ORGANIC GEOCHEMISTRY OF HUMIC ACIDS FROM A NEOGENE LIGNITE SAMPLE, BULGARIA

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Abstract

Humic substances naturally occur in Miocene/Pliocene-aged lignite at very high concentrations. Here biomarkers in the bitumen-free extract of humic acids from Thracian lignite, Bulgaria, are studied. Applying methods of organic geochemistry a broad range of compounds are isolated and characterised. Species are classified according to abundance, possible source input and diagenetic transformation. A feature of humic acids derived from Thracian coal is the extremely high content of 16α(H)Phyllocladane, ~60% of aliphatic fraction, or 1.6 wt.% of initial lignite. The high diterpenoids content, especially with abietane skeleton, proved the conifer contribution to the peat-forming helophytes, i.e. Cupressaceae s. str., Podocarpaceae, Araucariaceae, Taxodiaceae, Phyllocladus, Piceae. Tightly-trapped, linear long-chain fatty acids (FAs) are the main constituents of the acidic fraction of humic acids. Their distribution patterns indicate a dominant higher plant origin. The presence of αOH-FAs and hopanoid acids assumes bacterial activity in the plant material reworked. A hint for the input of plant biopolymers, i.e. cutin, suberin, is the relative high content of "even" carbon numbered ωOHFAs and α,ω-alkanedioic FAs. "Even" numbered short-chain ωOHFAs could originate from cutin-derived constituents of the needles of numerous species of gymnospermous families.

Key words: Thrace, lignite, humic acid, biomarker, fatty acid, palaeoenvironment.

1. Introduction

According to topographic, morphotectonic, lithologic and genetic characteristics Neogene coals in Bulgaria are divided into four coal-bearing provinces: Dacian, Thracian, Sofia, Strimon-Mesta (Šiškov, 1997). The Thracian is the largest one hosting the main energy resources of the country. Palaeobotanical data of the Thracian lignite supports the presence of Taxodiaceae issue remains (twigs, cones, wood, etc.) along with a floral composition being similar to this of the mires in temperate climates. The results from previous studies (Bechtel et al. 2005) indicated that the larger part of the Thracian coal deposits is derived from coniferous flora, which is the main source of resinous organic matter. The results confirmed a low Angiospermae/Coniferales ratio due to the high abundance of coniferous fragments in the lignite. The huge amounts of 16α(H)-Phyllocladane the variety of tricyclic diterpanes and their polar counterparts proved the gymnosperm contribution at a molecular level (Stefanova et al., 1995). There were also some chemical indicators for the presence of angiosperm debris in the source material (Stefanova et al., 2002).

Humic substances naturally occur in Miocene/Pliocene lignites at very high contents (~70%) and could be considered representative for coal organic matter. The aim of the present study was to as-

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sess biomarker assemblage of humic acid from Thracian coal-bearing province with the aim to enrich the information on coal precursors and maturation degree inasmuch humic substances are firmly involved in coal formation.

2. Geological setting

The Thracian coal-bearing province hosts three deposits, namely these of Maritza-East, Maritza-West (Early-Middle Miocene) and Elhovo (Pliocene) located in the Thracian depression, which is filled with sediments of the lower (Palaeogene) and upper (Neogene) coal-bearing molasse. Coal formation started in Late Oligocene and continued through Miocene into Pliocene. It took place in highly peneplaned coastal areas covered by eutrophic mires. Younger sedimentary sequences of varying thickness occur from west to east due to compensation from the formation of the Black Sea and gradual marine regression to this direction. The Maritza-East lignite deposit (maximum thickness 25 m) is a good example of the long-lasting peat accumulation in this region.

3. Methods

3.1 Bulk characteristics

One sample from Maritza-East lignite, seam "Troyanovo North", was extracted for humic acids (HA) preparation. Some rank parameters of Neogene coals in Bulgaria were published in Šiškov (1997). Briefly, Maritza East lignites were described by the following characteristics, in %: R_r– 0.20; W^r – 64.4; C^{daf} – 65.0, VM ^{daf} – 55.8 and calorific value in MJ/kg, Q^{daf} – 22.31.

