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A combined pyrolysis mass spectrometric and light microscopic study of peatified *Calluna* wood isolated from raised bog peat deposits

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Abstract—Stem wood of the Angiosperm *Calluna vulgaris* (Scotch heather), isolated at different depths from a selection of raised bog peat deposits, was chemically characterized using in-source pyrolysis mass spectrometry (Py-MS) and Curie-point pyrolysis gas chromatography mass spectrometry (Py-GC-MS). Light microscopy was performed to relate mass spectrometric characteristics with anatomical features. Peatified wood samples, isolated from increasing depth show a gradual decrease in carbohydrate content. This decrease is anatomically reflected in a selective removal of secondary cell wall material from the fibre-tracheids and wood parenchyma. During prolonged peatification a selective removal of hemicellulose sugars is observed, while a part of the cellulose fraction is preserved. This highly resistant cellulose is mainly located in the secondary cell walls of the vessels. The lignin macromolecule is preserved, but a gradual decrease in syringyl to guaiacyl ratio (S/G) is observed during peatification. Because no increase in catechol and phenolic compounds is observed, we conclude that S/G shifts are due to the removal of syringyl-rich secondary cell wall material and the retention of guaiacyl-rich compound middle lamella. Small chemical changes in the lignin macromolecule involve shifts in oxygen substitutions on the aliphatic side chains of the methoxyphenolics and the occurrence of aromatic acids.

Key words—pyrolysis gas chromatography mass spectrometry, platinum filament pyrolysis mass spectrometry, peatification, wood, raised bog, microscopy, *Calluna vulgaris* L. (Hull.)

INTRODUCTION

Raised bog ecosystems are widespread throughout north-western Europe and consist of a living top layer mainly dominated by Ericaceae, *Sphagnum* and *Eriophorum* species. Below the surface, thick deposits of partially degraded plant parts, intermingled with living roots from the top vegetation, are present. Ombrotrophic peat mainly accumulates in areas where the rate of plant growth exceeds the rate of plant decomposition. Complete decomposition is prevented in soils where waterlogging occurs. In north-west Europe, with its high rainfall, low evaporation and low temperatures, waterlogged soils are a common feature. Due to waterlogging, these aquatic deposits are poor in oxygen thus retarding oxidative biodegradation. A major factor contributing to the preservative conditions in raised bog deposits is the occurrence of *Sphagnum*, or Bog Moss. The fact that the ground water becomes highly acidic (pH 3), is due to the proton exchangibility of these plants. The acidic conditions of the soil inhibit bacterial growth and thus retard biodegradation processes. As a consequence, many plant parts are macromorphologically recognizable. However, van der Heijden *et al.* (1990, 1991) showed in preliminary studies that woody tissues isolated from raised bogs are chemically and

anatomically modified, although the anatomical degree of preservation of different woody tissues is highly tissue specific (van der Heijden *et al.*, 1994).

Most chemical work has been performed on woody or fossil leaf remains isolated from subtropical peat deposits or coal seams (Philp *et al.*, 1982; Hedges *et al.*, 1985; Stout, 1985; Wilson *et al.*, 1987; Hatcher, 1988; Hatcher *et al.*, 1982, 1988, 1989a, b; Durig *et al.*, 1988; Bates and Hatcher, 1989; Bates *et al.*, 1991), volcanic soils (Atalla *et al.*, 1988), or sand deposits with fossil forest remains (Blanchette *et al.*, 1991; Obst *et al.*, 1991). These studies especially focus on the effects of (an)aerobic biodegradation processes on the complex lignin macromolecule which is an important coal precursor. Many authors have shown that the aromatic constituents of coal are derived from defunctionalized lignin although information about the exact chemical pathways of anaerobic lignin degradation is scarce (Young and Frazer, 1987). More is known about the aerobic decay of lignin-derived standard compounds using aerobic fungi (Sarkanen, 1991; Umezawa and Higuchi, 1991), but it is difficult to relate the established chemical decomposition pathways with the decomposition of the lignin complex in natural environments.

Until now, no information is available about the chemical and anatomical modifications in woody

plant tissues submitted to biodegradation in raised bog peat deposits. Studies on these highly specific deposits mostly deal with peat mixtures (Boon *et al.*, 1986; Halma *et al.*, 1984) and specific classes of compounds, such as carbohydrates (Moers, 1989), humic acids (Meuzelaar *et al.*, 1977; Ertel and Hedges, 1984; Haider and Schulten, 1985) or lipids (Ekman and Ketola, 1981). The chemical analysis of complex peat samples does not yield any information about the effects of biodegradation on the plant tissue level. Therefore, microscale picking of peatified tissues from peat followed by chemical characterization, is of great importance in order to study the diagenetic changes in the plant cell walls due to decomposition processes. This approach yields more insight in the protective role of different plant biopolymers in the selective preservation and decomposition of plant tissues and thus increases the understanding of bias in the fossil record. For the chemical characterization of complex organic material on a microgram scale, analytical pyrolysis techniques in combination with mass spectrometry and gas chromatography mass spectrometry are excellent tools. Smeerdijk and Boon (1987) and Boon *et al.* (1989) developed this approach for analysis of selected peatified plant parts.

In this paper, an integrated study on anatomy and chemistry of peatified *Calluna vulgaris* L. (Hull.) wood is presented. Sub-recent wood samples, isolated from different depths in an Irish peat bog core are compared with recent and highly decomposed Holocene wood samples, obtained from a Dutch Holocene peat deposit. The samples are chemically characterized by in-source pyrolysis mass spectrometry using electron (Py-EI-MS) and ammonia ionization techniques (Py-NH₃CI-MS) and Curie-point pyrolysis gas chromatography mass spectrometry (Py-GC-MS). Chemical variations due to species differences are ruled out by studying one single plant species: *Calluna vulgaris*. Chemical variabilities due to variations in wood age are minimized by analysing twigs with more or less the same diameter (± 5 mm). In this way more insight is obtained of the chemical modifications of the biopolymers in the wood in relation with burial time and/or environmental conditions. The chemical data are related to light microscopic observations.

