Studies of Fossil and Modern Spore Wall Biomacromolecules using $^{13}$C Solid State NMR

ALAN R. HEMSLEY*, ANDREW C. SCOTT†, PATRICK J. BARRIE‡§ and WILLIAM G. CHALONER‡

* Department of Earth Sciences, University of Wales, Cardiff, PO Box 914, Cardiff, CF1 3YE, † Department of Geology, Royal Holloway University of London, Egham, Surrey, ‡ Department of Chemistry, University College London, Christopher Ingold Laboratories, 20 Gordon Street, London, WC1H 0AJ, UK

Received: 7 September 1995 Accepted: 22 January 1996

A range of Carboniferous lycophyte megaspore exines have been investigated using $^{13}$C magic-angle spinning nuclear magnetic resonance (MAS NMR) spectroscopy. Their composition differs considerably from sporopollenin obtained from an extant lycophyte. The differences observed result in part from varying degrees of diagenesis.

Fossil fern spores, gymnosperm megaspore-membranes and pollen have also been examined. These show a similar composition to the fossil lycophyte megaspores. The constituent material of all of these exines differs considerably from the sporopollenin obtained from comparable extant samples. Despite the changes in composition observed on fossilisation, differences in composition between the major groups of plants may be preserved to some extent in the fossil material. Walls of the fossil prasinophycean algal cyst *Tasmanites* have been examined and these show a greater similarity to fossil cuticle and algaenans than to sporopollenins.

The effect of oxidative maceration on fossil and modern sporopollenins has also been investigated. The main influence of oxidative maceration is the removal of unsaturated carbon environments such as aromatics; this causes fossil spores to be more susceptible to oxidative maceration than the modern exines. Heating of modern exine material models the alteration of exines by diagenesis. The changes that occur on heating an extant sample to 150–225 °C give a chemical composition that is similar to those of the fossil sporopollenins. © 1996 Annals of Botany Company

Key words: $^{13}$C solid state NMR, spores, pollen, fossil, Carboniferous lycopsids, ferns, pteridosperm, gymnosperm, oxidative maceration, heating, thermal maturation.

INTRODUCTION

Sporopollenin is the main biomacromolecule present in the outer wall (exine) of spores and pollen and its composition has long been a topic of interest. The present research was designed to investigate the chemical composition of a range of fossil and modern spore wall materials using $^{13}$C solid state NMR spectroscopy as the principal characterization technique. This work follows from an initial study (Hemsley et al., 1992) of fossil and modern sporopollenins and pursues some of the questions raised in the earlier article.

Each $^{13}$C nucleus in a sample resonates at a slightly different frequency when placed in a strong magnetic field depending on its chemical environment, and the resulting chemical shifts for different functional groups are now well-established. High-resolution NMR spectra may be obtained in the solid state by rapid rotation of the sample at an angle of 54°7° to the direction of the applied magnetic field (so-called magic angle spinning, MAS), and this technique has been widely used to study the composition of fossil fuels and precursor materials (Axelson, 1985; Wilson, 1987).

There are a number of questions concerning the chemical composition of fossil and modern sporopollenins that it was hoped would be clarified by the application of $^{13}$C solid state NMR spectroscopy. One major topic of interest is the extent to which the NMR spectrum might provide a ‘fingerprint’ characteristic of a plant’s systematic status, as this might prove particularly useful in studying fossil material of questionable origin. For instance, among the samples investigated are fossil algal cysts assignable to *Tasmanites* (Prasinophyceae); these have previously been considered as having been formed from sporopollenin (Brooks, 1971), while comparison of the NMR spectrum with those of the other spore wall biomacromolecules studied suggests a closer affinity either to algaenans or fossil cuticles. In this work we also compare the spectra of sporopollenin obtained from microspores and megaspores (including seed megaspore-membranes) from the same species of plant in order to see whether they have similar composition.

