (a & b) Chlorococcales, latest Eocene, England; (c) Prasinophyta, Permian, Australia, all transmitted light micrographs (for details see Collinson 1983; Collinson *et al.* 1994). Scale bar represents 10 μm in (a) & (b), 200 μm in (c).

Algal cell walls

Although higher plants are the focus of this review, for completeness brief reference is made here to algae. Algal cell walls (Fig. 1) are common constituents of sedimentary rocks. In some cases they may dominate the rock, as in the oil-shales of Eocene lake Messel, Germany, where the tiny cell walls of *Tetraedron* (Chlorophyta) produced a very fine-grained organic-rich sediment largely responsible for the exceptional preservation of an extensive fossil biota. The nonhydrolysable, insoluble, highly aliphatic substances in algal cell walls are generally termed algaenans (see review in De Leeuw & Largeau 1993). Associated observational and analytical evidence has revealed that they are selectively preserved and that they are major contributors to kerogen, i.e. 'sedimentary organic matter insoluble in common organic solvents' (Tegelaar *et al.* 1989b, p. 3103; see De Leeuw *et al.* 1991).

Cuticles

The major biochemical innovation represented by cuticle arose when plants first spread onto the land in the Silurian (see Selden & Edwards 1989). Cuticle is a specially modified, waterproof, protective covering found all over leaf and stem surfaces. In higher vascular plants the cuticle (or more strictly cuticular membrane) consists of a wax fraction (soluble in organic solvents) and an insoluble matrix. The matrix may consist entirely of the biopolyester cutin, but a highly resistant, highly aliphatic, non-saponifiable biomacromolecule named cutan is present in many plant cuticles [first reported by Nip *et al.* (1986) in the cuticle of modern *Agave* leaves (flowering plant, monocotyledon, Liliales, Agavaceae)]. Nip *et al.* (1986) and Tegelaar *et al.* (1991) used pyrolysis to study both modern cuticles (of 13 flowering plants, two conifers and *Ginkgo*) and fossil cuticles (or seven flowering plants and 11 other seed plants ranging in age from Permian to Miocene). Some of the modern cuticles did not contain cutan and these are very rarely preserved in the fossil record. In contrast, all the fossil cuticles analysed did contain cutan.

Most fossil 'leaves' are in fact cuticular envelopes (Fig. 2b) with (Fig. 2c) or without (Fig. 2b) residual material representing coalified mesophyll. These cuticular envelopes can be so concentrated and abundant as to form leaf-rich shales termed paper shales or paper coals. One such yielded the *Karinopteris* material (Fig. 3c). A cutan-like macromolecule is preserved in the stem cuticle of a Carboniferous lycopsid and in leaf cuticles of four genera of Carboniferous pteridosperms (Collinson *et al.* 1994; Van

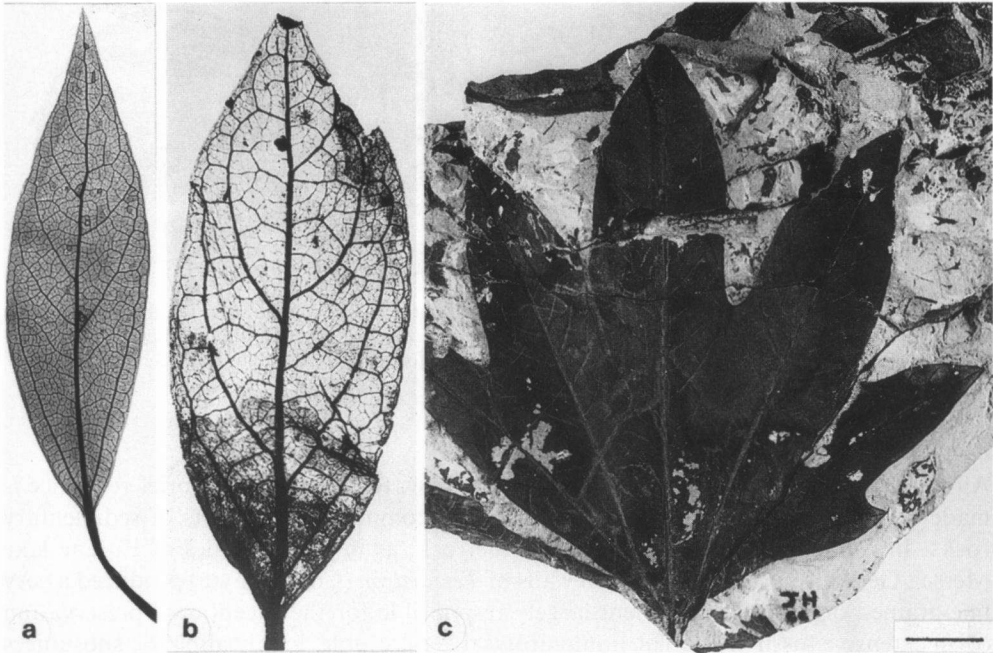


Fig. 2. The role of the resistant biomacromolecules cutan (in cuticles) and lignin (in xylem in veins) in the visual appearance of leaves. (a) Modern leaf of *Populus*, cleared resulting in translucency and similar appearance to fossil in (b), with only cuticular envelope and veins evident; (b) fossil *Diospyros*-like leaf consisting of resistant cuticle and veins only, hence translucency like modern cleared leaf; (c) fossil leaf of the *Macginitia* plant or 'Clarno-plane' (extinct genus of Platanaceae), a typical compression fossil where cuticular envelope still contains coalified mesophyll tissue giving the dark appearance with only major veins clearly evident. Sources: (a) in Jack Wolfe collections; (b) Eocene Australia, illustration courtesy of J. F. Basinger, reproduced with permission from Basinger & Christophel (1985); (c) Eocene, USA, illustration courtesy of S. R. Manchester, reproduced from Manchester (1986) with permission of the publisher, copyright 1986 University of Chicago. All rights reserved. For full references and further details see Collinson (1990). (a, b) transmitted light micrographs with (b) untreated apart from demineralization; (c) reflected light micrograph of hand specimen on rock. Scale bar represents 11.1 mm in (a), 5 mm in (b), 20 mm in (c).

Bergén *et al.* 1994a). One of these pteridosperm cuticles (Fig. 3c) shows preservation of detailed ultrastructural variation (Fig. 3d) whereas another (Fig. 3e) is quite uniform and amorphous under TEM. Cutan-like macromolecules also occur in the tegmen cuticles (Fig. 3b) of seeds (Van Bergén *et al.* 1994b; Van Bergén 1994) and in other thin translucent tissues in propagules (e.g. Fig. 3a) which vary in appearance under TEM. Therefore, there is no immediately obvious relationship between ultrastructure and presence of cutan. Cutans are clearly selectively preserved in the fossil record. They are major contributors to kerogen and, when subjected to appropriate experimental conditions that mimic diagenesis and catagenesis, they yield *n*-alkanes commonly found in oils (Tegelaar *et al.* 1989d).

Recently, Kögel-Knabner *et al.* (1994) showed that the leaf cuticle of the modern conifer *Picea* (spruce, Pinaceae) deviates markedly from other cuticles in containing a lignin-like polymer. N. L. Frewin, M. E. Collinson & J. W. De Leeuw (unpublished) documented another unusual variant in the cuticle of the seagrass *Thalassia* where the pyrolysate resembles that produced by bark (Frewin 1993).

