Biomarkers in the fossils from the Miocene-aged Chukurovo lignite, Bulgaria: sesqui- and diterpenoids

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Abstract. The attention is focused on the "Chukurovo" lignites of the Sofia coal-bearing province. The coal sediments are of Neogene age: petrological studies have indicated a Badenian-Sarmatian age, while the paleobotanical interpretations have indicated the Karpatian-Badenian, respectively Early-Middle Miocene boundary. Fossilized trees are common in the Chukurovo lignites, as well as petrified wood tissue consisting of stems and twigs impregnated by silica and other minerals and enriched in macroscopic organic matter.

In this study organic geochemistry is applied toward the identification of molecular indicators for the coal-forming fossil communities. Fractions isolated by sequential extraction from resinite were analyzed by GC and GC-MS to identify a variety of biomarkers. Diterpenoids receive special attention in the present study. Tri- and tetracyclic diterpenoids, the so-called "regular" diterpenoids, are discussed in detail. The combination of high sesqui-/diterpenoid contents is evidence for the input of higher plant residues (conifers), especially when accompanied by the diterpenoid dominance. Resins containing sesqui- and diterpenoids are produced by species of all contemporary conifer families, and hence conifers are a possible coal-forming vegetation in fossil swamps.

Key words: lignite, coal biomarkers, terpenoids

Introduction

The Chukurovo lignite is located 40 km SE of Sofia in a small valley of the Lozen Mountains (Fig. 1), and forms the part of Sofia coal-bearing province. On the basis of its maceral composition Šiškov (1997) assigned it to the A2 subgroup. The coals of this basin are of low rank and are extracted by opencast mining. The coal complex is represented by clays, sandy clays, and sandstone with 12–18 coal seams (8–12 of which are of industrial importance). The thickness of the coal layers change at various positions, often converting into coaly clays and sandy clays. The coal layers are covered with sandy clays and argillaceous sandstones.

The geological age of the coal sediments is Neogene, more precisely classified as Middle Miocene (Šiškov 1997). Petrological studies have indicated a Badenian-Sarmatian age, while the paleobotanical interpretations by Palamarev and Uzunova (1973), and Palamarev (1989) suggest the Early-Middle Miocene, respectively Karpatian-Badenian (Palamarev and Ivanov 2003).

The Chukurovo Basin is characterized by coal facies derived from a limnic ombrotrophic forest-swamp environment (Korten-ski 2002). Fossilized trees are abundant in the Chukurovo lignites, as are preserved fossils enriched in macroscopic organic matter. Palamarev (1989) proposed four types of phytocoenosis: aquatic, swamp, flood plain, and forest, i.e. mesophilous and mesohygrophilous. The composition of the latter supports a predominance of evergreen laurel and laurel-oak (angiosperm) communities during the Middle Miocene.

The biomarker assemblage of the macroscopically identified *Taxodium dubium* (Sternb.) Heer has recently

Figure 1. Location map of Chukurovo lignites.

been studied (Stefanova 2004). This macrofossil was botanically classified by Palamarev and Uzunova (1973), and Palamarev (1989) as one of the most widely distributed progenitors of the Chukurovo lignite. Twigs and shoots of these fossil conifers have been subjected to geochemical study. The aim was to depict the main biomarkers for the macrofossils, and to relate them to the conifer family Taxodiaceae. The results indicated that only phenolic diterpenoids, i.e. ferruginol, sugiol and totarol, were preserved in the macrofossils as chemosystematic markers for the phylogenetic and systematic comparison of the fossil taxa. Triterpenoids, steroids, and aliphatic lipids were also identified, but were considered to be present as contamination of the samples with organic sedimentary particles originating from detritus and waxes of angiosperm origin.

The aim of the present study is to follow the precursor-product relationship toward reconstructing the diagenetic transformations of the diterpenoids present in the Chukurovo lignites during coalification.

Materials and methods

The experiments were performed on handpicked resinite samples (about 10% of the coal). Additional information about the coal, its lithotypes, self-ignition ability, physical, and spectral characteristics, with a comparison to a large set of other coal samples of different ranks, can be found in the reports by Markova (2002) and Sotirov and Kortenski (2003). Their main characteristics can be briefly stated as follows:

Geological age: Middle Miocene, coal-bearing province: Sofia, coal-forming maximum: Neogene, $R_o = 0.20 \pm 0.02$ (%).

