Acta Bot. Neerl. 38(4), December 1989, p. 391–404

Molecular paleobotany of *Nyssa* endocarps

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SUMMARY

Fruit endocarps of three recent *Nyssa* species from southern Georgia and endocarps of three *Nyssa* species from the late-Oligocene Brandon lignite, VT. are characterized and compared using pyrolysis gas chromatography–mass spectrometry (PYGCMS), pyrolysis-mass spectrometry (PYMS and desorption chemical ionization mass spectrometry DCIMS), and microscopic techniques. PYGCMS and PYMS demonstrated that during the lignitization process almost all of the carbohydrate material is removed from the endocarp fibre walls. Some hexose oligomer residues do survive lignitization as levoglucosan was observed in the PYGCMS trace of the lignitic endocarp *N. fissilis* and mass peaks indicative for anhydrohexose oligomers were observed in DCIMS spectra of *N. fissilis* and *N. brandoniana*. The PYGCMS data on the mixed guaiacyl–syringyl lignin in the recent and fossil endocarp wall have very similar pyrolysis product distributions. The abundance of phenolic pyrolysis products with aliphatic side chains suggest a different less oxygenated lignin in the endocarps compared to the *Nyssa* xylem cell walls. In spite of the significant chemical changes which occur during the early coalification process considerable microscopic detail can be preserved. Some fibre cell walls even retained an anisotropic character which may be caused by preserved crystalline cellulose. The effect of storage conditions on the chemistry of the paleobotanical samples was investigated by PYMS and multivariate analysis. Fossil endocarps stored in glycerin/ethanol experienced some extraction of a soluble lignin-derived fraction whereas water-stored endocarps did not. The residues of water glycerol/ethanol stored samples have similar polyphenolic polymers.

Key-words: pyrolysis mass spectrometry, *Nyssa*, paleobotany, endocarp lignin, pyrolysis gas chromatography mass spectrometry.

INTRODUCTION

The relative contributions of the various plant parts to organic sediments are, in part, determined by their resistance to the natural decay processes which take place in soils and

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This paper is dedicated to Professor Dr T. van der Hammen on the occasion of his 65th birthday.
Part of this paper has been presented at the 13th International Meeting on Organic Geochemistry, Venice, September 1987.
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peats. Substances such as wood, pollen, and leaf cuticles are typically considered to be quite resistant to these processes and are thus often preferentially preserved in ancient (Stach *et al.* 1982) and recent (Brock *et al.* 1989; Dupont & Hooghiemstra, 1989) organic sediments. The resistant nature of lignified fruit endocarps, such as those produced by *Nyssa* sp. (tupelo gum), is often overlooked in spite of their conspicuous occurrence in many Tertiary deposits (Eyde & Barghoorn, 1963a; Dilcher & McQuade, 1967). Considering the volume in which these endocarps are observed in some recent peat-forming environments (e.g. the *Nyssa–Taxodium* swamps of southern Georgia, USA) their frequency in older sediments of similar origins, such as the Miocene German brown coals (Kirchheimer, 1957) and the late-Oligocene Brandon lignite, Vermont, USA (Eyde & Barghoorn, 1963a) is not surprising.

In spite of the considerable botanical interest in Tertiary *Nyssa* endocarps, there has been virtually no work focused on their chemistry. The present study was undertaken in order to compare the chemistry of lignitic *Nyssa* endocarps with those of their living equivalents. Such a study should yield insight into the molecular processes associated with the preservation of plant cell walls in the endocarps and their conversion into coal-forming entities. Analytical pyrolysis methods (pyrolysis mass spectrometry, PYMS, and pyrolysis gas chromatography–mass spectrometry, PYGCMS) were used to study the endocarps because these methods have been shown to be very successful in the characterization of woody materials (Windig *et al.* 1984; Obst, 1983; Boon *et al.* 1987; Boon, 1989), wood polysaccharides (Shafizadeh, 1984; Van der Kaaden *et al.* 1984; Pouwels *et al.* 1987; Pouwels *et al.* 1989a), lignins (Saiz-Jimenez & De Leeuw, 1986; Genuit *et al.* 1987; Faix *et al.* 1987) and coalified woods (Philp *et al.* 1982; Chaffee *et al.* 1984; Saiz-Jimenez *et al.* 1987; Stout *et al.* 1988). In the present study we have combined analytical pyrolysis with some microscopic examinations of the lignitic fruits in order to gain insight into the relationship between anatomical, chemical and optical properties of lignified cell walls.

MATERIALS AND METHODS

Sample collection, storage and preparation

Samples of lignitic *Nyssa* endocarps were recently collected from the 25 million-year-old lignite deposit near Brandon, Vermont, USA. The endocarps occurred throughout the deposit as well-preserved compactions, readily distinguished from the embedding matrix material. Details of this rather unique lignite deposit and its fossils have been presented elsewhere (Barghoorn & Spackman, 1950) and need not be reviewed here. A paleobotanic survey of the various *Nyssa* endocarps of the deposit made by Eyde & Barghoorn (1963a) was invaluable in our identification of the two species collected (*N. brandoniana* (Lesq.) Eyde and Barghoorn and *N. fissilis* (Lesq.) Eyde and Barghoorn). Samples of a third lignitic species, *N. lescurii* (C. H. Hitchcock) Perkins, present only in an overlying carbonaceous silt, were provided by Dr Bruce Tiffney of Yale University. For comparison, recent *Nyssa* endocarps were collected from the fruits of two species of tupelo gum trees found in southern Georgia (*N. aquatica* L. and *N. sylvatica* Marsh.). Among the *N. sylvatica* endocarps, two varieties were represented, i.e. *N. sylvatica* var. *sylvatica* Marsh. and *N. sylvatica* var. *biflora* Walt.

