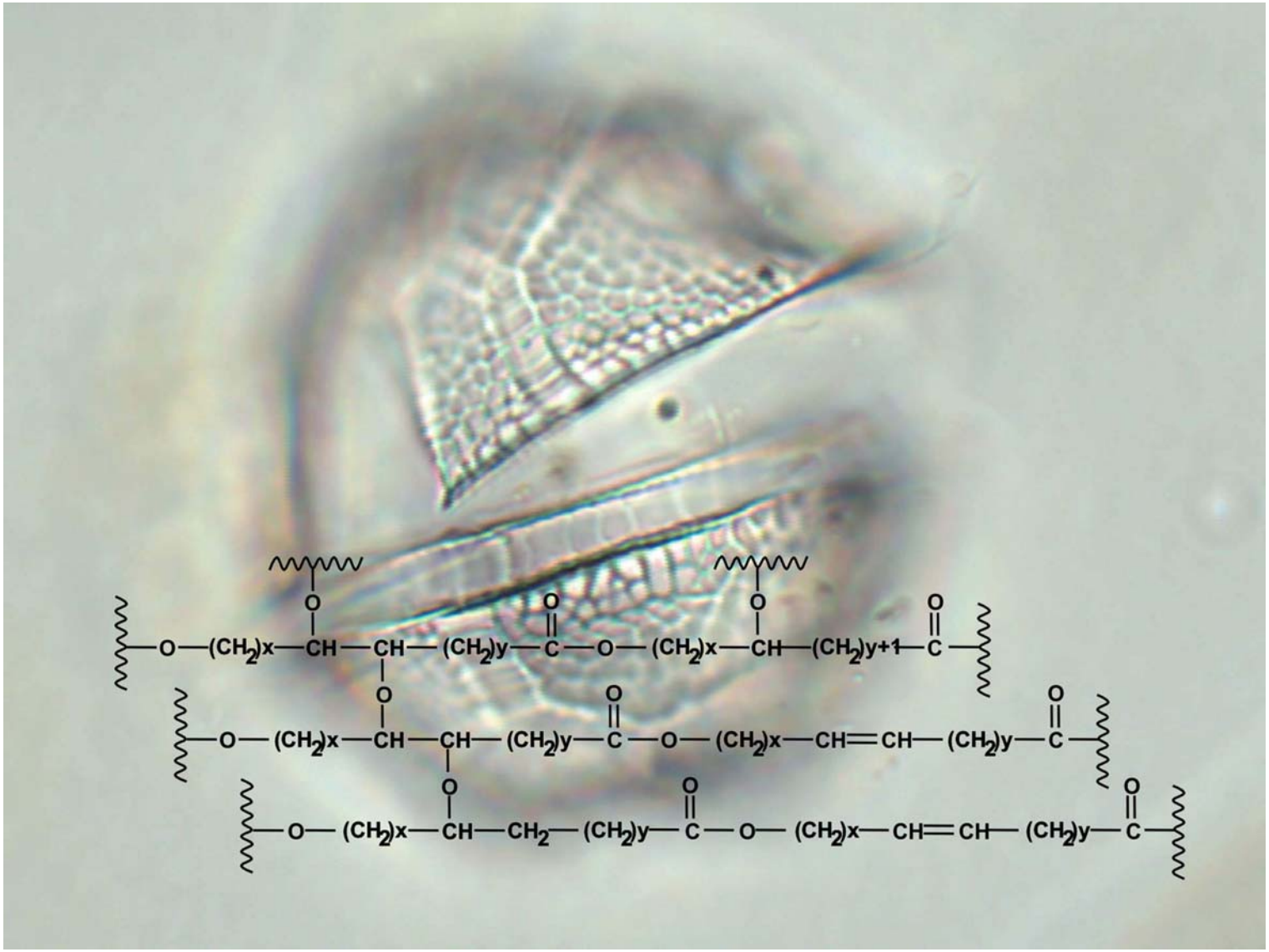

Photo. Theca of a freshwater dinoflagellate with a general chemical structure of chlorophyte algaenan (Photograph: G. Versteegh).

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Biomacromolecules of algae and plants and their fossil analogues

Jan W. de Leeuw[1,2,3,*], Gerard J. M. Versteegh[1,2,4] and Pim F. van Bergen[5]
*[1]Royal Netherlands Institute for Sea Research, 1797 SZ 't Horntje, Texel, The Netherlands; [2]Organic Geochemistry, Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584CD, Utrecht, The Netherlands; [3]Palaeoecology, Faculty of Biology, Budapestlaan 4, 3584CD, Utrecht, The Netherlands; [4]Hanse Wissenschaftskolleg, Lehmkuhlenbusch 4, 27753, Delmenhorst, Germany; [5]Shell Global Solutions International, Badhuisweg 3, 1031 CM, Amsterdam, The Netherlands; *Author for correspondence (e-mail: deleeuw@nioz.nl)*

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Abstract

A review of our current understanding of resistant biomacromolecules derived from present and past algae and higher plants is presented. Insight in the nature of recent and fossil macromolecules is strongly hampered by the difficulties in obtaining the material in pure and unaltered form. For the extant material, avoiding artificial condensation and structural alteration as a result of chemical isolation and purification of biomacromolecules requires constant attention. To date, several types of sporopollenin seem to occur. One type is characterised by oxygenated aromatic building blocks, in particular *p*-coumaric acid and ferrulic acid. The other type is thought to consist predominantly of an aliphatic biopolymer. In this review it is concluded that extant sporopollenin consists of the aromatic type, whereas the aliphatic component of fossil sporopollenin is due to early-diagenetic oxidative polymerization of unsaturated lipids. The cuticles of most higher plants contain the aliphatic biopolyester cutin. Additionally, cuticles of drought-adapted, mostly CAM plants, seem to contain the non-hydrolysable aliphatic biopolymer cutan. Only a very few algae are able to biosynthesize resistant, (fossilisable) cell walls: some Chlorophyta, Eustigmatophyta and Prasinophyta produce the aliphatic biopolymer algaenan. Some Dinophyta are also capable of producing algaenan cell walls. Additionally, some taxa produce highly resistant cyst-walls with a high proportion of aromatic moieties. For the morphologically well-preserved fossil material, contamination by organic particles other than the target taxon is hard to eliminate and can contribute to either the aliphatic or aromatic signal. Furthermore, post-mortem migration of aliphatic moieties into, and their condensation onto the macromolecule might occur, e.g. by oxidative polymerization. These phenomena hamper the evaluation of the aliphatic signature of fossil plant material and may for example explain the preservation of initially cutin-based cuticles or non-algaenan containing algae. The extent to which migration and *in situ* formation of aromatic moieties plays a role in modifying resistant algal macromolecules, notably under elevated temperature and/or pressure conditions, still remains an open question.

Introduction

To extend our understanding of present-day and future natural and anthropogenic climate change, detailed reconstructions of palaeo-environments (with limited or no human factor) and their changes through geological time are crucial. Such reconstructions may be based on instrumental,

historical and so-called proxy data. Instrumental data are reliable, but are very limited in time, i.e. at the most 200 years BP. Historical data can be retrieved for millennia but become less reliable with time. Ancient reconstructions rely on proxy data from dated sediment cores, ice cores or fossils. 'Direct' proxies concern environmental entities which are measured as such in the geological record, e.g. measurements of pCO_2 in air bubbles in ice cores. 'Indirect' proxies are entities analysed from sediments, ice cores and fossils formed during the time in question and transfer functions enable these entities to be used to reconstruct past environment and climate. Indirect biomarker proxies should be stable, difficult to degrade or mineralise and have locked-up environmental parameters such as temperature, salinity, humidity, redox potential, etc. Even in the case of partial degradation or mineralisation of biomarkers, ratios of biomarkers may serve as indirect proxies provided that the rate of partial diagenesis of the biomarkers making up the ratio is the same (e.g. Müller et al. 1998; Versteegh et al. 2000; Schouten et al. 2002).

To evaluate existing proxies or to develop new proxies based on organic compounds derived from algae and higher plants, a much better understanding of the origin, the structure, the function and diagenesis of specific low- and high-molecular-weight organic matter is crucial. This chapter therefore focuses on several aspects of molecular structures of resistant biopolymers in algae and higher plants and their fossilised counterparts and can be considered as an extension and up-date of a recent review by van Bergen et al. (2004).

Sporopollenin

Studies concerning the chemical structure of extant and fossilised spore- and pollen-walls have a long history. These studies have been reviewed recently by van Bergen et al. (1995, 2004) and by de Leeuw and Largeau (1993). Based on these reviews and the references cited therein it has been concluded that several types of extant sporopollenins of a variety of ferns, conifers and angiosperms may occur. Although no systematic chemical studies are known regarding relations of these structures with haploid or diploid sporophytes, or about different spore/pollen-wall layers and the different ways different plant groups produce spores and pollen.

One type is characterised by oxygenated aromatic building blocks, in particular *p*-coumaric and ferulic acid. The other type is thought to consist predominantly of an aliphatic biopolymer of unknown structure (e.g. Guilford et al. 1988; Hayatsu et al. 1988; Domínguez et al. 1999). Whether or not two or even more different types of sporopollenins exist – and the extent to which both types may occur in the same pollen-grain or spore, in separate layers or mixed – is not clear and difficult to determine because a multitude of different, incomparable methods have been used to separate the spore- or pollen-walls from the contents of the spores and pollen consisting of lipids, proteins, polysaccharides, etc. In virtually all cases this separation has been performed chemically, sometimes under very harsh conditions. The chemical methods applied may alter the structure of sporopollenin, may remove specific parts of the sporopollenin or may not be complete in removing non-sporopollenin constituents. This problem is approached by assuming that during burial and diagenesis sporopollenin behaves conservatively and that other non-sporopollenin constituents are selectively degraded and mineralised. This assumption is justified on the basis of the presence of perfectly morphologically preserved spore- and pollen-walls in sediments without microscopically recognisable remains of the contents of spores and pollen. In other words it was assumed that diagenetic processes represent the best (geo)chemical pathway to purify sporopollenin.

However it must be noted that the diagenetic 'purification' of sporopollenin probably depends on the preservation conditions. Yule et al. (2000) demonstrated through micro-FTIR that the spores of *Lycopodium clavatum* go through various phases of chemical degradation characterised by an initial relative increase in aliphatics followed by a FTIR signal more dominated by an aromatic signal. Especially oxidative conditions might affect the final chemistry of the remaining material as illustrated by Gabarayeva et al. (2003) who demonstrated that by successive chemical treatments starting with a relatively mild glacial acetic acid followed by oxidative potassium permanganate degradation isolates the exine of pollen but progressively erodes it specifically with the duration of the treatment. Though the permanganate

conditions may not have an equivalent under 'natural' conditions it illustrates that the sporopollenins investigated by Gabarayeva et al. (2003) can be regarded as inhomogeneous on a molecular level probably with respect to polymerisation grade of the monomeric components. So the degree of diagenetic purification and degradation, together with the wide variety of analytical techniques and samples analysed over time results in a extremely blurred insight into the actual chemistry of sporopollenin. Furthermore, there is no elaborate information on the effect of climatic conditions on the chemistry of sporopollenin, making it difficult to interpret the chemical data obtained from fossil materials solely.