EXPERIMENTAL

Study areas, stratigraphy, samples

Peat cores were taken from two different sites, namely Clarabog (Ireland) and Meerstalblok (the

Netherlands). The Clarabog peat deposit is situated in a depression south of the town of Clara, County Offaly, Ireland (sheet 15 Ord. Surv.; grid ref. 25/30 or 7°38'/53°19'). The bog is part of an undisturbed hummock/hollow complex with an intact surface vegetation. The core from which the wood samples were isolated was taken from the upper peat layers, up to 55 cm depth and is similar to the Clarabog 4 core used by van der Molen (1988). After sampling, the core was sealed and frozen until further use. In the core, three main strata can be recognized:

1. A 2–4 cm thick top layer of living *Sphagnum* species.
2. A 40 cm thick light brown layer of "non-decomposed" or slightly decomposed *Sphagnum* plants mixed with branches and roots of higher plants.
3. A 15 cm thick layer of dark brown, highly decomposed layer of *Sphagnum* plants, also mixed with Ericaceous remains (van der Molen, 1988, 1992).

The sharp transition from highly decomposed to non-decomposed peat is typical for many Irish raised bog deposits and is not related to the transition between aerobic and anaerobic decay conditions.

The Holocene wood samples were isolated from a dark *Sphagnum* peat (Schwarztorf) layer (240 cm below the surface), taken from the peat deposit Meerstalblok (52°41'N, 7°02'E), an area located in the central part of the Bargerveen, which is a natural bog reserve southeast of Emmen in the Eastern part of Drenthe Province (The Netherlands). Due to the fact that the deposit has been drained, aerobic conditions might prevail in this deposit and therefore more fungal infection could occur. The section was palynologically described by Dupont (1986).

The ages of the sub-recent peat layers were determined using pollen concentrations and pollen accumulation rates (van der Molen, 1988). The Holocene peat layer was dated using ¹⁴C dating techniques as well as the above mentioned pollen density dating (Dupont, 1986). Information on the samples is also shown in Table 1.

Anatomical procedures

Fresh and peatified samples were fixed in Craf III, subsequently dehydrated in an ethanol-*n*-butyl-alcohol series (10, 20, 40, 60, 80, 100%), embedded in glycol-methacrylate, sectioned into 5 μ m thick

Table 1. List of samples analysed by light-microscopy and analytical pyrolysis techniques

Sample	Sample location in the peat	Age
CB0	Recent wood collected from a living raised bog	1992 AD
CB20	20 cm depth from the subrecent peat bog	1927–1936 AD
CB34	34 cm depth from the subrecent peat bog	1878–1927 AD
CB47	47 cm depth from the subrecent peat bog	1772–1878 AD
CB52	52 cm depth from the subrecent peat bog	1772–1878 AD
MB	Collected from the Holocene peat deposit	ca 5000 BP

coupes with glass knives, stained with Schiff's reagent (Pearse, 1972; Scott and Peterson, 1979) and counterstained with toluidine blue (Sidman *et al.*, 1961).

Instrumentation

Platinum filament pyrolysis was performed on a JEOL DX-303 double focusing mass-spectrometer connected to a JEOL DA-5000 data system. Suspensions of homogenized samples in water were applied to the inert metal (Pt, Rh) loop of the desorption probe and dried. After insertion of the probe in the mass spectrometer, the loop was resistively heated at a rate of 16°C/s up to 800°C. The pyrolysis products were ionized under 16 eV electron impact conditions. The conditions for ammonia CI were: heating rate 13°C/s, 20 Pa ion-source pressure, acceleration voltage 2.2 kV. The mass range was set to 20–1000 a.m.u. during EI and 59–1000 during CI. The scan cycle time was 1 s. Data were processed using the Kratos analytical Mach3 software package (Kratos, Manchester, U.K.). For principal component analysis, a modified version of the Arthur package (Infometrix, Inc., Seattle, WA) was used. Samples were measured in triplicate or quadruplicate.

Mass peaks of carbohydrates and monomeric/dimeric pyrolysis products of lignin and carbohydrates, generated in the electron impact mode, were identified using Py-MS data of Pouwels and Boon (1990) and van der Hage *et al.* (1993), while DCI mass peaks of lignin and carbohydrates were identified using data of Tas *et al.* (1989), Pouwels and Boon (1990), Lomax *et al.* (1991a, b) and van Loon (1992).

Curie-point Py-GC-MS was performed on a HP 5890 series II gas chromatograph, connected to a Finnigan Incos 50 quadrupole mass spectrometer. A sample suspension in water was applied to a ferromagnetic wire with a Curie-point temperature of 610°C. The ferromagnetic wire was inserted into a glass liner and subsequently introduced into a FOM-4LX pyrolysis unit. The liner was flushed with argon to remove air and kept at a constant temperature of 180°C. The GC oven was kept at 30°C during pyrolysis and subsequently heated to 320°C with a temperature rise of 6°C/min. The pyrolysate was separated on a CP SIL-5 CB (25 m) fused silica capillary column (i.d. 0.32 mm, film thickness 0.41 µm). The end of the column was directly inserted in the ion source of the mass spectrometer and the compounds were ionized at 70 eV electron impact energy. The acceleration voltage was 1.0 kV. The scan time was 0.5 s with a mass range of 20–600 a.m.u. The data were processed, using the Kratos analytical Mach3 software package (Kratos analytical Ltd, Manchester, U.K.). Compounds were identified using a library of carbohydrate and lignin pyrolysis compounds, based on the work of Pouwels and Boon (1990), Boon *et al.* (1987) and van der Hage *et al.* (1993).