Isolation and fractionation of humic acids were described in a previous paper (Stefanova et al., 1993). To get an extractable portion (bitumen "free") of humic acids, about 6 g were extracted with chloroform under 100 atm at 80°C (3 x 20 min) (Dionex equipment). Acids and neutrals were separated from the bitumen-"free" fraction on a SiO_2/KOH column (McCarthy and Duthie, 1962). Hydrocarbons, ketones and esters were separated from the other neutrals applying liquid chromatography and using for elution diethyl ether/petroleumether mixtures of increasing polarity (Grasset and Amblès, 1998; Válková et al., 2009). Acids were identified as methyl esters. Hydroxyl FAs were determined as TMS ethers/methyl esters after classical methylation of the acid fraction with trimethylsilyldiazomethane (Hashimoto et al., 1981).

3.2. GC-MS study

The products were analyzed by capillary GC using a Hewlett-Packard 6890 GC (split injector, 250°C; flame ionization Detector (FID), 300°C) with a fused silica capillary column (SGE BPX 5%, 30 m length, 0.25 mm id., 0.25 μm film thickness) and helium as carrier gas. The GC was temperature programmed from 60 to 300°C at 5°C min-1 (isothermal for 20 min final time). The GC-MS analyses were performed on a Trace GC Thermo Finnigan coupled to a Thermo Finnigan Automass (with the same GC conditions). The MS was operated in the electron impact mode with a 70eV's ion source energy and the ion separation was operated in a quadripolar filter. The various products were identified on the basis of their GC retention times, their mass spectra (comparison with standards) and literature data. SIM was used for acids visualization: m/z 74 for linear fatty acids; m/z 98 for linear α,ω alkanedioic FAs; m/z 103 for αOH- and ωOH FAs; m/z 191 for hopanoic acids.

Table 1. Classification, abundance and source of biomarkers in neutral fraction (in rel.%).

Classification	Abundance	Higher plants	Bacteria	Uncertain
Acyclic alkanes:	19.37			
i.e. n-alkanes, ketones	19.06	17.98	?	1.08
isoprenoids	0.31	0.31	trace	-
Terpenoids:	77.88			
i.e. sesquiterpenoids	0.41	0.41	-	-
diterpenoids	68.75	68.75	-	-
sesterterpenoids	0.40	-	0.40	-
triterpenoids	8.32	-	0.32	-
Total	97.25			

4. Results

For Thracian lignite HAs "free" bitumen amounted 110 mg/g TOC. After fractionation on SiO$_2$/KOH column the material was separated into neutrals (~ 1/3 of the applied sample), acids (~ 1/3) and polars (~ 1/3).

4.1 Neutral fraction

The main homologue series GC-MS registered in the neutral fraction are:

- Hydrocarbons with long chains, nC$_{23}$÷nC$_{33}$, maximizing at nC$_{29}$, with strong "even" carbon numbers domination, CPI 2.8;

- Ketones with long chains, nC$_{23}$÷nC$_{33}$, with nC$_{29}$ in maximal content, CPI 4.0. One *iso*-ketone structurally related to phytol was registered as well;

- Diterpenoids were in a high preponderance due to the presence of huge amounts of tetracyclic diterpenoid 16α(H)-Phyllocladane (Str.I);

- Tri-, sesqui-, and sesterpenoids were in subordinating quantities compared to diterpenoids;

- Hopanoids (Hs), i.e. 17β-(H)22,29,30-trisnorhopane (H$_{27}$) and the range of H$_{29}$-H$_{31}$ 17β(H), 21β(H)-hopanes.

Table 1 presents a classification scheme, the abundance and possible sources of biomarkers in HA grouped according to Wang and Simoneit (1990).