To date, however, virtually all fossilised spore- and pollen-walls analysed consist of both aromatic and highly aliphatic moieties as revealed by pyrolytic and spectroscopic data (e.g. Schenck et al. 1981; van Bergen et al. 1993, 1995, 2004, subm.), even if their recent counterparts are almost exclusively of the aromatic type. To illustrate this phenomenon the pyrolysates of extant and fossil *Salvinia* megaspores are compared (van Bergen et al. 1993; Figure 1). In this particular example the extant megaspore material, exine and perispore, was dissected from mature plants and extracted extensively with organic solvents to remove low-molecular-weight organic matter, thus representing one of the very few extant spore wall isolations without extensive chemical treatments.

The gas chromatogram of the pyrolysate of the extant spore wall consists almost exclusively of peaks representing aromatic compounds that can be produced from a polymeric form of *p*-coumaric acid (see hereafter). Aliphatic compounds, i.e. C_{16} and C_{18} fatty acids (FAs), are present as minor compounds in the pyrolysate. It is not clear whether these aliphatic compounds originate from sporopollenin or represent membrane or other lipids associated with the material. In sharp contrast the pyrolysate of the fossil megaspore

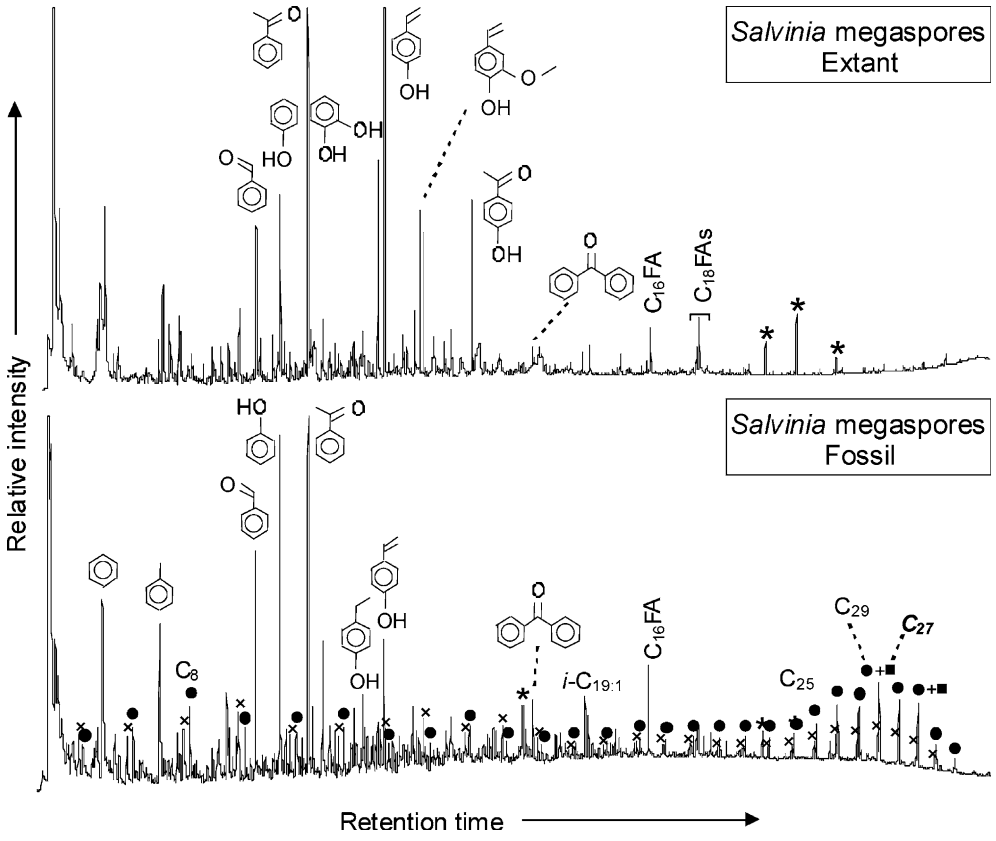

Figure 1. Comparison of pyrolysates of extant and fossil *Salvinia* megaspores. x = *n*-alk-1-enes, ● = *n*-alkanes, ■ = *n*-methylketones, * = contaminants (van Bergen et al. 1993).

wall material yields a strong aliphatic signal as indicated by the homologous series of alkane/alkene doublets. A number of aromatic compounds similar to those recognised in the modern material are still present, in particular, acetophenone and 4-vinylphenol; the latter most probably is a pyrolysis product of *p*-coumaric acid.

To explain the clear and sometimes dominant occurrence of aliphatic constituents in these and other fossilised spore- and pollen-walls it has been assumed that the corresponding or related extant sporopollenins do contain a (very) small portion of aliphatic building blocks that are selectively preserved at the cost of the aromatic constituents, i.e. cinnamic acids such as *p*-coumaric and ferulic acid, during burial and diagenesis. However, it cannot be excluded that the aliphatic constituent represents an aliphatic geopolymer produced during burial and diagenesis from low-molecular-weight lipids such as plant waxes (cf. Collinson et al. 1998) consisting of saturated and unsaturated hydrocarbons, alcohols FAs or from membrane lipids, consisting of saturated and unsaturated FAs, or, most likely, from unsaturated lipids present in the original spores and pollen that have become attached to the original sporopollenin serving as a matrix through oxidative cross linking.

Strong circumstantial evidence of such an oxidative cross linking of low-molecular-weight lipids is presented by a recent study of Versteegh et al. (2004) through a very detailed analysis of a dominantly present aliphatic constituent in morphologically very well preserved fossil algae (dinoflagellates). These algae did not contain a microscopically recognisable cell wall, thus indicating that the aliphatic constituent could not represent algaenan, a biopolymer present in several algae (van Bergen et al. 2004 for a review) and this paper. Based on extensive and detailed microscopical, chemical and spectroscopical analyses of these preserved dinoflagellates, Versteegh et al. (2004) had to conclude that a 'post-mortem polymerisation' of FA moieties from phospholipids, glycolipids or glycerol esters originally present in the cytoplasm by means of cross linking through ether bonds had taken place. Analogous to the migration and condensation of aliphatic lipids described for cavities in coals (Zhang et al. 1993), migration of additional aliphatic moieties from the surrounding environment taking part in the polymerisation process forming the preserved dinoflagellates can not be excluded either. Further detailed evidence for this oxidative cross-linking during diagenesis is described by Kuypers et al. (2002), in a study of Cretaceous black shales. In that study it has been evidenced that a substantial part of the kerogen of these shales consists of ether-linked isoprenoids derived from archaeal membranes as indicated by advanced pyrolysis GC-MS studies in combination with chemical degradation using RuO_4 oxidation. Furthermore, post-mortem formation of an ether cross-linked aliphatic biopolymer been observed in fossil arthropod cuticles (Briggs et al. 1995; Stankiewicz et al. 2000). In this case, the original chitin-based cuticles had been preserved very well morphologically, whereas the chitin was completely replaced by a full aliphatic non-hyrolysable geopolymer. Moreover, there are other indications that this oxidative lipid polymerisation during burial and diagenesis has been underestimated. For example, the oxidative polymerisation of triacylglycerols is a long known process, well known from the drying of linseed oil-based paints triggered by the autooxidation of unsaturated FAs (e.g. Blom, 1936; Fjällström et al. 2002), causes problems in vegetable oil storage and their use for diesel fuel engines (e.g. Srivastava and Prasad 2000) and is easily induced in the laboratory by keeping vegetable oil in sunlight (Versteegh et al. 2004). Finally, the absence of cutan in living *Ginkgo* suggests that fossil *Ginkgo* cuticles originate from oxidative cross linking of the less resistant, saponifiable organic entities originally suggested to be cutin (Mösle et al. 1997), later believed to be derived from cuticular lipids (Collinson et al. 1998).

The above clearly illustrates that the assumption that well-preserved fossil spore- and pollen-walls are highly representative for the chemistry of extant sporopollenin may be invalid, although further studies including FTIR and ^{13}C NMR spectroscopy are required to substantiate this. During burial and diagenesis the original biopolymer can change and/or newly generated geopolymers may be generated and may become closely associated with the original biopolymer or may even completely replace the original biopolymer without a change in morphology. Thus, these types of processes also indicate that chemical treatments of extant spore- and pollen-walls to separate sporopollenin from the cytoplasm constituents are rather problematic and have to be kept in mind

when interpreting results of sporopollenin studies based on the chemical separation of sporopollenin.

In a very recent study (Boom 2004) isolated megaspore walls of *Isoetes killipi* C. Morton were treated relatively mildly by sulphuric acid to remove proteins and polysaccharides and after extractions to remove low-molecular-weight lipids. The resistant material obtained was analysed using a series of complementary analysis methods such as Direct Temperature-resolved Mass Spectrometry (DT-MS), Curie-point pyrolysis-GC/MS (Py-GC/MS) and Fourier Transform-IR (FTIR). The data showed that the sporopollenin of these spores consists of polymerised *p*-coumaric acid because the analysis data were very similar to those of a synthetic *p*-coumaric acid-based dehydrogenation polymer (DHP) (Figure 2). The only difference between the synthetic polymer and the sporopollenin was explained by a small additional contribution of ferulic acid moieties in the *Isoetes* spore walls. The chemical treatment used in this particular case had not produced an aliphatic polymer.