RESULTS

Anatomy of recent, sub-recent and Holocene wood samples

The anatomical modifications in the cell wall system of the woody tissues are outlined here and have been described in detail elsewhere (van der Heijden *et al.*, 1994). Light microscopy photographs of a representative part of the secondary xylem from recent, sub-recent and Holocene wood samples are shown in Fig. 1. Due to the variability in degree of preservation of the cell wall system of the fibre-tracheids, it is possible to classify the different decomposition stages in several decomposition pathways. Two decomposition pathways are observed in the fibre-tracheids, namely A and B. Both pathways are schematically depicted next to the microphotographs. The numbers in the photographs point to the specific decomposition stages in the scheme. The first decomposition pathway (A) involves the selective degradation of the S₁ and S₂ layers and the preservation of the compound middle lamella and the S₃ layer. The second decomposition pathway (B) is characterized by a gradual thinning of the secondary cell wall, starting at the S₃ layer and progressing towards the primary wall. The two different decomposition pathways are quite often observed in the same wood sample. The main anatomical modifications in the peatified fibre-tracheids are summarized below.

The secondary xylem of recent *Calluna vulgaris* is of a rather simple structure consisting of different tissues, such as fibre-tracheids, wood parenchyma and vessels. In cross section, the fibre-tracheids consist of more or less square-shaped cells which are rather variable in size. The cell wall system is divided into a compound middle lamella (middle lamella and primary wall), a combined S₁ and S₂ layer and an inner S₃ layer. The wood parenchyma cells are arranged in uniseriate medullary rays consisting of cells which are radially elongated. The secondary wall thickenings are also divided in S₁, S₂ and S₃ layers. Observed in cross section, the recent vessels are rather small with only slightly secondary thickened cell walls.

The wood sample from 20 cm depth (CB20) shows some biodegradation, although most fibre-tracheids are well preserved. Most decomposed fibre-tracheids and wood parenchyma cells have been altered via decomposition pathway B, although decomposition pathway A is frequently present. The preserved S₃ layer of the wood parenchyma elements, decomposed via pathway A, are usually swollen. The secondary cell walls of the vessels are heavily swollen or well preserved. The lumen is sometimes filled with a glossy or granular precipitate. The infection with fungal hyphae is sparse and mainly occurs in the lumen of the vessels. The cell walls of even the most degraded elements are not stained.

The wood from 34 cm depth (CB34) resembles the CB20 sample in that most decomposed fibre-

Recent

CB20

CB34

CB52

Holocene (MB)

Fig. 1. Microphotographs of transverse sections of recent, sub-recent and Holocene wood and a schematic representation of the different decomposition stages. The numbers in the microphotographs correspond with the different decomposition stages such as shown in the scheme. ft = fibre-tracheids, v = vessel, wp = wood parenchyma. Scale bar = 10 μ m.

tracheids and wood parenchyma cells are degraded via decomposition pathway B. Fibre-tracheids and wood parenchyma cells with preserved secondary cell walls are also still present but appear less common than in the former sample. In contrast with the previous sample the lumen of the fibre-tracheids are

sometimes filled with a precipitate. The inner S_3 layer of the preserved fibre-tracheids and wood parenchyma cells are occasionally somewhat swollen. In non-artificially stained sections these swollen cell wall layers have a gold-brown stain. The secondary cell walls of the vessels are preserved or sometimes swollen.

Fig. 2. Time integrated pyrolysis low voltage electron impact mass spectra of recent (a), sub-recent (CB 47 cm) (b) and Holocene wood (c).

The wood sample isolated at 47 cm depth (CB47) is not depicted here, but it shows anatomical characteristics which are identical with the next sample.

The wood sample from 52 cm depth (CB52) shows mainly highly biodegraded cell walls. Sporadically secondary cell wall material is preserved. Most fibre-tracheids and wood parenchyma cells are degraded via decomposition pathway B. Fibre-tracheids and wood parenchyma, degraded via decomposition pathway A are also present, but are quite rare. The secondary cell walls of the vessels are usually well preserved or appear somewhat swollen. If the former tissue is decomposed, the secondary cell walls are usually gradually thinned.

Most fibre-tracheids and wood parenchyma cells of the Holocene wood (MB) are degraded via decomposition pathway A. This is in contrast with the former samples in which both cell types were mainly degraded via decomposition pathway B. Fibre-tracheids and wood parenchyma cells with preserved S₁ and S₂ layers are not observed. The vessels sometimes show a swollen secondary cell wall, although often no significant modifications are observed in this tissue.

Chemical analysis by pyrolysis (electron ionization) mass spectrometry (Py-EI-MS)

The anatomically investigated wood samples were analysed chemically by in-source Py-MS using electron impact ionization. The mass spectra of the recent, sub-recent (CB47) and the Holocene wood samples are depicted in Fig. 2(a-c). Principal component analysis was performed in order to determine correlations between the in-source Py-MS data of all the wood samples and to reveal chemical modifications invoked by the peatification process. Figure 3 shows a 1-D score map depicting the score values of the samples. Figure 4 shows the reconstructed mass spectra of the first principal component. The rotated first function (PC1, -40°) covers about 77% of the

total variance and describes the carbohydrate and lignin content in the wood samples. The reconstructed mass spectrum of the positive PC1 is characterized by monomeric pyrolysis products of lignin such as m/z 124, 137, 138, 150, 152, 164, 166, 178, 180 (guaiacyl) and m/z 154, 167, 168, 180, 182, 192, 194, 196, 198, 208, 210, 212 (syringyl) and dimeric pyrolysis products at m/z 272, 302, 328, 332, 358, 388, 418. The prominent masses at m/z 180 and 210 are assigned to the precursor monolignols coniferyl and sinapyl alcohol respectively (van der Hage *et al.*, 1994). The m/z 94 and 110 are assigned to phenol and dihydroxybenzene respectively. The reconstructed mass spectrum of the negative PC1 is characterized by intense mass peaks derived from pyrolysis products of polysaccharides, such as hexose (m/z 43, 57, 60, 73, 126, 144) and pentose sugars (m/z 85, 114). A low PC1 score indicates a higher carbohydrate content. From the score map it is evident that during increased peatification the amount of carbohydrates gradually decreases. The removal of carbohydrates is already evident in the sample at 20 cm depth, while the most prominent decrease in carbohydrate content is observed between CB47 and CB52 and between CB52 and the Holocene sample.

