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Resistant Biomacromolecules as Major Contributors to Kerogen [and Discussion]

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Resistant biomacromolecules as major contributors to kerogen

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SUMMARY

Current research concerning the chemical characterization of organic macromolecules present in well-preserved fossilized materials with known morphologies revealed by (electron) microscopic studies results in the recognition of unknown, resistant biomacromolecules in a variety of organisms. It is shown that highly aliphatic, non-saponifiable biomacromolecules in cell walls of algae (algaenans) have unique structures, probably as a result of different biosynthetic pathways and that they consist of *n*-alkyl-, isoprenoid and tricyclic alkyl units. It is also becoming clear that algaenans are structurally different from the highly aliphatic, non-saponifiable biomacromolecules occurring in plant cuticles (cutans), periderm tissue (suberans), some sporopollenins and in tegmens of seeds of water plants. All these types of aliphatic biomacromolecules are highly resistant and therefore selectively preserved in the geosphere. In particular, Type I and II kerogens consist mainly, in some cases exclusively, of these aliphatic biomacromolecules.

Polysesquiterpenoids and polyditerpenoids occur in fresh and fossil angiosperm and gymnosperm resins respectively and also show resistant behaviour in the geosphere. Some waxy crude oils contain large amounts of compounds derived from these substances after thermal cracking.

A completely new polyphenol type of biomacromolecule was encountered in several fossilized outer walls of seeds (testae) of water plants. Preliminary results indicate that this phenolic biomacromolecule is an alternative source of phenolic moieties in lignites and coals. The significance of lignin as a source of phenolic moieties in subsurface organic matter (e.g. vitrinites) is probably overestimated.

1. INTRODUCTION

The organic matter present in the subsurface of the Earth consists of two operationally defined fractions, the bitumen soluble in common organic solvents and the kerogen, which is insoluble in such solvents. The far greater part of the sedimentary organic matter (*ca.* 95%) consists of kerogen, even if oil accumulations are taken into consideration. Over the last fifty years or so research efforts on the molecular level mainly concentrated on the structural elucidation of low molecular mass (LMM) compounds present in crude oils and bitumens from sediments. The results of these studies have tremendously improved our knowledge of palaeo-environments and diagenetical pathways, and are applied in oil exploration. It has to be realized that in this way only a few percent of the organic matter in the subsurface has been analysed, simply because of the fact that soluble LMM organic compounds can be analysed relatively easily by means of standard methods such as gas chromatography–mass spectrometry (GC–MS). Consequently, our view of the molecular composition of organic matter in the geosphere has been very limited and probably also highly biased. During the last decade several analytical methods and techniques have become available to organic geochemists and other natural product chemists to characterize on a molecular level high molecular mass (HMM) insoluble organic substances such as kerogen and a variety of biomacromolecules. In most cases these analyses are indirect in that a 'demacromolecularization' step is performed before building blocks are identified by classical techniques. Demacromolecularization by specific chemical reactions (chemolysis) and flash-pyrolysis in combination with appropriate derivatizations, chromatographic separations and mass spectrometry are good examples of such an approach. Solid-state ^{13}C nuclear magnetic resonance (NMR) and Fourier transform infrared (FT–IR) spectroscopy are also useful tools, although the resolution of these methods is limited and information is obtained at only the functional group level.

As a result of the availability of these new methodologies and their application to investigate kerogens and unrecognized biomacromolecules, an alternative mechanism for the formation of kerogen has recently

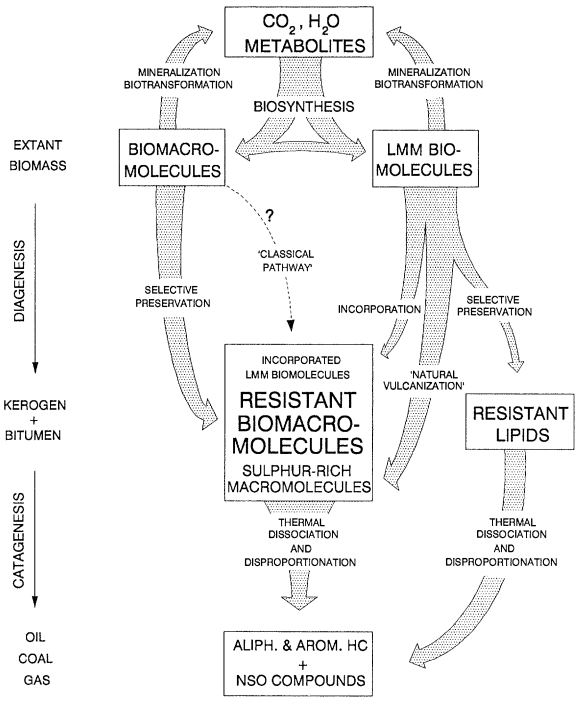

Figure 1. Proposed mechanism for kerogen formation describing the interrelations between the extant biomass, kerogen and fossil fuels. (After Tegelaar *et al.* 1989*a*.)