4.2 Acidic fraction

The acidic fraction was composed by linear long chain fatty acids (nFAs), their functionalized analogues α- (αOH-FAs), ω-(ωOH-FAs) hydroxy fatty acids and dicarboxylic fatty acids (α,ω-di-FAs). Their patterns of distributions are illustrated in Figure 1. The following acid series were mass-spectrometrically identified:

- linear long chain FAs, methyl esters (nC$_{12}$÷nC$_{34}$), nC$_{28}$ maximal, with "even" carbon number prevalence, 44.9 rel.% of acidic fraction (Str. III);

- linear long chain α,ω alkanedioic FAs, dimethyl esters (nC$_{16}$, nC$_{20}$÷nC$_{30}$) with a distribution similar to nFAs, 3.18 rel.% (Str. IV);

- αOH-FAs as 2-(trimethylsilyl)oxy, methyl esters, nC$_{22}$÷nC$_{27}$ (nC$_{23}$ in trace), 1.2 rel.% (Str. V);

- ωOH-FAs as trimethylsilyloxy, methyl ester derivatives, nC$_{12}$÷nC$_{28}$, strongly maximizing at nC$_{16}$, 3.32 rel.% (Str. VI);

- dehydroabietic acid, methyl ester, 0.8 rel.% (Str. VII);

- hopanoic acids, methyl esters, i.e. C$_{32}\alpha\beta$, C$_{30}\beta\beta$ and C$_{31}\beta\beta$, < 1 rel.% (Str. VIII).

Apart from the above mentioned acids some alkylated phenols were detected as well. They probably were produced during accelerated "free" bitumen extraction under pressure or are end products of diagenetic transformations of lignin moieties. One diterpenoid ketophenol, totarol 3-one (Str. II), was highly abundant (5.2 rel.%) in the acidic fraction.

4.3 Data analysis

In some papers dedicated to humic substances organic solvent soluble portion of humic acids was named hymatomelanic acids. It is still contestable were hymatomelanic acids a separate group of compounds or could be considered as lipids. Their origin received special attention in the study of Lehtonen et al. (2001), where the achievements in the field were discussed.

4.3.1 Neutral fraction

The aliphatic hydrocarbons in the bitumen-free fraction comprise long chain n-alkanes from nC$_{17}$ to nC$_{35}$, the major component being the nC$_{29}$ alkane. They show a clear odd/even carbon number predominance indicating a dominant plant input (Kolattukudy, 1976).

Terpenoids at different ratios depending on plant communities compose aliphatic fraction (neutrals). A striking feature of Thracian lignite HA is the extremely high content of 16(H)-Phyllocladane (~60% of aliphatics or 1.6% of initial coal).

The identified diterpenoids demonstrated the presence of Gymnospermae in the coal-forming plant community of Maritza-East lignite. Furthermore, phenolic ketoditerpenoid indicated a specific input of organic matter from conifers of the Cupressaceae/Taxodiaceae or Podocarpaceae as the most abundant trees in the coal-forming mire (Stefanova et al., 2002).

Actually, biomarker assemblage of humic acids resembled biomarker compositions of Maritza-East lignite lithothypes and their polar constituents already described in previous paper (Stefanova et al., 1995). All data support strong Gymnospermae contribution to the palaeomire.

4.3.2 Acidic fraction

Acids demonstrate some novelty concerning humic acids structure. Our results proved the high ability of humic acids to retain hydrophobic species. We suppose that functionalised fatty acids are integrated parts of humic acids inherited from precursors.

Fatty acids are common constituents of humic acids. Their forms of linkage have been studied by several researchers in a suite of papers devoted to humic acids in soils, peat, lignite, etc. (Amblès, 2001; Grasset et al., 2002; Guignard et al., 2005; Deport et al., 2006; Válkova et al., 2009). Except "free" fatty acids extraction, authors used thermochemolysis in the presence of different alkylation

reagents, i.e. TMAH, TEAAc, to distinguish strongly trapped and esterified FAs. It is worth noting that according to them the major part of FAs occurs as "free" constituents being trapped within the network.

