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Resin and wax biomarkers preserved in Miocene Cupressaceae s.l. from Bełchatów and Lipnica Wielka, Poland

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ABSTRACT. The shoots of four species of Cupressaceae s. l. from Miocene clays of Bełchatów and Lipnica Wielka, Poland, contain biomarkers which derive from the resins and waxes of the fossil conifers. Major compound classes are aliphatic lipids, terpenoids, and steroids. Some of the identified compounds are the diagenetic products of biolipids known from extant conifers, but several of the terpenoids and wax compounds survived diagenesis and are preserved unaltered.

All conifers analysed contain the aliphatic wax constituents *n*-nonacosan-10-ol and *n*-octacosane-10,19-dione. Major components in the extract of *Tetraclinis salicornioides* (Unger) Kvaček from Bełchatów are steroids and diterpenoids (ferruginol and derivatives). The diterpenoids dehydroabietic acid and abietic acid, its precursor, are dominant in the extract of the *Sequoia abietina* (Brongniart) Knobloch shoot from Bełchatów. Lignin degradation products (e.g. vanillin, vanillic acid) were also identified. The shoots of *Taxodium dubium* (Sternberg) Heer and *Glyptostrobus europaeus* (Brongniart) Unger from Lipnica Wielka contain *n*-nonacosan-10-ol and *n*-octacosane-10,19-dione as the major compounds and diterpenoids are present only in minor amounts or are not detectable.

The biomarker compositions of these macrofossils reflect their conifer character. The identified wax compounds and diterpenoids are known from extant conifers or are the diagenetic products of their biolipids. Abietic acid and isopimaric acid are common constituents of all conifers. Ferruginol and its derivatives are more specific markers, because these diterpenoids are known only from modern species of the Cupressaceae s. l., Araucariaceae and Podocarpaceae. Minor amounts of angiosperm derived triterpenoids were detected in all extracts. They are probably contamination from the embedding sediment matrix.

KEY WORDS: Cupressaceae, conifers, terpenoids, biomarkers, chemosystematics, gas chromatography-mass spectrometry, Miocene

INTRODUCTION

Biomarkers are organic molecules in sedimentary rocks which are derived from the biosynthesized compounds (natural products) of living organisms. During diagenesis, the precursor molecules may be altered to their respective diagenetic products. Despite various diagenetic alteration processes, the biomarkers still retain structures characteristic of their precursors and can thus be used as valuable markers for the determination of the biological source of organic material in geological samples (Streibl & Herout 1969, Simoneit 1986, Peters & Moldowan 1993). Previous investigations of biomarkers were focused on bulk samples of sediments and coals (e.g. Brassell et al. 1983, Schulze & Michaelis 1989, Chaffee et al. 1986, Simoneit op. cit., Wang & Simoneit 1990, Stefanova et al. 1995).

Biomarkers may also be used as chemical characters for the systematic assignment of fossil plants – palaeochemosystematics (Chaloner & Allen 1969). Due to their high preservation potential, classes of biomolecules such as steroids, terpenoids, cutins and lignins are suitable markers for chemosystematic studies of fossil plants (Eglinton & Logan 1991). For example, aliphatic wax constituents were used as chemosystematic markers for fossil angiosperm leaves (e.g. Logan et al. 1995, Lockheart et al. 2000), and characteristic lignin compositions were reported from the seeds and cone scales of several fossil and modern conifers (e.g. Stankiewicz et al. 1997). Terpenoids can be useful as chemosystematic markers in fossil conifers, because the terpenoids are common compounds in extant conifers and frequently preserved in fossil conifers (Thomas 1970). Since the distribution of terpenoids in extant conifers has been largely documented (e.g. Erdtman & Norin 1966, Hegnauer 1962, 1986, Otto & Wilde 2001) the terpenoid composition of fossil conifers can be compared to the terpenoid patterns in related extant taxa. The analysis of terpenoids in fossil conifers and related extant species by gas chromatography-mass spectrometry (GC-MS) was successfully applied in previous palaeochemosystematic studies on Tertiary and Cretaceous conifer fossils, such as leafy shoots and cones: Cretaceous *Sphenolepis pecinovensis* J. Kvaček (Otto et al. 1999); Eocene *Athrotaxis couttsiae* (Heer) Gardner, *Pinus palaeostrobus* (Ettingshausen) Heer, *Taxodium balticum* Sveshnikova & Budantsev (Otto & Simoneit 2001), Oligocene *Taxodium balticum* Sveshnikova & Budantsev (Otto et al. 1997), resin: Miocene *Cunninghamia miocenica* Ettingshausen (Vávra & Walther 1993), and wood: Pliocene *Taxodioxylon* sp. (Staccioli et al. 1993).

In the present study we analysed the extracts of the fossil plant remains from two localities of Polish Miocene to examine the preservation of biomarkers in Miocene conifer fossils. The systematic of conifers as applied in the present study follows the recent concept of the Cupressaceae s. l. (Gadek et al. 2000), including a monophyletic alliance comprising the former Cupressaceae (= Cupressaceae s. str.) and a number of isolated clades which were united before in the Taxodiaceae.

SAMPLES AND METHODS

GEOLOGICAL SETTING AND SAMPLING

The samples analysed for the present study (Tab. 1) were leafy shoots of *Tetraclinis salicornioides* (Unger) Kvaček and *Sequoia abietina* (Brongniart) Knobloch from Bełchatów (central Poland), and *Taxodium dubium* (Sternberg) Heer and *Glyptostrobus europaeus* (Brongniart) Unger from Lipnica Wielka (southern Poland) which had been stored in the Museum of Palaeobotany, W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM-P).

