A NOVEL POLYPHENOL BIOPOLYMER ISOLATED FROM FOSSIL SEEDS: AN ALTERNATIVE SOURCE FOR (ALKYL)PHENOL MOIETIES IN COALS.

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INTRODUCTION

During the last years several papers have been published on the chemical composition of recognizable higher plant remains in sediments (*e.g.* spores, pollen[1], cuticles[2,3], bark[4,5], wood remains[6]). It is thought that these tissues have survived in the geological record because they are comprised of highly resistant biopolymers such as cutan[3], suberan[4] and lignin[6], whose chemical structures are only slightly modified during sediment diagenesis.

Within this context seeds have hardly been studied. To some extent this is remarkable since they are often produced in large amounts. Alternatively, if few seeds are produced they can be of large size. Extremes in this respect are orchids with produce over one million seeds per fruit per year (each weighing 5 μg) and the Seychelles coconut with a production of perhaps ten seeds in a lifetime weighing 20 kg each. Perhaps more important from a geochemical point of view, seeds possess protective envelopes derived from the integuments of the ovule. They comprise the seed coat and include the testa and tegmen at least one layer of which often becomes tough and sclerotic at biological maturity. The testa is a product of the outer integument whereas the tegmen is produced by the inner integument occuring inside the testa. On morphological features the tegmen is comparable to a cuticle. The protective layers represent only a few percent of the total biomass of the produced seed. However, in most cases it are only the seed coats that are found in the geological record, because the outer layers of seeds contain resistant compounds to protect the genetic material against physical and chemical processes such as temperature and humidity changes and bacterial and fungal attacks. Hence, seeds, and particularly their resistant layers, have a large potential to enter the geosphere and may become selectively enriched.

In this study outer and inner layers (testae and tegmens, respectively) of Late Eocene seeds of *Stratiotes* (water soldier) and *Sabrenia* (fossil water lily) were analyzed by means of flash pyrolysis-gas chromatography-mass spectrometry and flash pyrolysis-gas chromatography in order to test the above mentioned hypothesis. The testa of the studied seeds is a sclerotic layer, whereas the tegmen is a translucent tissue comparable to a cuticle. The *Sabrenia* seeds are obtained from the Brembridge Marls Member, Bouldnor Formation, Hamstead Ledge, Isle of Wight. The *Stratiotes* seeds are obtained from the Totland Bay Member, Headon Hill Formation, Hordle Cliff, Hampshire. In addition a comparison is made between the pyrolysate of the *Sabrenia* testae and the Beulah Zap lignite[7]. This coal sample was obtained from the Sentinel Butte Formation of the Fort Union Group (Upper Palaeocene), Mercer County, North Dakota.

EXPERIMENTAL

The fossil testae and tegmens were dissected by hand. The samples were ultrasonically extracted with methanol. The residues were dried in a vacuum stove at 30°C.

Curie-point pyrolysis-gas chromatography (PY-GC) analyses were performed with a Hewlett-Packard 5890 gas chromatograph using a FOM-3LX unit for pyrolysis. The samples were applied to a ferromagnetic wire. The Curie temperature was 610°C. The gas chromatograph, equipped with a cryogenic unit, was programmed from 0°C (5 min) to 320°C (20 min) at a rate of 3°C/min. Separation was achieved using a fused-silica capillary column (25m x 0.32mm) coated with CP Sil-5 (film thickness 0.4 μm). Helium was used as the carrier gas.

The Curie-point pyrolysis-gas chromatography-mass spectrometry (PY-GC-MS) analyses wre performed using the same equipment and conditions as described above for the PY-GC conneted with a VG 70S mass spectrometer operated at 70 eV with a mass range m/z 40-800 and a cycle time of 1.8 s.