Maceral composition in vol%: for initial coal: huminite (H) 75, liptinite (L) 14, inertinite (I) 1, mineral matter (M) 10, for mineral matter free (mmf) – H 84, L 15, I 1.

Proximate analysis, %: $W^A = 8.8$, $A^d = 20.5$, $V^{dat} = 65.6$.

Ultimate analysis, wt%^{daf}: C – 67.9, H – 5.6, N –1.2, $S_{Org.}$ – 0.4, O^{diff} – 24.9.

Contents of oxygen functional groups, in mg/g: phenolic (-OH) – 1.8, carbonyl (> CO) – 0.9, carboxyl (-COOH) – 0.1.

According to Sotirov and Kortenski (2003) resinite is the most abundant liptinite maceral, reaching 10.4%. It occurs as spherical, oval, or elongated bodies mainly in the textinite, texto-ulminite, and eu-ulminite lumens. The other macerals in the liptinite group are present in negligible amounts compared to resinite (cutinite 0.13%, sporinite 0.13%, suberinite 1.86%, alginite 0.13%, liptodetrinite 1.6%, chlorophillinite 0.01%).

The chloroform extracts of the handpicked resinite samples were separated by absorption liquid chromatography in a SiO_2 column. The aliphatic fraction was isolated according to the protocol described in a previous paper (Stefanova and Magnier 1997).

The fraction was analyzed with a Perkin-Elmer Autosystem XL gas chromatograph equipped with a CP sil 5 CB column (0.22 mm x 30 mm, 0.25 µm film thickness), a flame ionization detector (300 °C), and a split/splitless capillary injector maintained at 300 °C and used in the splitless mode (valve reopened one minute after injection). After holding for 1 minute at 50 °C, the oven temperature was increased to 120 °C at 30°/minute, and then from 120° to 300 °C at 5°/minute, where the final isothermal hold was maintained for one hour at 300 °C.

The GC-MS analyses were performed with a Varian 300 chromatograph connected to an ITD 800 (Finnigan MAT) by a 2 m capillary interface heated to 300 °C. The other GC conditions were identical to those described above. The operating conditions for the Ion Trap were temperature 220 °C, ionization energy 80 eV, and Tscan 2s from 50 to 500 dalton. Peak identifications were done by comparison with the NIST library or literature data (Philp 1985).

Results

Resinite chloroform extract was separated into the following fractions: Aliphatics (eluent-hexane) – 5%, monoaromatics (hexane-toluene, 4:1) – 0.9%, polyaromatics (toluene) – 2.9%, polars (acetone) – 87.6%.

Our work concerned the aliphatic fraction. The GC and GC-MS data revealed the presence of sesqui- and diterpenoids (Tables 1 and 2). The formulae of the identified compounds are illustrated in Fig. 2. The distribution of compound classes in the samples under consideration was calculated based on GC separation. Contents were expressed in relative %:

sesquiterpenoids   6.18
diterpenoids   85.47
total   91.65.

Discussion

Sesquiterpenoids are present only in the resinite sample. They are typical coal biomarkers and are present in the resins and essential oils of higher plants (Simoneit 1986). Sesquiterpenoid biomarkers in combination with diterpenoids serve to tag plant resins in the geological record and connect them with certain classes of plant input. The precursors of most of sesquiterpanes are common components of essential oils and resins (Simoneit et al. 1986, Wang and Simoneit

Table 1. Structure of sesquiterpenoids

Peak	Molecular mass	Base peak [100%]	Structure	
a	206	163	Dihydrovalencene	Str. a
b	206	95	Longifolane	Str. b
c	202	132	Cuparene	Str. c
d	202	187	5,6,7,8 –Tetrahydrocadalene	Str. d
e	198	183	Cadalene	Str. e

1990). Sesquiterpenoids can occur as saturated, unsaturated, aromatic, and functionalized derivatives. The main sources for these sesquiterpenoids are Cupressaceae, Compositae, and Dipterocarpaceae, where the first is of the Gymnospermae plant family and the others are Angiospermae (Dicotyledonae) (Sukh Dev 1989).

Dihydrovalencene (a, all structures are given in Fig. 2), longifolane (b), and cadalene (e) were unequivocally identified in this sample. Cuparene (c) and cadalene structural types (d and e) were present in subordinate quantities. Nevertheless, cuparene is a very specific chemosystematic marker, as its derivatives have been reported only from modern species of the Cupressaceae and Taxodiaceae. These species have already been observed in another Neogene coal in Bulgaria, namely Thracian lignites (Stefanova et al. 2002).