Bełchatów

The Bełchatów Lignite Mine is located in central Poland between the rivers Warta and Pilica, ca. 15 km south of the town of Bełchatów (Fig. 1). Geologically, the mine is situated between two main structural units: the Szczecin–Łódź–Miechów composite syncline and the Foresudetic Monocline. The tectonic depression of Bełchatów, the “Kleszczów Graben”, is filled with Tertiary coal-bearing sediments (Stuchlik et al. 1990, Stuchlik & Szynkiewicz 1998, Szynkiewicz 2000). Our samples are derived from two different levels within the Miocene sequence. The first is the upper part of a lignitic-clayey unit which is probably of Middle Miocene (Sarmatian) or Late Miocene (Pannonian) age (Szynkiewicz 2000). Following an erosional contact with a characteristic layer of flint it is overlain by

Table 1. Sample list

Species	Locality	Age	Number
Tetraclinis salicornioides (Unger) Kvaček	Bełchatów	Middle to Late Miocene	KRAM-P 191/155a/b
Sequoia abietina (Brongniart) Knobloch	Bełchatów	Late Miocene	KRAM-P 217/263
Taxodium dubium (Sternberg) Heer	Lipnica Wielka	Middle Miocene	KRAM-P 124/125
Glyptostrobus europaeus (Brongniart) Unger	Lipnica Wielka	Middle Miocene	KRAM-P 124/113

Fig. 1. Location of Bełchatów Lignite Mine (1) and Lipnica Wielka (2) in Poland

a clayey-sandy unit which lower part is estimated to be of Late Miocene age (Worobiec unpubl.).

The sample of *Tetraclinis salicornioides* (Unger) Kvaček was recovered from the upper part of the lignitic-clayey unit in association with "Leaf flora A" (Tab. 2, Stuchlik et al. 1990) which is mainly representing mixed mesophytic and riparian forests and aquatic reservoir vegetation. *Sequoia abietina* (Brongniart) Knobloch was selected from palaeoflora KRAM-P 217 (Tab. 3, Worobiec, unpubl.) which occurs in the lower part of the clayey-sandy unit. Palaeoflora KRAM-P 217 is dominated by components of azonal swamp forests with elements of bush swamp and riparian forests.

Lipnica Wielka

The locality of Lipnica Wielka is situated in the Orawa-Nowy Targ Basin within the Podhale fault-trough (Western Carpathians) in southern Poland. It is placed among the High Beskids, the Klippen Belt and the Tatra Mountains. The Orawa-Nowy Targ Basin is a tectonic depression which formed during Neogene times and was enlarged in its north-eastern part in the Late Pliocene and Early Quaternary. The subsiding basin was filled with fluvial and lacustrine deposits (gravels, clays, sands and lignites) of Late Badenian to Pontian age reaching a thickness of up to 950 m (Klimaszewski 1972, 1988, Baumgart-Kotarba

Table 2. Fossil "Leaf flora A" of the Bełchatów Lignite Mine, Middle to Late Miocene (Stuchlik et al. 1990)

Coniferae gen.
Cupressaceae gen.
Pinus cf. *leitzii* Kirchheimer
Pinus palaeostrobus (Ettingshausen) Heer
Tetraclinis salicornioides (Unger) Kvaček
Acer sp.
Alnus cecropiaefolia (Ettingshausen) Berger
Alnus ducalis (Gaudin) Knobloch
Alnus sp.
Arceuthobium sp.
Batrachium sp.
Betula longisquamosa Mädler
Betula sp.
Betulaceae gen. et sp. div.
Carpinus grandis Unger emend. Heer
Carpinus betulus L. type
Carya serrifolia (Goeppert) Kräusel
Cedrela sarmatica E. Kovács
Ceratophyllum sp.
Decodon globosus (E.M. Reid) Nikitin
Eoeuryale brasenioides Miki
Fagus decurrens C. & E.M. Reid
Fagus silesiaca Walther & Zastawniak (as *Fagus attenuata* Goeppert)
Hamamelidaceae
Hypericum tertiaerum Nikitin
Liquidambar europaea A. Br.
cf. *Littorella* sp.
Ludwigia palustris (L.) Elliot *fossilis* Mai,
Microdiptera menzelii (E.M. Reid) Mai,
Myrica lignitum (Unger) Saporta s. l.
Nyssa ornithobroma Unger
Parrotia pristina (Ettingshausen) Stur
Phyllanthus sp.
Polygonaceae gen.
Proserpinaca reticulata C. & E.M. Reid
Prunus aff. *padus* L.
Quercus gigas Goeppert emend. Walther & Zastawniak (as *Quercus czeczottiae* Hummel)
Quercus pseudocastanea Goeppert
Quercus vel *Castanea*
Salix varians Goeppert
Salix sp.
Swida gorbunovii (Dorofeev) Negru
Trapa sp.
Ulmus carpinoides Goeppert
Ulmus sp.
Viscum miquelii (Geyler & Kinkelin) Czeczott
Viscum sp.
Vitis parasylvestris Kirchheimer
Zelkova ungeri Kováts
Zelkova sp.
Dicotyledones indet.
Carex sp.
Cyperaceae gen.
Cyperus sp.
Dulichium arundinaceum (L.) Britton *fossilis*
Dulichium vespiforme C. & E.M. Reid
Potamogeton sp. div.
Scirpus sylvaticus L. *fossilis*
Sparganium neglectum Beeby *fossilis*
Monocotyledones indet.

Table 3. Fossil leaf flora KRAM-P 217 of the Bełchatów Lignite Mine, Late Miocene (Worobiec unpubl.)

