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BIOMARKERS FROM THE TAXODIACEOUS CONIFER *SPHENOLEPIS PECINOVENSIS* KVAČEK AND RESIN FROM BOHEMIAN CENOMANIAN

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ABSTRACT. The biomarker composition in leaf compressions of *Sphenolepis pecinovensis* Kvaček (Taxodiaceae) and in isolated resin grains from the Cenomanian mudstones near Pecínov (Bohemia, Czech Republic) has been analysed to investigate the chemotaxonomy of the fossil conifer species and to evaluate the source of the resin. The extracts of the *Sphenolepis pecinovensis* shoot and the resin grains show similar patterns of terpenoid biomarkers indicating *Sphenolepis pecinovensis* as a likely botanical source for the resin. Some of the terpenoids are unspecific conifer biomarkers known from Recent conifers and fossil resins. More specific sesqui- and diterpenoids can be assigned to Cupressaceae, Taxodiaceae and in part also to Podocarpaceae, because their precursors have been identified only in Recent species of these families. The chemotaxonomy thus indicates a close relationship of *Sphenolepis pecinovensis* to the Taxodiaceae or the Cupressaceae.

KEY WORDS: *Sphenolepis*, Cretaceous, Bohemian Cenomanian, conifers, Taxodiaceae, Cupressaceae, terpenoids, biomarker, resin, biogeochemistry

INTRODUCTION

In Recent botany the distribution of secondary metabolites is – besides anatomy and morphology – commonly used for taxonomic approaches (e.g. Hegnauer 1962–1992). Important compound classes in chemotaxonomy are for example terpenoids, tannins, and proteins. The knowledge of characteristic compounds in fossil plants would also be useful as additional characteristics for their taxonomic assignment, the combination of isolated organs, the reconstruction of floral elements in sediments without macro- or microfossils and finally for the plant phylogeny (Thomas 1986, Otto *et al*. 1997a). Most of the organic molecules like nucleic acids or proteins are rapidly mineralized in sediments. The more stable lipids and macromolecules such as lignin or cutin though often are preserved in sediments and fossil plant remnants (e.g. Runnegar 1986, Logan *et al.* 1991, van Bergen *et al.* 1994).

Fossil lipid components, the biomarkers, have been described from numerous sediments, coals, crude oils and peats (Brassell *et al*. 1983, Simoneit 1986). Biomarkers show little or no change from their parent molecules in living plants and therefore can be assigned to certain biological precursor molecules (Thomas 1986, Peters & Moldowan 1993). The biomarkers encompass aliphatic molecules, e.g. n-alkanes, n-alkanols, fatty acids, and the cyclic terpenoids. Main sources for biomarkers are microorganisms and the waxes, woods and resins of higher plants (Logan *et al*. 1991).

In the present study the terpenoid biomarker composition of a fossil conifer species and isolated resin from the same strata have been investigated. Leaf compressions of *Sphenolepis pecinovensis* Kvaček (Taxodiaceae) and yellow resins grains (500–1000 μm) were collected from the Cenomanian Peruc-Korycany Formation of the Bohemian Cretaceous Basin (Kvaček 1997). The compressions of *Sphenolepis pecinovensis* represent branches with helically arranged leaves (Fig. 1), attached pollen cones and ovuliferous cones. Taxodiaceous assignment of this conifer is based on helically arranged leaves and attached *Sequoia*-like ovuliferous cones (Kvaček 1997). Organic geochemical analyses were applied to evaluate chemotaxonomical data of the fossil conifer species and to investigate whether the resin derived from the *Sphenolepis* plants or not.

EXPERIMENTAL

Sample material was collected from the Pecínov quarry, Babín Middle pit near Kladno (Czech Republic). The localitiy belongs to the Peruc – Korycany Formation

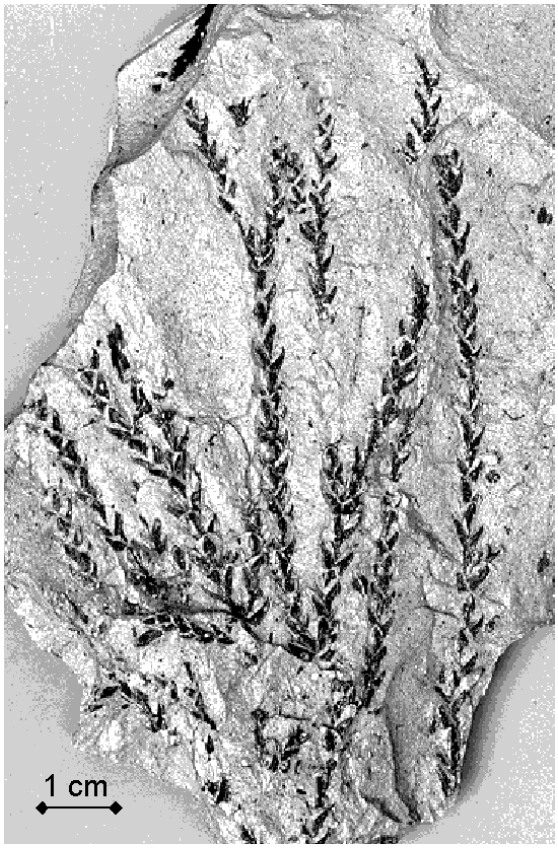


Fig. 1. *Sphenolepis pecinovensis* Kvaček vegetative shoot from Bohemian Cenomanian