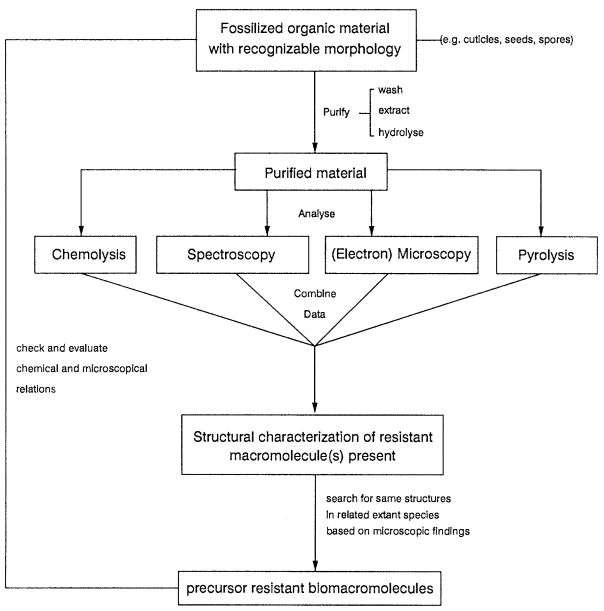

Figure 2. Research strategy for recognizing relations between resistant biomacromolecules and their fossilized counterparts.

been proposed (see figure 1). In contrast to previous ideas indicating that a random repolymerization and polycondensation of lipids with sugars and amino acids (released from polysaccharides and proteins, respectively) results in the formation of kerogen (Tissot & Welte 1984), this alternative is mainly based on a selective preservation of resistant biomacromolecules (see Tegelaar *et al.* (1989*a*) for a review). A number of investigations over the last years have indicated that highly resistant non-hydrolysable biomacromolecules are present in protective envelopes or exudates of extant organisms (e.g. Largeau *et al.* 1984; Goth *et al.* 1988; Nip *et al.* 1986; Van Aarssen *et al.* 1990, 1991*a*). These newly recognized biomacromolecules, although present in low amounts in the organisms themselves, are relatively enriched by several orders of magnitude during early diagenesis owing to the mineralization (conversion to CO_2 and H_2O) of other well-known biomacromolecules such as the polysaccharides and proteins making up the bulk of the original biomass.

Current research in this field focuses on the characterization of potentially resistant biomacromolecules present in kerogens and organisms which have gone unnoticed in the past. An effective research strategy to 'pick up' and characterize unknown, resistant biomacromolecules is schematically depicted in figure 2.

Detailed studies of sedimentary organic matter with microscopically recognizable morphologies (e.g. fossilized organisms or parts thereof, macerals) in most cases reveal the presence of relatively pure macromolecules because an organic fossil is only to be recognized if the underlying chemistry is basically intact. If this were not the case the chances of fossilization of organic matter entities are zero to begin with. We believe that Nature has done an excellent job in the purification of these resistant biomacromolecules via the selective degradation of other, less resistant LMM and HMM organic substances (Goth *et al.* 1988; de Leeuw & Largeau 1991).

In this paper, recent discoveries of resistant biomacromolecules are briefly reviewed and new preliminary data from ongoing research in our group are previewed.

2. MOLECULAR CHARACTERIZATION OF SEVERAL RESISTANT BIOMACROMOLECULES

(*a*) *Sporopollenins*

Earlier studies suggested that sporopollenins, the structural constituents in the outer walls of spores and pollen grains, were derived from carotenoids or carotenoid esters by partial oxidation (Brooks & Shaw 1978). More recent work, however, has demonstrated that oxygenated carotenoids do not contribute at all to sporopollenins and that their recognition earlier on must be ascribed to an incomplete purification of sporopollenin isolates (for a review, see de Leeuw & Lareau (1991)). Based on pyrolysis and ^{13}C NMR studies it is becoming clear that at least two totally different chemical types of sporopollenins exist; a highly aliphatic one that generates extended homologous series of *n*-alkanes, *n*-alk-1-enes and α, ω-alkadienes on pyrolysis, and a phenolic type releasing alkylphenols and benzaldehydes upon thermal stress (Schenck *et al.* 1981; Schultze Osthoff & Wierman 1987; Guilford *et al.* 1988; Wehling *et al.* 1989).