Sequoia abietina (Brongniart) Knobloch
Pinus cf. *spinosa* Herbst
Pinus sp. div.
Acer sp. div.
Aesculus cf. *hippocastanoides* Ilinskaya
Alnus gaudinii (Heer) Knobloch & Kvaček
Alnus menzelii Raniecka-Bobrowska
Alnus sp.
cf. *Betula* sp.
Byttneriophyllum tiliaefolium (Al. Braun) Knobloch & Kvaček
Cyrilla thomsonii Kräusel & Weyland
Diospyros anceps Heer
Kalmia cf. *saxonica* Litke
Myrica lignitum (Unger) Saporta s. l.
Pterocarya paradisiaca (Unger) Ilinskaya
Viscophyllum pliocenicum (Engelhardt) Mädler
"*Ficus*" *truncata* Heer sensu Bůžek
Dicotylophyllum sp. 3
Dicotylophyllum sp. 4
Dicotylophyllum sp. 5
Bambusa lugdunensis Saporta
Smilax cf. *protolancaefolia* Kolakovskij
Smilax cf. *weberi* Wessel in Wessel & Weber

Table 4. Fossil flora of the Lipnica Wielka outcrop, Middle Miocene

Taxodium dubium (Sternberg) Heer
Glyptostrobus europaeus (Brongniart) Unger
Sequoia abietina (Brongniart) Knobloch
Chamaecyparis cf. *pisifera* (Siebold & Zuccarini) Endlicher
Abies aff. *alba* Miller
Abies resinosa Mai
Abies sp. 1
Keteleeria sp.
Pinus strobus L. *fossilis*
Tsuga sect. *Eutsuga* L.
Acer sect. *Platanoidea* Pax
Actinidia argutaeformis Dorofeev
Alnus sp. 1
Alnus sp. 2
Aralia sp.
Broussonetia pygmaea (Dorofeev) Dorofeev
Caryophyllaceae gen.
Ericaceae gen.
cf. *Lactuca protomuralis* Mai
Lysimachia thyrsiflora Nikitin
Magnolia cor Ludwig
Meliosma wetteraviensis (Ludwig) Mai
Naumburgia subthyrsiflora (Nikitin) Nikitin
Parrotia reidiana Kirchheimer
Rubus laticostatus Kirchheimer
Schefflera dorofeevi Łańcucka-Środoniowa
Schisandra moravica (Mai in Knobloch) Gregor
Swida gorbunovi (Dorofeev) Negru
Viola neogenica Mai
Carex sp. div.
Cyperaceae gen.

1996). The results of palaeobotanical, geological and petrological investigations of the Neogene deposits in the Orawa-Nowy Targ Basin were summarized by Lesiak (1994). Macroremains of *Glyptostrobus europaeus* (Brongniart) Unger from Lipnica Mała (twigs, shoots, and needles) have before been studied chemically by pyrolysis (Alvarez Ramis et al. 1996, Almendros et al. 1999).

The section of the Lipnica Wielka outcrop was comprised of 2 m of gravel, sand, sandy clay, grey clay and brown coal. A tonstein layer in the brown coal at Lipnica Wielka was preliminary correlated with the Chodenice layers in the sub-Carpathian Foredeep and thus assigned to the lower part of the Late Badenian (Kosovian; Matl & Wagner 1985, 1987, Kołcon & Wagner 1991).

The fruit and seed flora from the Lipnica Wielka outcrop (Tab. 4) comprises a rich association of arborescent, shrubby and herbaceous elements. A remarkable diversity of conifers includes abundant remains of *Glyptostrobus europaeus* (Brongniart) Unger and *Taxodium dubium* (Sternberg) Heer (shoots, individual leaves and seeds).

Palynological investigations of sediments from several sections within the Orawa-Nowy Targ Basin including Lipnica Wielka permitted the reconstruction of the vegetation in a relatively extensive area during the Miocene (Tran 1974, Oszast & Stuchlik 1977). The low lying sites of humid-peaty soils with periodically stagnant water were covered by dense swamp forests with a prevalence of *Taxodium*, *Glyptostrobus*, *Cunninghamia*, *Nyssa* and *Sequoia*. Many intercalations of brown coal in the sections indicate that these associations persisted during the whole time of sediment accumulation. Slightly drier habitats in close vicinity of the swamps were carrying communities of Betulaceae, Myricaceae and Cyrillaceae with *Cercidiphyllum*, *Decodon* and *Liquidambar*. Along rivers, riparian forests with *Alnus*, *Populus*, *Pterocarya* and *Salix* were growing. More dry sites and the surrounding slopes were mostly covered by deciduous forests with *Acer*, *Fagus*, *Juglans*, *Liriodendron*, *Magnolia*, and *Tilia*. *Abies*, *Keteleeria*, *Pinus*, *Sequoia*, and *Tsuga* may have formed an association in the surrounding mountains. On sunny slopes, thermophilous low-growing oaks and several shrubs of the families Hamamelidaceae, Rhamnaceae, Rosaceae may have grown (Oszast & Stuchlik 1977).

The palaeofloras of the Orawa-Nowy Targ Basin were mainly composed of genera of a warm-temperate climatic zone comprising mostly arctotertiary elements. Based on palynological investigations, no plants representing a tropical climate have been found (Oszast & Stuchlik 1977). However, there are few palaeotropical elements among the fruits and seeds, like *Epipremnites reniculus* (Ludwig) Mai (Araceae), *Magnolia cor* Ludwig, *M. burseracea* (Menzel) Mai (both Magnoliaceae), *Spirematospermum wetzleri* Chandler (Zingiberaceae), *Toddalia* cf. *maerkeri* Gregor (Rutaceae), and *Urospathites cristatus* Gregor & Bogner (Araceae) (Lesiak 1994; pers. obs.).