The high intensities of coniferyl and sinapyl alcohol mass peaks (m/z 180, 210) in the peatified wood [Fig. 2(b) and (c)] point to an excellent preservation of β -O-4 linkages, which is the major linkage type between the phenyl propanoid units (Evans *et al.*, 1986; van der Hage *et al.*, 1993). However, the figures clearly demonstrate a significant shift in ratio between m/z 210 and m/z 180 during increased peatification, pointing to a modification in the syringyl-to-guaiacyl ratio (S/G). The S/G ratios, calculated from the intensities of a set of ions representing monomeric monomethoxy (m/z 137, 138, 140, 150, 151, 152, 164, 166, 178, 180) and dimethoxyphenolics (167, 168, 182, 192, 194, 196, 198, 208, 210, 212) are presented in Table 2. The data reveal a significant shift in the S/G ratio, which is already evident in the sample from 34 cm depth. The lowest ratio is observed in the wood from 47 cm depth (0.56). In the Holocene wood, the S/G ratio is rather high compared with the values of the deepest sub-recent samples. The observed S/G shifts, calculated from monomeric lignin pyrolysis products, is also evident in the mass region representing dimeric lignin pyrolysis products. Figures 5(a) and (b) show the in-source electron impact mass spectrum (m/z 250–500) of the native [Fig. 5(a)] and the sub-recent wood sample from 47 cm depth [Fig. 5(b)]. The masses at m/z 272, 302, 332, 328, 358, 388 and 418 are from dimeric pyrolysis products of lignin containing diguaiacyl, guaiacyl, syringyl and disyringyl units. Evans *et al.* (1986), Hempfling and Schulten (1990) and van der Hage *et al.* (1993) assigned these masses to phenylcoumaran, phenylcoumarone, biphenyl, stilbene and resinol types of structures. The substituents for these subunits are identical to those identified for

Fig. 3. Score plot of the first principal component function.

Fig. 4. Reconstructed mass spectra for PC1⁻ and PC1⁺ (-40° rotated).

Table 2. Yields of pyrolysis products from recent (CB0), sub-recent (CB20-C52) and Holocone wood (MB) as calculated from Py-EI-MS, Py-CI-MS data. nc = Not calculated

	CB0	CB20	CB34	CB47	CB52	MB
<i>Py-EI-MS</i>						
Syringyl/guaiacyl*	0.94 ± 0.02	0.99 ± 0.02	0.68 ± 0.02	0.56 ± 0.01	0.61 ± 0.03	0.77 ± 0.02
Phenols/guaiacyl and syringyl*	0.08 ± < 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.08 ± < 0.01	0.09 ± < 0.01
Catechol/guaiacyl and syringyl*	0.03 ± < 0.01	0.03 ± < 0.01	0.05 ± < 0.01	0.05 ± < 0.01	0.03 ± < 0.01	0.09 ± < 0.01
<i>Py-CI-MS</i>						
Hexose/Pentose*	1.92 ± 0.06	1.71 ± 0.12	1.81 ± 0.15	2.01 ± 0.03	2.20 ± 0.04	4.98 ± 0.31
Sinapyl/coniferylalcohol*	0.90 ± 0.02	1.13 ± 0.04	0.72 ± 0.02	0.47 ± 0.03	0.61 ± 0.03	0.92 ± 0.02
<i>Py-GC-MS</i>						
Syringyl/guaiacyl‡	1.12	1.09	0.58	0.37	0.42	0.65
Phenols/guaiacyl and syringyl‡	0.04	0.03	0.08	0.09	0.10	0.08
Catechols/guaiacyl & syringyl‡	0.07	n.c.	0.08	0.08	0.02	0.03
Methoxycatechols/syringyl‡	0.02	0.02	0.02	0.08	0.08	0.06
G-C3/G _{total} †	0.37	0.31	0.30	0.37	0.30	0.26
S-C3/S _{total} †	0.51	0.49	0.52	0.47	0.47	0.45
Total S and G with different functional groups:†						
ketones	13	13	12	15	22	19
aldehydes	27	26	13	21	19	13
alcohols	5	0	0	0	0	0
non-oxygenated	55	61	75	63	59	68

*Ratio's calculated from the summed ion intensities.

†Percentage of total integrated methoxyphenolic peak areas.

‡Ratio's calculated from the summed peak area's.

Fig. 5. Time integrated pyrolysis low voltage electron impact mass spectra (m/z 250-500) of recent (a) and sub-recent wood (b; CB47), showing characteristic ions for dimeric lignin pyrolysis products. ▲ = β -1-stilbene, □ = β - β -resinol types of structures. (The values in brackets represent the number of substituted methoxy groups on the dimer structures.)

monomeric pyrolysis products from lignin (Hempfling and Schulten, 1990). From the comparison of both mass spectra it is evident that dimers with a high degree of methoxy substitution (e.g. m/z 418 and 332) decrease in relative abundance while dimers with a low degree of methoxy substitution (e.g. m/z 272, 328) become higher in relative intensity, pointing to an enrichment of guaiacyl relative to syringyl during peatification. The percentage contribution of the intensities of these ions to the total intensity of the lignin dimer signal, has been calculated separately for each sample and is depicted in Fig. 6. A decrease in the S/G ratio in peatified wood was also observed by Hedges *et al.* (1985), Saiz-Jimenez *et al.* (1987), Iiyama *et al.* (1988), Stout *et al.* (1988), Bates *et al.* (1991) and Hatcher *et al.* (1989b). The latter author observed a relationship between S/G decrease and an increase in catechol and phenolic compounds. In Table 2, the ratios of phenolic to lignin and of catechol to lignin are presented, calculated from the ion intensities of non-methoxylated phenolics (m/z 94, 107, 108, 120, 122, 136), catechol (m/z 110) and lignin derived ions. The data do not show a significant change with increasing burial time.