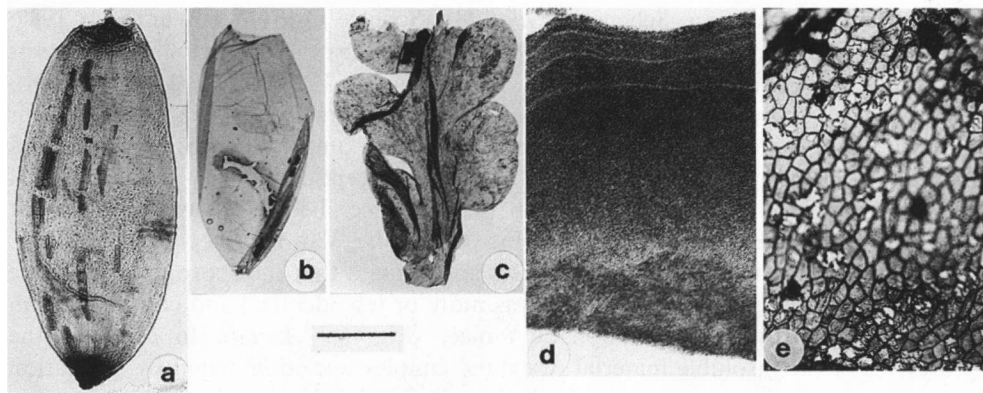


Fig. 3. Fossil leaf and seed cuticles containing the highly aliphatic biomacromolecules named cutans (as released from rock following demineralization, without further treatment). (a) Seed coat of *Typha*, latest Eocene; (b) tegmen of *Stratiotes* seed, latest Eocene; (c) leaf cuticle from portion of a pinna of the fern-like frond of the Carboniferous pteridosperm *Karinopteris*; (d) TS of cuticle from (c) showing preservation of varied ultrastructure including lamellate outer zone; (e) detail of upper cuticle from the fern-like frond of the Carboniferous pteridosperm *Alethopteris*, showing epidermal cell outlines. (a,b,c,e) Transmitted light micrographs; (d) transmission electron micrograph prepared as described in Collinson (1991). (For further details see Collinson 1983, Collinson *et al.* 1994, Van Bergen *et al.* 1994a,b, Van Bergen 1994) Scale bar represents 200 μm in (a) & (e), 1.4 mm in (b), 1.7 mm in (c), 0.17 μm in (d).

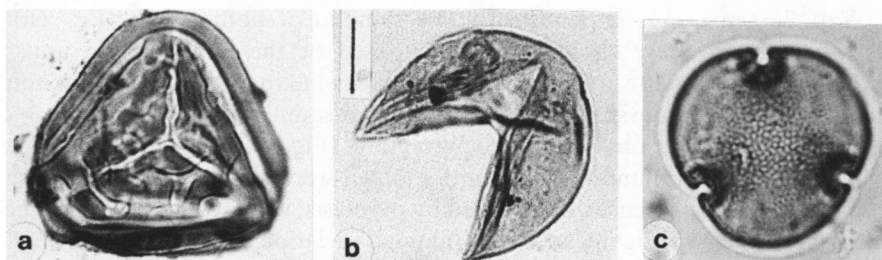


Fig. 4. Typical examples of fossil spore and pollen, the walls of which are considered likely to contain the biomacromolecule sporopollenin thus enabling their preservation as fossils. (a) Fern spore—*Polypodiaceosporites*; (b) Taxodiaceae (conifer) pollen—*Inaperturopollenites*; (c) *Tilia*-like flowering plant pollen—*Intratripopollenites*. Latest Eocene, England. Transmitted light micrographs (for details see Collinson 1983). Scale bar represents 10 μm .

Spore and pollen walls

Spores and pollen are represented in the fossil record only by their resistant walls (Fig. 4). Studies of sporopollenins, the macromolecular constituent of spore and pollen walls, have a long history. Nonetheless, the definitive chemical structure of this resistant macromolecule remains unclear (e.g. De Leeuw & Largeau 1993; Van Bergen *et al.* 1993).

Until recently, it was generally believed that the structural building units of sporopollenin were carotenoids and/or carotenoid esters (e.g. Brooks & Shaw 1968; Shaw 1971). However, later investigations indicated that oxygenated carotenoids do not contribute to sporopollenins (Herminghaus *et al.* 1988; Wehling *et al.* 1989) and that their recognition in early studies must be ascribed to incomplete purification of sporopollenin isolates (e.g. incomplete removal of non-resistant material; for a critical review see De Leeuw & Largeau 1993). Recent studies suggest that there are at least two chemically different types of sporopollenin. In one the main building blocks are

oxygenated aromatics (e.g. Schenck *et al.* 1981; Schulze Osthoff & Wiermann 1987; Herminghaus *et al.* 1988; Wehling *et al.* 1989), while in the other the main building units are thought to be aliphatic in nature (e.g. Guilford *et al.* 1988; Hayatsu *et al.* 1988).

Rarely fossil spores and pollen are preserved in clusters, either as a result of their botanical nature (water fern microspore massulae) or unusual depositional context (stamen clusters). Investigations of morphologically exceptionally well-preserved fossil clusters of water fern spores showed that the insoluble chemical constituent is most likely the highly resistant sporopollenin (Fig. 6a: *Azolla*, Van Bergen *et al.* 1993; P. F. Van Bergen *et al.*, unpublished). Pyrolysis demonstrated that the sporopollenin of the different water fern genera investigated was more or less identical and consisted of an oxygenated aromatic and an aliphatic moiety (Fig. 6a: *Azolla*). In contrast, the pyrolysates of the insoluble material of extant samples were dominated by aromatics. These aromatic moieties were shown to be lost during diagenesis, selectively enhancing the aliphatic moiety in the fossil material. In spite of this chemical alteration during diagenesis, the overall gross morphology of the spore walls remained unaltered.

Additional spectroscopic and chemolytic information (^{13}C NMR and RuO_4 oxidation, respectively) was obtained from pollen clusters from the fossil *Kurtzipites* (primarily composed of pollen walls; Fig. 5a–d), and microspore wall clusters from the fossil water fern *Azolla* (Fig. 5e–g). This suggested that the oxygenated aromatic and aliphatic moieties recognized previously (Fig. 6a: *Azolla*, Van Bergen *et al.* 1993) are actually two different parts of one monomeric unit linked via cross-linking moieties (P. F. Van Bergen *et al.*, unpublished). The samples of both *Azolla* (Fig. 6a) and *Kurtzipites* (Fig. 6b) were shown to be composed of these monomeric units and cross-linking moieties. However, the degree of cross-linking between the monomeric building blocks varied considerably between the two samples.

The chemical difference observed in the pyrolysates (Fig. 6a,b) cannot be explained by diagenetic and lithological influences as both samples were obtained from the same bed at the same site. The differences, as revealed by pyrolysis, were considered large enough to be of chemosystematic significance. The minimal variation between the sporopollenin of the three genera of water ferns (Van Bergen *et al.* 1993) indicates that the chemosystematic distinction is only significant at the level of order or probably higher. In significantly older material (e.g. Carboniferous) the chemical composition is strongly altered, especially when material has been subjected to high temperature and/or pressure during diagenesis. However, some chemosystematic signature is retained (e.g. between lycopsid and seed megaspores from the same horizon; Hemsley *et al.* 1995). Whether the differences recognized are characteristic for pollen versus spores, for flowering plants versus ferns, for seed plants versus pteridophytes, or for subsets of these groups, is as yet unknown (Van Bergen 1994).

Propagules

In contrast to most of the other plant entities discussed here, macromolecular resistant constituents of propagules (e.g. fruits and seeds) have been largely neglected. A few papers have been published about the biopolyester cutin, in the cuticular membranes surrounding fruits (Baker & Holloway 1970; Tegelaar *et al.* 1989c). In addition, Yatsu *et al.* (1983) and Ryser & Holloway (1985) examined the biopolyesters of extant seed coats of cotton (*Gossypium*), and Boon *et al.* (1989) described the chemical and anatomical properties of fossil and extant lignified endocarps (inner fruit walls) of *Nyssa*. Only the physically resistant outer coverings of propagules (seed coats and fruit