Hydrocarbons based on the cadinane skeleton are common constituents of resins and ambers, and of petroleum with a terrigenous input component (Simoneit et al. 1986, Otto and Wilde 2001). Longifolane has been identified in other fossil resins from Tertiary lignites, and is a characteristic constituent of the essential oils of Cupressaceae (order Coniferales) and some genera of the family Taxodiaceae.

Diterpenoids are distributed mainly in gymnosperms and in only a few angiosperms among contemporary plants (Sukh Dev 1989, Otto and Wilde 2001). The coal of the Chukurovo deposit is distinctive because the Taxodiaceae were the main species in the peat-forming paleo swamp, based on paleobotanical evidence (Palamarev and Uzunova 1973). The preponderance of the diterpenoids in the resinite provides chemical confirmation for the presence of conifers in the paleoplant taxa.

The GC-MS identification of diterpenoid structures is summarized in Table 2. Norpimarane (III) co-eluted with 16α(H)-Kaurane (IV), and the sum of their concentrations is the highest peak (34.1%). The total yield of diterpenoids

Table 2. Structure of diterpenoids

Peak	Molecular mass	Base peak [100 %]	Content [rel. %]	Structure	
1	254	239	1.00	19-Norabieta-3,8,11,13-tetraene	Str. I
2	272	257	0.35	$D^{8,9}$ - Sandaracopimaradiene	Str. II
3	274 262	123 233	Coelution 34.1	Norpimarane + 16α(H)-Kaurane	Strs III + IV
4	262	109	7.96	Fichtelite	Str. V
5	276	163	16.94	Abietane	Str. VI
6	274	123	0.72	16α(H)-Phyllocladane	Str. VII
7	270	255	3.32	Dehydroabietane	Str. VIII
8	268	197	0.22	2-Methyl-1-(4'-methylpenthyl)-9-*i*-propyl-naphthalene	Str. IX
9	252	237	12.69	Simonellite	Str. X
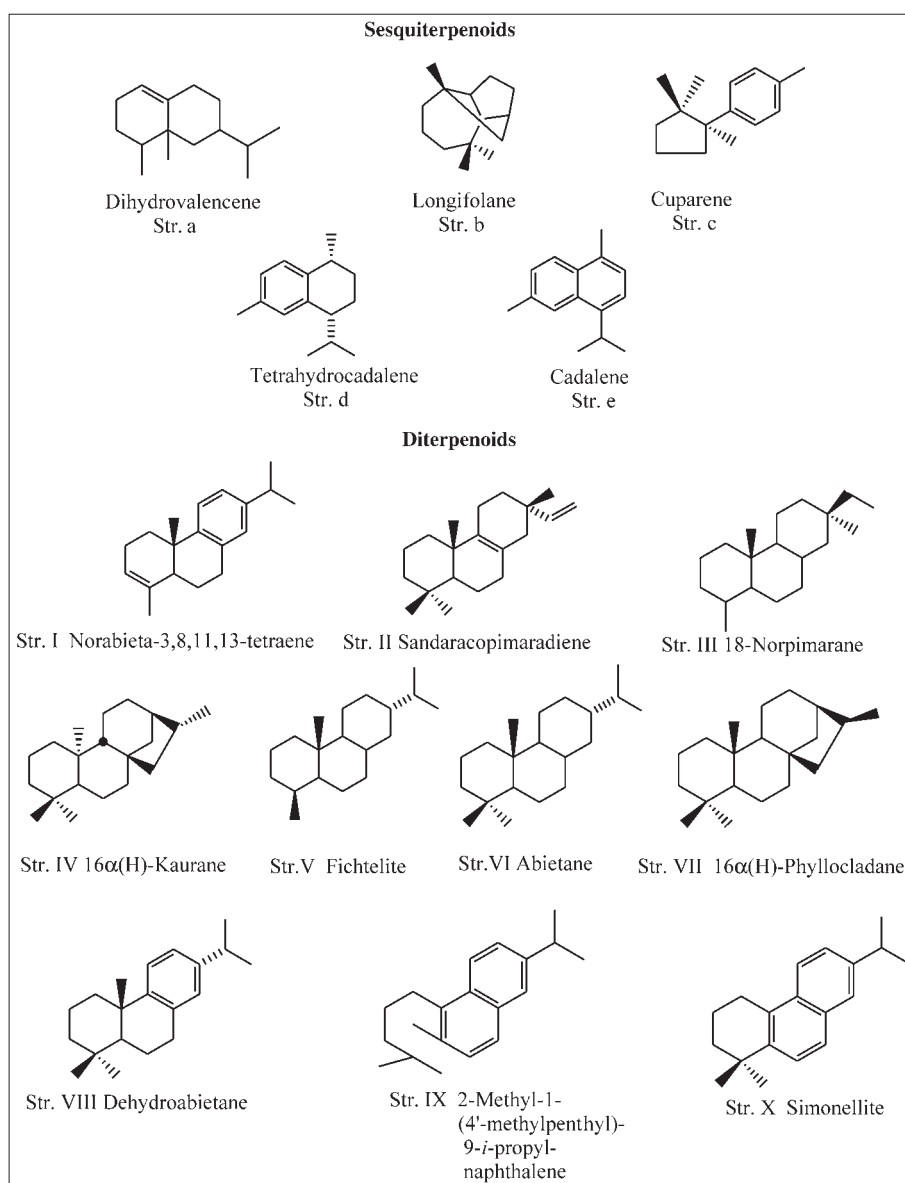
Figure 2. Structure of sesqui- and diterpenoids.