Chemical analysis by pyrolysis chemical ionization mass spectrometry (Py-NH₃CI-MS)

Modifications in polysaccharide composition due to peatification were studied by Py-MS using ammonia chemical ionization conditions. The use of ammonia in the ion source as a reaction gas suppresses the ionization of methoxyphenolic compounds, but selectively ionizes the monomeric and oligomeric pyrolysis products of polysaccharides. As a result, more specific information on the carbohydrate moiety is obtained. The investigated samples represent two extremes in degree of peatification, namely recent and peatified Holocene wood. In Fig. 7(a) and 7(b) the time integrated mass spectra

of recent and Holocene wood are shown. The mass spectra are characterized by mass peaks of ammoniated hexose sugar monomers (m/z 144, 162, 180, 222, 240), dimers (m/z 324, 342), trimers (m/z 402, 486, 504) and tetramers (m/z 666), which are common pyrolysis products from cellulose. Pentose sugar mass peaks, pyrolytically derived from hemicellulose are visible at m/z 132, 150 (monomers), m/z 264, 282 (dimers) and m/z 414 (trimers) (Tas *et al.*, 1989; Pouwels and Boon, 1990; Lomax *et al.*, 1991a, b; Boon, 1992). The presence of mono- and dimethoxyphenolic compounds, probably with a propenyl alcohol side chain is indicated by m/z 163, 198 and m/z 193, 228 respectively (Pouwels and Boon, 1990; van Loon, 1992; van der Hage *et al.*, 1993). Pseudomolecular ions of guaiacyl pyrolysis products are seen at m/z 151, 167, 170, 179, 184, 198, 200 (van Loon, 1992), while the identification of the pseudomolecular ions of syringyl pyrolysis products are still under investigation (van der Hage *et al.*, in press).

Mass chromatography performed on the pyrolysis products of cellulose (anhydrohexose ions: m/z 180, 162, 144), hemicellulose (anhydropentose ions: m/z 150, 132) and lignin (m/z 163, 193) [Fig. 7(c)], reveals that anhydropentose and lignin specific ions are generated at low pyrolysis temperatures, showing a maximum between scan 40 and 42 ($T = 405\text{--}432^\circ\text{C}$). The anhydrohexose ions are generated at higher pyrolysis temperatures, showing a maximum between scan 47–49 ($T = 500\text{--}526^\circ\text{C}$). Comparison of the total ion current of the recent [Fig. 7(a); inset] with that of the peatified wood [Fig. 7(b); inset], reveals a decrease in intensity in the low temperature range of the peatified wood mass spectrometric data. This is because of a selective depolymerization and solubilization of hemicelluloses which is due to biodegradation. The high intensity in the high temperature window of the total ion current and the high intensity

Fig. 6. Percentage contribution of some dimer ion intensities (m/z 272, 332, 328, 418) to the total ion intensity of all dimers. With increasing burial time, dimers with a high degree of methoxy substitution (m/z 418, 332) decrease in intensity, while the intensities of less substituted dimers (m/z 272, 328) increase. The compound structures of β -1-stilbene and β - β -resinol are shown in Fig. 5.

Fig. 7. Pyrolysis ammonia chemical ionization mass spectra of recent (a) and peatified wood (b). (c) shows the mass chromatogram of anhydrohexose (m/z 180, 162, 144), anhydropentose (m/z 150, 132) and lignin (m/z 163, 193) derived ions from the MS analysis of recent wood.

of monomeric and oligomeric hexose mass peaks (m/z 180, 162, 144, 342, 504, 666) in the mass spectrum, points to a partial preservation of cellulose in the peatified wood. No trimers of pentose sugars (e.g. m/z 414) are visible anymore.

In order to localize this resistant residual cellulose, thin cross sections of the Holocene wood were studied by means of cross-polarized light microscopy. With this technique the presence of microcrystalline cellulose in cell walls can be demonstrated (Stout and Spackman, 1987, 1989; Stout *et al.*, 1989). In the case of Holocene wood, birefringence is especially observed in the cell walls of the vessels (Fig. 8), indicating that the highly resistant microcrystalline cellulose is mainly located in this tissue.

The hexose to pentose sugar ratio was calculated for all samples (Table 2). From the data, it is clear that the ratios are more or less constant in the sub-recent samples (from 1.92 to 2.20), while the Holocene sample shows a prominent increase to 4.98.

The high intensity of m/z 163 and 193 in the mass spectrum of the peatified wood, representing the presence of coniferyl and sinapylalcohol respectively, indicates that β -O-4 linkages between the phenylpropanoid units are well preserved (van der Hage *et al.*, 1993). The calculated sinapyl to coniferyl alcohol ratios (Table 2) assure the S/G values of the Py-EI-MS data in that a similar trend is observed, although the sinapyl to coniferyl alcohol ratio of the MB sample is somewhat higher than expected.

Chemical studies by Curie-point pyrolysis gas chromatography mass spectrometry

The Py-GC-MS traces of the recent, sub-recent and Holocene wood samples are shown in Fig. 9. The peak numbers refer to the compounds listed in

Table 3. The pyrogram of the recent wood shows prominent peaks of low-molecular-weight pyrolysis products from polysaccharides (1–22, 26). In the peatified wood samples, a decrease in intensity of carbohydrate peaks is observed. The Py-GC-MS data confirm the Py-MS data in that a large part of the carbohydrate fraction is lost from the peatified wood as a result of biodegradation. A relative increase in anhydroglucose (46) yield is observed in the sub-recent samples from 20 and 34 cm depth, which might be attributed to an increase in transglycosidation reactions due to the removal of inorganics from the wood, which results in a higher recovery of anhydrohexoses. Dominant pyrolysis products from the mixed guaiacyl/syringyl lignin are guaiacol (25), vinylguaiacol (36), prop-2-enylguaiacol (44), vinylsyringol (50), formylsyringol (54), prop-2-enylsyringol (58) and acetylsyringol (59). The occurrence of methoxyphenolics with terminal hydroxyl groups on the propenyl side chain (coniferyl and sinapyl alcohol) are extremely low in abundance, while prop-2-enylsyringol (55, 58) and prop-2-enylguaiacol (41, 44) are dominant. The high abundance of these compounds is probably caused by thermal dehydration of coniferyl and sinapylalcohol in the glass liner. This leads to an underestimation of methoxyphenolics with a terminal hydroxyl group in the Py-GC-MS analysis compared with in-source Py-MS (van der Hage *et al.*, 1993). Non-methoxylated phenolics and catechols such as phenol (19), methylphenol (23, 24), ethylphenol (27), vinylphenol (30), 1,2-dihydroxybenzene (29) and dihydroxymethylbenzene (34) are present but in relative small quantities. The chromatograms of the wood samples isolated from different depths of the peat core reveal no significant changes in distribution of methoxyphenolic peaks. In

Fig. 8. Cross polarized light microscopy photograph (transverse section) of peatified Holocene wood, showing strong birefringence in the vessels. V = vessel. Scale bar = 30 μ m.