The lignitic *N. brandoniana* and *N. fissilis* were originally stored in water upon collection. Later in the laboratory, several specimens of each were transferred to solutions of glycerin and 50% ethanol (1/1; v/v) in accordance with normal paleobotanic preservation methods. The only samples of *N. lescurii* available were those from Dr Tiffney's

collection and all these had been stored in glycerin/ethanol for several years. Similarly, some samples of the three varieties of recent *Nyssa* endocarps were stored in water while others were stored in glycerin/ethanol solution. After several months of storage it was noticed that the glycerin/ethanol solutions in which the lignitic endocarps had been stored had turned amber in colour suggesting some extraction of material had taken place. In light of this it was decided to examine not only the chemical differences between the recent and lignitic fruits, but also to look at the chemical differences which may be introduced during the glycerin/ethanol storage of paleobotanic samples.

Some specimens of the lignitic samples stored in glycerin/ethanol were used to prepare microtomed thin-sections of each species for microscopic examination of the internal structure of the fruits. These thin-sections were examined in both plane-polarized light and crossed-polarized light using a Leitz microscope. The modern endocarps were examined only megascopically for their identification.

Analytical pyrolysis studies

For pyrolysis analysis, samples of the recent and lignitic endocarps, both water-stored and glycerin/ethanol-stored (after a thorough water washing), were freeze-dried and powdered with the aid of a steel file. Care was taken so that only the endocarp wall was sampled. From aqueous suspensions of 1 mg of homogenized sample, approximately 10 μg of sample were placed on a ferromagnetic wire using a micropipette.

Pyrolysis gas chromatography (PYGC) and PYGCMS were performed with a Carlo Erba 4200 gas chromatograph equipped with a flame ionization detector (FID) and a Packard 438S gas chromatograph combined with a JEOL DX-300 mass spectrometer linked to a JEOL DA-5000 data system. Curie-point pyrolysis took place in the FOM3-LX pyrolysis unit described previously (Boon *et al.* 1987). The pyrolysates were separated on a 30 m fused silica capillary column (i.d. 0·325 mm) coated with DB-1 (1·2 μm film thickness). The GC oven was programmed from 35°C (at time of pyrolysis) to 325°C at a rate of 4°C/min. Compounds were ionized at 70 eV. The acceleration voltage was 3 kV and the scan speed was 0·5 scans/s.

Curie-point PYMS was performed on a larger suite of samples using the FOM-auto-PYMS® system described previously by Boon *et al.* (1984). The conditions were as follows: Curie-point temperature 610°C, heated pyrolysis chamber (wall temperature about 220°C), expansion chamber temperature 200°C, temperature rise time 0·1 s, total heating time 0·8 s, mass range 25–220 a.m.u., scan speed 10 scans/s, total number of scans 200. Samples were analysed in triplicate. PYMS data were analysed via factor-discriminant analysis with the adapted ARTHUR program package described by Boon *et al.* (1984) and Hoogerbrugge *et al.* (1983).

In-source pyrolysis chemical ionization mass spectrometry DCIMS was performed with the JEOL DX-303 E/B mass spectrometer linked to a JEOL DA-5000 data system. The sample was pyrolysed from a resistive heated platinum/rhodium wire loop using a commercial desorption chemical ionisation probe. The ionization gas was ammonia.

RESULTS AND DISCUSSION

Anatomical considerations

The endocarps of seed plants occur in a variety of morphologies. By definition, an endocarp is the innermost layer of the pericarp, or fruit wall. The trees of *Nyssa* sp. produce

fruits, known as drupes, which have a fleshy covering over their oblong, lignified endocarps. The 1–4-cm fruits are produced abundantly by mature trees and drop in the fall of each year (Bonner, 1974). The fleshy coverings are quickly decayed while the resistant endocarp is left to contribute to the accumulating sediment or to be washed away. In this same manner it is presumed that the *Nyssa* trees of the Tertiary contributed hundreds of fruits each year to their surrounding sediments. The characteristic germination valve of the *Nyssa* endocarp has allowed for the easy recognition as well as rejection of many lignitic endocarps thought to belong to the Nyssaceae (Eyde & Barghoorn, 1963a). Figure 1a shows a photograph comparing a lignitic endocarp collected from the Brandon lignite (*N. brandoniana*) and the recent endocarp of *N. aquatica*. Both endocarps exhibit the characteristic germination valve on their dorsal sides and distinct longitudinal ribs. The preservation of these megascopic anatomical features emphasizes the durability of the *Nyssa* endocarps over geological time.

Taxonomic affinities between the Brandon lignite fossil species and the recent species were made by Eyde & Barghoorn (1963a) based upon morphological features such as: the nature of the valve margins, the arrangement of the vascular bundle traces, and the number of locules (i.e. cavities containing seeds).