Based on this and earlier results (e.g. Wehling et al. 1989; Mulder et al. 1992) and on re-inspection of other literature data concerning the oxidised aromatic type sporopollenin it may be concluded that sporopollenin consists of DHP type polymers based on *p*-coumaric acid and some ferulic acid, predominantly through ether linkages at the alpha, beta and C_4 carbon atoms and that the aliphatic constituent in fossilised spore- and pollen-walls is not part of the original

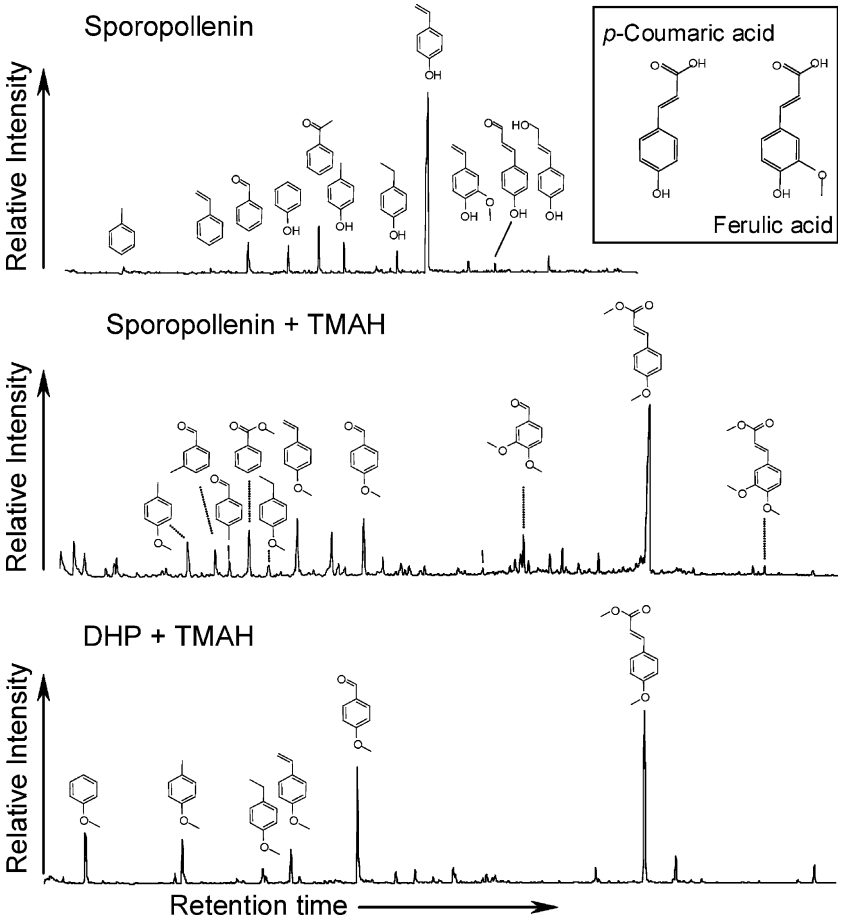

Figure 2. a. Pyrolysate of *Isoetes killipii* megaspore walls. b. Pyrolysate of *Isoetes killipii* megaspore walls with tetramethylammonium hydroxide (TMAH) coating. Upon thermolysis the TMAH methylates the oxygen radicals preventing secondary reactions of the pyrolysis products. c. Pyrolysate of a synthetic, *p*-coumaric acid-based, dehydrogenation polymer (DHP). The similarity of this pyrolysate with that of the *Isoetes killipii* megaspore walls confirms the high proportion of *p*-coumaric acid in the latter. (Adapted from (Boom 2004).

sporopollenin structure. Its highly variable presence and signature in many fossil spore- and pollen-walls may be due to the co-occurrence of the aromatic constituent representing the original sporopollenin, i.e. polymeric *p*-coumaric acid present as such or transformed during diagenesis, and an allochtonously-derived aliphatic component, i.e. a diagenetically produced geopolymer as indicated above. Depending on its availability, this aliphatic component may be derived from the entity itself and/or, from the outside, i.e. directly from the sediment.

This hypothesis implies that the relative proportion of *p*-coumaric acid or, more generally, cinnamic acids or their diagenetic counterparts present in fossil spore- and pollen-walls can not be used straightforwardly as a proxy for UV-B exposure as has been suggested earlier (Rozema et al. 2001, 2002). It is, however, interesting to investigate whether ratios of cinnamic acids or even the degree of esterified *p*-coumaric acid over ether-bound *p*-coumaric acid, can be used as UV-B proxies.

Cutin and cutan

The dominant, sometimes exclusive, presence of a non-hydrolysable aliphatic polymer in well-preserved and morphologically recognisable fossil cuticles led to the assumption that such a polymer occurs as a biopolymer labelled cutan, in a variety of extant plant cuticles (Figure 3; de Leeuw and Largeau 1993; van Bergen et al. 2004). This cutan should not be confused with cutin, a long and well-known biopolyester present in almost every plant cuticle (see hereafter). In several cases, however, the non-hydrolysable aliphatic polymer thought to represent the preserved and selectively preserved biopolymer cutan was only present in the fossil cuticles and not in the extant counterparts consisting of cutin only (Mösle et al. 1997; Collinson et al. 1998). As is the problem with sporopollenin (see above) the genesis of an aliphatic geopolymer replacing the cutin in the original cuticles has to be assumed (cf. Tegelaar et al. 1991; Collinson et al. 1998). In this particular case the most likely candidates to be transferred diagenetically into an aliphatic polymer are cutin (cf. Tegelaar et al. 1991) and/or leaf waxes (cf. Collinson et al. 1998), since these aliphatic constituents are part of the cuticle or very closely associated with it, respectively. Pyrolysates of fossil cuticles consist mostly of homologous series of alkanes and alkenes with carbon chain lengths up to C_{30} to C_{35} (Figure 3) indicating that leaf waxes are the preferred

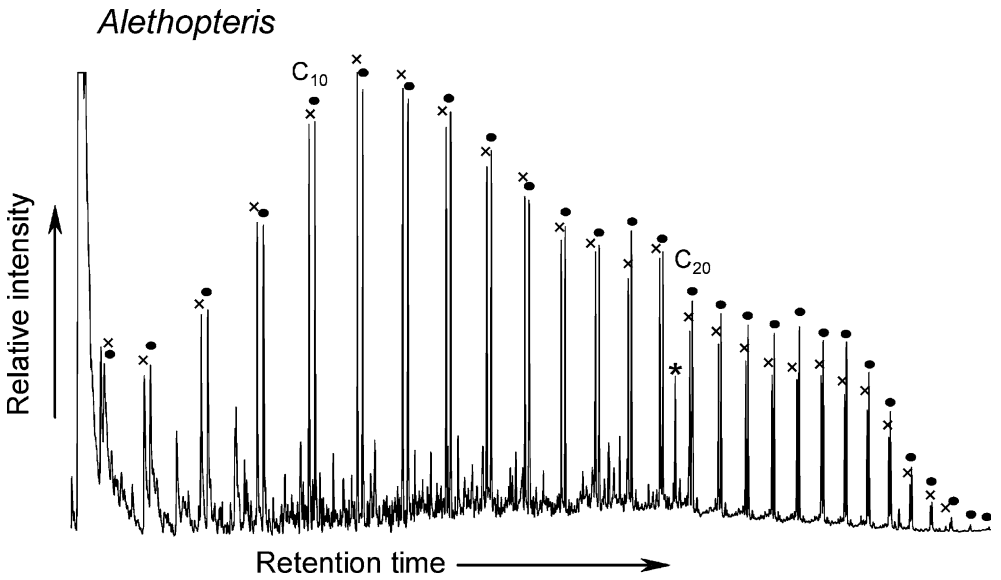

Figure 3. Pyrolysate of *Alethopteris lesquereuxi* pinnule cuticle (Carboniferous). x = *n*-alk-1-enes, ● = *n*-alkanes, * = contaminants (after Collinson et al. 1994).

candidates since the FA building blocks of cutin are C_{16} and C_{18} saturated and unsaturated FAs (e.g. Holloway 1982).

Once again, the problem is whether or not there exists a non-hydrolysable biopolymer cutan next to the biopolyester cutin in extant cuticles, also taking into consideration that chemical treatments performed to purify cutan from the cuticle matrix may lead to artefacts similarly to those mentioned above for sporopollenin. Until recently, rigourous studies of extant cuticles showed that the biopolymer cutan is indeed present in the cuticle of *Agave americana* and *Clivia miniata*, but that the presence of cutan previously reported in a number of other plant cuticles could not be confirmed (Collinson et al. 1998). Very recently however, cutan has been reported as a significant component of the cuticles in drought-adapted, mostly CAM plants (Boom 2004). Although these cutans were isolated chemically by extractions and acid- and base treatments no artificial aliphatic polymer was produced since other, non-drought-adapted plant cuticles treated identically, thus serving as blanks, yielded no cutan. Boom et al. (2005) speculate that the presence of cutan in the thick cuticles of these CAM plants serves as a physiological and chemical adaptation to survive drought conditions. Further studies have to show whether the presence of cutan in fossil plants cuticles can be used as indicators, i.e. proxies, for drought. These data imply that the selectively preserved biopolymer cutan must be discriminated from the diagenetically produced aliphatic geopolymer in fossil cuticles.

Several studies have indicated the presence of cinnamic acids, in particular *p*-coumaric and ferulic acid, as constituents of isolated extant and fossil cuticles from leaves and seeds from several plants (e.g. Deshmukh et al. 1964; Kolattukudy 1981; Holloway 1982; van Bergen 1994; McKinney et al. 1996; Collinson and van Bergen 2004; van Bergen et al. 2004). These aromatic components may be derived from the cell wall of the epidermis directly underlying the cuticle. Further studies have to reveal if these aromatic components play a role in the shielding of UV-B and if they can serve as proxies of UV-B irradiation in the palaeo-environment using well-dated fossilised cuticles (Rozema et al. 2001, 2002).

Resistant algal biomacromolecules

Micro-algae are diverse and abundant in aquatic environments. Most of them have no, or only very limited preservation potential. Nevertheless, a diverse and rich micro-algal fossil record of aquatic palynomorphs exists. This record is an important source of information for solving stratigraphic, palaeoenvironmental (e.g., palaeoclimatological) and evolutionary questions. For this purpose, almost solely the morphological characteristics of the fossils have been used, whereas their potential at the molecular level hardly has been exploited. As a result our knowledge of the structure, chemical diversity, function and diagenesis of the cell walls of algae is still very fragmentary. Improving this knowledge addresses directly the formation, transport and degradation of organic matter, which is central to our understanding of biogeochemical cycles, the formation of petroleum, gas and coal and the history of life and its environment. Below, an overview will be given on our current knowledge of cell wall biomacromolecules from micro-algae and their fossil geomacromolecular analogues.

Cell walls from extant micro-algae

Only the biomacromolecules of cell walls that are considered to fossilise will be discussed here. For cell walls of extant algae for which fossils may be unknown this implies that they resist the methods that palynologists have used to isolate fossil palynomorphs from sediments. In organic geochemical practice this means that the walls of extant algae must be able to resist base and acid hydrolysis. However, this criterion does not seem to hold since several fossil palynomorphs do not resist such harsh hydrolytic treatments.