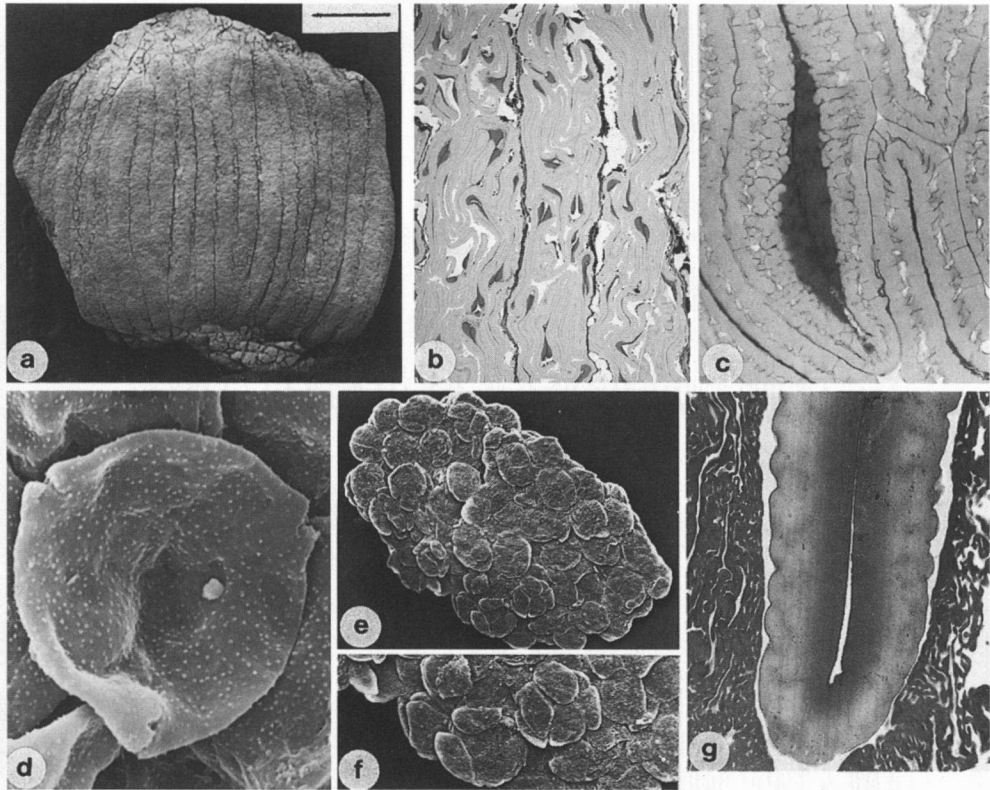


Fig. 5. Clusters of fossil flowering plant pollen and fossil water fern microspores contained in massulae. These large units are amenable to chemical analyses (as are megaspores) and have enabled the chemical nature of the resistant biomacromolecule sporopollenin to be elucidated (Fig. 6). (a)–(d) *Kurtzipites* extinct flowering plant pollen; (e)–(g) *Azolla* microspore massulae; both from Cretaceous/Tertiary boundary, Teapot Dome, Wyoming, USA. (a) Complete pollen-sac cluster; (b) section through several pollen sacs crowded with mature pollen grains; (c) detail of pollen grains in (b); (d) single pollen grain; (e) & (f) microsoral clusters of numerous microsporangia, each containing 6–8 microspore massulae, the initial segregation of which is evident in (f); (g) TS of portion of one microspore exine surrounded by massula filaments (for further details see Collinson 1991, 1992; Van Bergen *et al.* 1993; Van Bergen 1994). (a), (d)–(f) SEM; (b), (c), (g) TEM. Scale bar represents 400 μm in (a), 10 μm in (b), 2.3 μm in (c), 6.3 μm in (d), 670 μm in (e), 333 μm in (f), 0.67 μm in (g).

walls) are normally preserved in the fossil record (Collinson 1980; Chandler 1964). For this reason several studies have been undertaken recently on the biomacromolecules present in extant and fossil propagule walls (Van Bergen 1994; Van Bergen *et al.* 1994b–e). Both chemical and microscopic changes occurring during fossilization were monitored by subjecting most samples to both microscopic (LM, SEM and TEM) and chemical analysis (mainly pyrolysis, as well as solid state ^{13}C NMR and chemolysis using CuO oxidation).

In most cases the propagule walls studied contained a sclerotic outer covering (sclerotic endocarp or sclerotesta; Fig. 7) and a translucent inner layer (translucent testa or tegmen; Fig. 3b). In extant samples these two distinct layers are firmly attached, whereas they are often easy to separate in fossil material. This emphasizes the importance of studying fossil samples in order to obtain insight into the macromolecular constituents of these structures in the modern material.

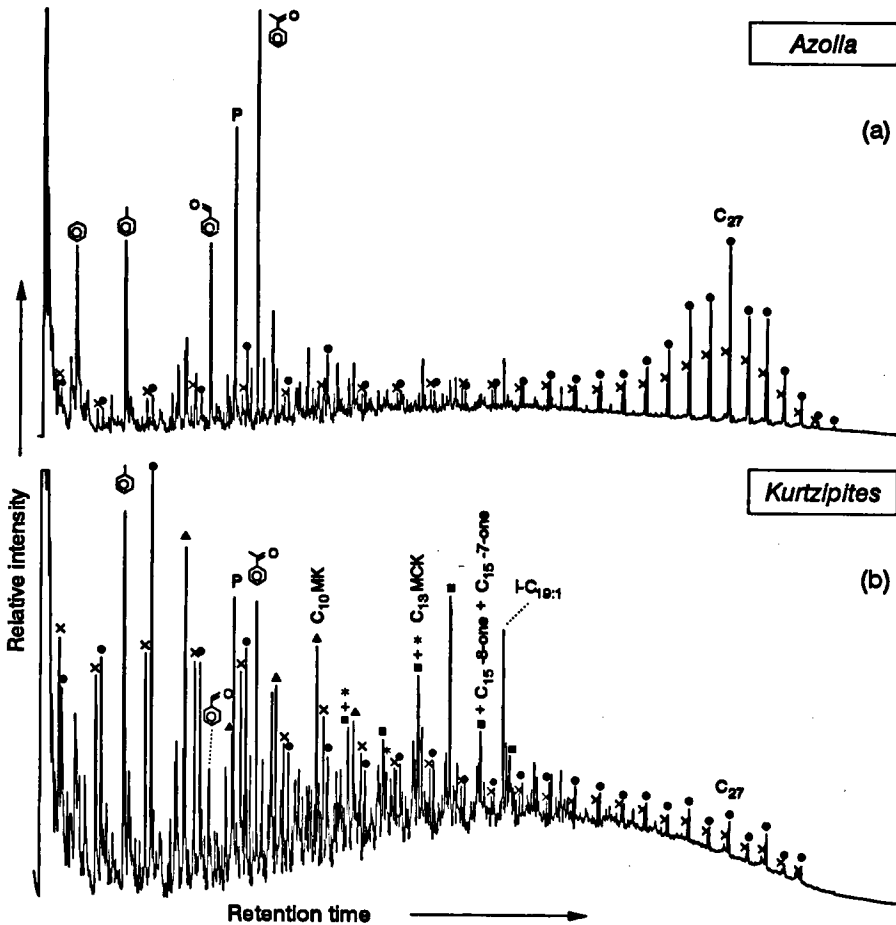


Fig. 6. Py-GC traces (Curie-temperature 610°C) of (a) microspore massulae of fossil *Azolla* (Fig. 5e-g); (b) pollen clusters of fossil *Kurtzipites* (Fig. 5a-d); both Teapot Dome, K/T boundary (Van Bergen *et al.* 1993). ●, *n*-alkanes; x, *n*-alk-1-enes; ▲, 2-alkanones (methylketones; MK); *, 5-alkanones; ■, 6-alkanones; C₂₇, heptacos-1-ene and heptacosane; C₁₀MK, 2-decanone; C₁₃MCK, 6-tridecanone and 5-tridecanone; P, phenol.