For example, a transverse section through the typically bilocular lignitic *N. fissilis* is shown in Fig. 1. On close examination of some specimens of *N. fissilis* the arrangement of vascular bundles is revealed along the ridge crests. Since the only modern *Nyssa* fruit with this arrangement is *N. aquatica* it is not unreasonable to infer an evolutionary affinity between *N. aquatica* and *N. fissilis* (Eyde & Barghoorn, 1963a). The thick endocarp wall (about 5 mm) and the septum (which divides the two locules) are also notably apparent in Fig. 1b. It is interesting to note that no remnants of seeds are evident and that the pressures associated with burial have closed the cavities of the locules but have left the endocarp wall itself seemingly uncompacted. Thus, megascopically the lignitic endocarps generally appear quite intact.

Microscopically the endocarp walls of all the lignitic *Nyssa* fruits appear very fibrous and dense. Figure 1c shows a photomicrograph of the endocarp wall of *N. fissilis*. The anastomosed nature of the fibres is apparent as they can be seen to form a dense network of cells not unlike those which would be expected in recent endocarp walls (Eyde & Barghoorn, 1963b). When these fibres are viewed in crossed-polarized light they exhibit a feeble birefringent effect. As birefringence is usually attributed to the presence of crystalline cellulose in the cell walls of plants (O'Brien & McCully, 1981) it suggests that the lignitic endocarp fibre walls have retained at least some of this crystalline character.

Pyrolysis gas chromatographical studies

The PYGC-FID traces for recent *N. aquatica* and lignitic *N. fissilis* endocarp are shown in Fig. 2a and b. These two species were chosen for a detailed comparison because of their proposed evolutionary link mentioned previously. Shown in Fig. 2c is the PYGC-FID trace for recent *N. aquatica* secondary xylem or wood. Table 1 lists the corresponding compounds in the three chromatograms identified by PYGCMS analysis under similar conditions. Identification of the compounds was based on comparison with the mass spectral data from previous studies (Pouwels *et al.* 1987, 1989a; Boon *et al.* 1987; Van der Kaaden *et al.* 1984; Ohnishi *et al.* 1975; Shafizadeh *et al.* 1979).

The most notable chemical difference between the lignitic endocarps and the recent samples is the virtual absence of general polysaccharide marker peaks occurring in the lignitic endocarp. The PYGC shown Fig. 2b is virtually devoid of peaks in the elution