Figure 3 shows the gas chromatogram of the flash pyrolysate of well preserved earliest Palaeocene *Azolla* (water fern) massulae. A fossil massula is the common outerspore member (perispore) surrounding several separate microspore exines (spore wall). Apart from some substances added during the sampling procedure,

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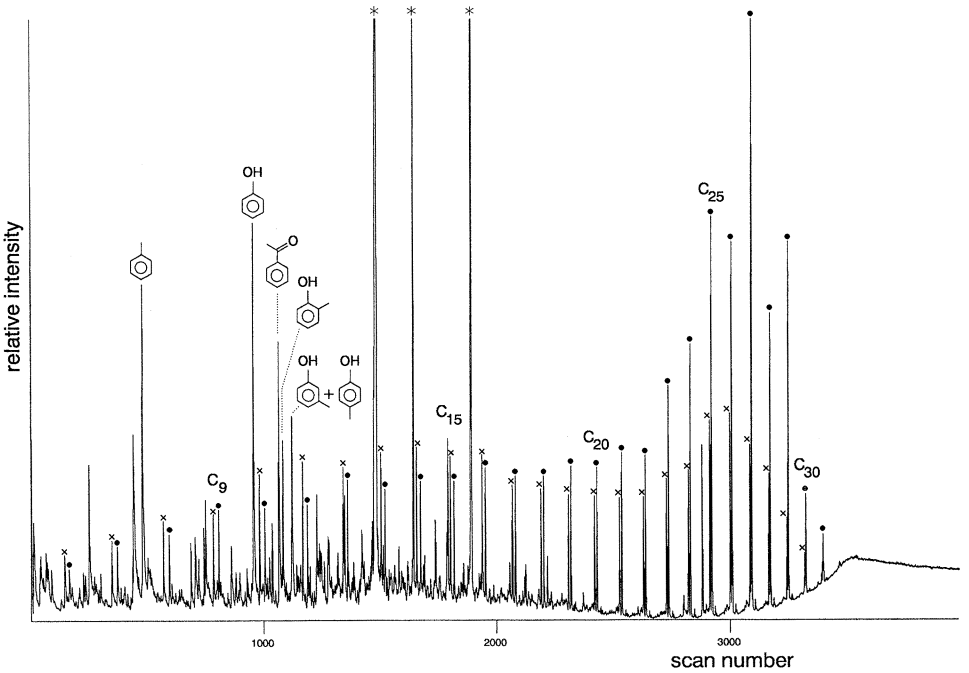

Figure 3. TIC of the pyrolysate of *Azolla* massulae with microspores. Curie temperature 610 °C. Some standards were added during the sampling procedure and are marked *. *n*-Alkanes and *n*-alk-1-enes are marked • and × respectively. (After Van Bergen *et al.*, in preparation.)

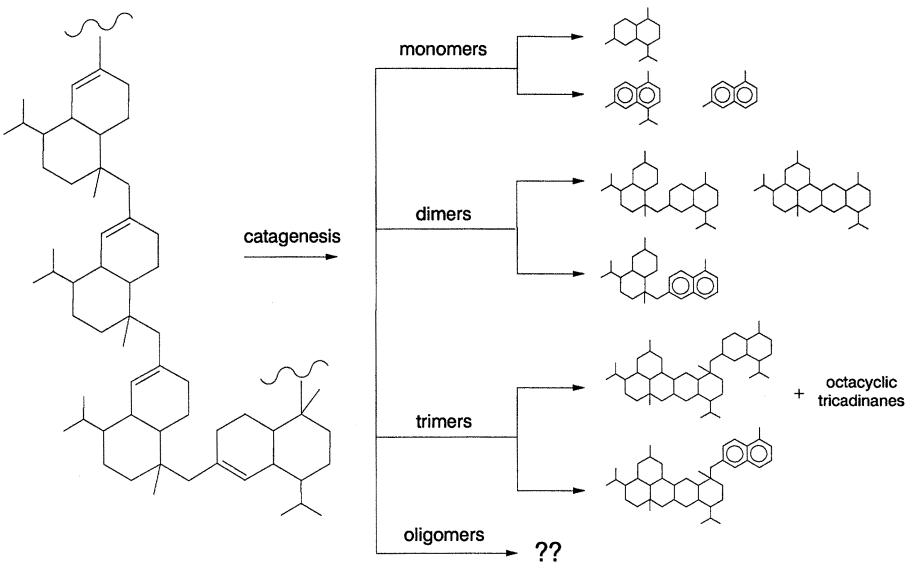

Figure 4. Proposed catagenetic pathway of polycadinene. (After Van Aarssen *et al.* 1991 *a*.)

the pyrolysate is dominated by homologous series of *n*-alkanes and *n*-alk-1-enes. A significant amount of aromatic pyrolysis products are however present as well. Whether these two series of pryolysis compounds represent the microspore exines as well as the perispores or that one of these series is associated only with either the exines or the perispore is currently under investigation (Van Bergen *et al.*, in preparation).