RESULTS AND DISCUSSION

The pyrolysates of the fossil tegmens of both *Stratiotes* and *Sabrenia* seeds are dominated by series of n-alkanes and n-alk-1-enes. In addition, the pyrolysate of the *Sabrenia* tegmens shows the distinct presence of isoprenoids. Prist-1-ene is the most abundant compound. Since δ-tocopherol was also detected, this compound is thought to be the precursor of the observed isoprenoids[8]. The n-alk-1-enes and n-alkanes detected in pyrolysates of the tegmens are thought to be derived from a highly aliphatic biopolymer comparable to cutan[3].

On the contrary, the main compounds in the pyrolysate of the fossil testae of *Stratiotes* and *Sabrenia* are phenol and C_1 and C_2 alkylated phenols (Table 1). In addition, some methoxyphenols and benzenediols (mainly benzene-1,2-diols) are present. The abundance of these latter compounds is very low in the pyrolysate of the *Stratiotes* testae. The identification of these compounds was confirmed by comparison of mass spectra and relative retention times with those of authentic standards. The methoxyphenols present in these pyrolysates are not derived from lignin, since the distribution of these compounds in lignin pyrolysates is completely different[6]. The phenols and benzenediols detected are thought to be pyrolysis productes of a novel polyphenol biopolymer.

The seeds of *Stratiotes* as well as *Sabrina* are produced under or near the water surface. In these environments fungal activities are prominent. Since phenols are known to possess fungicidal properties[9], it is thought that the outer layer (testa) contains a polyphenol biopolymer as an additional protective shield for the genetic material. Hence, physical as well as chemical protection is conferred by the sclerotic testa.

As mentioned earlier the tegmen occurs inside the testa as a cuticle based on biological features. This similarity of tegmen and cuticle is supported by pyrolysis data since a highly aliphatic biopolymer comparable to cutan has been recognized as a constituent of tegmens.

The distribution patterns of the phenols and benzenediols in the pyrolysates of the testae are remarkably similar to those in the pyrolysate of the Beulah Zap lignite (Table 1; Fig. 1). This indicates that selective preservation of this novel biopolymer may be an alternative for the proposed demethoxylation of lignins[10] to explain the presence of phenol moieties in coals. Whether these phenol moieties in coal are derived from testae only remains to be seen, because this novel biopolymer may also occur in other tissues of water plants not yet investigated.

CONCLUSIONS

The two anatomically different seed layers of fossil *Stratiotes* and *Sabrenia*, tegmen and testa, respectively, show remarkable differences in chemical composition. The tegmens are mainly comprised of an aliphatic biopolymer comparable to cutan whereas the sclerotic testae contain a novel polyphenol biopolymer.

The highly similar distribution pattern of phenols and benzenediols observed in both pyrolysates of testae and lignites strongly indicates that selective preservation of the novel biopolymer may be an alternative for the proposed demethoxylation of lignins to explain the presence of phenol moieties in coals.

REFERENCES

1. Schenck, P.A., de Leeuw, J.W., Van Graas, G., Haverkamp, J. and Bouman, M. (1981), In: Organic Maturation Studies and Fossil Fuel Exploration 1981 (ed. J.Brooks), Academic Press, London. 225-237.
2. Nip, M., Tegelaar, E.W., Brinkhuis, H., de Leeuw, J.W., Schenck, P.A. and Holloway, P.J. (1986), Org.Geochem., 10: 769-778.
3. Tegelaar, E.W., de Leeuw, J.W., Largeau, C., Derenne, S., Schulten, H-R., Muller, R., Boon, J.J., Nip, M. and Sprenkels, J.C.M. (1989), J.Anal.Appl.Pyrolysis, 15: 29-54.
4. Tegelaar, E.W., Hollman, G., Van der Vegt, P., de Leeuw, J.W. and Holloway, P.J. (1990), In: Resistant biomacromolecules in morphologically characterized constituents of kerogen: a key to the relationship between biomass and fossil fuels (ed. E.W.Tegelaar). PhD thesis, University of Utrecht.
5. Wilson, M.A. and Hatcher, P.G. (1988), Org.Geochem., 12: 539-546.
6. Saiz-Jimenez, C. and de Leeuw, J.W. (1986), Org.Geochem., 10: 869-876.
7. Smith, K.L. and Smoot, L.D. (1990), Prog.Energy Combust.Sci. 16: 1-53.
8. Goossens, H., de Leeuw, J.W., Schenck, P.A. and Brassell, S.C. (1984), Nature, 312: 440-442.
9. Waller, G. (1987), Allelochemicals: Role in agriculture and forestry. Am.Chem.Soc.Symp.Series 330.
10. Senftle, J.T., Larter, S.R., Bromley, B.W. and Brown, J.W. (1986), Org.Geochem. 9: 345-350.