$^{13}$C solid state NMR spectroscopy also allows structural changes that occur upon chemical treatment to be probed. Oxidative maceration is frequently used in the separation of spores from coals, and so knowledge of its effect on spore structure is desirable, particularly as it is known that fossil material will eventually ‘dissolve’ in Schulze’s solution (a saturated solution of potassium chlorate in nitric acid, see Traverse, 1988), or in fuming nitric acid. This work also studies chemical changes that occur during heating in the presence of oxygen, which may well be related to the
changes that occur during fossilisation. Of particular interest are whether the changes in composition as a result of diagenesis (changes that occur in sediments and trapped organic materials prior to their consolidation) are gradual or abrupt, at what temperature particular alterations in composition occur, and how far diagenesis may blur the characteristics of sporopollenin from any particular source plant.
MATERIALS

Modern spores and pollen and seed membranes

*Lycopodium clavatum* L. (Lycophyta) spores obtained from a commercial source (BDH). *Anemia* sp. (Filicophyta) spores obtained from plants at the Royal Botanic Gardens, Kew, UK. *Pinus sylvestris* L. (Coniferophyta) pollen collected from local trees in Surrey. *Dioon edule* Lindley (Cycadophyta) pollen obtained from plants at the Royal Botanic Gardens, Kew, UK. *Cycas circinalis* L. (Cycadophyta) megaspore-membrane from seeds collected by WGC at the Marie Selby Botanic Garden, Florida, USA.

Fossil spores, pollen and seed membranes

*Zonalesporites* sp. (Lycophyta, Fig. 3) from the Kaluga lignite deposit, Kurovskaya Mine, Moscow Coal Basin, Russia, (Viséan, Lower Carboniferous); see Dijkstra and Piéart (1957). *Lagenicula crassicauleata* Zerndt (Lycophyta, Fig. 1) from limestone, Foulend, Berwickshire, UK (late Tournaisian, Lower Carboniferous) see Scott and Meyer-Beutha (1985). *Lagenicula subpilosa* f. major Dijkstra ex. Chaloner (Lycophyta, Fig. 2) from limestone, Pettycur, Fife, Scotland, UK (Viséan, Lower Carboniferous) see Rex and Scott (1987). *Lycospora chaloneri* Scott and Hemsley (Lycophyta, Fig. 6, see Scott and Hemsley, 1993a) from a cone of *Flemingites scottii* (Jongmans) Thomas and Brack-Hanes. Location and matrix as above. *Tuberculatisporites* sp. (Lycophyta, Fig. 5) from Bed 20f, Middle Coal Measures (shale), Swillington Brickpit, Yorkshire, UK. (Westphalian B, Upper Carboniferous) see Scott (1978). Spores from *Botryopteris globosa* Darrah (Filicophyta) from Coal Ball, West Mineral Kansas, USA (Univ. Kansas 11072) see Phillips (1980). *Schoepfiapollesites* sp. (Pteridospermophyta) from *Dolerotherca*, Upper Carboniferous (Pennsylvanian), Mazon Creek, Illinois, USA (PP 27832 Field Museum of Natural History, Chicago, Illinois; see Peppers and Pfefferkorn, 1970). Megaspore-membranes of seeds (Pteridospermophyta, Fig. 4) extracted from calcareous ash, Oxroad Bay, Scotland, UK (late Tournaisian, Lower Carboniferous); see Bateman and Scott (1990).

Fossil cuticle and algal cyst

*Tasmanites* sp. (algal cyst) obtained from Tasmanite Coal, La Trobe, Mersey River, Northern Tasmania, Australia (Permian) see Newton (1875). (RHBNC Geology Collection R.1657). *Eskdalia* sp. (Lycophyta, cuticle) from the Russian Paper Coal, Tovakova. British Geological Survey Collection (Kidston Collection 1299) (Lower Carboniferous); see Wilson (1931), Thomas (1968) and Collinson *et al.* (1994).

METHODS

All spores and pollen obtained from living plant material used in this study were acetylated using the method outlined by Erdtmann (1960). The material was saturated with glacial acetic acid before acetylosis (2 min duration) and returned to this after treatment. Each sample was then rinsed six times with acetone to remove most of the acetic acid. This treatment causes some acetylation of the sporopollenin to occur, resulting in an increase in peak intensities at 21 and 173 ppm of the $^{13}$C NMR spectrum (see Hemsley, Barrie and Scott, 1995; Figs 7a and 9a therein).