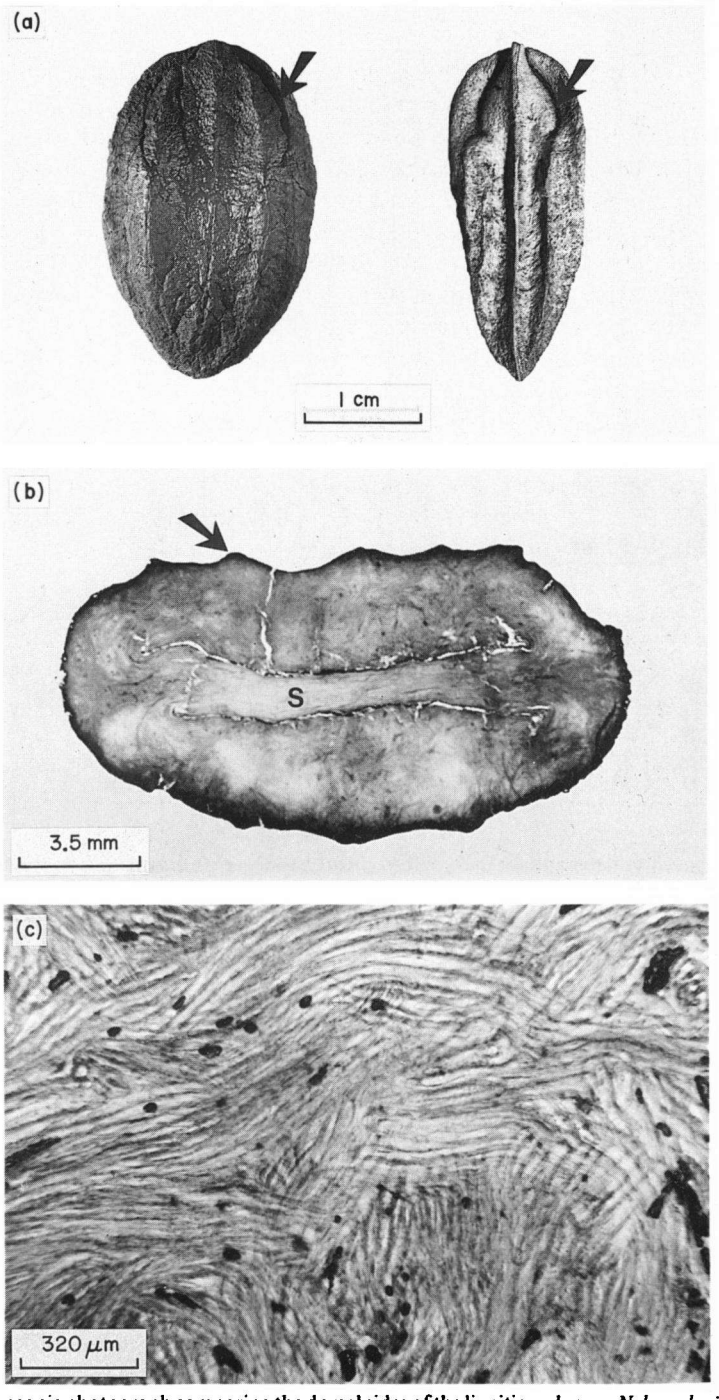

Fig. 1. (a) Megascopic photograph comparing the dorsal sides of the lignitic endocarp *N. brandoniana* (left) to the recent *N. aquatica* endocarp (right). Characteristic germination valves. (b) Transmitted-light photomicrograph of a transverse section through a specimen of the lignitic endocarp *N. fissilis*. (↑) Denotes one of the longitudinal ridges along which vascular bundles occurred. S denotes the intact septum which divides the two collapsed locules. (c) Transmitted-light photomicrograph of a lignitic endocarp *N. fissilis* showing the dense, anastomosing nature of the fibres. This network arrangement is not unlike that which would be found in a recent endocarp and no doubt serves to enhance the structural durability of the endocarp. These fibres exhibit a feeble birefringence when viewed in crossed-polarized light.

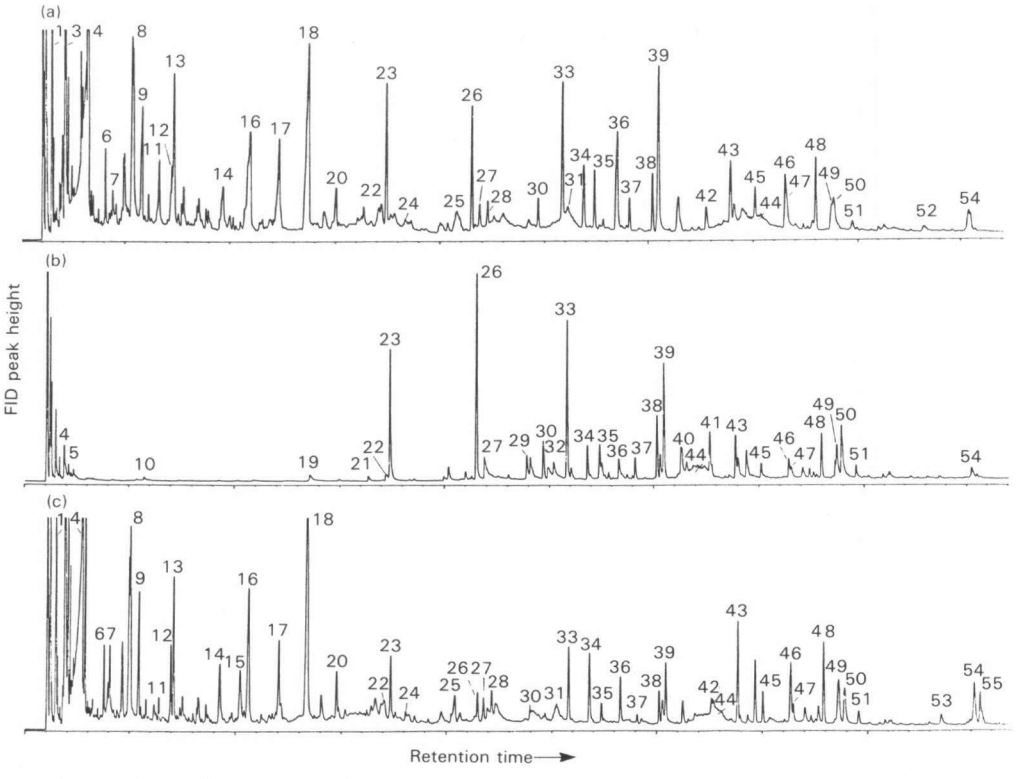

Fig. 2. Pyrolysis gas chromatograms for the recent *N. aquatica* endocarp (a), the lignitic *N. fissilis* endocarp (b), and the recent *N. aquatica* wood (c). Peak numbers correspond to those listed in Table 1.

range of polysaccharide dissociation products (the first half of the chromatogram). Compounds typically found in the pyrolysates of polysaccharide and native wood, such as 2,3-butandione (peak 3a), but-3-enal-2-one (7), 3-hydroxypropanal and (3H)-furan-2-one (8), (2H)-furan-3-one (11) and 2-furfural (13) were abundant in the recent endocarp but were not observed in the lignitic endocarp. Likewise, the pentose sugar markers from hemicelluloses such as 3-hydroxy-2-penteno-1-5-lactone (18) and an unidentified pentose marker compound (17) and the hexose sugar markers, 2-angelicalactone (16), 2-methyl-3-hydroxy-pyran-4-one (24) and the 1,4-dideoxy-D-glycero-hex-1-enopyranos-3-ulose (31) were absent from the lignitic endocarp (Fig. 2b) and abundant in the recent endocarp (Fig. 2a). It is interesting to note, however, the distinct presence of the cellulose marker, levoglucosan (i.e. 1,6-anhydroglucopyranose (49) in the lignitic endocarp. This suggests that at least some cellulose remnants must have survived the conversion to lignite.