Linear long chain FAs are the main constituents of the acidic fraction of humic acids, amounting about a half of GC amenable compounds. The distribution pattern of "free" nFAs presents a long mode maximizing at nC$_{28}$ with strong "even" carbon numbered dominance. Such a distribution indicates a dominant higher plant origin. In the samples under study there were not unsaturated and branched chain FAs.

One order less of magnitude was the content of α,ω -alkanedioic FAs with "even" carbon numbered dominance. Sources of long members could be suberin and cutin from higher plants or products of FAs microbial ω-oxidation. A hint for the input from plant biopolymers is the relative high content of "even" numbered ωOH–FAs (Fig.1). Actually hydroxyl FAs are the second in abundance. Allard (2006) published a comparative study on the chemical compositions of humic acids extracted from soil, agricultural soil and lignite deposits based on the data for bound lipids, carbohydrates and amino acids compositions. ωOH–FAs were determined only in soil humic acids being completely absent in lignite HAs. It should be emphasized that humic acids from Maritzsa-East lignite containing a high content of xylite-rich lithotype, are comparable with these of soil organic matter. "Even" numbered short-chain ωOH–FAs (C$_{12}$,C$_{14}$,C$_{16}$) have been reported as characteristic cutin-derived constituents of needles of numerous species of gymnospermous families (Oros et al., 1999). The data coincides well with the conclusions based on the diterpenoids assemblage assuming predominant gymnospermous contribution. The input of suberin is indicated by the presence of linear long-chain FAs, α,ω–alkanedioic FAs, ωOH–FAs and long-chain alcohols (not described here as registered in humin), all suberin building blocks (Kolattukudy, 1980).

α–OH–FAs are in a lowest content compared to the other linear FAs. According to Cranwell (1982) they are by-products of the *a*-oxidation of FAs and are not source specific biomarkers.

The presence of hopanoid acids assumed bacterial activity in the reworking process of the plant material. The dominance of ββ hopanoid acids is in agreement with the immaturity of the lignite sample.

5. Conclusions

Biomarker assemblage of humic acids from Maritza-East lignite, Bulgaria, proved a strong gymnospermous contribution to the palaeomire. A feature of the Thracian lignite HAs was the extremely high content of 16α (H)–Phyllocladane (~60 % of aliphatics or 1.6 % of initial coal). The identified diterpenoids demonstrated the presence of Gymnospermae in the coal-forming plant communities. Phenolic ketoditerpenoids indicated a specific input of organic matter from conifers of the Cupressaceae/Taxodiaceae or Podocarpaceae as the most abundant trees in the coal-forming mire.

Tightly–trapped linear long-chain FAs were the main constituents of the acidic fraction of humic acids. Their distribution pattern indicated a dominant higher plant origin.

The presence of hopanoid acids assumed bacterial activity in the plant material reworked. A hint for the input from plant biopolymers, i.e. cutin, suberin, was the relative high contents of "even" carbon numbered ωOH–FAs and α,ω–alkanedioic FAs.

"Even" numbered short-chain ωOH–FAs could originate from cutin-derived constituents of needles of numerous species of gymnospermous families.

6. Acknowledgement

Appendix: Structures cited in the text
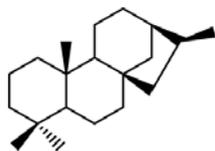
Str.I. 16α(H)-Phyllocladane

Str.II. Totarol-3-one

$CH_3(CH_2)_nCOOH$

Str.III. Fatty acid

$HOOC(CH_2)_nCOOH$
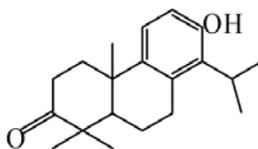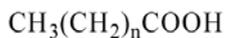
Str. IV. α,ω Dicarboxylic acid

$$\overset{\displaystyle OH}{CH_3(CH_2)_n\overset{|}{C}H - COOH}$$
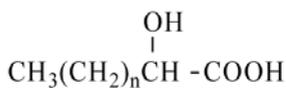
Str. V. αOH Fatty acid