There has been considerable confusion on the relation between wall ultrastructure and the occurrence of such 'fossilisable' algal walls. The walls of extant and fossil algae often consist of several layers. For the Chlorophyta, the presence of a trilayered (trilaminar) outer wall is often associated with the presence of hydrolysis resistant walls. However, there is no strict relationship between both features. Species that lack a hydrolysis resistant wall may have a trilaminar wall and *vice versa* (for a discussion see, de Leeuw and Largeau 1993).

Furthermore, the different methodologies of isolating the walls of extant algae have led to considerable confusion on the chemical wall structure. This is largely related to artificial polymerisation of the cell contents induced by some isolation methods (Brunner and Honegger 1985; Gelin et al. 1997; Allard et al. 1998). Isoprenoids (e.g. carotenoids), which are highly abundant in many algae, easily become incorporated in these artefacts with the result that isoprenoids have been claimed to be important building blocks of resistant algal cell walls (e.g. Burczyk 1987a, b; Derenne et al. 1996; Kokinos et al. 1998). Despite these problems, there are also early studies e.g. on *Botryococcus braunii* (Berkaloff et al. 1983) and *Tetraedron minimum* (Goth et al. 1988), suggesting that such walls are highly aliphatic, i.e. consist of non-cyclic carbon chains which have been cross-linked. Careful re-examination, with more recent technology avoiding the co-analysis of condensed cytoplasm (e.g. by breaking the cell walls prior to chemical treatment), (Brunner and Honegger 1985; Allard et al. 1998; Blokker et al. 1998a) did not confirm this but instead demonstrated either the absence of a resistant wall or the presence of a highly aliphatic cell wall composed of unbranched, but cross-linked carbon atoms, termed algaenan. Consequently, claims in the older literature that a given taxon produces resistant macromolecules, should be evaluated with due consideration. Although not all algae have been subject to such re-examination yet, all evidence suggests that resistant isoprenoid-based biomacromolecules are not produced by modern plants (algae and higher plants).

To date, only two biochemical pathways seem to lead to resistant algal walls (and resistant plant macromolecules in general): (I) the acetate–malate pathway (leading via lipid-synthesis to algaenans, cutin and cutan), and (II) the phenylpropanoid pathway (leading to e.g. sporopollenin, and probably dinosporins). However, we have to acknowledge that only a very limited portion of the living and fossil algae has been studied for the presence and composition of acid and base resistant cell walls. Most species belong to the Chlorophyta and most are from fresh water environments. The marine realm, with the richest and longest fossil record, has hardly been exploited. New pathways leading to fossilisable biomacromolecules may therefore still await discovery. Despite this bias it is clear that only a few living taxa are capable of producing such walls (Table 1).

Table 1. Microalgae and their fossil palynomorphs.

Bacillariophyta	– (but + + record of opal skeletons)
Chlorarachniophyta	–
Chloromonadophyta	–
Chlorophyta	+ (mainly freshwater Chlorococcales and mesospores of Zygnematales)
Cryptophyta	–
Dinophyta	+ + (mainly marine, very abundant long and diverse record)
Euglenophyta	+ (but very rare)
Eustigmatophyta	–
Haptophyta	– (but + + for record of $CaCO_3$ skeletal elements)
Prasinophyta	+
Xanthophyta	–
Acritarcha	+ + (mainly marine, many Palaeozoic and earlier taxa. Polyphyletic)

Fossilised walls of micro-algae

From the above it is clear that the presence of cell contents hampers the isolation and analysis of cell walls of living algae. This problem is absent for analysis of fossil palynomorphs. However, three other difficulties complicate the evaluation of fossil algal walls. First, isolating pure, monotypic assemblages is difficult. The unusually high abundance of *Pediastrum* fossils (60% of the palynomorphs) in a late Miocene sediment led to the conclusion that the *Pediastrum* walls contain aromatic or partly aromatic compounds (Sinninghe Damsté et al. 1993) contradicting later analyses indicating an aliphatic wall for this taxon (Blokker et al. 2000). Second, the lack of recent counterparts, as is the case for the Acritarcha. Third, the transformation of the original biomacromolecule into a geomacromolecule which would provide an alternative explanation to contamination for explaining the results on the Miocene *Pediastrum* walls. At least, if we assume that aromatic moieties 'invaded' the originally aliphatic biomacromolecule *post-mortem*. Despite these problems, very close morphological *and* chemical correspondence does occur between fossil algae and their living counterparts, e.g. for *T. minimum* (Goth et al.

1988). This demonstrates that algaenans can survive relatively unchanged in sediments for millions of years.

Compared with algal diversity in present time, the fossil record is severely incomplete. Some algal groups seem to leave no, or almost no, fossils, e.g. like the Euglenophyta (Gray and Boucot 1989). Others leave lipids (e.g. Eustigmatophyta) and/or biominerals (e.g., Bacillariophyta and Haptophyta) but are unknown as fossil palynomorphs. However, some extant Eustigmatophyta and Chlorophyte taxa without known micro-fossil record have been shown to produce hydrolysis resistant walls. It seems likely that their fossil cell walls are present in the sediments but have not been recognised for a lack of morphological characteristics. The ubiquitous presence of 'ultralaminae' with an aliphatic nature in sediments corroborates with this idea (Derenne et al. 1991). Of the algal groups that have been identified from the fossil record, the palynomorphs of Prasinophyta and Chlorophyta are known from the Proterozoic (Knoll 1992, 1996) and continue to the present day. Their fossil records are, however, not very diverse (Batten and Grenfell 1996; Batten 1996; Guy-Ohlson 1996; van Geel and Grenfell 1996; Wicander et al. 1996). Only one, still existing, group of algae, the dinoflagellates, is known to have given rise to a diverse record of largely marine microfossils since the early Mesozoic (Fensome et al. 1999). A second rich and diverse group of fossil palynomorphs is the Acritarcha. This group consists entirely of taxa with an unknown biological affinity but is considered to include a large proportion of palynomorphs from micro-algae. Acritarchs have been found in Precambrian and younger strata. They reached particularly high diversity during the Palaeozoic (Strother 1996). It must be noted that the fossil record from lacustrine environments is much more limited that that from marine environments so that the preservation potential of fresh water algae (notably Chlorophyta) must be underestimated.

Algaenans

Algaenans of extant micro-algae

Algaenans (Tegelaar et al. 1989) represent a series of acid and base-resistant aliphatic biomacromolecules. It is important to note that other compounds may be associated with the algaenan e.g. isoprenoids in the case of *B. braunii* race L (Bertheas et al. 1999) or sugars in the case of *Coelastrum sphaericum* (Rodríguez et al. 1999) but they are removed upon hydrolysis. The aliphatic nature suggests that algaenans are biosynthesised via the acetate/malate pathway which leads to FAs. Algaenans appear widespread in Chlorophyta and have been detected in some Eustigmatophyta and a member of the Dinophyta but have not been detected in Bacillariophyta or Haptophyta (Table 2). The walls of some Prasinophyta and the pellicles of several Dinophyta have also been reported to be resistant (e.g. Morrill and Loeblich III 1981; Aken and Pienaar 1985) but information on the wall chemistry is too sparse to infer that they consist of algaenan. For the few more closely analysed algae, three general algaenan structures have been proposed. For most Chlorophyta for which structural information is available, the building blocks consist of linear C_{22} to C_{34} even-numbered carbon chains with functional groups at the α, ω, ω^9 and sometimes ω^{18} positions. In the algaenan the functional groups cross-link the monomers with ether and ester bonds (Blokker et al. 1998a, 1999). However, the algaenan of the Chlorophyte *B. braunii*, (at least race A) seems to be based on unsaturated aliphatic aldehydes and unsaturated hydrocarbons with on average 40 carbon atoms. Here, the monomers are cross-linked by acetal and ester bonds (Simpson et al. 2003). The third algaenan type proposed is produced by the Eustigmatophyta. Here, the building blocks are probably mid-chain (ω^{15} to ω^{18}) C_{28} to C_{36} diols and C_{30} to C_{32} alkenols, as well as C_{25}, C_{27}, and C_{29} (poly)unsaturated free hydrocarbons which in the algaenan are cross-linked with mid-chain ether bonds (Gelin et al. 1997). Algaenans are among the most frequently studied resistant algal macromolecules. They are mostly isolated from actively growing cultures. The active metabolism implies that the algaenan walls must contain pores to exchange compounds with the outer environment. Despite such pores, algaenan walls have been shown to form an effective barrier for extracellular enzymes (e.g. Atkinson Jr et al. 1972; Syrett and Thomas 1973) and detergents (Biedlingmaier et al. 1987; Corre et al. 1996).

During a phase of quiescence, the reduced metabolic activity allows for much less exchange with the outer environment. These cyst walls could

Table 2. Occurrence and composition of resistant biomacromolecules in recent algae.

Taxon	Biomacromolecule	Latest Reference(s)
Bacillariophyta (Diatoms)		
Chaetoceros calcitrans (CCAP1010/5)	–[a]	(Gelin et al. 1999)
Chaetoceros muelleri (CS-176)	–	(Gelin et al. 1999)
Nitzschia palea (UTEX1813)	–	(Gelin et al. 1999)
Skeletonema costatum (CCAP 1077/1b)	–	(Gelin et al. 1999)
Chlorophyta (green algae)		
Bulbochæte sp.	? non-sugar-based resistant 'chitinous' outer wall layer (filamentous taxon)	(Tiffany 1924)
Botryococcus sudeticus (braunii A race, UTEX572)	algaenan: non-isoprenoid polyaldehyde network (poly-botryal) C_{40} average chain length	(Metzger and Largeau 2002; Simpson et al. 2003)
Botryococcus braunii (SAG 30.81)	algaenan: C_{32} di-unsaturated α, ω-dialdehyde polymer	(Blokker et al. 2000)
Botryococcus braunii (B race)	algaenan: aliphatic polymer $< C_{31}$ chains	(Kadouri et al. 1988)
Botryococcus braunii (L race Yamoussoukro strain)	algaenan similar to A race, but isoprenoids attached to algaenan backbone	(Bertheas et al. 1999)
Brachiomonas submarina (CCAP7/2b)	–	(Gelin et al. 1997)
Chlorella[b]*ellipsoidea* (CCAP211/1a)	–	(Burczyk et al. 1999)
Chlorella emersonii (CCAP211/8p)	algaenan: C_{26} & C_{28} FA & OH; C_{30} & C_{32}-OH-FA & α, ω diols	(Allard and Templier 2001)
Chlorella fusca (several strains)	+[c]	(Burczyk et al. 1999)
Chlorella nana (type strain)	+ acetolysis resistant, multiple sporopollenin layers	(Rascio et al. 1979)
Chlorella marina (CCAP211/27)	–	(Allard and Templier 2000)
Chlorella minutissima (Utex2219)	algaenan	(Allard and Templier 2000)
Chlorella minutissima marina (Utex2341)	–	(Allard and Templier 2000)
Chlorella pyrenoidosa (A-24)	–	(Burczyk et al. 1999)
Chlorella saccharophila (1 str. + CCAP211/1a)	–	(Burczyk et al. 1999)
Chlorella sorokiniana (2 str. + CCAP211/8k)	–	(Burczyk et al. 1999)
Chlorella sorokiniana (Utex1230)	algaenan	(Zelibor Jr et al. 1988)
Chlorella spaerckii (CCAP211/29A)	–	(Gelin et al. 1997)
Chlorella vacuolatus (CCAP211/8b)	algaenan: up to C_{27}polymethylenic chains	(Derenne et al. 1992a; Burczyk et al. 1999)
Chlorella vulgaris (4 str. + CCAP211/1e)	–	(Burczyk et al. 1999)
Chlorococcum sp. (CCAP11/62)	–	(Gelin et al. 1997)
Cladophora glomerata	? non-sugar-based resistant 'chitinous' outer wall layer (filamentous taxon)	(Wurdack 1923)
Coccomyxa dispar	+ acetolysis, K_2CrO_4 (30%) resistant (free living taxon)	(Honegger and Brunner 1981)
Coccomyxa glaronensis	+ (lichen phycobiont)	(Brunner and Honegger 1985)
Coccomyxa tirolensis	+ (lichen phycobiont)	(Brunner and Honegger 1985)
Coelastrum proboscideum var.*dilatatum* (UTEX282)	+acetolysis resistant	(Marchant 1977)
Coelastrum reticulatum (SAG8.81)	algaenan: mainly polymer of $\Delta\omega^9$ $C_{30}\omega$-OH FA	(Blokker et al. 2000)
Coelastrum sphaericum var. *dilatatum*	algaenan: polymethylenic chains (with amido groups ?)	(Rodríguez and Cerezo 1996)
Cylindrocapsa geminella	– filamentous taxon	(Tiffany 1924)