The polyphenolic component of the fibre walls of the lignitic endocarp yielded a variety of methoxyphenolic compounds previously identified in the pyrolysates of milled wood lignin (Boon *et al.* 1987), and of native and fossil woods (Stout *et al.* 1988; Saiz-Jimenez *et al.* 1987). Many types of guaiacyl (2-methoxyphenols) and syringyl (2,5-dimethoxyphenols) derivatives occur in the PYGCs of both the recent and lignitic endocarps but differences are evident when the distribution patterns are compared with literature data and with wood from *Nyssa* (see Fig. 2a and c). Both tissues yielded about the same profile of carbohydrate pyrolysis products while the distribution of lignin-derived pyrolysis products differed

considerably. For example, the endocarp (Fig. 2a) not only yielded more lignin markers than the secondary xylem (Fig. 2c), but also the different lignin marker ratios strongly suggest that the endocarp lignin is of a different nature than the wood lignin. The endocarp lignin consisted predominantly of the guaiacyl-type monomers (peaks 23, 26, 33, 39) while the wood's lignin consisted of more equal proportions of guaiacyl and syringyl-type monomers with the syringyl markers (peaks 43, 46, 48, 54, 55) being even slightly more dominant. Also noteworthy is the predominance of methoxyphenols with aliphatic side chains (peaks 26, 30, 33, 35, 37, 38, 39, 42, 43, 45, 47, 48) in the data of the endocarp walls compared to the wood. In the light of these differences the similarities in the methoxyphenol distribution pattern of the recent and fossil endocarp are striking. This similarity points to a high degree of preservation of the endocarp phenolic polymer system, which is contrary to what is observed in fossil wood samples. Pyrolysis gas chromatography of fossil woods often display a shift from dimethoxy to monomethoxy and desmethoxy compounds which is considered as a criterium for the modifications in the wood lignin macromolecule during peatification and early coalification (Hatcher *et al.* 1981; Chaffee *et al.* 1984; Russell & Barron, 1984; Saiz-Jimenez *et al.* 1987; Stout *et al.* 1988).

The differences between the *Nyssa* endocarp and its wood suggests that the lignin in the endocarps is less oxygenated than the lignin present in the wood. Such a difference must be the result of a different linkage pattern of the lignin monomers with less ether bonds and more carbon–carbon bonds in the endocarp lignin. It could be argued from an evolutionary point of view that the endocarps, in an attempt to protect the seeds they enclose(d) better, developed a different lignin structure, perhaps less susceptible to microbial attack.

Pyrolysis chemical ionization mass spectrometry

Recent work on PYMS of cellulose and other polysaccharides under chemical ionization conditions DCIMS (Boon, 1989; Pouwels *et al.* 1989b; Tas *et al.* 1989) has shown that anhydrosugar oligomers are generated in the thermal dissociation process. These oligomers can be demonstrated by PYMS experiments in the ion source under conditions which minimize wall contacts and employ soft ionization conditions. Use of ammonia as the reaction gas greatly suppresses ion formation of phenols, but selectively ionizes the sugars (Boon, 1989). The presence of levoglucosan in the PYGCMS data of the *N. fissilis* suggested that anhydrosugar monomers could be present as such in the sample rather than the result of the thermal dissociation of a fossil glucose polymer; this hypothesis was tested. Figure 3a and b show the pyrolysis chemical ionization mass spectra of the fossil endocarp of *N. fissilis* and *N. brandoniana.* The mass peak at m/z 180 is the ammonia adduct ion of the levoglucosan (MW 162). In the cellulose spectrum (Pouwels *et al.* 1989) the higher homologues of the anhydroglucose oligomers evolved by pyrolysis are shown by mass peaks in the series m/z 180, 342, 504, 666 with mass increments of 162 a.m.u. from anhydroglucose units. This same mass peak series is present in the sample of the fossil endocarps although the ions are more prominent in the case of *N. brandoniana.* Many of the other mass peaks, e.g. m/z 163 and 193 are also observed in milled wood lignin samples analysed under these conditions.

The presence of ions indicative for levoglucosan and its homologues demonstrates a derivation by pyrolysis from a glucan oligomer or polymer, in this case most probably cellulose. Cellulose oligosaccharides can apparently survive the conversion to lignite, probably because of an adequate protection by polyphenolic materials against enzymatic degradation and perhaps also due to the impermeability of parts of the endocarp cell wall (see Fig. 1c) during early peatification.

Table 1. Pyrolysis products identified by pyrolysis capillary gas chromatography mass spectrometry in recent and lignitic endocarps