EXTRACTION AND GC-MS ANALYSES

The fossil plant material was scraped off the sediment with a solvent-washed scalpel, transferred to glass vials and pulverized with a spatula. The samples were sonicated three times for 10 min with dichloromethane:methanol (1:1; v/v). The solvent extract was filtered and concentrated under blow-down with dry nitrogen gas. Aliquots of the total extracts were converted to trimethylsilyl derivatives by reaction with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine for 3 h at 70°C to derivatize functional groups.

Gas chromatography – mass spectrometry (GC-MS) analyses of the total and the derivatized total extracts were performed on a Hewlett-Packard model 6890 GC coupled to a Hewlett-Packard model 5973 MSD. A fused silica capillary column coated with DB5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used. The GC operating conditions were as follows: temperature isothermal at 65°C for 2 min, increase from 65 to 300°C at a rate of 6°C min^{-1} with final isothermal hold at 300°C for 20 min. Helium was used as carrier gas. The sample was injected splitless with the injector temperature at 300°C. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV and scanned from 50 to 650 dalton. Data were acquired and processed with HP Chemstation software. Individual compounds were identified by comparison of mass spectra with literature and library data, comparison with authentic standards and interpretation of mass spectrometric fragmentation patterns.

RESULTS

COMPOSITION OF THE FOSSIL CONIFER EXTRACTS

The extracts of all samples analysed contain soluble organic compounds. Aliphatic wax constituents, terpenoids, steroids, and lignin degradation products are the major compound classes. Series of the aliphatic *n*-alkanols (v) and *n*-alkanoic acids (+) are present in all conifer extracts analysed (Figs 2, 3). They are common wax constituents of all higher plants (Tulloch 1976, Baker 1982). *n*-Nonacosan-10-ol (a1) and *n*-octacosane-10,19-dione (a2) are major compounds in the samples from Lipnica Wielka and also present in the fossil conifers from Bełchatów. The secondary alcohol *n*-nonacosan-10-ol (a1) is known as a wax compound of higher plants (Knoche et al. 1968, Baker 1982, Barthlott et al. 1998). The biomarker composition of these fossil conifers is discussed next.

Cupressaceae s. l.

Cupressoideae

Tetraclinis salicornioides (Unger) Kvaček from Bełchatów

The extract of *Tetraclinis salicornioides* from Bełchatów contains diterpenoids, steroids, and triterpenoids (Fig. 2; Tab. 5). The diterpenoids are comprised of a series of phenolic abietanes (d1-d4). Ferruginol (d1; 93.4 % relative abundance) and sugiol (d2) are bioterpenoids and have been preserved unaltered. 6,7-Dehydroferruginol (d3) and 12-hydroxysimonellite (d4) are interpreted as diagenetic products of ferruginol and its derivatives. Major compounds in the extract are the steroids. β-Sitosterol (st1; 42 %) is the biological precursor for the diagenetic products stigmastanol (st2), and stigmast-4-ene (st3) which are generated by reduction of the double bond or elimination/rearrangement reactions of the hydroxy group in β-sitosterol, respectively (Mackenzie et al. 1982). The extract also