(*b*) *Resins*

It has been shown recently that the so-called Damar resins of the Angiosperm family Dipterocarpaceae contain a polycadinene as a major biomacromolecule (Van Aarssen *et al.* 1991 *a*, *b*). This substance is highly resistant and makes up a considerable part of kerogens with oil potential in S.E. Asia (Van Aarssen *et al.* 1991 *a*). Upon catagenesis of such kerogens a large number of aliphatic and aromatic mono-, di- and oligomeric cadinenes are formed (figure 4). These compounds are frequently encountered in large amounts in crude oils from S.E. Asia (Van Aarssen *et al.* 1991 *a*). Flash pyrolysis studies have shown that polysesquiterpenes are also present in U.S. coals (Meuzelaar *et al.* 1991) and in the 50 million years (Ma) old Messel oil shale kerogen in Germany (J. J. Boon, personal communication) indicating the occurrence of Dipterocarpaceae or other angiosperms with similar resins in palaeoenvironments other than those in S.E. Asia.

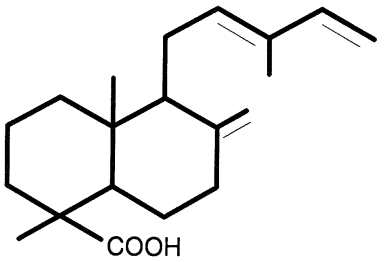

Figure 5. Structure of communic acid; the labdane skeleton is indicated in bold.

Gymnosperm resins contain macromolecules built up from diterpenoids with labdane carbon skeletons (figure 5). It has been suggested that communic acid or related compounds present as free compounds in the fresh resin polymerize under the influence of light or oxygen. New data, resulting from pyrolytic and spectroscopic investigations of Australian and Chinese fossil resins, seem to indicate more precisely how such polymerizations take place and which compounds are involved. Based on these new data a standing controversy in the literature concerning the presence or absence of methyl ester groups in natural monomeric units making up the *Agathis*-type resin macromolecules seems to be settled; such methyl esters are absent in these resins and their presumed presence is probably a result of misinterpretation caused by an insufficient extraction of LMM substances (Anderson *et al.* 1989; Wilson *et al.* 1990; Van Aarssen *et al.*, in preparation).

(*c*) *Cutans and suberans*

Highly resistant aliphatic biomacromolecules other than the polyesters cutin and suberin, are omnipresent in extant and fossil cuticles and periderm tissues of higher plants and are called cutans and suberans respectively (Tegelaar *et al.* 1991*a*; Nip *et al.* 1986). In a recent paper, data are presented that indicate that the palaeobotanical record of cuticles is heavily biased towards taxa originally possessing a significant amount of cutan in their cuticular matrix (Tegelaar *et al.* 1991*b*).

Complementary pyrolysis and spectroscopic data of cutans have indicated that, apart from the dominating *n*-alkyl moieties, polysaccharide units also contributed to the cutan structure (Tegelaar *et al.* 1989*b*). Current research has, however, demonstrated that after a more rigid purification procedure cutans consist almost exclusively of *n*-alkyl units linked together via relatively stable ether-bonds (figure 6). The ether linkage was revealed after chemolytic experiments with specific ether cleavage reagents like BCl_3 (A. Jenisch, personal communication) although the quantities of released products were unexpectedly low. The released components consisted almost entirely of C_{27}, C_{29} and C_{31} homologues.

Cutans are highly resistant and have been shown to be a likely source for aliphatic components, especially *n*-alkanes, in waxy crude oils (Tegelaar *et al.* 1989*d*) and for *n*-alkyl moieties in kerogens and coals (Nip *et al.* 1989).