Fossil material from calcareous matrices was extracted using 10% HCl, picked by hand and stored in acetone before drying. Material from siliceous samples was extracted by maceration with 10% HF, washed thoroughly with water, picked and stored in acetone. Material from the Moscow lignite was macerated gently with concentrated HNO$_3$ to facilitate release from the matrix and thereafter treated as above.

Some samples of *Lycopodium* and *Lagenicula* were subjected to oxidation by treatment with concentrated HNO$_3$ saturated with KClO$_3$ (Schulze’s solution) for 30 min. The *Lycopodium* was initially introduced to a small quantity of dilute HNO$_3$ (2 ml) before making up to 50 ml with the above solution. This procedure was necessary to reduce the problem of the highly exothermic reaction on initial contact with the full strength solution. After oxidation, samples were rinsed with water, then with acetone a number of times, and finally air dried in a desiccator.

The samples to be subjected to heating were prepared for study, dried and placed in open crucibles in a muffle furnace for 1 h. The chosen temperatures of 150, 225 and 300 °C were kept constant for this period. Air was admitted to the samples during heating.

$^{13}$C solid state NMR spectra were obtained on a Bruker MSL-300 spectrometer using cross-polarization (CP), high power decoupling and magic-angle spinning. Spectra were normally accumulated overnight (16 h), and processed using a line-broadening factor of 50–100 Hz for the modern material, and up to 200 Hz for the fossil material. All spectra were acquired under identical conditions, namely using 1 ms contact time and 1 s recycle delay at spinning speeds of 4.5–5 kHz. The $^1$H 90° pulse was 4 $\mu$s. Chemical shifts are given in parts per million (ppm) relative to external TMS. In order to alleviate the problem of overlapping spinning sidebands, the TOSS sideband suppression sequence (Dixon *et al.*, 1982) was used. It is recognized that both CP and TOSS are not necessarily fully quantitative, but these experimental conditions are required to give reasonable quality spectra in an acceptable time on the very small amounts of fossil material available (down to 2 mg in some cases, see Hemsley *et al.*, 1995).

Aromaticities of the fossil samples were measured by simple integration taking the aromatic $^{13}$C environments to resonate between 165 and 90 ppm. Before integration, care was taken with the phase correction (so that the baseline at $>$ 200 ppm and $<$ 0 ppm was flat). For a few samples an empirical polynomial baseline correction was then applied.

RESULTS

*Lycopodites* spores (Fig. 7)

Figure 7 illustrates six NMR spectra obtained from samples of sporopollenin of lycophyte origin. Extant *Lycopodium* (Fig. 7a) exhibits a spectrum which we have found to be typical of a sporopollenin (Hemsley *et al.*, 1993) and which is
similar to that illustrated by Guilford et al. (1988). Each distinct $^{13}$C chemical environment gives rise to a peak at a characteristic chemical shift. The peaks can loosely be assigned according to spectral region: 10–50 ppm indicates aliphatic hydrocarbon environments; 50–105 ppm indicates oxygenated aliphatic groups; 110–160 ppm indicates unsaturated carbon environments (olefins/aromatics); 160–180 ppm indicates carboxylate groups (acids/esters).

The remaining spectra shown in Fig. 7b–f, have all been obtained from fossils. These differ considerably in composition from the untreated Recent spore wall material (unlike the IR sporopollenin spectra of Brooks, 1971) but are relatively similar to each other. The lower four spectra (Fig. 7c–f) are remarkably consistent despite differing in sedimentary history and diagenetic influence. Lagenicula crassiaculeata and L. subpilosa f. major (Fig. 7c and d) are both from calcareous rocks and the spores possess excellent three-dimensional preservation (see Figs 1 and 2). Despite this, as with the other fossil sporopollenins discussed below, the exine material has undergone substantial modification involving relocation or loss of oxygen and an increase in aromatic content (in a process akin to coalification). Hence the spectra consist of two broad peaks corresponding to the aromatic and aliphatic carbons. The aromatic peaks are clearly compound with contributing peaks at 126, 138 (due to bridgehead carbons) and 152 ppm (due to phenolic carbons).