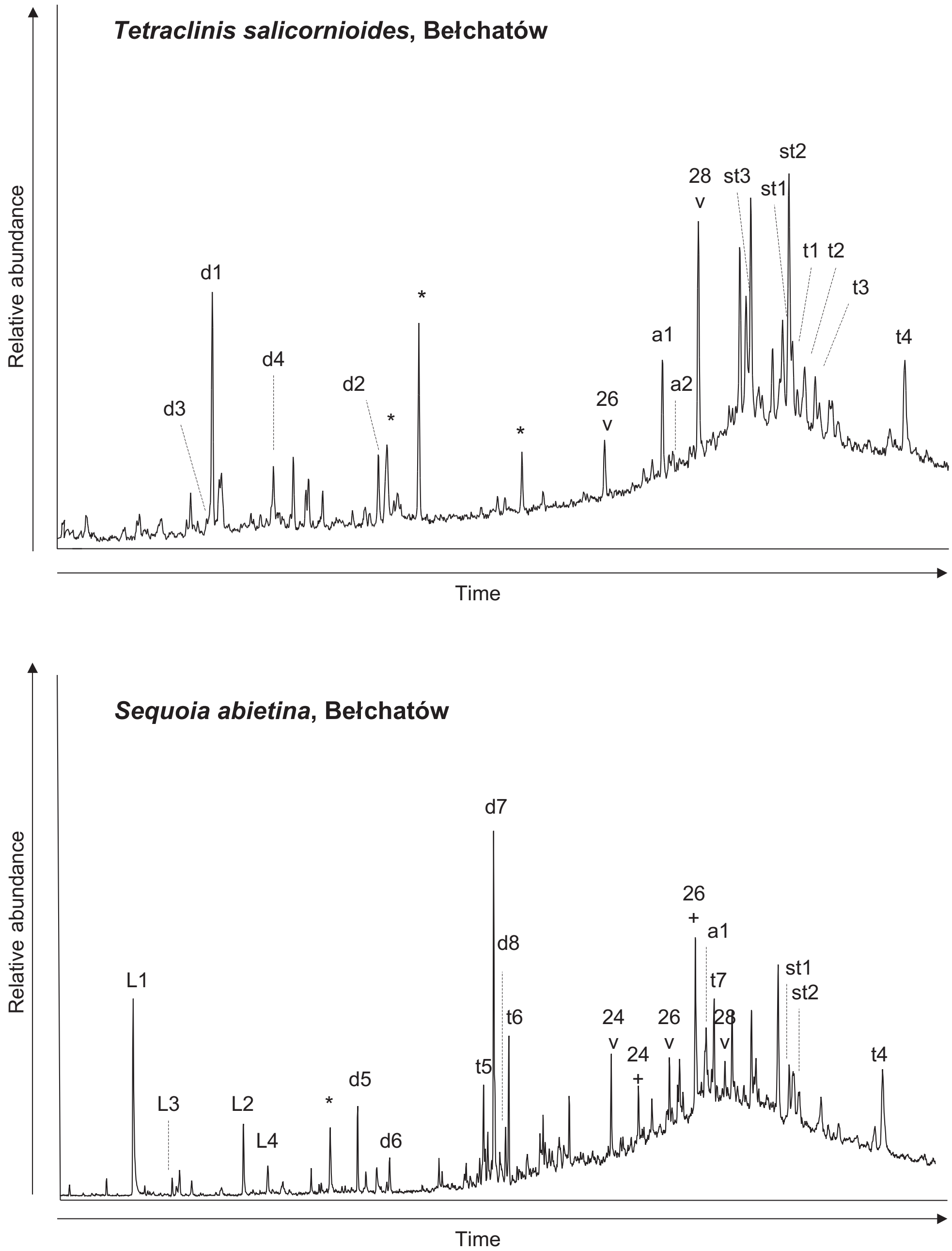

Fig. 2. GC-MS traces of the derivatized (TMS) total extracts of the fossil conifer shoots from Bełchatów, Middle to Late Miocene. Peak annotation see Tab. 5. v – *n*-alkanol, + – *n*-alkanoic acid, * – contamination

contains the triterpenoids β-amyrin (t1), β-amyrone (t2), α-amyrin (t3), and friedelin (t4).

Sequoioideae

Sequoia abietina (Brongniart) Knobloch from Bełchatów

Lignin degradation products, diterpenoids, steroids, and triterpenoids were identified in the extract of *Sequoia abietina* from Bełchatów (Fig. 2). The benzyl derivatives vanillin (L1), vanillic acid (L2), 3-hydroxybenzoic acid (L3), and 3,4-dihydroxybenzoic acid (L4) are degradation products of lignin. Diterpenoids with the abietane skeleton (d5-d8) are the predominant components in the extract. The aromatic abietane derivatives 18-norabieta-8,11,13-triene (d5), 1,2,3,4-tetrahydroretene (d6) are the diagenetic products of various abietane type precursors. Dehydroabietic acid (d7; 100%) is the oxidation product of the biosynthesized abietic acid (d8) which is also present in the extract (17.7%). The dominance of dehy-