(*d*) *Algaenans*

Algaenans are highly aliphatic, non-hydrolysable resistant biomacromolecules occurring in outer cell walls of several species of very common green algae such as *Botryococcus braunii*, *Tetraedron minimum* and *Scenedesmus* species (Tegelaar *et al.* 1989*c*; Largeau *et al.* 1990*a*; Derenne *et al.* 1991*a*). The most studied algaenans are those of races A, B and L of *B. braunii*. An extended review of the excellent work done on the structural elucidations and biosyntheses of the LMM and HMM lipids of the several races of *B. braunii* has recently been published (Metzger *et al.* 1991). Based on pyrolysis and spectroscopic studies of labelled and unlabelled

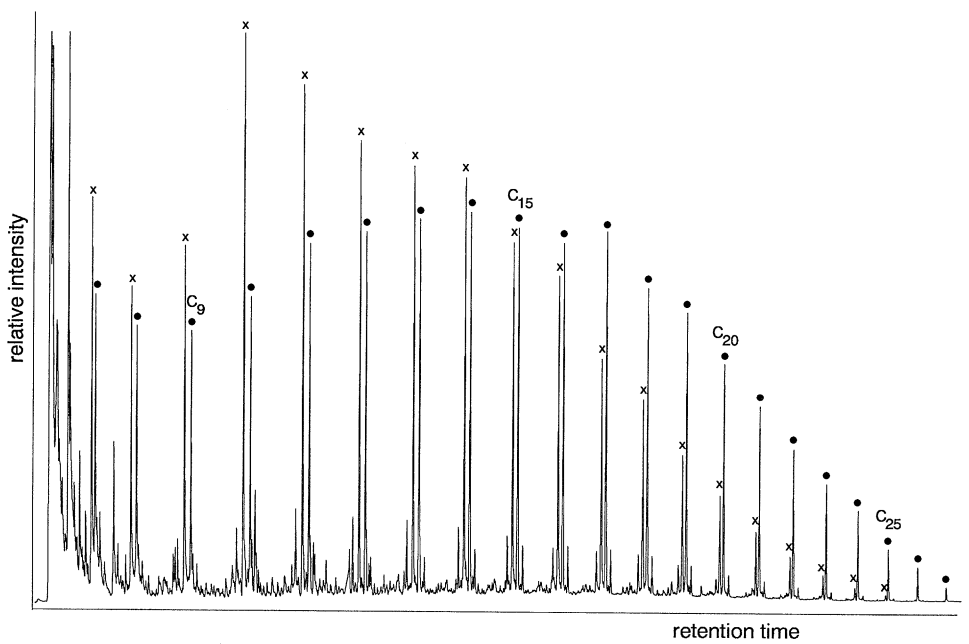

Figure 6. Py-GC-FID trace of the cuticular residue of *A. americana*. Curie temperature 770 °C. *n*-Alkanes and *n*-alk-1-enes are marked • and × respectively.

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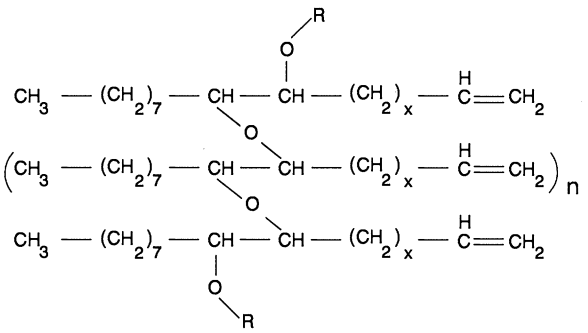

Figure 7. A possible schematic structure of an outer cell wall biomacromolecule of *B. braunii* race A. $n = ?$; $x = 15$, 17 or 19; $\times = C_{18}$–C_{30} carbonyl groups with an even number of carbon atoms or C_{24}, C_{26} alkyl groups. (Modified after Metzger & Largeau (1991).)

algaenans and on rigid structural identifications of soluble oligomeric lipids using cultured algae, structural units have been proposed for the algaenans of *B. braunii* races A, B and L. A possible schematic structure of an outer cell wall biomacromolecule of *B. braunii* race A, slightly revised compared with the one proposed by Metzger *et al.* (1991) as a result of ongoing studies (Gatellier *et al.*, in preparation), is presented in figure 7. The chemical differences observed between the three races are dramatic. The less studied race L biosynthesizes an algaenan consisting of isoprenoid (lycopadiene) units probably linked together via ether bridges, whereas the races A and B biosynthesize algaenans derived from ether-bound unbranched C_{27}, C_{29} and C_{31} alkenes and alkadienes. The LMM lipids also show major differences for the three races: unbranched long-chain alkadienes and alkatrienes in race A, the so-called highly branched botryococcenes in race B and lycopadiene in race L (Metzger *et al.* 1991). Although it was believed that the algaenans of races A and B are very similar, more recent detailed investigations of algaenans of cultured *B. braunii* and their fossilized counterparts show that considerable differences may exist. The algaenans of other algae are less studied. Comparison of the flash pyrolysates of the algaenans of *B. braunii* races A or B with that of *Tetraedron minimum* shows that they are clearly different. The putative building blocks of the *T. minimum* algaenan may consist of limited series of mono-unsaturated ω-hydroxy fatty acids, substances encountered in the extractable lipids of *T. minimum* but not in the races of *B. braunii* (Rijpstra *et al.*, in preparation). It is becoming evident that algaenans of different species or even races are chemically quite different from each other and from cutans and suberans, although all these biomacromolecules are highly aliphatic in nature and non-saponifiable. It is also interesting to note that all these aliphatic biomacromolecules are probably biosynthesized via completely different biochemical pathways.