of the Bohemian Cretaceous Basin (as defined by Čech *et al*. 1980). Palynological data indicate late middle Cenomanian age of this Formation (Pacltová 1977). The Pecínov locality provides the best opportunity to follow the continental/marine sedimentation of the Peruc-Korycany Formation (see Uličný & Spicáková 1996, Uličný *et al*. 1997). The lower parts (units 1 and 2), dominated by an angiosperm *Myrtophyllum geinitzii* Heer, represent fluvial sedimentation. The middle part (unit 3), dominated by *Frenelopsis alata* (K. Feistmantel) Knobloch, represents salt marsh setting. The upper part is developed in marine facies with a thin layer containing terrestrial flora rich in the conifer *Sphenolepis pecinovensis*. Its compressed shoots, cones and isolated resin grains are preserved in a dark mudstone (Kvaček 1997).

Approximately 5 cm (3.2 mg) of a vegetative shoot of *Sphenolepis pecinovensis* was scraped off the sediment, and 3 mg of resin grains were handpicked from the sediment. The shoot as well as the resin were seperately sonicated twice in organic solvents dichloromethane and methanol (1:1) for 5 min. The total extracts were then derivatised to protect functional groups (Otto *et al*. 1996). Separation and detection of individual compounds was performed on a gas chromatograph Fisons GC8000 coupled to a quadrupole mass spectrometer Fisons MD800. The gas chromatograph was fitted with a 25 m fused silica capillary column (SE 54; 0.25 mm i.d.). Oven temperature was programmed from 80 to 300 °C at a rate of 4 °C/min with final isothermal conditions for 20 min. Helium was used as carrier gas. Individual biomarkers were identified by comparison with published mass spectral data.

RESULTS AND DISCUSSION

The extracts of the *Sphenolepis* leaves and the resin show similar patterns of biomarkers (Fig. 2). Some sesqui- and diterpenoids have been identified by comparison of their mass spectra with published data (Enzell & Ryhage 1967, Philp 1985). According to their basic structures, the sesquiterpenoids identified in the extracts belong to the cedrane, cadinane, and cuparane classes (Table 1). The identified diterpenoids belong to the tricyclic classes of pimaranes and abietanes.

Dominant in the first part of the chromatograms of both extracts is a sesquiterpenoid of the cadinane class (cadinadiene), while an abietane (norabietatriene) shows

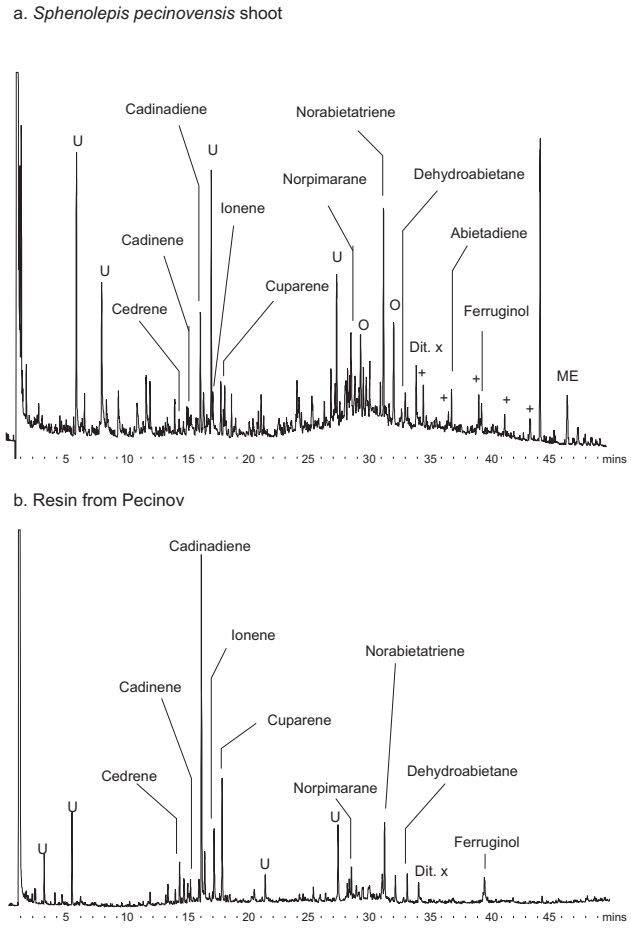
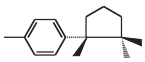


Fig. 2. Gas chromatograms of the extracts of Cenomanian (a) *Sphenolepis pecinovensis* shoot and (b) isolated resin from Pecinov (Czech Republic). + – n-alkanes, o – fatty acids, ME – methyl ester, IS – internal standard, U – unknown terpenoids