Fig. 9. Py-GC-MS traces of recent, sub-recent (CB20-CB52) and Holocene wood samples (MB). The peak numbers refer to the compounds in Table 3.

Table 3. List of identified pyrolysis products from recent, sub-recent and Holocene wood

N.R.	M +	Compound Name	Structure	Sample
1	86	1,4-pentadiene-3-one		1,2,3,4,5
2	74	3-hydroxypropanal		1,2,3,4,5
3	84	(3H)-furan-2-one		1,2,3,4,5
4	102	pyruvic acid methyl ester		1,2,3,4,5,6
5	84	2(H)-furan-3-one		1,2,3,4,5,6
6	90	2,3-dihydroxypropanal		1,3,4
7	96	3-furaldehyde		1,2,3,4,5
8	98	3-(hydroxymethyl) furan		1,2,3,4,5,6
9	82	2,4-pentadienal		1,2,3,4,5,6
10	96	2-furaldehyde		1,2,3,4,5,6
11	116	1-acetyloxypropane-2-one		1,3,4
12	98	2-hydroxymethylfuran		1,2,3,4,5
13	96	cyclopent-1-ene-3,4-dione		1,2,3,4,5
14	84	(5H)-furan-2-one		1,2,3,4,5
15	110	2-acetylfuran		1,3,4
16	98	2,5-dihydro-5-methylfuran-2-one		1,2,3,4,5
17	98	2,3-dihydro-5-methyl-furan-2-one		1,2,3,4,5,6
18	110	5-methyl-2-furaldehyde		1,2,3,4,5,6
19	94	phenol	P	1,2,3,4,5,6
20	114	4-hydroxy-5,6-dihydro-(2H)-pyran-2-one		1,2,3,4,5,6
21	112	3-hydroxy-2-methyl-2-cyclopentene-1-one		1,2,3,4,5,6
22	112	2-hydroxy-3-methyl-2-cyclopentene-1-one		1,2,3,4,5
23	108	2-(or 3-)methylphenol	P-C	1,3,4,5,6
24	108	4-methylphenol	P-C	1,3,4,5,6
25	124	2-methoxyphenol	G	1,2,3,4,5,6
26	126	3-hydroxy-2-methyl-(4H)pyran-4-one		1,3,4,5
27	122	ethyl-phenol	P-C-C	1,3,4,5,6
28	138	methylmethoxyphenol	G-C	1,2,3,4,5,6
29	110	1,2-dihydroxybenzene	Ca	1,2,3,4,5,6
30	120	vinylphenol	P-C=C	1,4,5,6
31	140	2-methoxy-4-hydroxyphenol	G-OH	1,2,3,4,5,6
32	124	dihydroxymethylbenzene	Ca-C	1,6
33	152	2-methoxy-4-ethylphenol	G-C-C	1,2,3,4,5,6
34	124	dihydroxymethylbenzene	Ca-C	1,6
35	134	propenylphenol	P-C=C-C	6
36	150	2-methoxy-4-vinylphenol	G-C=C	1,2,3,4,5,6
37	154	2,6-dimethoxyphenol	S	1,2,3,4,5,6
38	164	2-methoxy-4-(prop-1-enyl)phenol	G-C-C=C	1,2,3,4,5,6
39	166	2-methoxy-4-propylphenol	G-C-C-C	1,2,3,4,5,6
40	152	2-methoxy-4-formylphenol	G-CHO	1,2,3,4,5,6
41	164	2-methoxy-4-(prop-2-enyl)phenol (cis)	G-C=C-C	1,2,3,4,5,6
42	168	2,6-dimethoxy-4-methylphenol	S-C	1,2,3,4,5,6
43	166	2-methoxy-4-ethanalphenol	G-C-CHO	1,2,4,5
44	164	2-methoxy-4-(prop-2-enyl)phenol (trans)	G-C=C-C	1,2,3,4,5,6
45	166	2-methoxy-4-acetylphenol	G-CO-C	1,2,3,4,5,6
46	162	anhydroglucose		1,2,3,4,5,6
47	180	2-methoxy-4-(propane-2-one)phenol	G-C-CO-C	1,2,3,4,5,6
48	182	2,6-dimethoxy-4-ethylphenol	S-C-C	1,2,3,4,5,6
49	168	2-methoxy-4-carboxyphenol	G-COOH	5,6
50	180	2,6-dimethoxy-4-vinylphenol	S-C=C	1,2,3,4,5,6
51	178	2-methoxy-4-(prop-1-en-3-one)phenol	G-CO-C=C	1,2,4,5,6
52	194	2,6-dimethoxy-4-(prop-1-enyl)phenol	S-C-C=C	1,2,3,4,5,6
53	196	2,6-dimethoxy-4-propylphenol	S-C-C-C	3,5
54	182	2,6-dimethoxy-4-formylphenol	S-CHO	1,2,3,4,5,6
55	194	2,6-dimethoxy-4-(prop-2-enyl)phenol (cis)	S-C=C-C	1,2,3,4,5,6
56	180	2-methoxy-4-(1-hydroxy-prop-2-enyl)phenol (cis)	G-C=C-COH	1
56A	192	syringol (S-C ₃ H ₃) derivative		1,2,3,4,5,6
57	196	2,6-dimethoxy-4-ethanalphenol	S-C-CHO	1
58	194	2,6-dimethoxy-4-(prop-2-enyl)phenol (trans)	S-C=C-C	1,2,3,4,5,6
59	196	2,6-dimethoxy-4-acetylphenol	S-CO-C	1,2,3,4,5,6
60	178	2-methoxy-4-(prop-2-enal)phenol	G-C=C-CHO	1,2,3,4,5,6
61	180	2-methoxy-4-(1-hydroxy-prop-2-enyl)phenol (trans)	G-C=C-COH	1
62	210	2,6-dimethoxy-4-(propane-2-one)	S-C-CO-C	1,2,3,4,5,6
62A	210	2,6-dimethoxy-4-(propyl-3-one)	S-CO-C-C	1,2,3,4,5,6
63	198	2,6-dimethoxy-4-carboxyphenol	S-COOH	5,6
64	208	2,6-dimethoxy-4-(prop-1-en-3-one)	S-CO-C=C	1
65	212	2,6-dimethoxy-4-(1-hydroxy-propyl)phenol	S-C-C-C-OH	1,3
66	210	2,6-dimethoxy-4-(1-hydroxy-prop-2-enyl)phenol (cis)	S-C=C-COH	1
67	208	2,6-dimethoxy-4-(prop-2-enal)phenol	S-C=C-CHO	1,2,3,4,5,6
68	210	2,6-dimethoxy-4-(1-hydroxy-prop-2-enyl)phenol (trans)	S-C=C-COH	1
69	256	C _{16:0} fatty acid		1,3,4,5,6
70	284	C _{18:0} fatty acid		1,3,4,5,6
71	272	α,β -diguaiacyl ethene		1,2,3,4,5,6