Peak	M^+	Compound name	Source
1	58	Acetone	PS
2	68	Furan	PS
3a	86	2,3-Butandione	H
3b	70	Unknown (C_4H_6O*)	?
4	60	Acetic acid	PS
5	78	Benzene	
6	86	Tetrahydrofuran-3-one	H
7	84	But-3-enal-2-one	PS
8a	74	3-Hydroxypropanal	H
8b	84	(3H)-Furan-2-one	H
9	102	Pyruvic acid methyl ester	H
10	92	Toluene	
11	84	(2H)-Furan-3-one	H
12	82	2,4-Pentadienal	H
13	96	2-Furfural	PS
14	84	(5H)-Furan-2-one	H
15	98	β-Angelicalactone	H
16	98	α-Angelicalactone	H
17	114	Unknown pentose marker (114,86)	P
18	114	3-Hydroxy-2-penteno-1,5-lactone	P
19	94	Phenol	
20	112	2-Hydroxy-3-methyl-2-cyclopenten-1-one	PS
21	108	*o*-Cresol	
22	108	*m*/*p*-Cresol	
23	124	Guaiacol	L
24	126	2-Methyl-3-hydroxy-(4*H*)-pyran-4-one	H
25	116	Unknown (116,43)	PS
26	138	Methylmethoxyphenol	L
27	110	1,2-Dihydroxybenzene	
28	126	5-Hydroxymethyl-2-furfural	H
29	140	2-Methyoxy-1,4,-dihydroxybenzene	
30	152	Guaiacylethane	L
31	144	1,4-Dideoxy-D-glycero-hex-1-enopyranos-3-ulose	H
32	124	Methyl-1,2-dihydroxybenzene	
33	150	*p*-Vinylguaiacol	L
34	154	Syringol	L
35	164	3-Guaiacylprop-1-ene	L
36	152	Guaiacylaldehyde	L
37	164	3-Guaiacyl-prop-2-ene (*cis*)	L
38	168	Methylsyringol	L
39	164	3-Guaiacyl-prop-2-ene (*trans*)	L
40	166	Guaiacylethanone	L
41	180	3-Guaiacylpropan-2-one	L
42	182	Syringylethane	L
43	180	Syringylethene	L
44	162	Levoglucosan	H
45	194	3-Syringylprop-1-ene	L
46	182	Syringylaldehyde	L
47	194	3-Syringylprop-2-ene (*cis*)	L
48	194	3-Syringylprop-2-ene (*trans*)	L
49	196	Syringylethanone	L

Table 1. (Continued)

Peak	M+	Compound name	Source
50a	178	3-Guaiacylprop-2-enal	L
50b	180	3-Guaiacylprop-2-enol	L
51	210	Syringylpropan-2-one	L
52	212	3-Syringylpropan-1-ol	L
53	210	3-Syringylprop-2-en-1-ol (*cis*)	L
54	208	3-Syringylprop-2-enal (*trans*)	L
55	210	3-Syringylprop-2-en-1-ol (*trans*)	L

*Based on high resolution mass spectrometric data.
PS, general polysaccharide marker; P, pentose marker; H, hexose marker; L, lignin marker.

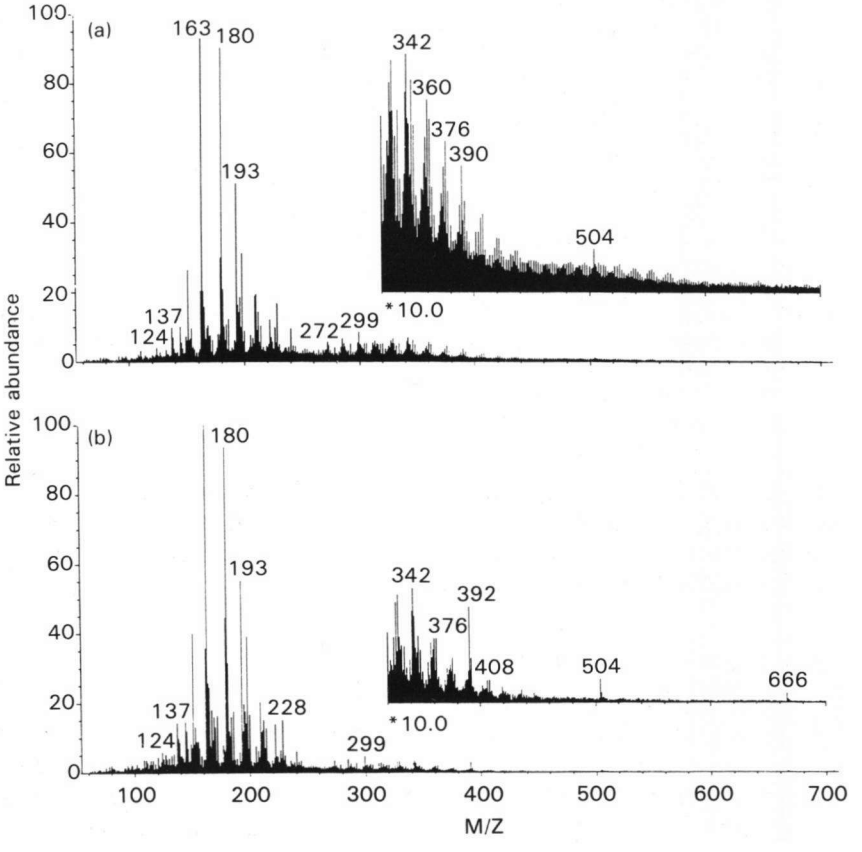

Fig. 3. In-source pyrolysis chemical ionization mass spectrum of fossil endocarp from *N. fissilis* (a) and *N. brandoniana* (b).