Lycospora chaloneri (Figs 6 and 7e) are the microspores produced within the same cone as Lagenicula subpilosa f. major and these give a similar $^{13}$C spectrum as does the sample of Tuberculatisporites (megaspore of Sigillaria, Figs 5 and 7f). The Tuberculatisporites result is important as the sample was extracted from a siliceous shale, unlike the other fossil samples discussed so far. Aromaticity values (the ratio of aromatic carbon to total carbon content) measured from the NMR spectra are given in Table 1. It thus appears that
Table 1. Measured aromaticities of the fossil samples. (Aromaticity = aromatic peak area/total carbon area)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aromaticity %</th>
<th>Organ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonalesporites</td>
<td>37</td>
<td>Carb. lycopsid megaspore</td>
</tr>
<tr>
<td>Lagenicula crassiacauleata</td>
<td>43</td>
<td>Carb. lycopsid megaspore</td>
</tr>
<tr>
<td>L. subpilosa f. major</td>
<td>48</td>
<td>Carb. lycopsid megaspore</td>
</tr>
<tr>
<td>Tuberculatisporites sp.</td>
<td>50</td>
<td>Carb. lycopsid megaspore</td>
</tr>
<tr>
<td>Lycospora chaloneri</td>
<td>53</td>
<td>Carb. lycopsid microspore</td>
</tr>
<tr>
<td>Botryopteris globosa</td>
<td>56</td>
<td>Carb. fern microspore</td>
</tr>
<tr>
<td>Schopfipollenites sp.</td>
<td>45</td>
<td>Carb. pteridosperm pollen</td>
</tr>
<tr>
<td>Seed megaspore membrane</td>
<td>33</td>
<td>Carb. pteridosperm seed</td>
</tr>
<tr>
<td>Tasmanites</td>
<td>15</td>
<td>Permian algal cyst</td>
</tr>
<tr>
<td>Eskdalia</td>
<td>10</td>
<td>Carb. lycopsid cuticle</td>
</tr>
</tbody>
</table>

Fig. 8. $^{13}$C NMR spectra obtained from a living fern spore (a) and a fossil fern spore (b). The living spore spectrum shows a distinctly different composition from that of *Lycopodium* whilst the fossil fern has a spectrum similar to the fossil lycophytes, but with a relatively higher aromatic signal.

the diagenetic change produced in the sporopollenin is independent of the nature of the matrix in the samples examined here.

*Zonalesporites* (Figs 3 and 7b) differs from the four fossil spores mentioned above in retaining evidence of the oxygenated aliphatic (55–95 ppm) and carboxyl peaks.
(160–180 ppm) present in the extant lycophyte sporopollenin (Fig. 7a). This fossil material has been subjected to partial oxidative maceration which might affect composition slightly. However, from the results given below, the effect of this treatment is unlikely to alter these two additional features. This example suggests that there is less corruption of the chemical identity of the original sporopollenin retained in material of very low rank (i.e. not subjected to high temperatures associated with deep burial).

**Fern spores (Fig. 8)**

The spectrum obtained from the extant fern spores is different from that of *Lycopodium*. Most noticeable is the significant reduction in the number of aliphatic carbon environments (compare the region 20–35 ppm in Figs 7a and 8a). The lower proportion of aliphatic material present in the extant fern spores may be retained to some degree during diagenesis, as manifest by the spectrum of fossil fern spores (Fig. 8b), in which the aromatic peak intensity is higher than that of the aliphatic peak (unlike those of the fossil lycophytes shown in Fig. 7). This results in the higher aromaticity values for the fossil fern spores presented in Table 1, and this may provide a basis by which spores of ferns might be distinguished from those of lycophytes. It should, however, be borne in mind that aromaticity may also be affected by thermal history as is demonstrated below.

**Gymnosperm pollen and seed megaspore-membranes (Fig. 9)**

Gymnospermous plants produce sporopollenin in the form of a pollen wall, and also as the seed megaspore...
membrane around the female gametophyte. This latter structure is interpreted as the relictual megaspore wall, retained from an evolutionary precursor in which the megaspores were freely dispersed (Chaloner and Pettitt, 1987).