Recent = 1, CB20 = 2, CB34 = 3, CB47 = 4, CB52 = 5, MB = 6.

P = phenol, Ca = catechol, G = monomethoxyphenol (guaiacyl), S = dimethoxyphenol (syringyl).

order to trace possible lignin-degradation trends in the peatified wood, calculations were performed using the peak areas of specific classes of pyrolysis products. The syringyl to guaiacyl (S/G), phenol/lignin, catechol/lignin and methoxycatechol/syringyl ratios are calculated from the summed peak areas of phenol, catechol, guaiacyl and syringyl derivatives, while the degree of side chain reduction is determined by dividing the summed areas of guaiacyl or syringyl pyrolysis products with a C_3 side chain with the total guaiacyl or syringyl peak area ($G-C_3/G_{total}$, $S-C_3/S_{total}$) (van der Hage *et al.*, 1993). The percentage contribution of different functional groups (alcohols, ketones, aldehydes) substituted on the aliphatic side chain of the lignin pyrolysis products is determined from the summed peak areas of a specific group, normalized to the total peak area of guaiacyl and syringyl. The data of different calculations are presented in Table 2. The ratio which expresses the degree of side chain preservation ($G-C_3/G_{total}$, $S-C_3/S_{total}$) does not show a significant trend with increasing depth, indicating that side chain cleavage does not occur during the first stages of peatification. A significant decrease is observed in the S/G ratios, which is already evident in the wood sample from 34 cm depth. The latter data confirms the Py-EI-MS results. The phenol/lignin ratio does not show a significant increase. Chemical changes of the lignin molecule involve small changes in oxygen functionality such as a slight increase in ketones (from 13 to 19%), a decrease of aldehydes (from 27 to 13%), while the alcohols are absent (Table 2). The decrease in aldehydes is partially due to a decrease of 2-methoxy-4-ethanal-phenol (43) and 2,6-dimethoxy-4-ethanalphenol (57), while the increase in ketones is due to a significant increase in 2-methoxy-4-acetyl phenol (45) in the pyrolysis data (Fig. 9). The occurrence of a low signal of vanillic and syringic acid (49 and 63) in CB-52 and the Holocene sample may indicate that a small amount of the phenyl propane monomers is oxidatively cleaved between C_α and C_β (Chen and Chang, 1983). These compounds are degradation products of lignin and are easily ethanol extractable (van der Heijden *et al.*, unpublished results).

DISCUSSION

The anatomical studies reveal two main decomposition pathways. Decomposition type A, might be attributed to soft rot fungi which cause boreholes and solubilize the S_1 , S_2 layers of the secondary wall, leaving the compound middle lamella and the S_3 layer intact. Decomposition type B might be induced by the activity of white rot fungi, which first erode the secondary cell wall and finally the compound middle lamella (Wilcox, 1970). The white rot fungi usually degrade and solubilize the lignin macromolecule, leaving the polysaccharide fraction more or less intact (Blanchette *et al.*, 1990; Mulder *et al.*, 1991). In our

samples, no severe lignin degradation has been observed, while the compound middle lamella is preserved in almost all fibre-tracheids. Also because of the fact that no fungal remains are visible in cells decomposed via decomposition pathway B, we suggest that the latter decomposition pathway is induced by bacteria. From the anatomical observations it is evident that decomposition pathway B is most prominent in the CB47, CB52 and CB34 samples, while decomposition pathway A is dominant in the Holocene material. The CB20 wood sample shows an intermediate pattern. The loss of secondary cell wall material is already present in the sample from 20 cm depth, indicating that biodegradation processes on the cell wall level are already prominent in the top layer of the peat. With increasing depth a progressive loss of secondary cell wall material is observed. This loss is directly related with a decrease in carbohydrate content, which confirms the idea that structural disintegration is directly related with the loss of carbohydrates (Barghoorn and Spackman, 1950). The loss of cell wall material is most prominent in the samples from 52 cm depth. At this depth anaerobic conditions prevail, which indicates that anaerobic degradation of carbohydrates is an important process in waterlogged raised bog deposits. The fact that carbohydrates are degraded more rapidly than lignin is also observed by other authors in more heterogeneous samples using NMR, analytical pyrolysis and wet chemical techniques (Philp *et al.*, 1982; Hatcher *et al.*, 1982, 1989a, b; Hedges *et al.*, 1985; Ryan *et al.*, 1987; Wilson *et al.*, 1987; Hatcher, 1988; Stout *et al.*, 1988, 1989; Bates *et al.*, 1991; Obst *et al.*, 1991; Rollins *et al.*, 1991).