Draparnaldia plumosa	− filamentous taxon	(Wurdack 1923)
Dunaliella tertiolecta	algaenan	(Zelibor Jr et al. 1988)
Dysmorphococcus globosus (UTEX65)	− lorica vegetative cells	(Porcella and Walne 1980)
Elliptochloris bilobata	+ (lichen phycobiont)	(Brunner and Honegger 1985)
Elliptochloris sp.	+ (lichen phycobiont)	(Brunner and Honegger 1985)
Microsopra willeana	− filamentous organism	(Tiffany 1924)
Myrmecia biatorellae	− (lichen phycobiont)	(Brunner and Honegger 1985)
Myrmecia reticulata	− (lichen phycobiont)	(Brunner and Honegger 1985)
Myrmecia sp.	− (lichen phycobiont) the resistant walls reported by König and Preveling (1980) may be considered artefacts	(Brunner and Honegger 1985)
Nannochloris sp. (CCAP 251/2)	−	(Gelin et al. 1997)
Nanochlorum eucaryotum	algaenan: up to C_{30} polymethylenic chains (salt water taxon)	(Derenne et al. 1992a)
Oedogonium crassum amplum	? unidentified non-sugar-based resistant outer wall layer (filamentous taxon)	(Wurdack 1923)
Oedogonium irregulare	? non-sugar-based resistant 'chitinous' outer wall layer (filamentous taxon)	(Wurdack 1923)
Oocystus solitaria (SAG 83.80)	−	(Blokker 2000)
Pediastrum braunii (SAG 43.85)	algaenan: polymer of mainly C_{30} & $C_{32}\omega$-OH FA	(Blokker 2000)
Pediastrum boryanum (SAG N.N.)	algaenan: polymer of mainly $\Delta\omega^9$ C_{30} & C_{32}, $\Delta\omega^{9,19}$ C_{30}, $\Delta\omega^{9,18}$ C_{32} ω-OH-FA	(Blokker et al. 1998b)
Pediastrum duplex ()	+ 'sporopollenin-like'	(Gunnison and Alexander 1975)
Pediastrum kawraiskyi (SAG35.81)	algaenan: C_{30} & C_{32} ω-OH-FA	(Blokker 2000)
Phycopeltis epiphyton	+ acetolysis and K_2CrO_4 (30%) resistant (subaerial taxon)	(Good and Chapman 1978)
Prototheca wickerhami	+ (but terpene units need verification)	(Puel et al. 1987)
Prototheca moriformis (CCAP263/2)	−	(Atkinson Jr et al. 1972)
Prototheca portoricensis (CCAP263/3b)	+	(Atkinson Jr et al. 1972)
Prototheca zopfii (CCAP263/5)	+	(Atkinson Jr et al. 1972)
Pseudochlorella pyrenosidosa	−	(Brunner and Honegger 1985)
Pseudochlorella sp.	−	(Brunner and Honegger 1985)
Pseudodidymocystis planctonica (SAG40.98)	+ acetolysis resistant	(Hegewald and Deason 1989)
Pseudodidymocystis fina	+ acetolysis resistant	(Hegewald and Deason 1989)
Pseudoschroederia punctata (UTEX LB2490)	− not acetolysis resistant	(Hegewald and Deason 1988)
Pseudotrebouxia corticola (UTEX909)	+ reported but probably −, see *Trebouxia* (taxa are lichen phycobionts)	(König and Peveling 1984)
Pseudotrebouxia impressa (UTEX893)	+ reported but probably −, see *Trebouxia* (taxa are lichen phycobionts)	(König and Peveling 1984)
Scenedesmus armatus (Paris)	algaenan	(Allard and Templier 2000)
Scenedesmus longus (Indiana Cult. Coll. 614)	+ acetolysis and boiling 6N NaOH resistant	(Staehelin and Picket-Heaps 1975)
Scenedesmus communis (CCAP 276/4b)	algaenan: C_{26} & C_{28} FA & OH; C_{30} & C_{32}-OH-FA & α, ω diols polymer of mainly $\Delta\omega^9$ C_{30} ω-OH-FA	(Allard and Templier 2001) (Blokker et al. 1998b)
Scenedesmus obliquus (3+ strains)	algaenan	(Zelibor Jr et al. 1988; Burczyk et al. 1999)
Scenedesmus pannonicus (Utex77)	algaenan (=CCAP276/4a)	(Allard and Templier 2000)
Scenedesmus subspicatus (Göttingen)	algaenan	(Allard and Templier 2000)
Staurastrum sp.	+ but lignin-like	(Gunnison and Alexander 1975)
Sorastrum spinulosum (SAG B40.81)	algaenan: $\Delta\omega^9$ C_{30} & $C_{32}\omega$-OHFA	(Blokker 2000; Blokker et al. 2000)
Stichococcus bacillaris (CCAP379/32)	−	(Gelin et al. 1997)
Trebouxia spp.	− the resistant walls reported by König and Preveling (1980, 1984) may be considered artefacts (taxa are lichen phycobionts)	(Honegger and Brunner 1981) (Brunner and Honegger 1985)

Table 2. (Continued).

Taxon	Biomacromolecule	Latest Reference(s)
Trentepholia sp. (UTEX1227)	− (both free living and as lichen phycobiont)	(Brunner and Honegger 1985)
Tribonema bombycina	− filamentous organism	(Tiffany 1924)
Tribonema urticulosa	− filamentous organism	(Tiffany 1924)
Tribonema minus	− filamentous organism	(Tiffany 1924)
Tetraedron minimum (Göttingen)	algaenan: C_{26}, C_{28} FA; some C_{30}-C_{34} ω-OH FA, minor aromatics	(Goth et al. 1988; Allard and Templier 2001)
	polymer of mainly $\Delta\omega^9$ C_{32} & C_{34} ω-OH-FA	(Blokker et al. 1998b)
Vaucheria geminata	− filamentous taxon	(Wurdack 1923)
Zygnemataceae	− filaments of *Debarya decussata, Mougeotia* (5 spp.), *Spirogyra* (23 spp.) species names are listed below in section 'spores and resting stages'	(Tiffany 1924)
Zygnema cruciatum	− wall of filaments made of pectose and cellulose	(Wurdack 1923)
Zygnema sp.	? NMR spectrum does not support algaenan (filamentous organism)	(Zelibor Jr et al. 1988)
Zygnema spp.	− species are *collinsianum, decussatum, ericetorum, insignis, pectinatum* and *stellinum*	(Tiffany 1924)
Dinophyta (Dinoflagellates)		
Alexandrium acatenella (ML524)	−	(Morrill and Loeblich III 1981)
Alexandrium catenella (ML497; ML525)	−	(Morrill and Loeblich III 1981)
Alexandrium monilatum (ML487)	−	(Morrill and Loeblich III 1981)
Alexandrium tamarense (6 strains)	−	(Morrill and Loeblich III 1981)
Alexandrium tamarense (ML440)	+ acetolysis resisant	(Morrill and Loeblich III 1981)
Amphidinium carterae (UTEX1561)	−	(Morrill and Loeblich III 1981)
Amphidinium corpulentum (UTEX1652)	−	(Morrill and Loeblich III 1981)
Amphidinium operculatum (ML137; ML143)	−	(Morrill and Loeblich III 1981)
Ceratocorys horrida (ML496)	−	(Morrill and Loeblich III 1981)
Crypthecodinium cohnii (UTEX1649)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Ensiculifera loeblichii (UTEX1595)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Fragilidinium heterolobum (ML491)	−	(Morrill and Loeblich III 1981)
Heterocapsa illdefina (ML495)	−	(Morrill and Loeblich III 1981)
Heterocapsa niei (UTEX1654)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Heterocapsa pygmaea (4 strains)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Heterocapsa triquetra (ML532)	−	(Morrill and Loeblich III 1981)
Heterocapsa triquetra (NEPCC89)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Gloeodinium montanum (UTEX1651)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Gonyaulax diegensis (UTEX1992)	−	(Morrill and Loeblich III 1981)
Gonyaulax grindleyi (UTEX1956)	−	(Morrill and Loeblich III 1981)
Gonyaulax grindleyi (ML511)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Gonyaulax sphaeroidea (UTEX1947)	−	(Morrill and Loeblich III 1981)
Gonyaulax spinifera (ML502)	−	(Morrill and Loeblich III 1981)
Gymnodinium catenatum (CS301-theca)	algaenan: up to C_{35} *n*-alkyl units, maximum at C_{14}	(Gelin et al. 1999)
Gymnodinium sp. (UTEX1654)	−	(Morrill and Loeblich III 1981)