Table 1. Terpenoids identified in the extracts of Cenomanian *Sphenolepis pecinovensis* and the distribution of their possible precursor compounds in extant conifers (References see in the text)

Compound	Structure	Structural class	Distribution of possible precursors in extant conifers
Sesquiterpenoids			
Cadinene		Cadinane	all families
Cadinadiene		Cadinane	all families
Ionene		degradation product of various classes	all families
Cedrene		Cedrane	Cupressaceae, Taxodiaceae
Cuparene		Cuparane	Cupressaceae
Diterpenoids			
Norpimarane		Pimarane	all families
Norabietatriene		Abietane	all families
Dehydroabietane		Abietane	all families
Abietadiene		Abietane	all families
Ferruginol		phenolic Abietane	Cupressaceae, Taxodiaceae, Podocarpaceae

high concentrations in the middle part. Analytical data of the hitherto unknown terpenoid x (molecular weight 286, main fragment m/z 271) indicate a diterpenoid alcohol that probably has been preserved unchanged. Some chain-like aliphatic biomarkers (n-alkanes, fatty acids, methyl ester) originating from epicuticular waxes occur additionally to the terpenoids in the extract of the *Sphenolepis* shoot while the terpenoid compounds are concentrated in the resin extract.

Composition and relative abundance ("fingerprint") of individual biomarkers in the extracts of the shoot of *Sphenolepis pecinovensis* and the isolated resin show a great correspondance. The terpenoid biomarkers thus indicate *Sphenolepis pecinovensis* as the likely botanical source for the resin.

CHEMOTAXONOMICAL ASPECTS

The biomarkers still have their characteristic skeletons and thus can be assigned to their possible biological precursors that are present in extant plants. In Table 1 all the terpenoids hitherto identified in *Sphenolepis pecinovensis* are listed together with the distribution of their possible biological precursors in extant conifers (Kariyone 1964–1983, Karrer 1958, Erdtman & Norin 1966, Karrer *et al.* 1977, Hürlimann & Cherbuliez 1981, Sukh Dev 1989, Glasby 1991 and literature cited there).

The possible precursors of ionene and the cadinanes, pimaranes, and abietanes that have been identified in the extracts have a wide distribution among species of all conifer families. These terpenoids therefore can only be used as unspecific conifer biomarkers.

Terpenoids of the cuparane and cedrane skeleton have also been reported from some Tertiary fossil resins (Grantham & Douglas 1980). Cuparene and cedrene are highly specific sesquiterpenoids, because terpenoids of these structural classes are known among the extant conifers only from Cupressaceae and Taxodiaceae (cedranes) or Cupressaceae (cuparanes) (Erdtman & Norin 1966, Grantham & Douglas 1980). Cuparene and cedrene occur in extant conifers together with their associ-

ated alcohols (cedrol, cuparenol) that are easily degraded to cedrene and cuparene (Grantham & Douglas 1980). Cedrene and cuparene therefore are slightly degraded or unchanged preserved biomarkers characteristic for Taxodiaceae and Cupressaceae.

The phenolic terpenoid ferruginol that has been identified in the extracts probably survived the diagenesis without degradation. Ferruginol has been isolated before from lignites (Baset *et al*. 1980) and the Oligocene Taxodiaceae *Taxodium balticum* (Otto *et al.* 1997b). The distribution of ferruginol among the extant conifers seems to be limited to species of the Cupressaceae, Taxodiaceae and Podocarpaceae (Kariyone 1964–1983, Erdtman & Norin 1966, Karrer *et al*. 1977).

In summary, the extracts of *Sphenolepis pecinovensis* contain besides degraded unspecific conifer biomarkers (cadinanes, abietanes, ionene, norpimarane) several slightly degraded or unchanged preserved terpenoids that are characteristic for certain groups of families or even a single family. Possible prescursors of these biomarkers obeserved in the fossil conifer are hitherto known only from extant Cupressaceae, Taxodiaceae and Podocarpaceae (ferruginol), in Cupressaceae and Taxodiaceae (cedrene) or only in Cupressaceae (cuparene). Extant species of Cupressaceae and Taxodiaceae contain very similar patterns of terpenoids (Erdtman & Norin 1966, Thomas 1970). The identified biomarkers thus indicate a close relation of *Sphenolepis pecinovensis* to the Taxodiaceae or the Cupressaceae which is in accordance to the assignment of *Sphenolepis* to taxodiaceous conifers (Kvaček 1997).

CONCLUSIONS

The terpenoid composition in the Cretaceous conifer *Sphenolepis pecinovensis* yielded information about the chemotaxonomy of the fossil species. The identified biomarkers indicate a close relation of *Sphenolepis pecinovensis* to the Taxodiaceae or Cupressaceae.

Biomarker investigation has allowed the assignment an isolated resin to a likely plant source. The similarity of the biomarkers in the fossil *Sphenolepis* shoot as well as in the resin indicates *Sphenolepis pecinovensis* as a possible botanical source for the resin.

Future work will attempt to detect characteristic terpenoids (or their diagenetic products) known from extant plants in several organs of the same fossil species especially in genera whose possible affinity is with Cupressaceae and Taxodiaceae.

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