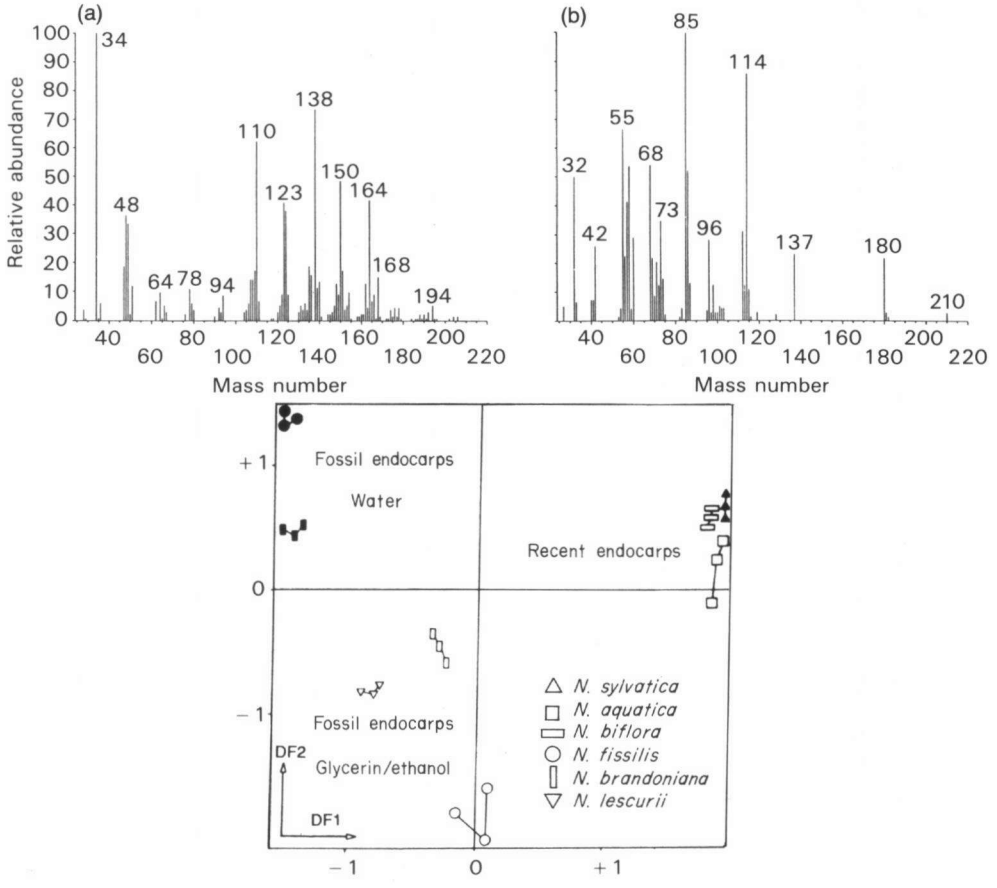

Fig. 4. DF1/DF2 score map produced by the multivariate analysis of PYMS spectra of recent and lignitic endocarps and corresponding DF1 positive (a) and negative (b) spectra. Closed symbols indicate storage in water and open symbols storage in glycerin/ethanol. (△) *N. sylvatica*, (□) *N. aquatica*, (▭) *N. biflora*, (○) *N. fissilis*, (▯) *N. brandoniana*, (▽) *N. lescurii*.

The analytical observations match the microscopic observation that a feeble birefringence was observed in the fibre walls of the lignitic endocarp suggesting that a correlation exists between the relative amount of carbohydrate material retained in the lignitic endocarp walls and the relative degree of birefringence displayed by these walls.

Comparative studies with pyrolysis low voltage mass spectrometry

After the comparisons between recent wood and endocarps from *N. aquatica* and lignitic *N. fissilis*, a larger suite of fruit endocarps was studied by Curie pyrolysis low voltage electron impact mass spectometry. With this method the pyrolysates of the samples are represented in a highly condensed form as one spectrum (PYMS). These spectra can be compared relatively easily with multivariate techniques when they are plotted on a so called 'discriminant score map', a diagram expressing the difference in chemical composition between samples as an euclidean distance between points on the map (Fig. 4).

Multivariate analysis was performed on the PYMS spectra generated for three recent and five lignitic *Nyssa* endocarp samples. Some of these samples had been stored in water and others in glycerin/ethanol.

The discriminant function (DF) DF1/DF2 score map produced in this analysis is shown in Fig. 4 with the mass spectra representing the positive (a) and negative (b) first discriminant functions (DF1). The discrimination represented in DF1 accounts for 92% of the characteristic variance between the samples while DF2 accounted for an additional 5·6%. This suggests that the chemical differences imposed during the conversion to lignite far exceed the variations which existed between the different species. The DF1 positive spectra are dominated by the presence of electron impact (EI) mass peaks considered to be characteristic of the pyrolysis products of pentosans (m/z 85, 112, 114) and other carbohydrates (m/z 32, 43, 57, 60, 73, 98). In addition, the mass peaks m/z 137, 180 and 210 represent a few lignin-derived pyrolysis products which are unique for the recent samples. In light of the previous results (PYGCMS in Fig. 2, DCIMS in Fig. 3 and the photoionization PYMS data of Genuit, 1986) it is not surprising that the DF1 positive spectra has separated the three recent *Nyssa* endocarps from the six lignitic endocarps due to the former's relative enrichment with respect to carbohydrates. The six lignitic endocarps displayed negative DF1 score values due to their relative enrichment of mass peaks representing various markers of the aliphatic lignin (m/z 124, 138, 150, 154, 164, 168, 194) and of reduced organic sulphur compounds (m/z 34, 48, 64, 66). The relatively intense mass peak m/z 110 represents dihydroxybenzene, which is measured at higher sensitivity under low voltage EI conditions.