Gymnodinium sp. (FCRG47)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Gymnodinium sp. (LG161)	–	(Morrill and Loeblich III 1981)
Gyrodinium resplendens (UTEX1655)	–	(Morrill and Loeblich III 1981)
Gyrodinium dorsum (LG21)	–	(Morrill and Loeblich III 1981)
Lingulodinium polyedrum (red tide; ML492)	+ acetolysis resistant but may be contaminated; strain not tested	(Morrill and Loeblich III 1981)
Ostreopsis siamensis (ML538)	–	(Morrill and Loeblich III 1981)
Oxyrrhis marina (LG91)	–	(Morrill and Loeblich III 1981)
Peridinium balticum (UTEX1563)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium cinctum (CCAP1134/2)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium cinctum f.*ovoplanum* (UTEX1336)	+acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium foliaceum (UTEX1688 & 2006)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium gatunense (UTEX2051)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium volzii (UTEX2175)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium willei (ML488)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Peridinium willei (UTEX2028)	–	(Morrill and Loeblich III 1981)
Prorocentrum cassubicum (UTEX1596)	–	(Morrill and Loeblich III 1981)
Prorocentrum mexicanum (CS28-theca)	–	(Gelin et al. 1999)
Prorocentrum micans (CS28-theca)	–	(Gelin et al. 1999)
Prorocentrum minimum (ML5 & 403)	–	(Morrill and Loeblich III 1981)
Pyrocystis lunula Schütt	+ acid, base and acetolysis resistant	(Swift and Remsen 1970)
Pyrocystis pseudonocticula Murray	– (in fact not explicitly mentioned)	(Swift and Remsen 1970)
Pyrocystis fusiformis Murray (ML493)	–	(Swift and Remsen 1970; Morrill and Loeblich III 1981)
Scrippsiella gregaria (UTEX1948)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Scrippsiella trochoidea (FCRG72; ML540)	+ acetolysis resistant	(Morrill and Loeblich III 1981)
Woloszynskia coronata (CCAP1117/2)	+ acetolysis resistant	(Morrill and Loeblich III, 1981)
Zooxanthella micro-adriatica (ML395 & 406)	–	(Morrill and Loeblich III, 1981)
Eustigmatophyta		
Nannochloropsis sp. (CCAP849/7)	similar to *N. salina* (also functionality at C_9 ?)	(Gelin et al. 1997)
Nannochloropsis granulata (CCMP529)	algaenan	(Gelin et al. 1999)
Nannochloropsis oculata (CS179)	algaenan	(Gelin et al. 1999)
Nannochloropsis salina (CCAP849/4)	C_{28}-C_{36} alkyl diols & alkenols, $C_{25,\ 27,29}$ alkenes; functionalities around C_{15}	(Gelin et al. 1997)
Haptophyta		
Emiliania huxley (NIOZ)	–	(Gelin et al. 1999)
Phaeocystis sp.(North Sea)	–	(Gelin et al. 1999)
Prasinophyta		
Tetraselmis chui (CS28)	–	(Gelin et al. 1999)
Spores and resting stages		
Chlorophyta		
Chlamydomonas monoica (UTEX220)	algaenan: C_{22}-C_{30} *n*-OH & *n*-FA & α, ω OH-FA & di-FA polymer	(Blokker et al. 1999)
Chlamydomonas geitleri (Ettl 1966/3,	+ acetolysis resistant	(Zárský et al. 1985)
Debarya decussata	? see *M. calcarea*	(Tiffany 1924)

Table 2. (Continued).

Taxon	Biomacromolecule	Latest Reference(s)
Dunaniella sp. (aplanospores)	algaenan	(Blokker 2000)
Dysmorphococcus globosus (culture?)	+ acetolysis resistant	(Porcella and Walne 1980)
Haematococcus pluvialis (akinetes)	algaenan: similar to *C. monoica*	(Blokker 2000; Montsant et al. 2001)
Mougeotia calcarea	? but, wall contains discontinuously non-sugar-based 'chitinous'-deposits	(Tiffany 1924)
Mougeotia laevis	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Mougeotia genuflexa	? see *M. calcarea*	(Tiffany 1924)
Mougeotia quadrangulata	? see *M. calcarea*	(Tiffany 1924)
Mougeotia robusta	? see *M. calcarea*	(Tiffany 1924)
Mychonastes desiccatus	+ acetolysis resistant	(Hull et al. 1985)
Sirogonium melanosporum	+ acetolysis, HCl (3%)–KOH (2%)–H_2SO_4 (65%) resistant	(Hull et al. 1985)
Spirogyra acanthomorpha	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Spirogyra calospora	+ acetolysis resistant	(Ashraf and Godward 1980)
Spirogyra crassa	+ acetolysis resistant	(Ashraf and Godward 1980)
Spirogyra gracilis	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Spirogyra hassallii	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Spirogyra jatobae	+ acetolysis, HCl (3%)–KOH (2%)–H_2SO_4 (65%) resistant	(Hull et al. 1985)
Spirogyra longata	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Spirogyra sp. (zygospores)	algaenan: (presence of suberin/cutin like monomers needs confirmation)	(Blokker 2000)
Spirogyra spp. (7 unnamed species)	+ acetolysis resistant	(Ashraf and Godward 1980)
Spirogyra submarginata	+ acetolysis resistant	(Ashraf and Godward 1980)
Spirogyra submaxima	+ acetolysis, HCl (3%)-KOH (2%)-H_2SO_4 (65%) resistant	(Hull et al. 1985)
Spirogyra weberi	+ acetolysis, HF (40%) and K_2CrO_4 (30%) resistant	(de Vries et al. 1983)
Spirogyra spp.	? see *M. calcarea*, species are *S. catenaeformis, circumlineata, crassa, daedala, ellipsopora, flavescens, fluviatilis, fovealata, hydrodictya, illinoiensis, inflata, insignis, irregularis , laxa, majuscula, novae-angliae, protecta, rectangularis, stictica, tenuitissima, varians, velata* and *weberi*	(Tiffany 1924)
Zygnema (spp.)	? see *M. calcarea*. Species are *Z. collinsianum, decussatum, ericetorum, insignis, pectinatum* and *stellinum*	(Tiffany 1924)
Dinophyta (dinoflagellates)		
Lingulodinium polyedrum (GpES19-cyst)	aromatic	(Kokinos et al. 1998), this study
Scrippsiella ramonii (Naples)	aromatic + aliphatic	This study
Scrippsiella sp.	complex including aromatic and aliphatic moieties	(Hemsley et al. 1994)
Prasinophyta		
Prasinocladus marinus	–	(Shaw 1971)
Pyramimonas pseudoparkeae	acetolysis resistant walls supposed to be 'sporopollenin': needs confirmation	(Aken and Pienaar 1985)

[a]For all taxa with a '–' indication a resistant biomacromolecule could not be evidenced.[b]*Chlorella* taxonomy based on Huss et al. (1999). The status of *C. fusca* and *C. pyrenoidosa* in Burczyk et al. (1999) however remains unclear.[c]For all taxa with a '+' indication, assessment of the presence of a resistant wall is based on old methodology and the presence of such a wall needs to be verified.

help to survive dryness during aerial dispersion or dry periods (e.g., *Haematococcus pluvialis*), and/or they could be more resistant to attack if the cysts form a 'seed bank'. Clearly, the constraints set to cell walls of metabolically active and quiescent algae may be very different. Nevertheless, for the Chlorophyta, this does not seem to have had an effect on the wall chemistry. All resting stages analysed also have algaenan walls. The possible presence of aromatic moieties in the algaenan of *Spirogyra* sp. mesospores, however, needs further investigation (Blokker 2000). Interestingly, in extant algae, algaenans have almost exclusively been detected for fresh water species. The highly aliphatic (plastic like) algaenan may function as a relatively water-proof layer. Such a layer might be an important adaptation for fresh water species or species that live in smaller enclosed habitats; it enables them to spread from one place to another (by wind, birds etc.) and to resist periods of dryness. Most marine species would not necessarily need this.

Fossil algaenans and algaenan-likes

Only a few fossil algal-walls have been shown to closely resemble their Modern algaenan counterparts. These are all derived from the Chlorophyta (Table 3). To what extent did, the hypothesised migration of aliphatic moieties into the algaenan and the process of oxidative polymerisation attaching the aliphatic moieties to the algaenan (Versteegh et al. 2004) (analogous to the process proposed for changing sporopollenin and cutan above) turn the original algal wall into its present state? Due to the aliphatic nature of the algaenan itself, these processes usually remain unnoticed with current methodology. Seen in this light, the mainly aliphatic nature reported for some Proterozoic acritarchs (Arouri et al. 1999) should be interpreted with care. The additional presence of aromatic and (unusual) amide groups in the walls of these acritarchs, generate questions on the purity of the material, the extent to which the acritarch geomacromolecule still represents the original biomacromolecule and the degree to which this original biomacromolecule was aromatic. For this very old material, a second process, like the removal of aliphatic moieties from the acritarch wall at elevated temperature and pressure conditions may additionally have influenced the wall composition. For obvious reasons, this second process has been subject to intense study in relation to understanding oil and gas formation (e.g. Combaz 1971; Rullkötter, 1993) and will therefore not be discussed further.

The acritarch *Gloeocapsomorpha prisca* (see, Wicander et al. 1996 for an overview) is the principal component of middle Ordovician marine oil shales (kukersites). The taxonomic position of *G. prisca* has been a matter of considerable debate. On the basis of morphological comparison with modern organisms, it has been assigned mostly to the Cyanobacteria and Chlorophyta, notably the fresh water species *B. braunii* which when cultured under salt stress produces structures that are morphologically similar to those of *G. prisca* (Derenne et al. 1992b). Recent chemical analyses (Blokker et al. 2001; Lille 2003) indicate an aliphatic wall composed of 1,3-benzediol (resorcinol) building blocks with mainly C_{15}, C_{17}, and C_{19} alkyl side chains. Resorcinols are known from a variety of higher plants, mosses, fungi, bacteria. They have also been identified as free lipids from *Botryococcus* (with C_{25}–C_{29} alkyl chains) (Metzger and Largeau 1994). Upon salt stress the contribution of phenols to *Botryococcus* pyrolysates increases (Derenne et al. 1992b) but if these phenols were derived from resorsinols or not, remains to be investigated. Fossil *Botryococcus* differ from *G. prisca* in lacking evidence for an aliphatic macromolecular wall structure and the shorter chain lengths of the *n*-alkyl resorcinol building blocks of the fossil macromolecule (Blokker et al. 2001).