The insoluble chemical constituents of all extant sclerotic layers (testa and endocarp), except that of *Nelumbo*, were shown to be lignin-celluloses (Fig. 8a) or lignin-hemicelluloses (Van Bergen *et al.* 1994c-e; Van Bergen 1994). The sclerotestae of the water lily samples analysed contain dicotyledon lignin as revealed by their characteristic pyrolysis products (e.g. Saiz-Jimenez & De Leeuw 1986), guaiacyl (2-methoxyphenols) units (G-units) and syringyl (2,6-dimethoxyphenols) units (S-units). In contrast, the endocarp of the extant *Sparganium* and the whole seed coat of *Stratiotes* (Fig. 8a) contained monocotyledon lignin characterized by the presence of both G- and S-units, in addition to coumaryl (phenols; P) units and, in the case of *Sparganium*, ferulic acid (e.g. Ralph & Hatfield 1991). The biomacromolecule of the extant *Stratiotes* was distinct, however, because of the relatively high amounts of 4-hydroxybenzoic acid (Fig. 8a: Van Bergen *et al.* 1994c). The pyrolysate of the extant seed coats of *Typha* also revealed the typical monocotyledon lignin markers similar to those found in the pyrolysate of the extant *Sparganium*. This was somewhat unexpected because these

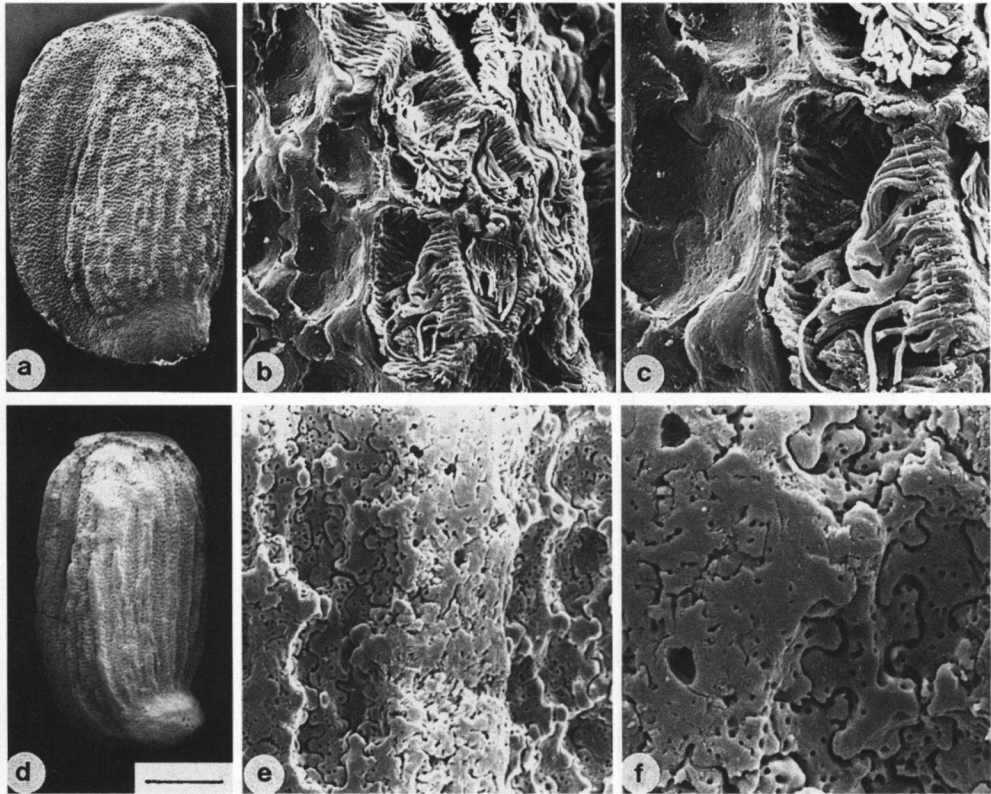


Fig. 7. Sclerotic outer seed coats of fossil *Stratiotes* seeds showing variation in morphological preservation associated with variation in preservation of the lignin biomacromolecule, as shown in Figs 8–10. (a)–(c) The morphology of the seed coat is exceptionally well-preserved including the outer tissues of fine ‘mucilaginous’ cells; (b)–(d) in contrast, the morphology of the seeds is still clear enough to permit generic identification but there have been micromorphological changes including loss of the outer tissue, separation of sclereids and widening of sclereid cell lumina. In both cases the original lignin-type macromolecule has been severely altered to a polyphenol-type macromolecule, in the latter the degree of alteration is extreme. (a),(d) External view of half seed after germination; (b),(c),(e),(f) details of sclerostoma surfaces. Latest Eocene, England (for further details see Hooker *et al.* 1995). All SEM. Scale bar represents 1.7 mm in (a) & (d), 40 μm in (b) & (c) & (f), 50 μm in (e).

seeds (Fig. 3a) have no apparent sclerotic seed coat layers. However, the outermost layer of the *Typha* seed coat is cellular, not merely cuticular (Van Bergen 1994). The extant *Typha* sample also contained abundant aliphatic moieties in its pyrolysate (Van Bergen 1994). (There is additional information on lignins in the section on secondary xylem.)

In sharp contrast to the results from the sclerotic water lily seed coats, the data from the sclerotic fruit wall of the water lily *Nelumbo* revealed an insoluble constituent which was identified as a tannin-polysaccharide (other than cellulose) complex (P. F. Van Bergen *et al.*, unpublished). The exceptional chemical composition of this propagule wall, which lacks lignin, was interpreted as the reason why these propagules are absent in the fossil record (P. F. Van Bergen *et al.*, unpublished). This is in spite of the exceptionally high longevity of the modern propagule (Williamson & Schneider 1993 and references cited therein). Another sclerotic propagule with insoluble constituents of distinct chemical composition is that of *Raphanus*. In this case the biomacromolecule in

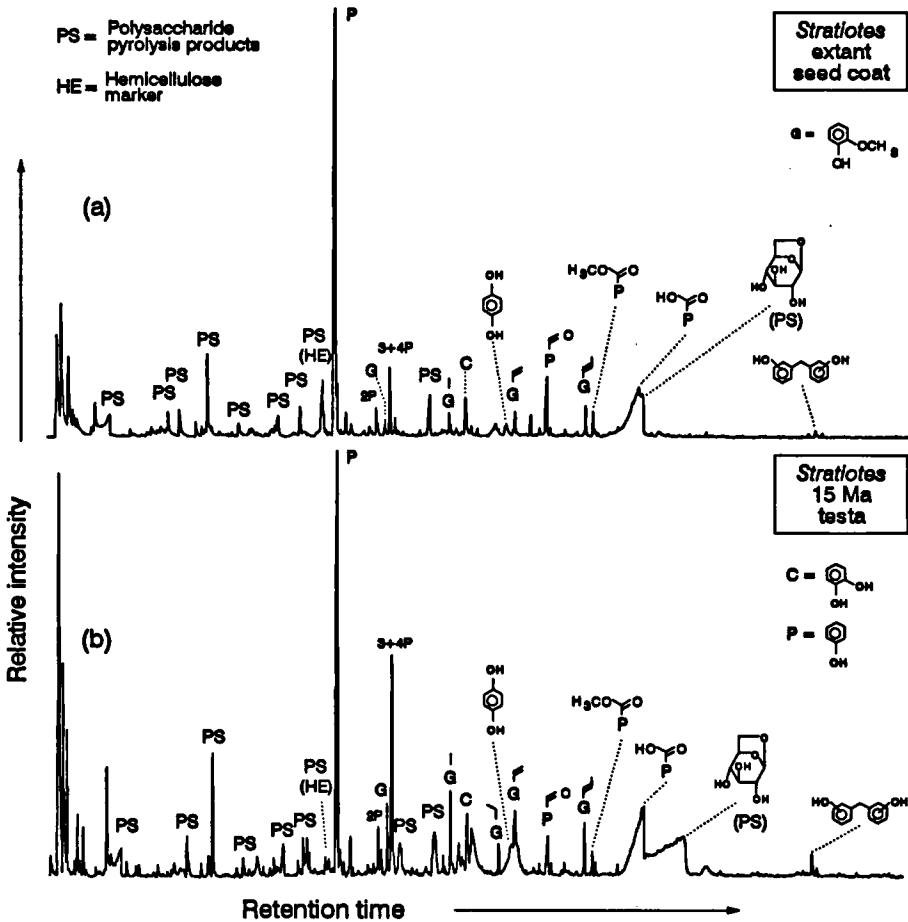


Fig. 8. Py-GC traces (Curie-temperature 610°C) of (a) seed coat of extant *Stratiotes aloides*; (b) testa of the Miocene *Stratiotes kaltennordheimensis* (Van Bergen 1994). 2P, 2-methylphenol; 3+4P, co-eluting 3- and 4-methylphenol.

the seed coat may be responsible (P. F. Van Bergen & R. P. Evershed, unpublished data) for the well-preserved state of other chemical compounds, such as nucleic acids, in desiccated counterparts about 1400 years old (O'Donoghue *et al.* 1994).