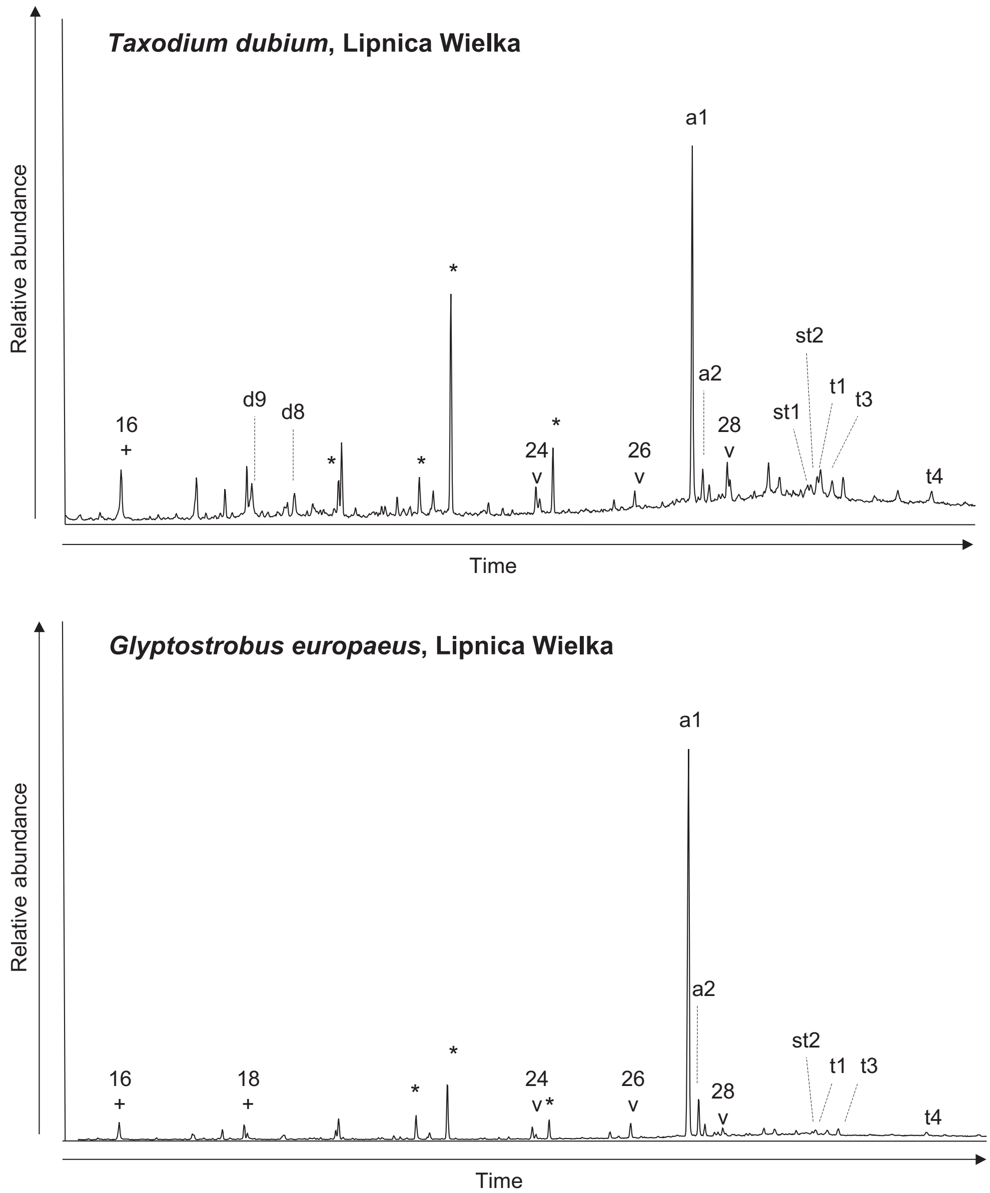

Fig. 3. GC-MS traces of the derivatized (TMS) total extracts of the fossil conifer shoots from Lipnica Wielka, Middle Miocene. Peak annotation see Tab. 5. v – *n*-alkanol, + – *n*-alkanoic acid, * – contamination

droabietic acid and the minor amount of abietic acid reflect the significant resin oxidation during the fossilization processes (Otto & Simoneit 2001). The steroids β-sitosterol (st1) and stigmastanol (st2) are present only in minor amounts. The triterpenoids β-amyrin (t1) and friedelin (t4) also occur in the *Sequoia abietina* extract. Diagenetic products of triterpenoids are des-A-olean-12-ene (t5) and des-A-lupane (t6) which are generated by the elimination of ring A of oleanane and lupane derivatives, respectively, and the monoaromatic lupane derivative 24,25-dinorlupa-1,3,5(10)-triene (t7) (Trendel et al. 1989).

Taxodioideae

Taxodium dubium (Sternberg) Heer from Lipnica Wielka

The shoots of *Taxodium dubium* from Lipnica Wielka yielded only a few terpenoids and steroids (Fig. 3). The aliphatic wax constituent *n*-nonacosan-10-ol (a1; 100%) is the predominant biomarker. Abietic acid (d8) and isopimaric acid (d9) were identified as the only diterpenoids. The steroids β-sitosterol (st1) and stigmastanol (st2) and the triterpenoids β-amyrin (t1), α-amyrin (t3), and friedelin (t4) were present.

Table 5. Soluble compounds identified in the extracts of Miocene conifer fossils. Abbrevations: Tet. – *Tetraclinis salicornioides*, Seq. – *Sequoia abietina*, Tax. – *Taxodium dubium*, Gly. – *Glyptostrobus europaeus*. Relative abundance normalized to major peak = 100

Peak	Compound name	Composition	Relative abundance			
			Bełchatów		Lipnica Wielka	
			Tet.	Seq.	Tax.	Gly.
	Aliphatics					
a1	*n*-Nonacosan-10-ol	$C_{29}H_{60}O$	50.7	1.4	**100.0**	**100.0**
a2	*n*-Octacosane-10,19-dione	$C_{28}H_{54}O_2$	3.2		11.4	9.7
	Diterpenoids					
d1	Ferruginol	$C_{20}H_{30}O$	93.4			
d2	Sugiol (7-Ketoferruginol)	$C_{20}H_{28}O_2$	28.7			
d3	6,7-Dehydroferruginol	$C_{20}H_{28}O$	2.6			
d4	12-Hydroxysimonellite	$C_{19}H_{24}O$	28.7			
d5	18-Norabieta-8,11,13-triene	$C_{19}H_{28}$		25.9		
d6	1,2,3,4-Tetrahydroretene	$C_{18}H_{22}$		9.8		
d7	Dehydroabietic acid	$C_{20}H_{28}O_2$		**100.0**		
d8	Abietic acid	$C_{20}H_{30}O_2$		17.7	10.2	
d9	Isopimaric acid	$C_{20}H_{30}O_2$			17.6	
	Lignin degradation products					
L1	Vanillin	$C_8H_8O_3$		86.0		
L2	Vanillic acid	$C_8H_8O_4$		26.4		
L3	3-Hydroxybenzoic acid	$C_7H_6O_3$		5.2		
L4	3,4-Dihydroxybenzoic acid	$C_7H_6O_4$		12.6		
	Steroids					
st1	β-Sitosterol	$C_{29}H_{50}O$	42.1	13.3	1.2	
st2	Stigmastan-3β-ol	$C_{29}H_{52}O$	**100.0**	18.5	5.8	0.7
st3	Stigmast-4-ene	$C_{29}H_{50}$	60.3			
	Triterpenoids					
t1	β-Amyrin	$C_{30}H_{50}O$	34.9	17.4	10.6	0.8
t2	β-Amyrone	$C_{30}H_{48}O$	39.2			
t3	α-Amyrin	$C_{30}H_{50}O$	24.9		8.3	0.8
t4	Friedelin	$C_{30}H_{50}O$	56.6	49.7	5.6	
t5	des-A-Olean-12-ene	$C_{24}H_{40}$		32.8		
t6	des-A-Lupane	$C_{24}H_{42}$		43.6		
t7	24,25-Dinorlupa-1,3,5(10)-triene	$C_{28}H_{42}$		18.9		

Glyptostrobus europaeus (Brongniart) Unger from Lipnica Wielka

The extract of *Glyptostrobus europaeus* from Lipnica Wielka also shows the wax components *n*-nonacosan-10-ol (a1) and *n*-octacosane-10,19-dione (a2) as the major biomarkers (Fig. 3). The steroid stigmastanol (st2) and the triterpenoids β-amyrin (t1) and α-amyrin (t3) were observed only in minor relative abundances. Diterpenoids were not detected.