The NMR spectrum obtained from pollen (Fig. 9a) from Dioon edule (a cycad) illustrates a spectrum that appears intermediate between the lycophyte and fern spores. In this material, the unsaturated carbon environments are rather less clearly defined and form less of the sporopollenin composition than in the ferns or lycophytes. A fossil gymnosperm pollen (a medullosan Schopfipollenites) exhibits a spectrum similar to that of the fossil lycophytes with a comparable aromaticity (Fig. 9b).

Extant cycad seed megaspore-membranes show a spectrum (Fig. 9c) similar to that produced by the extant pollen, but a number of additional oxidized aliphatic components are present (65 and 99 ppm) whilst the peak at 52 ppm (present in the pollen) is absent. The fragility of these extremely thin sporopollenin membranes and their degree of attachment to the surrounding seed wall layers, makes it difficult to be certain that all of the non-sporopollenin material has been removed even after acetolysis. It is possible that some of the differences between the megaspore membrane and the pollen are a result of retention of some of these adjacent layers in the former.

Despite this potential problem, it is clear that the higher proportion of aliphatics present in the extant membrane occurs also in the fossil material of a pteridosperm seed megaspore-membrane (Fig. 9d) which has an aromaticity of only 33%. This observation lends support to the suggestion that the relative proportion of aromatics to aliphatics is
retained to some degree in the fossil material despite diagenesis.

The spore wall of Tasmanites (Fig. 10)

Figure 10a illustrates the $^{13}$C NMR spectrum obtained from the *Tasmanites* sp. cyst wall. Members of this genus have been attributed to the algal group Prasinophyceae (Wall, 1962), now Pterospermataceae (Chlorophyta) (Guy-Ohlson and Boalch, 1992), and were reported to consist of sporopollenin by Brooks and Shaw (1971) who found that the infra-red spectrum from their wall material was the same as that from spores and pollen. From our spectra it is immediately evident that of *Tasmanites* is very different from the NMR spectra of both the modern and fossil spores and pollens. The bulk of the material is aliphatic with only a small proportion being of aromatic composition. This corresponds quite closely to a spectrum obtained from an alga (Zelibor et al., 1988) or a fossil cuticle (Fig. 10b), in this case that of *Eskadalia*, a lycophyte cuticle from the Lower Carboniferous of Russia. (Collinson et al., 1994).

This result strongly suggests a closer similarity of tasmanite walls to algal or cuticular material as opposed to sporopollenin. This striking difference in composition suggests that NMR characteristics should distinguish between prasinophycean algal cysts and the sporopollenin of archegoniate plants.

Oxidative maceration of spores (Fig. 11)

Figure 11 illustrates four spectra, two obtained from acetylated *Lycopodium* and two from the fossil *Lagenicula*
c. Pinus sylvestris pollen heated to 225°C

Fig. 12. $^{13}$C NMR spectra showing the effects of heating (for 1 h) on the chemistry of extant pollen walls. Acetolysed pine pollen (a) shows a typical spectrum, but as this is heated (b to d) the spectrum begins to resemble (at low temperatures) low rank fossil material (b) and at higher temperatures, greatly altered material (c and d). (Compare b to Fig. 7b, and c to Fig. 7c–f).

crassiaculeata. The unoxidized samples (Fig. 11a and c) are the same as those in Fig. 7 and are repeated here for immediate comparison with the oxidatively macerated material. Macerated Lycopodium (Fig. 11b) clearly illustrates that the main effect of this treatment is the removal of the unsaturated carbon environments which resonate between 110 and 150 ppm. The spectrum is barely altered in other respects.

The spectrum of oxidised fossil sporopollenin (Fig. 11d) shows a significant loss of aromatic material (120–160 ppm) as a result of the treatment. The relatively poor signal-to-noise ratio is a result of the very small amount of sample analysed that survived the macerative treatment. It can be seen that it is the unsaturated carbon environments that are attacked by oxidative maceration in both modern (Fig. 11b) and fossil (Fig. 11d) sporopollenins, but that proportionally more material is lost from the fossil (in this case, aromaticity decreases from 48 to 26%). Benzoic acid was recovered from the oxidizing solution which is a likely derivation from the aromatic component of the sporopollenin.