Moreover, in the light of the widespread occurrence of resorcinols in organisms their occurrence in *Botryococcus* forms no argument to consider *G. prisca* a member of the Chlorophyta. Resorcinols are produced via the polyketide or acetogenic pathway (Kozubek and Tyman 1999). Depending on the phylogenetic position of *G. prisca* this could represent a third pathway in algae for the biosynthesis of resistant macromolecules. However, Blokker et al. (2001) indicate that *G. prisca* may have produced resorcinols for UV and/or microbial protection. They further suggest that the high reactivity of the resorcinols favours their polymerisation, e.g. catalysed by oxygen or trace metals, and that the fossilised macromolecule may have been formed *post-mortem*. Instead of representing a third biosynthetic pathway, this would provide a

Table 3. Composition of resistant biomacromolecules in fossil algae.

Taxon	Biomacromolecule	Age	Latest Reference(s)
Acritarchs			
Reduviasporonites	mainly aliphatic and minor aromatic components filamentous taxon	P/T boundary	(Foster et al. 2002)
Gloeocapsomorpha prisca	algaenan: mainly C_{21} & C_{23} *n*-alkyl resorcinol polymer	Ordovician	(Blokker et al. 2001; Lille, 2003)
Leiosphaeridia	aliphatic	Silurian and Ordovician	(Kjellström 1968)
Multifronsphaeridium peliorum	manly aliphatic, also aromatic and amide groups	Neoproterozoic	(Arouri et al. 1999)
Species A	manly aliphatic, also aromatic and amide groups	Neoproterozoic	(Arouri et al. 1999)
1.*Tanarium* sp. A	1–3 acanthomorph, multilayered fibrillar wall		
2. *Hocosphaeridium scaberfacium*	4–6 no wall layering		
3. *Alicesphaeridium medusoidum*	1–6, no pyrolysates available, but IR and Raman	Neoproterozoic	(Arouri et al. 2000)
4. Species C2	Spectroscopy indicates a highly ordered aromatic		
5. *Chuaria circularis*	biopolymer which can also be ascribed to over-		
6. *Leiosphaeridia* sp.	maturity of the organic matter.		
Chlorophyta			
Botryococcus braunii	aliphatic, like recent representatives (isoprenes questionable)	Permian to Recent	(Derenne et al. 1994; 1997)
Tetraedron minimum	aliphatic, like recent representative	Eocene (Messel 45 Ma)	(Blokker et al. 2000)
Pediastrum	aliphatic, like recent *P. boryanum*	Early Holocene (10 ka)	(Blokker et al. 2000)
Coelastrum reticulatum (inferred)	aliphatic, like recent representative	Early Holocene (10 ka)	(Blokker et al. 2000)
Prasinophyta			
Tasmanites	polymer of aliphatics, (+ isoprenoids and aromatics?) Permian		(Collinson et al. 1994; Greenwood et al. 2000)
Dinophyta	Biomacromolecule purity[a]	Age	Reference
Brigantedinium spp.	aliphatic/aromatic 83	± E/Oboundary	(Dammers 2003)
Chiropteridium spp.	aliphatic/aromatic 95(97)	Oligocene	This study
Deflandrea sp.	aliphatic/aromatic 74(75)	Middle Eocene	(Warnaar 2001)
Enneadocysta sp.	aliphatic/aromatic 89(92)	Middle Eocene	(Warnaar 2001)
Nematosphaeropsis labyrinthus	aromatic 63	± 77500 y	(Dammers 2003)
Manumiella druggii	aliphatic/aromatic >75	± K/T boundary	This study
Palaeoperidinium spp.	aliphatic/aromatic 88	± K/T boundary	(Dammers 2003)
Polysphaeridium zoharii	aromatic 76	± 50000 y	(Dammers 2003)
Spiniferites sp.(D3)	aliphatic/aromatic (>50%)	± K/T boundary	(van Mourik 2000)
Thalassiphora sp.	aliphatic/aromatic 42(60)	Middle Eocene	(Warnaar 2001)
Trithyrodinium evittii (D2; D4)	aliphatic/aromatic (95; 90%)	± K/T boundary	(van Mourik 2000)
Mixed gonyaulacoids[b] (D1; GMB-A)	aliphatic/aromatic? 73	± K/T boundary	(van Mourik 2000; Warnaar 2001)
'Dinocasts'	aliphatic C_{16} and C_{18} 100 fatty-acid-based geopolymer	Eocene	(Versteegh et al. 2004)

[a]Purity indication denotes the percentage of cysts of the indicated taxon on organic particles (between brackets the percentage of dinoflagellate cysts on organic particles).
[b]The mixture includes *Areoligera senonensis, Cribroperidinium* sp., *Glaphyrocysta perforata, Hystrichosphaeridium* sp., *Spiniferites ramosus*.

second example of oxidative polymerisation of algae, analogous to the recent findings of Versteegh et al. (2004). The possibility that the organism excreted the resorcinols to form a protective layer, by oxidative polymerisation, can not be ruled out though.

Tasmanites, represents the only fossil member of the Prasinophyta studied for its wall composition. Pyrolysis of an extracted Tasmanite coal, almost entirely consisting of *Tasmanites*, by Collinson et al. (1994) and nuclear magnetic resonance (NMR) analysis of HF treated and picked *Tasmanites* specimens (Hemsley et al. 1993) indicate that these fossils predominantly feature alkane/alkene doublets in the GC-FID trace and thus, that the *Tasmanites* walls could be made of algaenan, confirming the early suggestion of Kjellström (1968) that the walls consist of long chains of aliphatic saturated hydrocarbons. However, Greenwood et al. (2000) report that the solvent extracted walls are 'comprised of ubiquitous *n*-alkane/alkene, parent and alkyl aromatics, and tricyclic terpenoids' on the basis of GC-MS analysis. They also noted that the untreated parent Tasmanite oil shale has the same composition as *Tasmanites*. We attribute this discrepancy to the higher ionisation efficiency of aromatics and isoprenoids compared to aliphatics, resulting in underrepresentation of the latter upon mass spectrometry. Therefore, we infer that *Tasmanites* indeed largely consists of aliphatic moieties. Moreover, the formation of aromatic moieties from isoprenoids since the Permian may explain the aromatic signature of the material. The absence of acid or base hydrolysis treatments of the *Tasmanites* furthermore leaves the possibility that the isoprenoids became attached to the wall macromolecule *post mortem* via ester bonds. Analogous to all other fossil and recent walls of algae analysed we suggest that the isoprenoids did not form cell wall constituents. Greenwood et al. (2000) suggest on the basis of the same composition of parent Tasmanite and *Tasmanites* that the isoprenoids are wall derived, we rather would like to propose the opposite, i.e. that the isoprenoid moieties have become attached to the *Tasmanites* wall. Based on the results of Kuypers et al. (2002), it can be speculated that apart from *Tasmanites*-derived also the degradation resistant archaeal isoprenoid membrane lipids have become oxygen cross-linked and contributed to the macromolecular isoprenoid constituent in these samples.

Dinosporins

Dinosporin of extant dinoflagellates

In contrast to the algaenan wall of the motile stage of the dinoflagellate *Gymnodinium catenatum*, (Gelin et al. 1999) dinoflagellates seem to be able to produce a completely different kind of wall for their resting cysts, called dinosporin (Fensome et al. 1993). There is only very limited information on recent dinoflagellate cyst walls. The walls of *L. polyedrum* are reported to be relatively condensed and predominantly aromatic, compositionally distinct from 'sporopollenin', unrelated to the walls of green algae (algaenan) and that the isoprenoid tocopherol as an important monomer (Kokinos et al. 1998). Upon re-analysis using the methodology of Blokker et al. (1998a; Figure 4) these conclusions are confirmed with the exception that no evidence of isoprenoids is observed in the pyrolysates. The discrepancy may be attributed to the phosphoric acid treatment (3 weeks) during the cyst wall isolation procedure by Kokinos et al. (1998) which has been shown to produce artefacts (see for a review, Allard et al. 1998; Allard and Templier 2000). Our pyrolysates show few, short chain (C_6-C_{10}), carbon chains and a dominance of mono- to poly-methylated aromatic fragments. Longer chain alkyl benzenes (e.g., butyl-benzene) are virtually absent as is any evidence of alkane/alkene doublets which is so characteristic for algaenans. Clearly, the cyst walls consist of a dense aromatic network in which few short alkyl chains are intercalated (dinosporin) which is, in agreement with Kokinos et al. (1998), totally different from the aliphatic algaenans. Considering the highly aromatic nature of the cyst biomacromolecule, we propose the phenylpropanoid pathway as the most logical one for the synthesis of its precursors. NMR analysis of *Scrippsiella* sp. cysts suggests for this taxon a very complex cyst wall macromolecule with also a substantial aliphatic component (Hemsley et al. 1994). Our preliminary analysis of the transparent cyst-walls from a culture of the peridinioid *Scrippsiella ramonii* confirms this. Both aromatic and aliphatic moieties are observed. Isoprenoid moieties are, however, absent (Figure 5). A series of alkane/alkyl doublets (up to C_{18}) and their corresponding ketones (not shown) are present. Interestingly, the aliphatic and aromatic moieties overlap in the sense