The analysis of fossil material revealed that lignin-cellulose complexes were chemically modified during diagenesis whereas the gross morphology and, in most cases, the micromorphology of the sclerotic tissue remained well-preserved (Van Bergen 1994; Van Bergen *et al.* 1994c-e). The extent to which chemical modifications occur is considered to depend on a variety of parameters including depositional setting, and composition of the surrounding matrix and/or overburden. The lignin-cellulose complex of sclerotic testae from subfossil samples (*Brasenia* sp., c. 100 000 years; *Nuphar* sp. c. 2 Ma) was shown to retain a similarity to that of their extant counterparts, particularly the lignin constituent (Van Bergen 1994; Van Bergen *et al.* 1994c). The carbohydrate composition, on the other hand, is altered, especially the more labile hemicellulose (P. F. Van Bergen *et al.*, unpublished). It is noteworthy, however, that samples as old as 15 Ma (Miocene

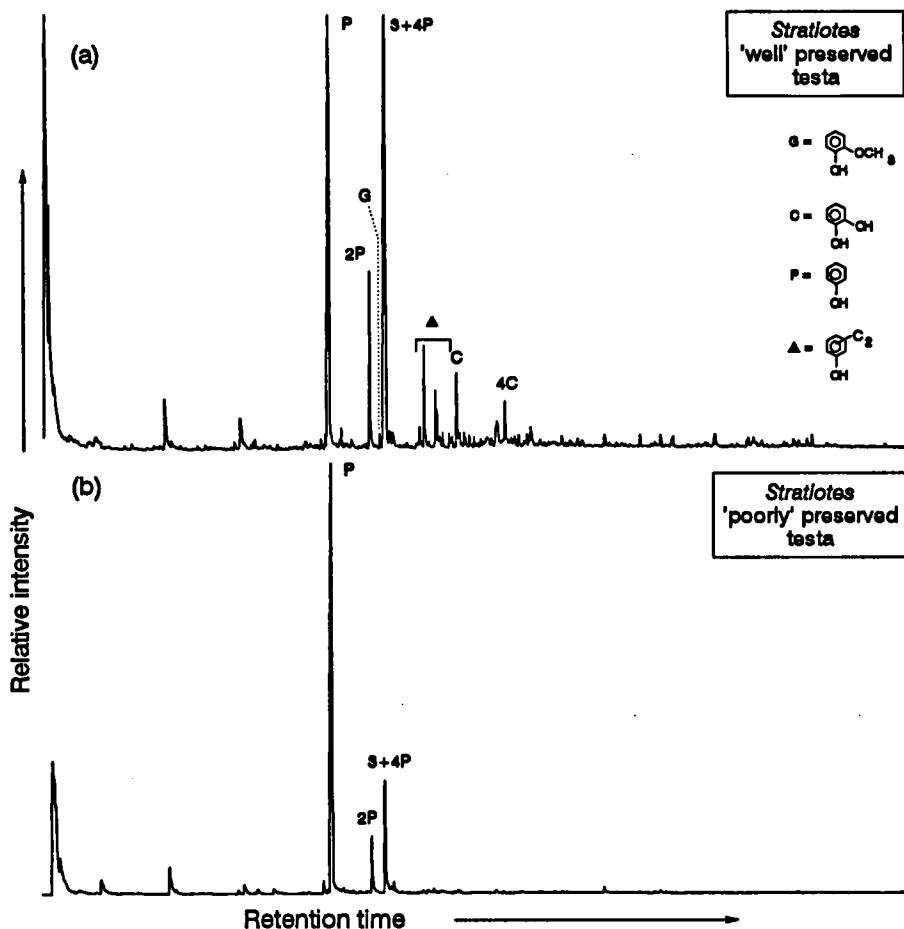


Fig. 9. Py-GC traces (Curie-temperature 610°C) of (a) well-preserved testa of the Eocene *Stratiotes headonensis*; (b) poorly-preserved testa of the Eocene *Stratiotes headonensis* (Van Bergen *et al.* 1994c). 2P, 2-methylphenol; 3+4P, co-eluting 3- and 4-methylphenol; 4C, 4-methyl-1,2-benzenediol.

Stratiotes; Fig. 8b), may still show remarkable similarity in both lignin and carbohydrate composition to extant material (Fig. 8a). In the special case in question, the relatively little overburden at the site was considered to explain the low degree of alteration (Van Bergen 1994). In contrast, biomacromolecules present in older (Eocene) sclerotic samples have undergone more drastic chemical alteration, causing the loss of all carbohydrates as well as severe modification of the lignin biomacromolecule (Van Bergen *et al.* 1994c,d). This led to the formation of 'simple' polyphenolic macromolecules ('well-preserved' *Stratiotes*, Fig. 9a: Van Bergen *et al.* 1994c,d). In the most extreme case pyrolysis of the macromolecule released almost exclusively phenol ('poorly' preserved *Stratiotes*, Fig. 9b: Van Bergen *et al.* 1994c; Hooker *et al.* 1995). The gross morphology of the seed coat (Fig. 7) remained intact, however, even though micromorphological alterations were observed.

Van Bergen *et al.* (1994d) showed that the chemical composition of sclerotic tissue from coarser-grained sediments is generally better preserved than that from fine-grained sediments. Despite chemical alterations during diagenesis, variation between

sclerotic samples of different genera revealed by pyrolysis could still be of use for chemosystematic purposes (cf. 4-hydroxybenzoic acid in the fossil Miocene *Stratiotes*, Fig. 8b; Van Bergen 1994).

Lignin-containing plant remains are generally believed to be the major contributors to coals (Hatcher *et al.* 1994 and references cited therein). The pyrolysates of lignites and brown coals are often similar to those of the sclerotic propagule tissues. Thus, understanding the chemical modifications taking place in these morphologically well-defined entities is of essential importance to understanding the process of coalification. The coalification of sclerotic materials involves the removal of polysaccharides followed by the alteration of the lignin structure. The three-dimensional morphology of the sclerotic layers remains intact (Van Bergen *et al.* 1994c) suggesting that these transformations involve bond-breaking and simultaneous bond-making.

The translucent layers have only been analysed in fossil samples to date (Van Bergen *et al.* 1994b,d,e; Van Bergen 1994). The chemical and microscopic analyses of these samples [tegmens (*Stratiotes*, Figs 3b, 10a), translucent testae and fossil seed coat (*Typha*, Figs 3a, 10b)] clearly showed that these layers are actually cuticles (Van Bergen *et al.* 1994b,e; Van Bergen 1994). The macromolecules recognized in these plant structures all show a highly aliphatic nature, as revealed by the alkanes and alk-1-enes in the pyrolysates (Fig. 10), similar to those recognized in leaf cuticles (Nip *et al.* 1986; Tegelaar *et al.* 1991). However, the chemical differences revealed by pyrolysis (Fig. 10), particularly among monocotyledonous samples, are significant enough to be used for chemotaxonomy at least at family level (Van Bergen 1994). It appears, based on the available data and analyses of subfossil translucent layers [*Brasenia* sp. c. 100 000 years; and *Nuphar* sp.], that these macromolecules remain chemically unaltered upon fossilization (P. F. Van Bergen *et al.*, unpublished). Moreover, variation in lithology does not affect the chemical composition (Van Bergen *et al.* 1994d). The apparent stability of these translucent layers, which contain aliphatic biomacromolecules, contrasts strongly with the sclerotic layers, which contain aromatic biomacromolecules (lignin) (see above). Comparison between fossil (Figs 3a, 10b) and extant *Typha* samples showed that only the two layers that revealed an aliphatic signal upon pyrolysis became fossilized. This is another clear demonstration of the selective preservation of highly aliphatic macromolecules (see Tegelaar *et al.* 1989b).