Modelling diagenesis by heating

Acetolysed pine pollen (Fig. 12a) was heated for 1 h at 150 °C (Fig. 12b), 225 °C (Fig. 12c) and 300 °C (Fig. 12d) in each case. The resulting spectra show considerable changes from those of the unheated pollen sample. The sample subjected to 150 °C shows four main peaks (29, 71, 129, 170 ppm). Homogenization of both the aliphatic and aromatic components has occurred resulting in the loss of fine structure from the spectrum. A small oxygenated aliphatic peak is retained but the bulk of oxygenated
After heating at 225 °C (Fig. 12c), only a single aliphatic and aromatic peak remain and the spectrum closely resembles those from most of the fossil samples investigated; the observed aromaticity is 54%.

The sample heated at 300 °C (Fig. 12d) shows a major change from the above spectra. Here, virtually all of the aliphatic component has been lost and the remaining material is almost entirely aromatic in composition as seen in fossil samples significantly affected by diagenesis (i.e. above the lignite rank, Davis et al., 1988).

**DISCUSSION AND CONCLUSIONS**

The above results provide information regarding the effects of diagenesis on the chemistry of spore and pollen walls. It is evident that exine material from vascular plants differs only slightly between certain major groups (compare the *Lycopodium, Anemone, Dioon, Cycas* and *Pinus*) and that the differences observed previously may have been influenced by different preparatory treatment of the samples (Guilford et al., 1988; Hemsley et al., 1992, 1993).

The fossil material is considerably altered from the original sporopollenin, exhibiting a redistribution or loss of oxygen and a significant increase in aromatic content. The results from the diagenetically altered spores are very similar to sporinite samples investigated by Axelson (1985) and Davis et al. (1988). In none of the fossil sporopollenins are there clear indications of any of the distinctive characteristics of the original sporopollenin that would enable us to make a close taxonomic assignment. Despite this, differences in aromaticity of fossil sporopollenins may provide the basis for assignment to a major plant group. Low rank material may retain more information but even this is lacking in distinctive characteristics. There are no apparent differences between the exine material derived from micro- and megaspores from the same plant, suggesting that no change in the plants synthesis of sporopollenin has accompanied the evolution of distinct, separate male and female morphology.

A feature of the spectra of fossil vascular plants is that the observed aromaticity is about 50% which is similar to that of a low-rank coal. Only in the fossil megaspore-membrane does the aromaticity differ greatly from 50% and is perhaps more similar to the cuticular material of *Eskdalia*.

NMR analysis of tasmanite cysts clearly demonstrates a closer affinity with algaenans and cuticular material (*Eskdalia*). The observed aromaticity is far lower than that of the sporopollenins. This ratio is evidently characteristic for these two different groups of biomacromolecule.

Oxidative maceration of sporopollenins results in the removal of unsaturated carbon environments in the range of 110–150 ppm, both in modern and fossil material. However, in the fossil material (prior to oxidative maceration) the aromatics within the appropriate range account for a much greater proportion of the exine material. This greater proportion of susceptible material explains why fossil spores can be ‘dissolved’ in Schulze’s solution whilst modern *Lycopodium* retains its integrity for a considerably longer period under the same conditions.

Pine sporopollenin heated in the presence of air shows a number of striking differences from the acetolysed, unheated sample. The sample heated to 150 °C (Fig. 12b) shows a remarkably similar spectrum to that obtained from the low rank *Zonalesporites* (Fig. 7b) suggesting that this degree of heating is a reasonable model for the diagenesis experienced by such a sample. One of the first effects of heating seems to be the homogenization of the aromatic peaks into a single broad-based peak. This has also been observed at the lower temperature of 100 °C during an acetolysis treatment lasting for 3 h as opposed to the 2 min of acetolysis used here (Hemsley et al., 1993). Hayatsu et al. (1988) illustrate NMR spectra of heated sporopollenins and their sample subjected to 150 °C for 3 months is similar to that shown here. It is apparent that, beyond a critical interval, the length of time for which the sample is heated is of no importance. More critical is the maximum temperature attained. Heating to 225 °C clearly approximates the degree of diagenesis experienced by the majority of fossil material shown here whilst heating to 300 °C has a dramatic effect on the sporopollenin, removing or altering almost all of the aliphatic component and probably simulating an extreme case of diagenesis. If changes in fossil sporopollenin attributed to diagenesis are indeed the result of past heating, it may be possible to ascertain, on the basis of an NMR spectra, the maximum temperature attained.