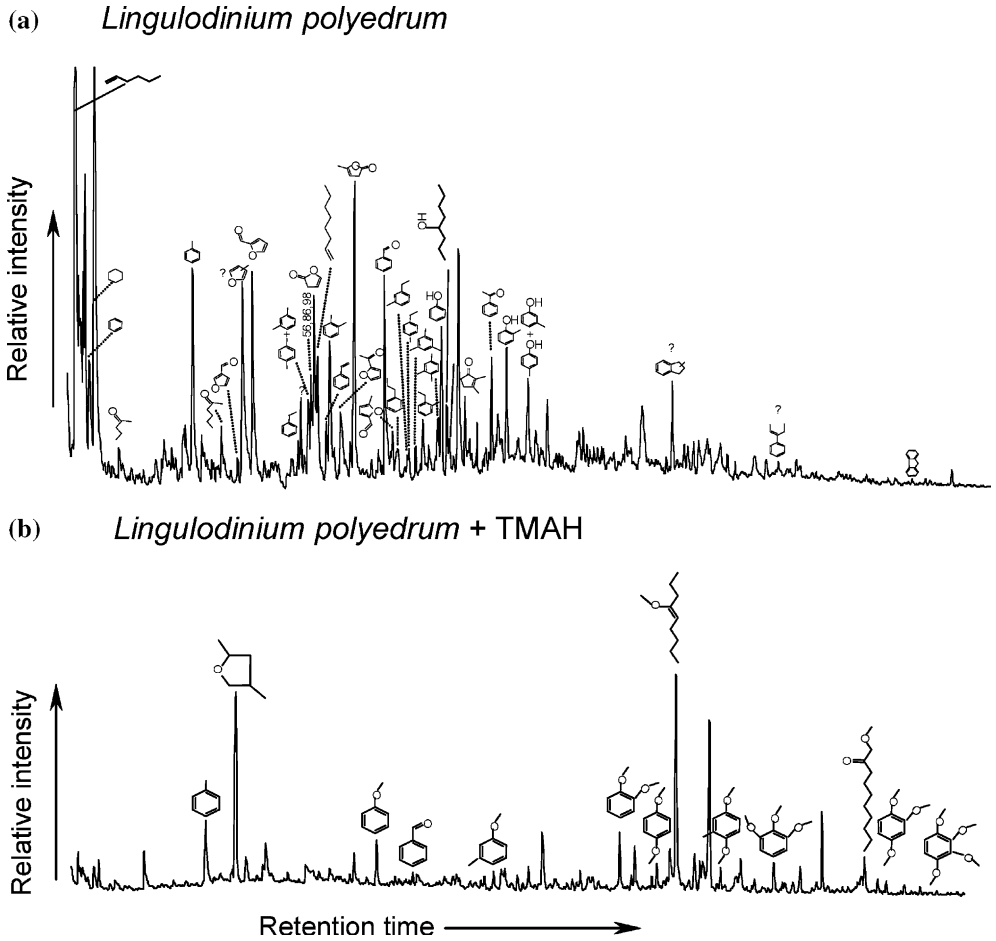

Figure 4. a. Pyrolysate of *Lingulodinium polyedrum* cyst walls from cultured material. b. Pyrolysate of *Lingulodinium polyedrum* cyst walls using tetramethylammonium hydroxide (TMAH). Upon thermolysis the TMAH methylates the oxygen radicals preventing secondary reactions of the pyrolysis products. Both panels demonstrate the aromatic nature of the walls. Note also the absence of isoprenoid moieties.

that also a series of alkyl-benzenes (up to C_7) is present but there is no trace of resorcinols. These latter observations suggest that the aliphatic and aromatic moieties are not organised in separate layers of the cyst wall but are mixed. However, the extent to which the alkyl-benzenes can be formed from aromatic and aliphatic pyrolysis products still remains to be investigated and thus can not be excluded either. Finally, C_{14} and C_{16} FAs are prominent in the pyrolysate whereas the C_{18} FA is absent. This absence of the C_{18} FA makes contamination unlikely as well as a contribution of free, dinoflagellate derived, lipids or their salts (Hartgers et al. 1995) since the C_{18} FA is also prominent in the free lipid extract. Upon pyrolysis with TMAH the C_{14} and C_{16} FAs reappear prominently. However, a series of distally unsaturated and α, ω-dicarboxylic acids are also present suggesting that the aliphatic moieties are part of the biomacromolecular network and represent FA monomers bound to the macromolecule.

The chemical and geological stability of the cysts informs us on a different aspect of dinoflagellate cyst walls. In marine palynology, strong acids, acetolysis and base treatment are avoided as much as possible upon processing of dinoflagellate assemblages from sediments. The reason is that although most gonyaulacoid cysts (the group to which *Lingulodinium* belongs) resist such treatments, they destroy many protoperidinioid cysts (the group to which *Scrippsiella* belongs), notably the brown-walled taxa (e.g. Dale

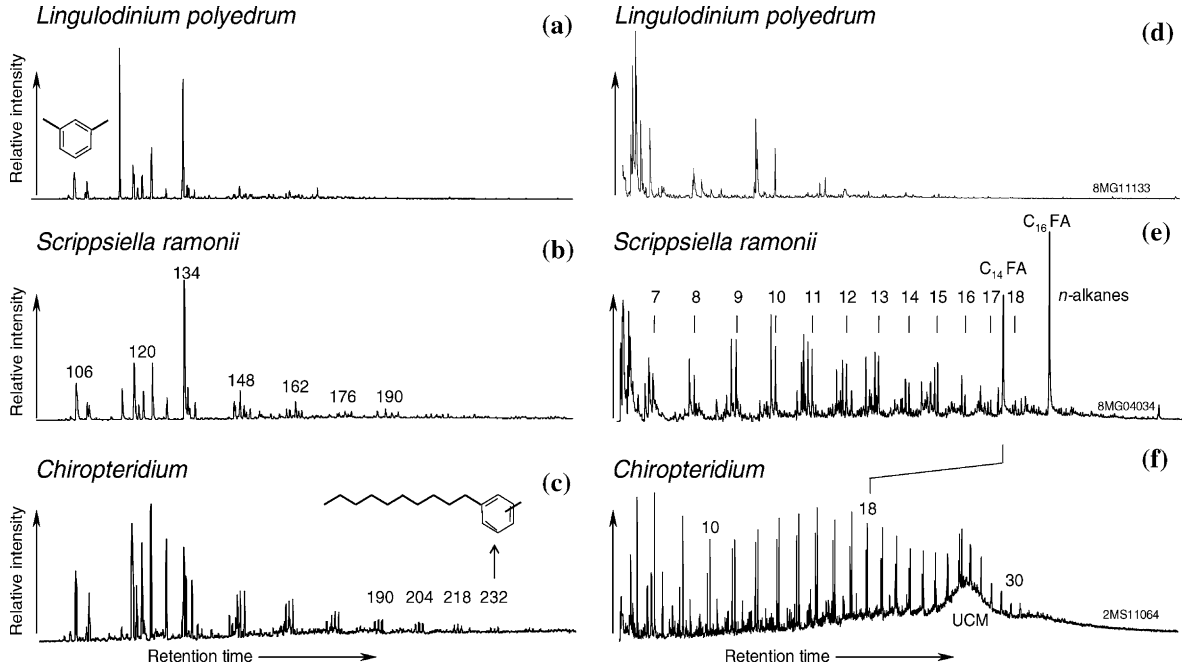

Figure 5. Pyrolysate mass chromatograms of a, b. *Lingulodinium polyedrum* cysts from cultures, c, d. *Scrippsiella ramonii* cysts from cultures, e, f. *Chiropteridium* cysts from Oligocene sediments. m/z 105 (a,c,e) showing the methyl-, alkyl-benzenes and m/z 55 + 57 (b,d,f) showing the aliphatic moieties, notably alkene/alkane doublets. Numbers in panels c, e refer to the M^+ of the corresponding components. FA = fatty acid, UCM = unresolved complex mixture.

1976; Turon 1984; Schrank 1988; Marret 1993; Hopkins and McCarthy 2002; and pers. obs.). This suggests that the gonyaulacoid macromolecule consists of a high proportion of carbon and ether-linked building blocks whereas the building blocks of protoperidinioid macromolecules are much more ester linked. Interestingly, in the sediments, the resistance of cysts to oxidation parallels their resistance to chemical treatment in the laboratory (Zonneveld et al. 1997, 2001; Versteegh and Zonneveld 2002).

On the basis of the above we conclude that there are probably two variables influencing dinosporin composition, the proportion of aliphatic *vs*. aromatic moieties and the proportion of ether- and carbon-bonds vs. ester-bonds. We further hypothesise that these two variables covary and relate to cyst preservation such that the chemically and/or geologically more resistant cysts (c.f. *L. polyedra*) have more aromatic moieties and more ether-bonds connecting these moieties than the less resistant ones (c.f. *S. ramonii*). However, this hypothesis urgently needs further testing. A major problem in achieving this is that many organic-cyst forming dinoflagellates are notoriously difficult to culture and that inducing cyst formation in culture in sufficient is technically challenging.

We speculate that the presence of aromatic moieties in the cyst wall relates to the fact that they are resting cysts. Some taxa are known to survive in the sediments for more than a decade (Lewis et al. 1999). The cysts are metabolically almost completely inactive (Binder and Anderson 1990) but simultaneously have to protect themselves against bacterial and fungal attack. The aromatic moieties may function as toxins, similar to flavins and tannins in higher plants. Interestingly, this implies that a positive correlation may exist between the survival period of the encysted organism in the sediment, the chemical composition of the cyst wall and its fossilisation potential.

Fossil dinosporin

In recent years several attempts have been made to obtain pure dinoflagellate cyst fractions from sediments, starting in the early 70s (Combaz 1971). In a few cases, high purity was obtained (Table 3).

Pyrolysis of the purest, base- and acid-hydrolysed, fraction with 96% of the Gonyaulacoid *Chiropteridium* displays a mixture of aliphatic and aromatic moieties and no isoprenoids. The pyrolysate resembles that of *S. ramonii* but the aliphatic fragments continue to longer chain lengths; alkane/alkene doublets and their corresponding ketones to C_{30} and the alkylbenzenes up to C_{11}. Furthermore, the FAs are absent. The purity of the sample strongly suggests that the cyst walls are composed of both aliphatic and aromatic moieties. Upon pyrolysis with TMAH, a series of saturated and unsaturated FA moieties is formed, sharply dropping of at C_{18}. The importance of a high purity is illustrated by the observation that analysis of less pure samples yielded highly aliphatic products with the aromatic contribution being strongly dependant on the amount of little black blocky entities in the sample (van Mourik 2000; Warnaar 2001). These black bits yielded predominantly phenols and not only simple aromatics. Clearly, more analyses are needed on pure fossil cyst fractions before and after artificial maturation to elucidate the nature of the fossil cyst walls.

Since the only extant micro-algae known to produce aromatic walls are dinoflagellates, the basically aromatic wall composition of several acantomorph Neoproterozoic Acritarcha has been used to argue that these Acritarcha are related to the dinoflagellates (Arouri et al. 2000). This corroborates with earlier suggestions, based on acritarch morphology (e.g. Sarjeant 1978; Butterfield and Rainbird 1998; Leppig and Montenari 2000), the fossil record of dinosteroids (e.g. Moldowan et al. 1996; Talyzina et al. 2000) and molecular phylogeny (Javaux et al. 2003) that the dinoflagellates originate in the Neoproterozoic.

However, it must be stressed that alternative interpretations are still feasible since the highly aromatic nature of the Neoproterozoic acritarchs may also be related to maturity levels (Arouri et al. 2000), or even convergence.

Concluding remarks

It is clear that the analysis of recent and fossil resistant biomacromolecules requires extreme care especially with respect to the purification procedures and maturation. For the extant material, avoiding artificial condensation and oxidative polymerisation of cytoplasm and ester-bound moieties requires constant attention. Notably addition of aliphatic moieties may occur. For the fossil material, contamination by organic particles other than the target taxon is hard to eliminate and can contribute to either the aliphatic or aromatic signal. Furthermore, *post-mortem* migration of aliphatic moieties into, and their condensation onto the macromolecule might occur as well. These things hamper the evaluation of the aliphatic signature of fossil plant material. The extent to which migration and *in situ* formation of aromatic moieties plays a role in modifying resistant algal macromolecules, notably under elevated temperature and/or pressure conditions, still remains an open question.

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