Table 1: Major identified peaks in pyrolysates of *Sabrenia* testae and the Beulah Zap lignite (Fig. 1)

1.	Phenol
2.	2-Methylphenol
3.	2-Methoxyphenol
4.	3-Methylphenol and 4-Methylphenol
5.	2,4-Dimethylphenol
6.	4-Ethylphenol
7.	4-Methyl-2-methoxyphenol
8.	Benzene-1,2-diol
9.	C_3-phenol
10.	4-ethyl-2-methoxyphenol
11.	4-methyl-benzene-1,2-diol

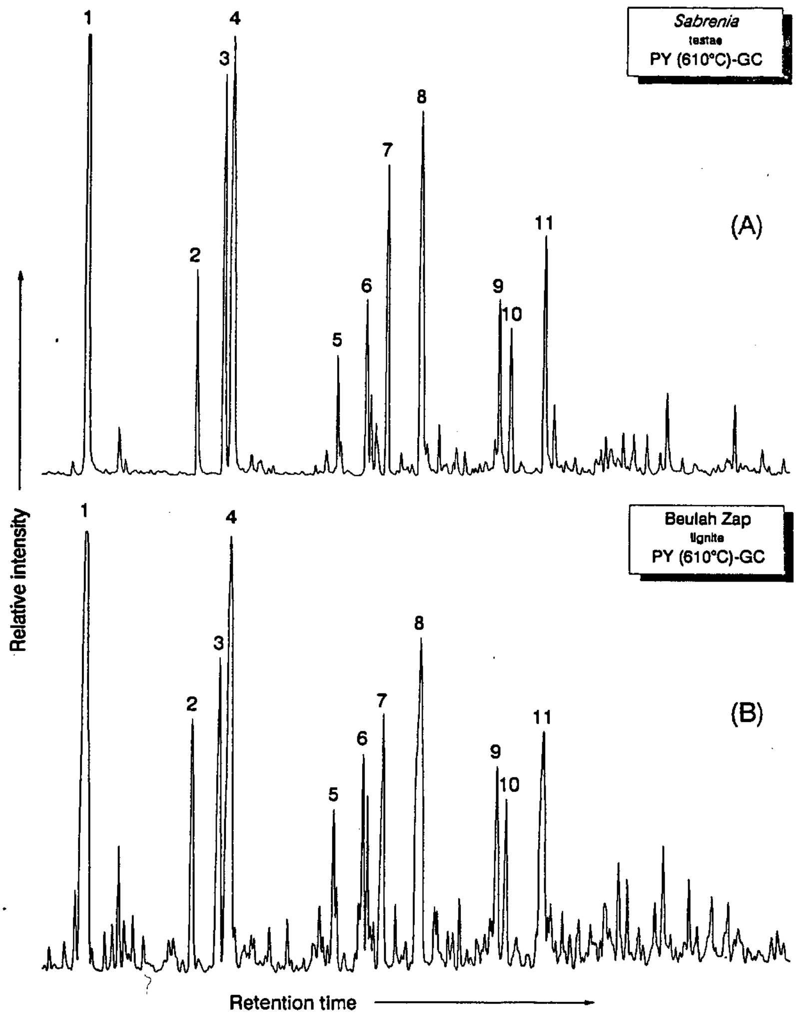

Fig. 1: Partial gas chromatogram of flash pyrolysates of *Sabrenia* testae (A) and the Beulah Zap lignite (B).