The highly characteristic series of products generated during pyrolysis of these algaenans has enabled their recognition in many mature and immature Type I and II kerogens. In many so-called Torbanites and Coorongites, as well as in the famous 50 Ma-old Messel kerogen in Germany and in a Miocene lacustrine sediment from Spain, the kerogens are thought to consist almost exclusively of fossilized algaenans of one algal species (Largeau *et al.* 1986; Derenne *et al.* 1988; Goth *et al.* 1988; Sinninghe Damsté *et al.*, in preparation; Gatellier *et al.* in preparation), indicating the selective preservation of these biomacromolecules as such or slightly altered even under oxic depositional conditions. In flash pyrolysates of Ordovician source rocks rich in a microscopically recognizable fossil species known as *Gloeocapsomorpha prisca* dominant series of *n*-alkanes, *n*-alk-1-enes, and α,ω-alkadienes with similar and specific chain length distributions are present. There are strong indications that these pyrolysis products are generated from a fossilized algaenan derived from *G. prisca* and consisting of C_{16} and C_{18} building blocks with an original double bond at the C_9 or C_{10} position (Derenne *et al.* 1990; Douglas *et al.* 1991). Although many speculations have been made in the past concerning the origin of *G. prisca*, recent investigations seem to indicate a very strong relationship between the extant *B. braunii* race A and the fossil species *G. prisca* (Zalessky 1917; Reed *et al.* 1986; Derenne *et al.* 1991*b*). Electron microscopical investigations of *B. braunii* cultured under marine conditions show identical cell wall structures to the fossil remains of the Ordovician *G. prisca* (Derenne *et al.* 1991*b*). Current investigations are aimed at establishing whether these microscopical similarities are supported by similar or identical chemistries.

Oil shales consisting almost entirely of remains (cysts) of Tasmanaceae (closely related to present *Pachysphaera*) are called tasmanites. Pyrolysis of tasmanites and of isolated cysts generates series of *n*-alkanes and *n*-alk-1-enes but also tricyclic diterpenoids (Philp *et al.* 1982), suggesting that diterpenoids can also be building blocks of algaenans (Sinninghe Damsté *et al.* in preparation).

Raynaud *et al.* (1988) noticed that a large number of kerogens that were classified heterogeneous and amorphous by light microscopy actually contained very thin ultralamellar structures when investigated by transmission electron microscopy. These observations were further confirmed by Largeau *et al.* (1990*a*, *b*) in source rocks and oil shales. The latter authors named these structures ultralaminae and discovered identical structures in outer cell wall layers of many common freshwater green algae like *Scenedesmus* species and in a few marine algae. Chemical comparisons of the ultralaminae from extant species with those present in kerogens, using pyrolysis and spectroscopic methods, clearly indicated that the ultralaminae present in many type I and II kerogens are selectively preserved laminae of outer wall biomacromolecules present in these green microalgae. These resistant substances represent a type of algaenans other than those mentioned above, because the pyrolysates contained, apart from homologous series of *n*-alkanes and *n*-alk-1-enes, a highly characteristic series of *n*-alkylnitriles ranging from C_{12} to C_{32} (Derenne *et al.* 1991*a*). The authors suggest that these alkylnitriles are generated from alkylamide moieties present in the biomacromolecules. Microscopic differences between outer walls of these algae and those of *B. braunii*, *T. minimum* and