CHEMOSYSTEMATIC VALUE OF THE IDENTIFIED BIOMARKERS

Aliphatic compounds like *n*-alkanols and *n*-alkanoic acids are common in the waxes of all higher plants and thus nonspecific chemosystematic markers (Tulloch 1976, Baker 1982, Otto et al. 1997, Barthlott et al. 1998, Otto & Simoneit 2001). *n*-Nonacosan-10-ol (a1) and octacosane-10,19-dione (a2) which were detected in all samples are observed in the waxes of higher plants (Knoche et al. 1968, Baker 1982, Barthlott et al. 1998). **The lignin degradation products** which were observed in the extract *of Sequoia abietina* (Fig. 4) are nonspecific, because they derive from various phenolic lignin monomers which are abundant among the higher plants. The identified **steroids** β-sitosterol (st1) and its diagenetic products stigmastanol (st2) and stigmast-4-ene (st3) (Fig. 5) are also nonspecific markers, because the biological precursor β-sitosterol is

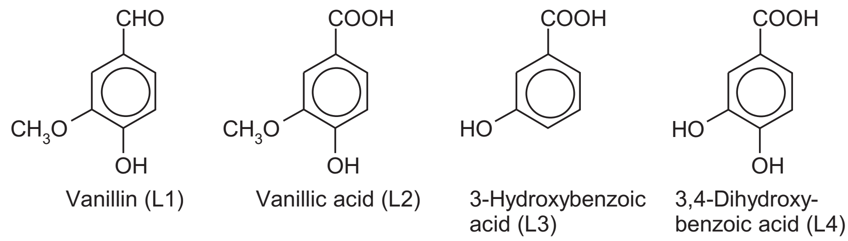

Fig. 4. Structures of the identified lignin degradation products (L1-L4)

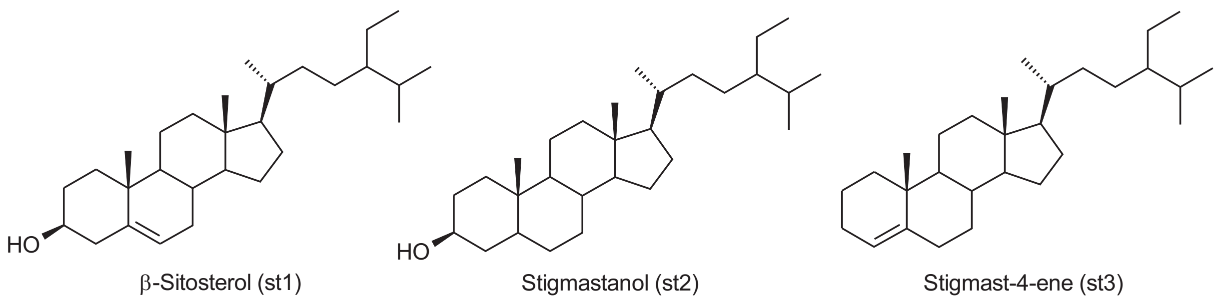

Fig. 5. Structures of the identified steroids (st1-st3)

widely distributed among the plant kingdom (Brassell et al. 1983, Oros & Simoneit 1999).

The **diterpenoids** in the samples analysed belong to the structural classes of abietane and isopimarane (Fig. 6). Isopimaric acid and the "normal" (non-phenolic) abietane derivative abietic acid are common in the resin and wood of extant conifers, and thus nonspecific conifer markers (Karrer 1958, Karrer et al. 1977, Hegnauer 1962, 1986, Otto & Wilde 2001). Phenolic abietanes like ferruginol (d1) and sugiol (d2) are characteristic chemosystematic markers, because they are known among the conifers only from extant species of the Cupressaceae s. l., Araucariaceae and Podocarpaceae, and are especially abundant in the Cupressaceae s. l. (Erdtman & Norin 1966, Thomas 1986, Hegnauer 1962, 1986, Otto

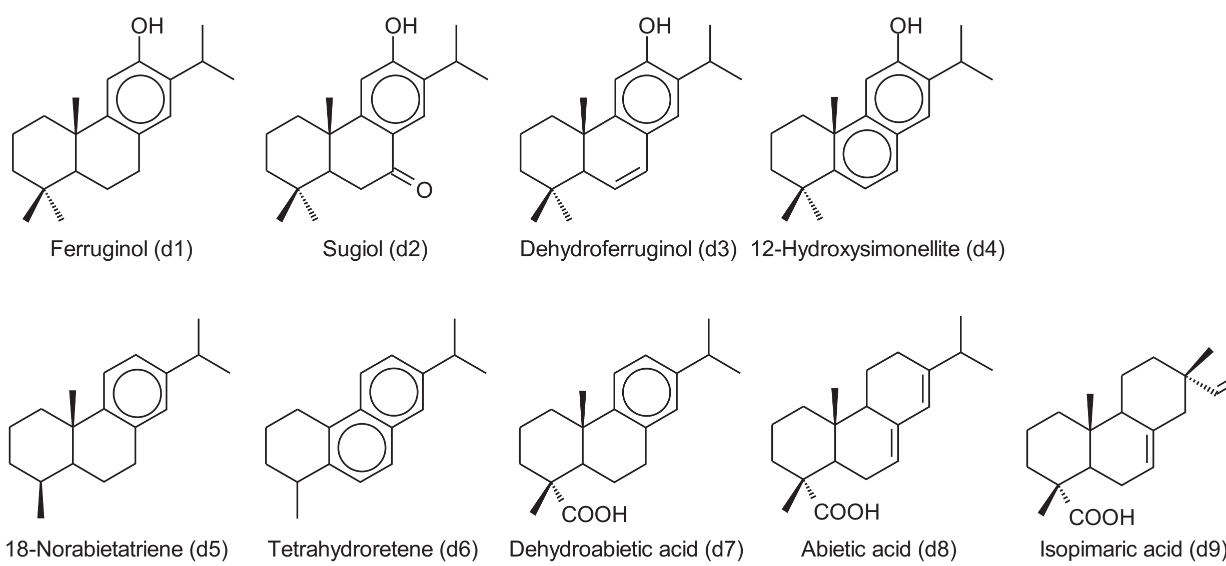

Fig. 6. Structures of the identified diterpenoids (d1-d9)