Table 3. Common compounds of tri-/tetracyclic diterpenoids and their occurrence in conifers *

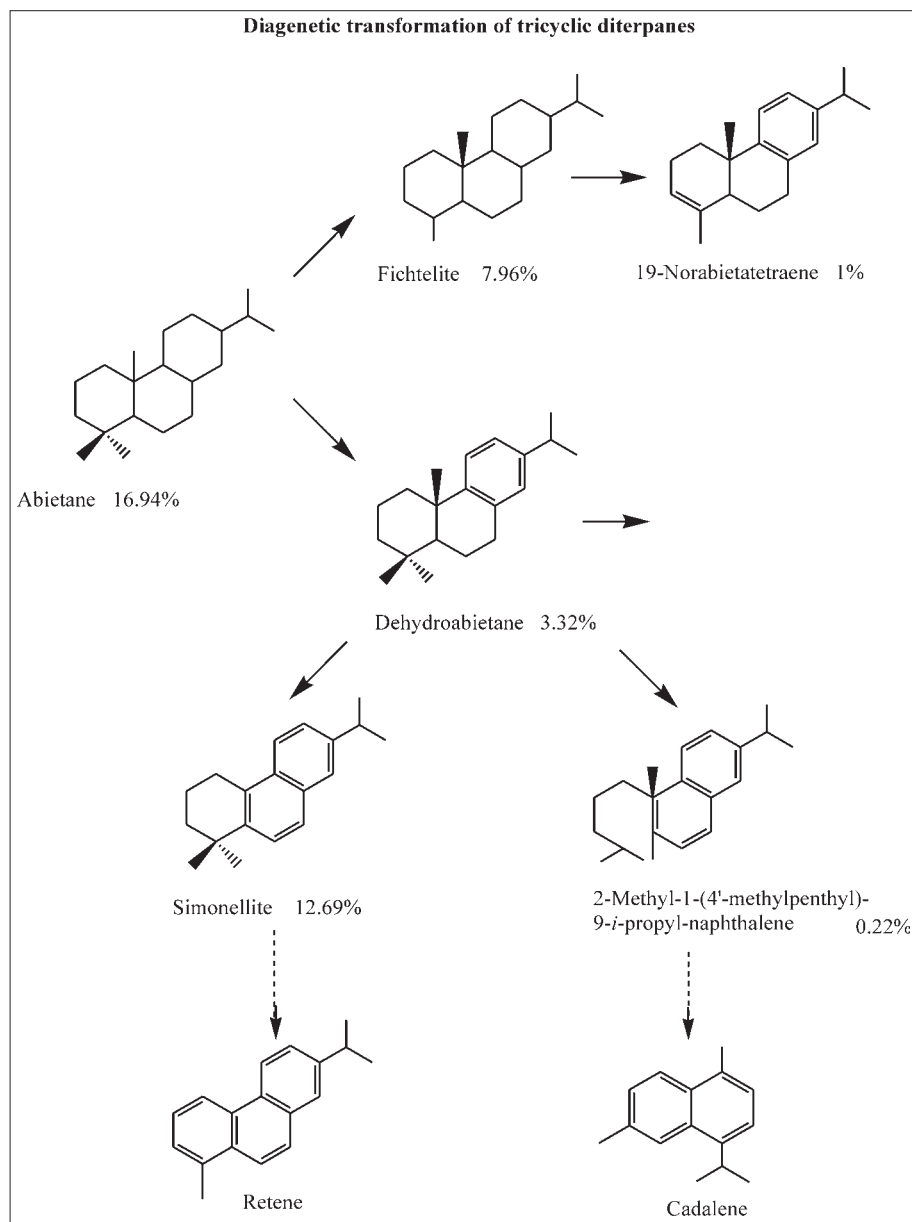| Structural class	Common compounds	Occurrence
Normal abietanes	Abietane, fichtelite, simonellite	All families, except Phyllocladaceae
Kauranes	Kaurenes	All families, except Taxaceae
Phyllocladanes	Phyllocladenes	Cupressaceae s.s. and Podocarpaceae