In the sub-recent samples a constant hexose/pentose ratio was observed pointing to the fact that an almost equal amount of hemicellulose and cellulose is removed during the first stages of peatification. In contrast with the sub-recent samples, a selective preservation of hexose sugars over pentose sugars is seen in the Holocene sample. In spite of the existence of a large time gap between the sub-recent and Holocene wood, we postulate that the residual hemicellulose fraction in the sub-recent wood will be (an)aerobically further degraded prior to the cellulose fraction. The preferential degradation of hemicellulose over cellulose during long term degradation is observed by other authors as well (Hedges *et al.*, 1985; Stout *et al.*, 1989; Blanchette *et al.*, 1991).

The occurrence of birefringence in the vessels of the Holocene wood and the occurrence of oligomeric anhydrohexose sugars in the mass spectrum is indicative for the presence of microcrystalline cellulose. In contrast with the vessels, the fibre-tracheids show a distinct loss of crystalline cellulose. Given *et al.* (1948) suggested that preservation of cellulose in peats is probably due to the fact that a part of the cellulose is chemically altered without being depolymerized and that cellulase enzymes no longer recognize the

material as cellulose. However, the specific location of resistant cellulose in the vessel cell walls suggests that cellulose is not biodegraded and protected by another mechanism. Although investigations on the physical/chemical nature of these protection mechanisms are scarce, we postulate that a fraction of cell wall polysaccharides are protected from enzymatic scission due to packaging in condensed lignin or by hydrophobic layers.

The Py-EI-MS and the Py-GC-MS data reveal a significant change in the S/G ratio with increasing depth. Hatcher (1988) and Hatcher *et al.* (1982, 1988, 1989a, b) explained this phenomenon by proposing a mechanism in which syringyl is converted to guaiacyl by demethylation and dehydroxylation, thus enriching the guaiacyl yield. Guaiacyl is then converted to catechols and phenols. Young and Frazer (1987) and Frazer and Young (1986) experimentally proved the occurrence of demethylation processes, proposing the mechanism as a simple reaction in which the methoxygroups are used as C₁ substrate for acetogenic bacteria. In our data, no significant increase in methoxycatechol, catechol and phenolic compounds is observed which indicates that no demethylation and dehydroxylation processes occur in raised bog deposits. We therefore propose that the decrease in the S/G ratio is directly related with the removal of syringyl-rich secondary cell wall material and the preservation of guaiacyl-rich compound middle lamella. This idea has also been proposed by Hedges *et al.* (1985), and is supported by the results obtained by Terashima (1990). The latter author points to the fact that the secondary cell walls of wood contain more syringyl lignin than the compound middle lamella, which is dominated by highly condensed guaiacyl lignin. In our samples, a significant S/G shift is already observed in the CB34 sample, which shows severe decomposition of secondary cell wall material.

Compared with the deepest sub-recent sample, the Holocene material shows a higher S/G and sinapyl to coniferyl alcohol ratio. This discrepancy between sub-recent and Holocene wood might be induced by differences in biotic and abiotic conditions in the peat environment, resulting in different decomposition pathways. We indeed observed major anatomical differences between the sub-recent and Holocene samples; the fibre-tracheids of the Holocene wood showed a preservation of S₃ layers (decomposition pathway A), while the S₃ of most sub-recent fibre-tracheids are decomposed via decomposition pathway B. Although no information exists on the lignin composition of the S₃ cell wall layer, we postulate that the high syringyl contribution of the Holocene wood is due to a selective preservation of syringyl rich S₃ cell wall layers.

The Py-GC-MS data point to other chemical modifications in the lignin macromolecule. The depletion of mono- and dimethoxy-4-ethanal phenol in the sub-recent and Holocene wood indicates a chemical modification in the precursor of this pyro-

lysis product. Other modifications are indicated by the presence of small amounts of carboxylated methoxyphenolics and the increase of 2-methoxy-4-acetyl phenol, which suggest that some side chain oxidation has occurred (Saiz-Jimenez *et al.*, 1987). This is not reflected in a change in the yield of pyrolysis products with an intact C₃ side chain. From this, we conclude that the residual lignin consists of a reservoir of intact lignin and a shell of biodegraded lignin.

CONCLUSIONS

- Biodegradation processes in raised bog deposits already occur in the top layers of the peat. These processes are mainly mediated by fungi and bacteria.
- In the fibre-tracheids and the wood-parenchyma, two anatomical decomposition pathways are discerned:
 1. The S₁, S₂ layers are biodegraded, while the S₃ layer and the primary wall are preserved. This pathway is thought to be induced by soft rot fungi (decomposition pathway A).
 2. The secondary wall is gradually degraded from the lumen outwards, probably mediated by bacteria (decomposition pathway B).
- The selective removal of carbohydrates corresponds anatomically with the removal of secondary cell wall material (S₁, S₂, S₃) from fibre-tracheids and wood-parenchyma.
- The carbohydrate contents tend to drop with increasing burial time of the wood.
- During the very first stages of peatification, cellulose and hemicellulose are removed from the wood at equal rates. When peatification proceeds, hemicellulose is preferentially degraded over cellulose.
- A highly resistant microcrystalline cellulose is preserved in the vessel cell walls of the peatified Holocene wood, shielded by an "unknown" protection mechanism, which may involve packaging by lignin.
- A decrease in the syringyl-to-guaiacyl ratio is already observed in the very first stages of peatification, which is attributed to the selective removal and solubilization of syringyl-rich secondary cell wall material (S₁, S₂, S₃).
- The fact that Holocene wood shows a higher S/G ratio than the deepest sub-recent sample is probably due to the preservation of syringyl-rich S₃ layers in the Holocene fibre-tracheids.
- Although some side chain oxidation has occurred in the lignin molecule, no decrease in amount of pyrolysis products with a C₃ side chain is observed. The residual lignin is postulated to be a reservoir of intact lignin surrounded by a shell of biodegraded lignin.
- Changes in the lignin molecule comprise modifications of oxygen functionality on the aliphatic

side chain of the methoxyphenolics such as a reduction in aldehydes and a slight increase in ketones.

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