There is significant spread among the lignitic endocarps along the DF1 negative scores. This spreading apparently corresponds to the manner in which the fossils had been stored prior to pyrolysis rather than their taxonomy. The water-stored fossils attained higher DF1 negative score values than the glycerin/ethanol-stored fossils suggesting the latter were depleted in 'endocarp polyphenolics'. Though less important statistically, the separation displayed in the DF2 direction was also apparently due to the manner in which the samples had been stored prior to pyrolysis. Examination of the mass spectra representing DF2 (not shown) suggested that this separation was again due to variations in the carbohydrate and phenolic markers. Organic sulphur peaks were apparently not influenced by the manner of storage. It is important to note that the manner of storage seems only to have influenced this ratio in the lignitic endocarps as the differentially stored recent endocarps are not separated in DF1/DF2. Recalling that the lignitic endocarps stored in glycerin/ethanol produced an amber-coloured solution after several months of storage, while the recent endocarps did not, suggests that these samples had, in part, been extracted. The extraction of a polymeric, though soluble, polyphenolic fraction from the glycerin/ethanol-stored lignitic samples seems most likely, thus producing a higher DF1 score value for these samples in the discriminant map. Though perhaps not immediately apparent, this observation can yield valuable information with respect to lignin's modification during the conversion of recent endocarps to coalified endocarps. The intact lignin was not susceptible to extraction suggesting a possible connection to the hemicellulosic polysaccharides. However, during coalification this polysaccharide portion of the polymeric framework is hydrolysed to the point where portions of the endocarp lignin molecule are rendered soluble in the glycerin/ethanol storage solutions. Further studies employing various organic solvents will address the nature of the extractable material further. We conclude that the glycerin/ethanol-stored fossil samples are chemically modified and do not wholly reflect the original chemistry of the lignitic endocarps. This also

implies that museum specimens like *N. lescurii* stored for a longer time under these conditions cannot be used for detailed molecular studies. Despite this unfortunate conclusion we can deduce from this survey that there are no great differences in polyphenolic composition of the samples because they are qualitatively similar and appear to use similar molecular protection mechanisms of the endocarp polysaccharides.

CONCLUSIONS

The durability of fruit endocarps over geological time is demonstrated by the relatively high degree of preservation of the anatomical features of *Nyssa* endocarps from the late-Oligocene Brandon lignite, VT. The preservation of detailed structure in these endocarps is remarkable considering the chemical changes they have undergone during their conversion to lignite.

The PYGCMS of a recent *N. aquatica* endocarp and a lignitic *N. fissilis* endocarp reveals that most of the carbohydrate material has been removed from the lignitic endocarp. However, a small levoglucosan peak indicates that some hexose residues have survived the conversion to lignite. DCIMS demonstrates that monomeric and oligomeric anhydroglucoses are present in the pyrolysate of the fossil endocarp pointing to the survival of glucose polymer chains. These residues may have retained some degree of crystallinity as is shown by the birefringence observed microscopically for these endocarps.

A PYGCMS comparison of *N. aquatica* fruit endocarp to secondary xylem reveals significant differences with respect to their lignin chemistry. The fruit endocarp and the wood pyrolysate show, as expected, a number of guaiacyl and syringyl compounds but the endocarp methoxyphenol profile is significantly enriched in guaiacyl-type lignin markers with an aliphatic side-chain. Similar distributions of guaiacyl and syringyl compounds are found in the fossil endocarps pointing to a well-preserved lignin which resists modification under peatification and lignification conditions. The main difference between the wood and the endocarp lignin appears to be the degree of oxygenation of the polymer. The less oxygenated and more aliphatic lignin in endocarps may aid in their resistance to decay and may similarly have aided the Tertiary *Nyssa* species.

Multivariate analysis of a Curie-point PYEIMS survey of recent and lignitic endocarps from several species reveals that the removal of carbohydrates and the preservation of lignin dominates the lignitization conversion process in general. Proposed taxonomic affinities between recent and lignitic *Nyssa* sp. are obscured because chemical differences due to the nature of storage were imposed on the data of lignitic samples. Those fossil specimens which had been stored in a glycerin/ethanol solution experienced some extraction of a soluble polymeric lignin fraction. Despite this storage problem no major differences in the phenolic mass peak profile were evident between water-stored specimens and residues remaining after glycerin/ethanol storage.

ACKNOWLEDGEMENTS

The authors thank Ms A. Tom, Mrs B. Brandt-de Boer and Mr G. B. Eijkel for their technical assistance. Dr B. Tiffney (Yale University) is acknowledged for his gift of the *N. lescurii* endocarp sample. This work was performed as a co-operative project between the Coal Research Section of the Pennsylvania State University and the FOM Institute for Atomic and Molecular Physics. This research is part of the research programme of Fundamenteel Onderzoek der Materie (FOM) with financial support from the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO).

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