* – after Otto and Wilde (2001)

Figure 2. Scheme for the possible diagenetic pathway for diterpenoid transformation.

nor quantities of partial aromatized abietane structures are also found due to carry-over from the aromatic fractions: Dehydroabietane, 3.32% (VIII), 2-methyl-1-(4'-methylpenthyl)-6--isopropyl-naphthalene, 0.22% (IX), and highly abundant simonellite, 12.7% (X) and norabieta-3,8,11,13-tetraene (I). The study of aromatic and NSO fractions will further detail the composition of aromatized and functionalized diterpenoids.

The progenitors of tetracyclic diterpanes are particularly abundant in the leaf resins of conifers (Otto et al. 1997). Kaurenes occur in high amounts in waxes of the Araucariaceae family (Coniferales), while phyllocladanes are proposed as markers for the Podocarpaceae family and simonellite/retene (aromatized tricyclic diterpenoid structures) for Pinaceae (Otto et al. 1997).

The combination of high contents of sesqui-/diterpenoid contents is evidence for the input of higher plant residues (conifers), especially when accompanied by the diterpenoid dominance. Resins containing sesqui- and diterpenoids are produced by species of all contemporary conifer families, and in especially large amounts by Pinaceae and Araucariaceae. Otto et al. (1997) advised that phyllocladane be related only to the whole Coniferales group and not to individual plant families. Bechtel et al. (2003a, b) also support the assertion that the taxonomical differentiation between conifer families based on the diterpenoid hydrocarbon composition is in most cases not possible. However, the relationship of plant type, i.e. flowering plants, conifers, ferns, mosses, etc, and diterpenoids is often applied. Nevertheless, high contents of abietane, pimarane, beyerane, kaurane, and phyllocladane are indications for conifers.

On the basis of molecular data and paleobotanical observations by Otto and Wilde (2001) the following fossil vegetation is proposed in Table 3. The possible diagenetic pathways of diterpenoid transformations with coalification are schematically illustrated in Fig. 3. The "regular"

amounts to 85.5% of the resinite extract based on GC analysis, and 24.31% of the aliphatic fraction from the bitumen.

"Regular" abietane skeletal type diterpenoids are represented by fichtelite (V) and abietane (VI). These compounds are often found in pine wood that has been submerged in peat (Simoneit 1986). The "regular" abietanes occur in all conifer families except Phyllocladaceae (Otto and Wilde 2001). The "phenolic" abietanes, i.e. ferruginol, sugiol, etc., are expected in the aromatic/polar fraction. Mi-

diterpenoids are highly abundant in the resinite sample. Regardless of the low maturity of the coal, the aromatization processes have already proceeded and simonellite and cadalene are amenable to identification.

In the near future, our studies will be devoted to aromatic and polar constituents of the extracts in which more molecular indicators for coal-forming vegetation paleo-communities are expected.

Conclusions

Based on the molecular composition of the biomarkers from the Chukurovo lignite it is concluded that the precursor swamp should have contained Pinaceae and Taxodiaceae, the dominant gymnosperms within the Pinophyta. There are biomarker indications for Cupressaceae as well. The molecular data confirm the paleobotanical and petrographic observations, and enrich our knowledge on the lignite extractable portion.

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