Periderm

The term periderm is used to refer to the outer layers of woody plants (bark of branches, stems and roots). The chemical composition of periderm has only been investigated in a small number of taxa. Tegelaar *et al.* (1995) demonstrated that the periderm of *Betula* contains a non-hydrolysable polymethylenic type macromolecule called suberan, which is similar to cutan. This suberan macromolecule is thought to be a major constituent of suberinite, the name given to fossilized outer bark tissue that occurs as a maceral of coal (Tegelaar *et al.* 1995). A similar macromolecule occurs in the periderm of a Carboniferous arborescent lycophyte (Fig. 11a) (Collinson *et al.* 1994). The presence of a highly aliphatic macromolecule (suberan) in periderm versus lignins in wood has important implications in studies of decomposition, peatification and coalification. The chemistry renders the former an 'oil-prone' tissue whilst the latter is not. There is a tendency to assume that tissues composed of thick-walled cells would have been lignified in extinct plants. The initial results suggest that this is not true for

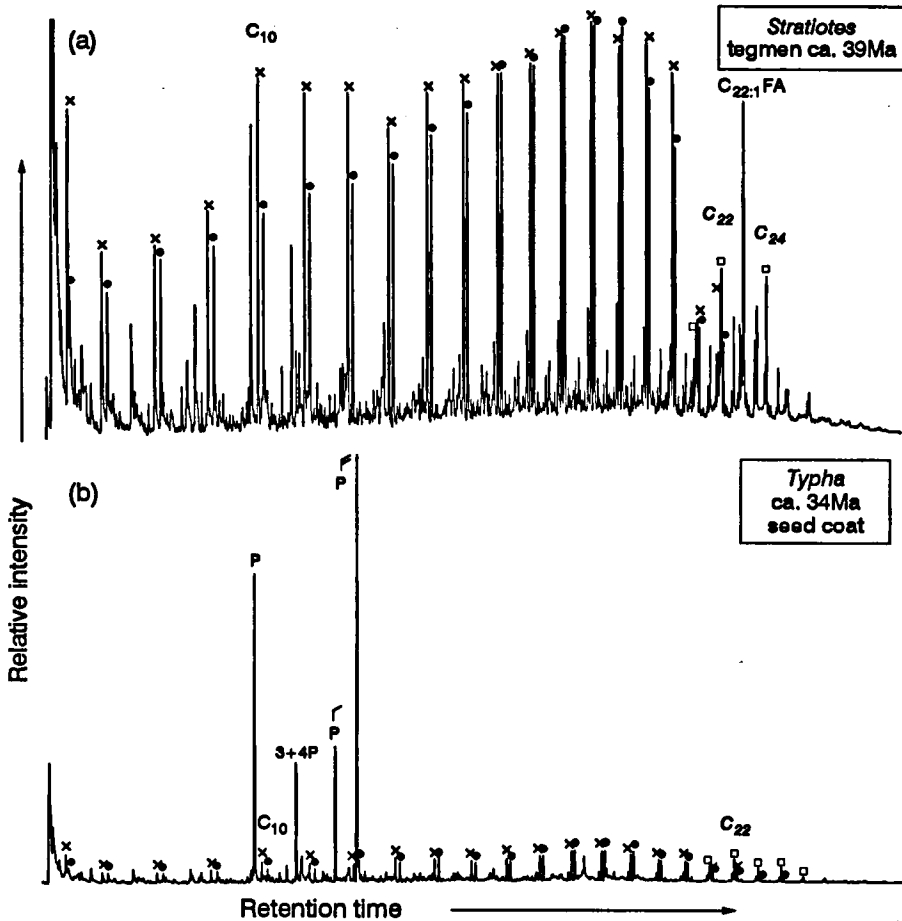


Fig. 10. Py-GC traces (Curie-temperature 610°C) of (a) tegmen of the Eocene *Stratiotes headonensis*; (b) seed coat of the Eocene *Typha latissima* (Van Bergen 1994). P, phenol; 2P, 2-methylphenol; 3+4P, co-eluting 3- and 4-methylphenol; ●, *n*-alkanes, x, *n*-alk-1-enes; □, methylketones; C₂₂, 2-docosanone; C₁₀, dec-1-ene and decane; C_{22:1}FA, docosenoic acid.

the main tissue of structural support (periderm) in arborescent lycophytes. This has implications for studies of plant biomechanics as well as for the oil-prone nature of 'woody' coals.

Secondary xylem

The cell walls of secondary xylem vessels in the woody tissues of both gymnosperms and angiosperms are composed primarily of lignin-cellulose complexes. The distribution of lignins, however, is much wider; they occur in nearly all vascular plants. The decay-resistant properties of this biomacromolecule, more than any other, account for the abundance of plant material in the fossil record. There are three distinct types of lignin (Sarkanen & Ludwig 1971; see De Leeuw *et al.* 1995). Gymnosperms are characterized by guaiacyl units, whereas angiosperms contain guaiacyl as well as syringyl units (Sarkanen & Ludwig 1971; Saiz-Jiminez & De Leeuw 1986; Ralph &

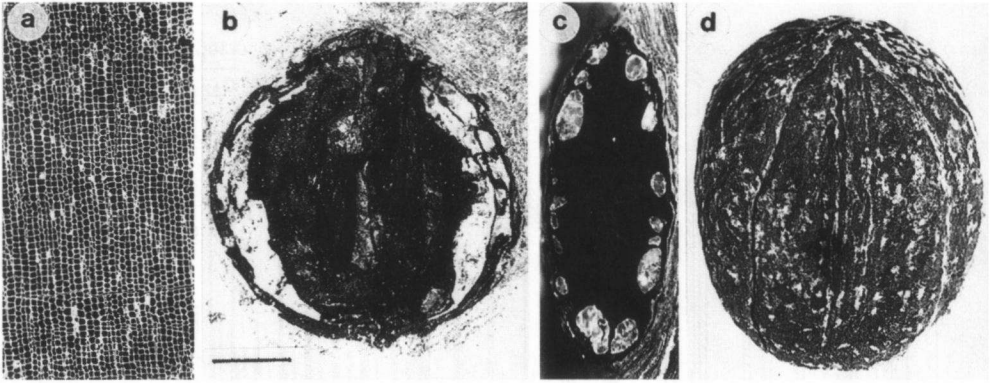


Fig. 11. (a) Fossil lycophyte periderm tissue which contains a highly aliphatic biomacromolecule similar to suberan from modern periderm: *Diaphorodendron*, a Carboniferous arborescent lycophyte, specimen prepared as a coal ball peel and photographed using transmitted light with dark field illumination (for further details see Collinson *et al.* 1994). Scale bar represents 1 mm. (b)–(d) Fossil fruits of mastixoid Cornaceae which contain resin *in situ* in secretory canals. (b), (c) canals (contents pale in illustration) in LS and TS respectively; (d) an entire fruit. This particular resin contains macromolecules of the polysesquiterpenoid type with the sesquiterpenoid cadinane skeleton as their basic structural unit. This composition was previously thought to occur only in members of the Dipterocarpaceae. (For further details see Van Aarssen *et al.* 1994.) All reflected light micrographs. Scale bar represents 2.5 mm.

Hatfield 1991). Amongst the angiosperms the lignin of monocotyledons and legumes contains both the guaiacyl and syringyl units in addition to *p*-hydroxyphenyl units (e.g. Ralph & Hatfield 1991). The proportion of these different types of lignin in marine sediments allows the source of organic material (e.g. terrestrial versus marine) to be estimated. Diagenesis, however, may heavily alter lignin in sedimentary rocks (Van Bergen *et al.* 1994c,d) depending on the nature of the lithology, and the amount of overburden. Lignin is a major contributor to some kerogens. It is also considered to be the main precursor for vitrinite (Hatcher *et al.* 1994). There is insufficient space to treat the very substantial literature on lignin here (see reviews in De Leeuw & Largeau 1993; De Leeuw *et al.* 1995).

Secretions

In addition to plant tissues, plant secretions can be preserved in the fossil record. Most of these are resins (Fig. 11b–d) (Anderson *et al.* 1992; Van Aarssen *et al.* 1990, 1994), but insoluble tannins (Wilson & Hatcher 1988) and rubber (Mahlberg & Störr 1989) are occasionally found. Fossil resins are subdivided into three main types based on their macromolecular constituents (e.g. Anderson *et al.* 1992). However, analyses of botanically/anatomically well-described samples is a prerequisite in order to avoid erroneous interpretation of the origin of the fossil resin based solely on chemical analyses (Van Aarssen *et al.* 1994; Van Bergen *et al.*, in press; for a review see Anderson & Crelling, in press).