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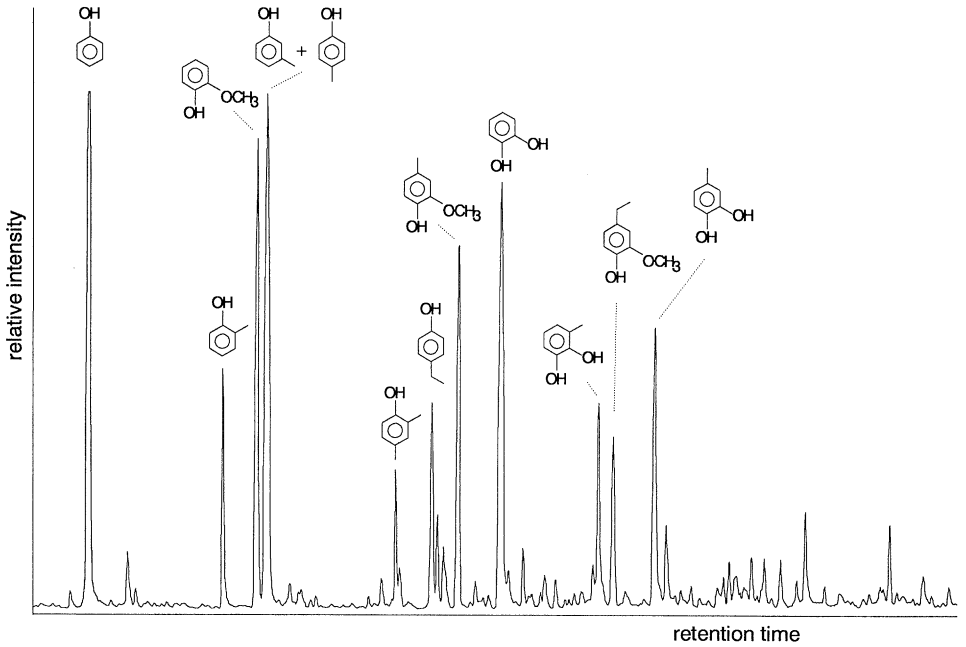

Figure 8. Partial GC trace of the pyrolysate of fossil testae of *Sabrenia*. Curie temperature 610 °C. (After Van Bergen *et al.*, in preparation.)

G. prisca also exist: the resistant outer cell walls of the latter ones are very thick (*ca.* 1000 nm) whereas the ultralaminae represent very thin outer walls (*ca.* 15 nm).

The detailed study of algal cell wall biomacromolecules as well as their fossilized counterparts seems to be a very interesting and fascinating area of research not only for organic geochemistry and other earth-related sciences but also for natural product chemistry and biochemistry.

(*e*) *Polyphenols in testae and lignites*

At present, well preserved fossil seed coats of several water plants (Collinson 1983, 1988), such as water soldier (*Stratiotes*) and an extinct water lily (*Sabrenia*), are being examined by Py-GC–MS, FT–IR and solid state ^{13}C NMR. In both cases it was easy to dissect the seed coat inner layer (tegmen) from the outer seed coat layer (testa). The GC–MS analysis of the pyrolysates indicated that the tegmen consists almost entirely of a highly aliphatic biomacromolecule similar to cutans present in higher plant cuticles and that the testa is composed of a phenolic macromolecule (Van Bergen *et al.*, in preparation). The pyrolysate of the latter consisted of relatively large amounts of phenol, methylphenols, dimethylphenols, some dihydroxybenzenes and a few methoxyphenols (see figure 8). These phenolic pyrolysis products are completely different from those observed upon pyrolysis of extant and fossil lignins and they cannot be associated with tannins. Consequently it is concluded that testae of water plant seeds and possibly other plant tissues consist of an as yet unknown resistant polyphenol. Perhaps such a biomacromolecule has a bacteriostatic or fungistatic function because it is in direct contact with the surrounding water. Comparisons of the highly characteristic pattern of phenols in the pyrolysates of testae with those in flash pyrolysates of a number of lignites like the Beulah Zap lignite of Upper Palaeocene age (Sentinel Butte Formation; Mercer County, North Dakota, U.S.A.) show striking similarities. We therefore speculate that this polyphenol is highly resistant and is thus selectively preserved in lignites and brown coals. This may imply that phenol units in these types of sediments cannot simply be ascribed to an origin of lignin or partly altered lignins. An alternative origin from testae may actually explain the presence of phenolic pyrolysis products with alkyl and hydroxyl substitution patterns completely different from those of lignin building blocks (Sentfle *et al.* 1986; Nip *et al.* 1991).

3. EPILOGUE

Based on the recently obtained data as described above, it may be concluded that the chemical analysis of well-preserved fossils with an organic matrix seems to be an efficient way to recognize and identify resistant biomacromolecules and their biological precursors. However, accumulating knowledge of the presence and nature of resistant biomacromolecules in extant organisms allows for a better understanding of diagenetic and catagenetic pathways of organic matter in sediments and enables the reconstruction of palaeoenvironments in a more reliable and detailed fashion.

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Discussion

R. P. Ambler (*Institute of Cell and Molecular Biology, Division of Biological Sciences, University of Edinburgh, U.K.*) Some molecules that end up in kerogen are presumably immunogenic *in vivo*. Has Dr de Leeuw had success with looking for epitopes in the material left in kerogen?