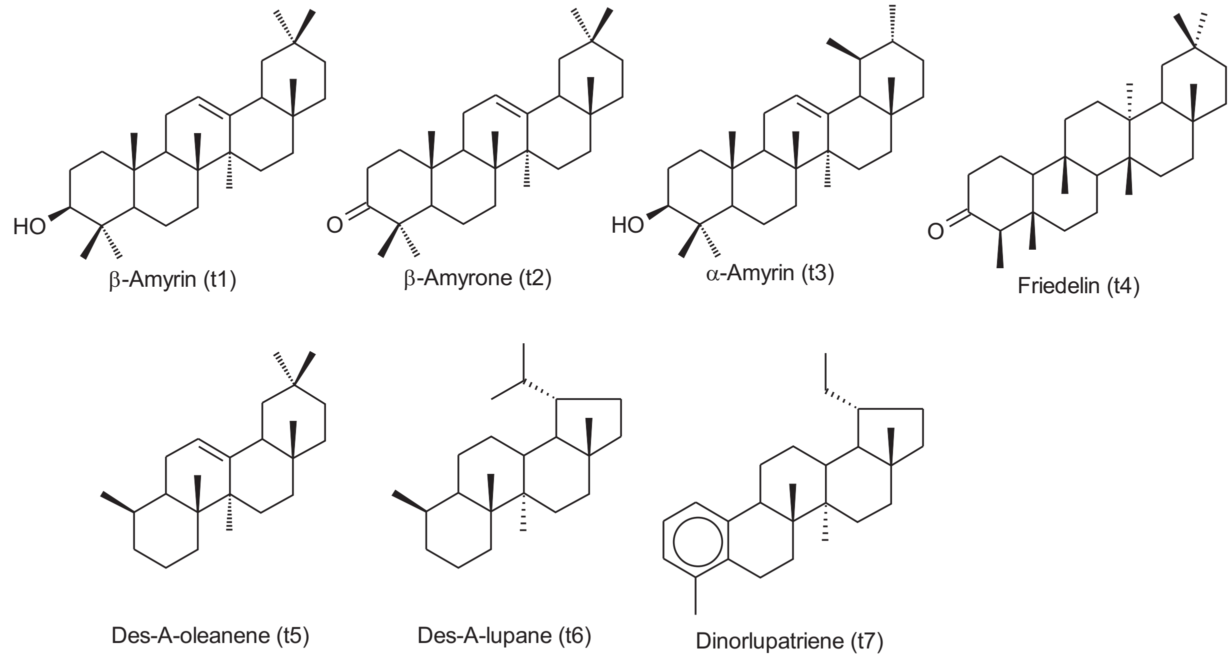

Fig. 7. Structures of the identified triterpenoids (t1-t7)

& Wilde 2001). The presence of ferruginol (d1) and sugiol (d2) in *Tetraclinis salicornioides* from Bełchatów thus reflects its systematic assignment to the Cupressaceae s. l.

The major part of the diterpenoids is closely associated with the woody tissues or resins of the conifer fossils, and their migration into the sediment and/or into other plant fossils can probably be excluded. The observed diterpenoid patterns thus represent probably the original terpenoids of the fossil conifers analysed. In contrast, the **triterpenoids** of the oleanane, ursane, and lupane classes are prevailing in surface waxes or the easily degraded leaves of angiosperms. The presence of the triterpenoids derived from angiosperms (Fig. 7) in the extracts of the fossil conifers is probably due to contamination of the samples with organic sedimentary particles (detritus and wax particles) of angiosperm origin. Angiosperm derived triterpenoids were previously observed also in the extracts of Eocene conifer shoots from Zeitz, Germany (Otto & Simoneit 2001).

CONCLUSIONS

The extracts of the Miocene conifer shoots analysed contain aliphatic wax constituents, terpenoids, steroids, and lignin degradation products. Most of the identified biomarkers are diagenetic products of biosynthetic compounds, but some terpenoids and steroids survived diagenesis unchanged. The presence of unaltered biomarkers and the low degree of degradation of the diagenetic products indicate the excellent preservation of the samples. The biomarkers in the fossil conifers from Bełchatów are better preserved than those in the samples from Lipnica Wielka.

The observed biomarker compositions are in accordance with the botanical assignment of the macrofossils to the conifers. *n*-Nonacosan-10-ol, which is known from extant conifers, was identified in all samples analysed. Some of the identified diterpenoids are nonspecific conifer markers, such as abietic acid and derivatives in *Sequoia abietina* or isopimaric acid in *Taxodium dubium*. *Tetraclinis salicornioides* contains phenolic abietanes (ferruginol, sugiol) which are characteristic for the conifer families Cupressaceae s. l., Araucariaceae and Podocarpaceae.

The steroids and lignin degradation products are nonspecific markers for higher plants, but are common constituents of conifers. The presence of angiosperm derived triterpenoids in the conifer extracts is probably due to contamination from angiosperm derived sedimentary particles.

The terpenoids are valuable markers for

chemosystematic studies of fossil conifers. The analyses of extracts using gas chromatography-mass spectrometry is a suitable method for the investigation of solvent extractable biomarkers in fossil plants.

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