An important observation is that the effect of heating sporopollenin in air is completely different from that of oxidative maceration; the former increases the aromatic proportion of the substance in question while the latter causes this to diminish.

**Diagenetic alteration of sporopollenin and spore colour**

There has been significant interest in the alteration of sporopollenin (spores, sporinite) during diagenesis especially with regard to thermal alteration. Several authors have noted both physical and chemical changes in sporopollenin during natural and artificial thermal maturation (e.g. Durand, 1980; Sengupta, 1980; Piéart, Postiau and Roism, 1980; Schenck et al., 1981). The NMR results presented here indicate particularly that aliphatic groups are lost or reorganized and that aromatic groups increase (relatively) during thermal alteration. Whilst there has often been significant chemical alteration of the sporopollenin there appears, at least at moderate temperatures, to be less alteration in the spore wall ultrastructure (Scott and Hemsley, 1993 b) despite changes in the overall dimensions of the palynomorph (e.g. Piéart et al., 1980). For the most part, ultrastructure appears to be affected more by pressure and lithology than moderate heating (Scott and Hemsley, 1993 b).

One of the most obvious physical changes in spores caused by thermal maturation is that of a change in colour (reviewed by Marshall, 1991). It is generally recognized that spores change from colourless to yellow, orange, brown and finally black with increasing thermal maturation. This phenomenon has been widely used for interpreting the
degree of maturation of kerogen in a rock (Durand, 1980). Many groups of palynomorphs have been used in this way and some colour scales use not only spores (sensu lato), but cysts of dinoflagellates and acritarchs (the latter used in pre-Devonian assemblages).

The results of our NMR study suggest that there may be problems in assuming that colour changes in other groups of palynomorph bear any constant relationship to colour changes in archegoniate spores. The initial chemical composition of the resistant walls are different from those of at least one kind of planktonic cyst, and changes during diagenesis are likely to be equally different. There is no reason to suppose that the degree of colour change in an organic wall made of sporopollenin (containing both aliphatic and aromatic components) and of algaenan (largely aliphatic components) is the same. However, if the same kind of palynomorph is compared, i.e. spore, pollen or acritarch, then results are probably comparable (van Bergen and Kerp, 1990). We suggest therefore that care be exercised in equating spore and algal (tasmannite, dinoflagellate and acritarch) colour changes. This may be of particular importance in pre-Silurian rocks where there is no vitrinite, which means that comparisons with a standard reflectance scale cannot be made.

The darkening in colour of spores and pollen during diagenesis has resulted in the necessity of ‘cleaning’ them (i.e. making them less opaque) for light microscopic studies. Dark brown or black samples are generally oxidised to a lighter colour. A significant problem has concerned very dark spores which, when oxidatively macerated, disintegrate (Marshall, 1980). In the cases of spores which have undergone intense diagenesis (maturation) aromatic structures dominate. When attacked with an oxidising acid, the subsequent removal of aromatics leaves insufficient material to retain the structure, causing it to fall apart. We believe therefore, that no chemical technique is likely to cause very mature spores to revert to yellow whilst still remaining intact (without special measures; Marshall, 1980).

ACKNOWLEDGEMENTS

We thank the British Geological Survey (Eskdale), M. Oshurkova (Zonalesporites), C. Maples and the staff of the herbarium, University of Kansas (Botryopteris), P. Crane, Field Museum of Natural History, Chicago (Schopfipollenites), the Royal Botanic Gardens, Kew, UK, and the Marie Selby Botanic Garden, Florida, for the supply or loan of material. We also thank D. Butler for running many of the samples on the ULIRS solid state NMR facility at UCL. This work was supported by a grant under the NERC biomolecular palaeontology special topic (to WGC and ACS), for which we record our grateful thanks.

LITERATURE CITED