J. W. de Leeuw. As you know attempts have been made, for instance by Gerard Muyzer, to use immunological techniques and methods to recognize intact biomacromolecules in recent sediments. These attempts have been rather successful. There were, however, strong indications that the immunological reactions were due to the presence of hydrophobic maybe partly water-soluble constituents in the very recent sediments investigated. When the kerogens mature they become increasingly hydrophobic in nature and, as a consequence of that, I do not believe that immunological methods have a great potential in those cases.

S. Macko (*Department of Environmental Sciences, University of Virginia, U.S.A.*). The significance of Dr de Leeuw's findings is obvious to understanding the generation of petroleum. However, we need to consider the overall picture of preservation of organic matter; the fact that kerogens contain nitrogen (probably 2–4%) which is likely based originally on amino acids. If we assume that 3% is amino acid nitrogen with a c/n ration for protein of about 4, then roughly 12% of the carbon of the kerogen is derived from amino acids; also 6% is oxygen from amino acids and about 1% is hydrogen from amino acids. Therefore a total of about 25% of the kerogen could be amino acid in origin. If one considers this along with the carbohydrate preservation, perhaps 50% of a kerogen could be unrelated to lipid preservation.

J. W. de Leeuw. Although every kerogen is unique on an average base I think that Dr Macko's estimation of *ca.* 3% nitrogen in the 'average' kerogen is much too high, especially when we refer to kerogens of less immature sediments. Moreover, I'm not sure that the nitrogen left has an origin from proteins or amino acids. Bound porphyrins and chlorophylls and the recently encountered long-chain alkylnitriles in pyrolysates of kerogens are examples of alternative source of nitrogen in kerogens. We have to keep in mind once again that kerogens are the products of drastic selective preservation. As a consequence we can see enrichment of several orders of magnitudes of relatively stable nitrogen-containing biochemicals that are minor components in the original organisms when compared with the (labile) proteins and amino acids. I should also like to refer to sulphur in kerogens. It has been shown very clearly that the organic sulphur constituents in kerogen have no relation whatsoever with sulphur-containing amino acids or proteins. With respect to oxygen the situation is ever more complex. I am of the opinion that the oxygen in kerogens is mainly derived from phenolic macromolecules such as lignins, tannins, etc. with additional contributions of structural polysaccharides and oxygen-containing lipids. Such an origin actually explains very well the relative high oxygen content in immature Type III kerogens. I do not think that oxygen from amino acids plays a significant role.

G. B. Curry (*Department of Geology and Applied Geology, University of Glasgow, U.K.*). Dr de Leeuw said that β-carotene is associated with, but not a component of, sporopollenin. In the light of the result discussed elsewhere in this symposium showing that an intracrystalline carotenoprotein is responsible for shell colour (Curry *et al.*, this symposium), is it possible that the β-carotene association with sporopollenin has a similar role in coloration?

J. W. de Leeuw. Dr Curry's suggestion is certainly an interesting one. To the best of my knowledge nothing is known about the possible role of carotenoids with respect to colour changes of spores and pollen during diagenesis. There are, however, strong indications that poly-unsaturated components such as carotenoids may undergo internal cyclization and aromatization upon diagenesis processes which could be related to colour changes.

G. Eglinton (*Organic Geochemistry Unit, School of Chemistry, University of Bristol, U.K.*). Some of the biopolymers Dr de Leeuw showed had terminal unsaturation in vinyl groups. It could be possible to expect these to undergo reactions during diagenesis, e.g. to copolymerize and hence extend the three-dimensional nature of the polymers; in addition, to undergo further reactions such as protonation or attachment of thiophenes and aromatic molecules.

J. W. de Leeuw. Yes, the functionalized groups present in resistant biomacromolecules are certainly prone to alteration of some sort during diagenesis. As observed in the case of *G prisca*-rich materials the original functional groups in the macromolecules may have been hydrogenated or replaced by more stable thiophene or benzene groups. It is feasible that

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original functional groups also have triggered reactions between the monomeric units resulting in additional cross-linking and consequently even more stability and resistance.

J. R. Maxwell (*Organic Geochemistry Unit, School of Chemistry, University of Bristol, U.K.*). Dr de Leeuw mentioned the important and highly resistant aliphatic polymeric materials in algae and bacteria. Does he know if there is any evidence for such materials in bacteria?

J. W. de Leeuw. Current research by Largeau and colleagues indicates that some types of bacteria contain resistant biomacromolecules. They are, however, not aliphatic in nature.

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