THE PRESERVATION OF ANIMAL CUTICLES IN THE FOSSIL RECORD

Shells, bones and teeth, the remains of tissues that are biomineralized in life, comprise the vast majority of animal fossils. Tissues that lack a biomineralized

skeleton, on the other hand, are normally easily degraded and leave no trace. This simple distinction between 'hard' and 'soft' tissues, however, conceals a wealth of complexity. 'Soft' tissues range from decay-resistant recalcitrant material like the sclerotized (i.e. strengthened by cross-linking; see Muzzarelli 1977) cuticles of some arthropods to labile tissues like muscle that are degraded very rapidly. Labile tissues are actually far more commonly fossilized than is generally realized but, in order to be preserved, they must be replicated in authigenic minerals (commonly calcium phosphate) at a very early stage before details of the morphology decay (e.g. Briggs *et al.* 1993). Soft tissue mineralized in this way has not been systematically explored as a possible source of original organic material (DNA has been unsuccessfully sought: D. M. Martill, personal communication); it has normally been assumed to be absent. More recalcitrant tissues, on the other hand, are known to survive as organic material (reviewed by Butterfield 1990). Cuticles of arthropods, for example, have been isolated by acid preparation of a range of marine and terrestrial sediments, from rocks at least as old as Cambrian and Silurian respectively (Butterfield 1990, 1994; Jeram *et al.* 1990), although these have yet to be analysed. The organic skeletons of graptolites, isolated by similar means from Palaeozoic sediments, have been a vital source of data on their fine structures (e.g. Crowther 1981). However, these ancient cuticles have been largely ignored so far by organic geochemists. They are insoluble in organic solvents and are generally assumed to have been altered by diagenetic changes into macromolecular material that is stable in the sedimentary record (see Allison & Briggs 1991). The methods used in their investigation are similar to those described above for plants.

The most decay resistant animal tissues are those that are 'structural' in function, such as cuticles and jaws. Although they may be biomineralized with calcium salts, in many cases the organic material is simply strengthened by sclerotization. In addition to the relatively abundant examples of arthropod cuticles and graptolite periderm, organic sponge skeletons, scolecodonts (the jaws of polychaete worms), and squid pens may be organically preserved. Organic preservation may also be a common feature of exceptional morphological preservations, so-called Konservat-Lagerstätten (Butterfield 1990).

Arthropods are a major component of marine communities and they represent a significant proportion of biomass in the oceans (they are also an important element of terrestrial communities). There is evidence of the preservation of fossil arthropods as an essentially *organic* residue in a range of environments from marine to non-marine (Butterfield 1990, 1994; Briggs & Clarkson 1989; Shear & Kukalova-Peck 1990). This is a reflection of the properties of the sclerotized or tanned chitinous organic exoskeleton (Muzzarelli 1977). A preliminary study of decay in the shrimp *Crangon* in sea-water inoculated with bacteria demonstrated that chitin is selectively preserved (Briggs & Kear 1994; Baas *et al.* 1995). Preliminary analysis of well preserved fossil shrimp cuticles from the Carboniferous of Scotland and Jurassic of Bavaria (Baas *et al.* 1995), using pyrolysis, reveals little similarity to fresh or decayed cuticle. The pyrolysate of the fossil cuticle reveals a homologous series of alkanes and alkenes (Fig. 12) indicating a substitution of chitin by more resistant organic matter. Further research is necessary to determine the nature of the diagenetic changes that had led to a kerogen-like substance rich in aliphatic biomacromolecules, but it is likely that the source is plant material (Baas *et al.* 1995).

The record of Lower Palaeozoic planktic communities is dominated by extinct colonial organisms known as graptolites (Phylum Hemichordata). Even though the

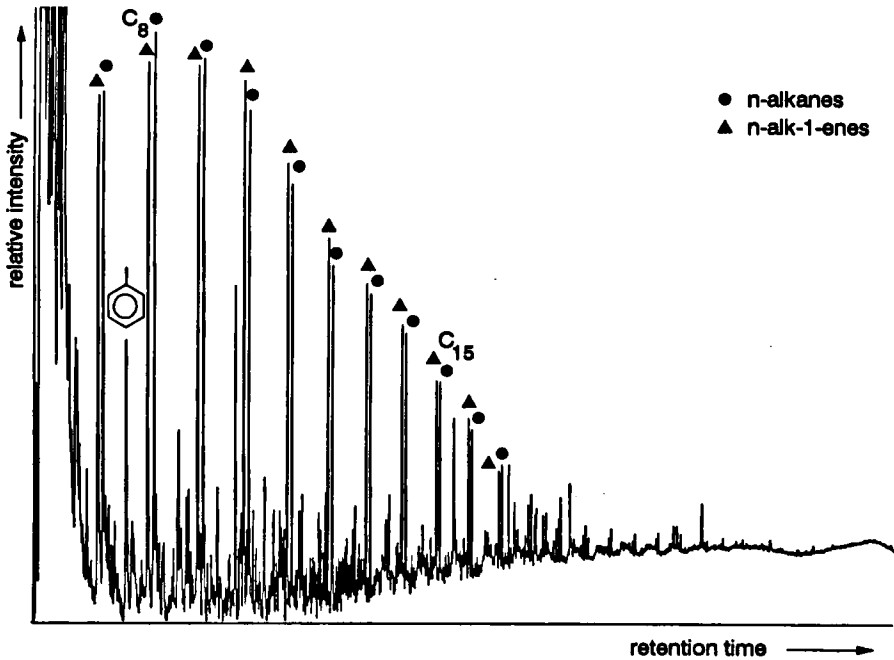


Fig. 12. Py-GC trace (Curie-temperature 610°C) of a cuticle sample of the shrimp *Pseudotealliocaris* from the Carboniferous at Willie's Hole in southern Scotland (reprinted from Baas *et al.* 1995, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington OX5 1GB, UK).

skeleton was not biomineralized, their remains are enormously abundant, particularly in deeper water mudstones, to the extent that 'graptolitic shales' is a familiar lithological descriptor. Although the graptolites became extinct at the end of the Palaeozoic, they have close living relations in the living pterobranchs (*Rhabdopleura*, *Cephalodiscus*) with a cuticle (periderm) that is essentially identical morphologically. On the basis of comparisons of the ultrastructure of the periderm in the living and fossil forms (Towe & Urbanek 1972; Crowther & Rickards 1977; Crowther 1981), their composition has been interpreted as essentially proteinaceous.

Decay experiments and observations on *Rhabdopleura* demonstrated that the periderm and stolon are highly resistant to decay, remaining intact for months while the zooids decay in days (Briggs *et al.* 1995). Pyrolysis gas chromatography (Py-GC) and pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) of the periderm confirmed that proteinaceous organic matter is a major constituent of the periderm of this living form (Briggs *et al.* 1995) (Fig. 13a). Although some amino acid analyses of graptolites have proved positive (Foucart & Jeuniaux 1966), more recent tests (see Crowther 1981, p. 23) have failed to confirm the presence of protein in graptolite periderm. Py-GC-MS analysis of graptolites from the Silurian of Cornwallis Island in Arctic Canada and the Ordovician *Viola* limestone of Ohio confirmed that the protein making up the original periderm could not be detected (see Towe 1980). The trace, like that of the fossil shrimps, suggested diagenetic replacement with aliphatic biomacromolecules (Briggs *et al.* 1995) (Fig. 13b). In this case the source cannot have been higher plants, although algal cells walls are a possibility.

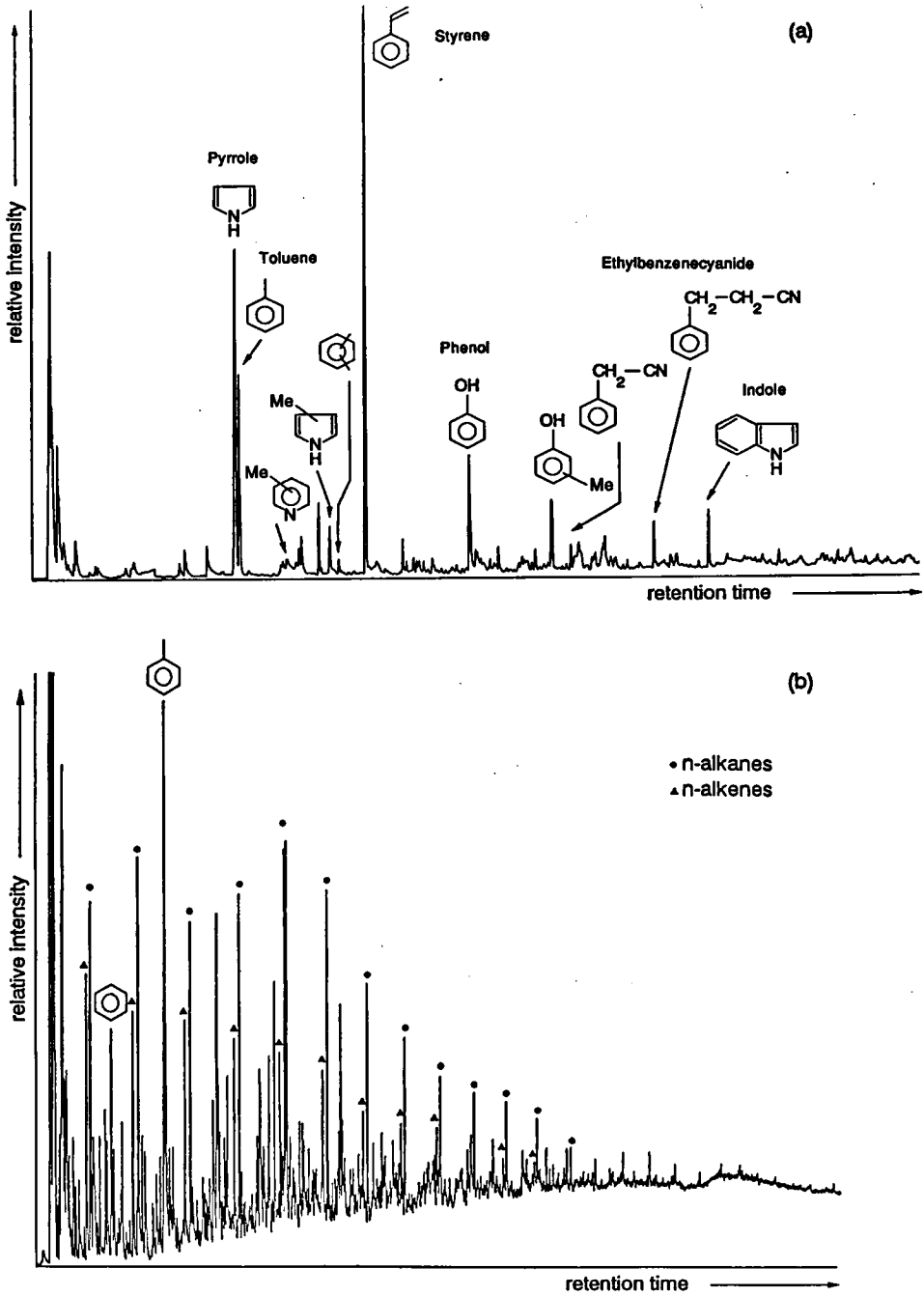


Fig. 13. Py-GC traces (Curie-temperature 610°C) of: (a) the periderm of the extant hemichordate *Rhabdopleura*—the identity of the major pyrolysis products are indicated by their chemical structures (the highest peak, styrene, is a contaminant introduced during work up procedures); (b) the periderm of the Ordovician graptolite *Amphigraptus* sp. from the Viola Limestone Formation of Oklahoma (from Briggs *et al.* 1995).

FUTURE DIRECTIONS

There is enormous scope for future work on resistant biomacromolecules in several directions. Some of the more obvious targets for research are as follows.

Characterization of sporopollenin

The chemical composition of sporopollenins, the resistant constituents of spore and pollen walls, is still a matter of debate after almost 100 years of research! Combined microscopic, spectroscopic, chemolytic and pyrolytic techniques need to be applied to a range of well-characterized fossil spores and pollen wall material from different ages which have undergone relatively little diagenetic alteration. Ideally, genera or species which have close extant counterparts and a long fossil record should be investigated, to trace variation and/or transformation through time.

Characterization of extant and extinct tissues and organs

Although our present understanding of resistant biomacromolecules in plants is very limited, they are clearly much more variable than previously thought. Analyses of biomacromolecules must be combined with microscopic studies of ultrastructure, so that the anatomical location of the molecules detected can be identified. A survey of the macromolecules present in cuticles is needed in order to establish if chemical distinctions are between cuticles from different organs (seeds, leaves, etc.) or from different taxa (families, genera, etc.). The macromolecular components of cuticles may reflect evolutionary history, systematic relationships, environmental influence, leaf form and structure, or other factors. The unusual cuticle of the seagrass *Thalassia*, for example, might be related to its submerged marine habitat, but there is no equally obvious possible explanation for the lignin-like polymer in *Picea* cuticle compared with those of *Amentotaxus*, *Sciadopitys* or *Ginkgo* (three other non-flowering, seed plant, trees) all of which have mixed cutin/cutan composition (Tegelhaar *et al.* 1991). Chemical variation in cuticles will influence many factors of geological and biological significance, e.g. preservation potential and contribution to kerogen. There has been no systematic survey of cuticles from any one plant group, vegetation type, geological period or preservational category. At present we have only minimal understanding of the variation in resistant biomacromolecules within them, but it is clearly much greater than previously thought.

The translucent layers in propagules, which are morphologically similar to cuticles, show the largest diversity in chemical composition within these organs (Van Bergen 1994). Thus, a systematic survey of cuticular layers in fossil and extant fruits and seeds is necessary to examine the level at which they are distinct. The function of the translucent layer, containing a resistant, highly aliphatic macromolecule similar in nature to cutan, inside a layer containing a lignin–cellulose or lignin–hemicellulose, also remains to be unravelled.

Chemosystematic investigations

The chemical variation among the small number of monocotyledon fruits and seeds analysed indicates a large potential for chemosystematic investigations of both the lignin and the highly aliphatic biomacromolecules (Van Bergen 1994). Chemical diversity within monocotyledon lignins seems to be greater than the overall variation known within gymnosperms and dicotyledons combined. A survey of the macromolecular composition of various lignin-containing tissues (vascular and sclerenchymatous tissues, seed and fruit

walls) from different monocotyledonous genera, families and orders is required so that the implications for monocotyledon phylogeny and systematics can be considered.

Taphonomic processes

The factors controlling the preservation of biomacromolecules are poorly understood. Observations on seeds (Van Bergen *et al.* 1994b–d), for example, suggest that highly aliphatic macromolecules, like cutan, are largely unaffected by diagenesis or enclosing lithology whilst lignin-like macromolecules are strongly affected. Tegelaar *et al.* (1991) showed bias against cuticles lacking cutan in the fossil record. Variation in, and relative abundance of, resistant biomacromolecules in plants and their constituent parts has a major impact upon fossilization potential. This is exemplified by the water lilies. Their seed coats are normally composed of lignin-celluloses, and are common in the fossil record (Collinson 1980). *Nelumbo* propagules, on the other hand, are not known as fossils because the fruit wall lacks lignin and is composed of a more readily degradable tannin–polysaccharide complex (P. F. Van Bergen *et al.*, unpublished). Similarly, the chemical composition of lignin in sclerified outer layers is heavily altered, whereas the aliphatic macromolecules, in the translucent layers, remained intact. This suggests that fruit wall and seed coat layers containing resistant highly aliphatic macromolecules have a higher preservation potential compared with those composed of aromatic macromolecules (Van Bergen 1994). Fruits and seeds lacking both lignin and a highly aliphatic macromolecule will probably not be preserved as fossils. The biases resulting from such differences must be considered in the course of investigations based on the occurrence of plants in the fossil record, such as studies of plant evolution, ancient climate (leaf physiognomy), vegetation, and animal/plant interactions.

Biomacromolecules in animal fossils

The nature of organic remains of animals found in the fossil record is much less well known than that of plants. Further investigation is required to establish to what extent the decay-resistant macromolecular remains (e.g. cuticles) of different animals retain a diagnostic signature through degradation and diagenesis. Preliminary studies indicate that diagenetic replacement is an important process in their preservation. The source for that replacement remains to be determined and, specifically, which if any plant group might be involved. Likewise, the extent to which the degree of morphological detail that survives reflects the chemistry of the fossil is unknown. Finally, we do not know whether or not animal remains contribute significantly to kerogen.

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