$OH-CH_2(CH_2)_nCOOH$

Str. VI. ω OH Fatty acid
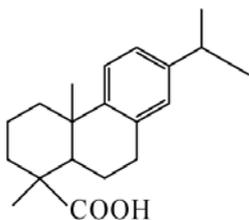
Str. VII. Dehydroabietic acid

Str. VIII. ββC$_{32}$ Hopanoid acid

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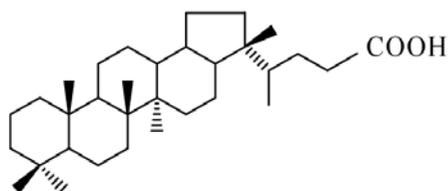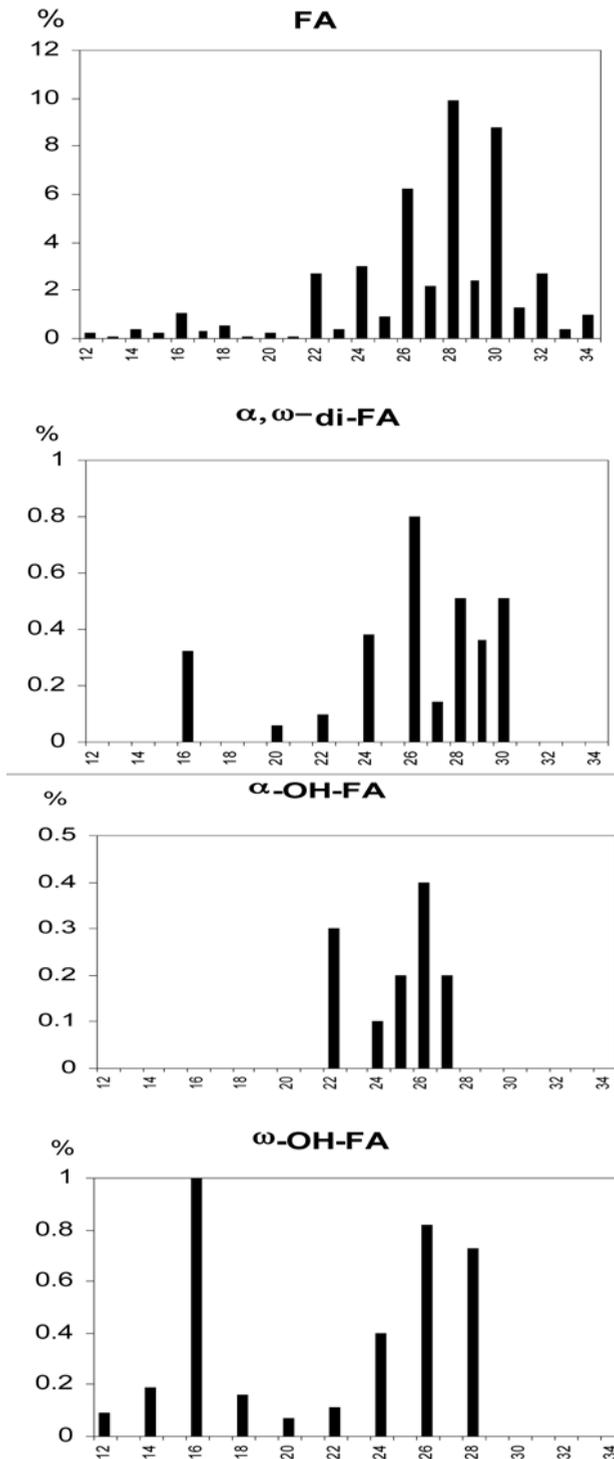
Fig. 1: Patterns of distributions of fatty acids (X axis numbers correspond to carbons in FAs chains).

7. References

Allard, B. 2006. A comparative study on the chemical composition of humic acids from forest soil, agricultural soil and lignite deposit. Bound lipid, carbohydrate and amino acid distributions. *Geoderma* 130, 77-96.

Amblès, A. 2001. Methods to reveal the structure of humic substances. In: Hofrichter, M., Steinbüchel, A. (Eds) *Biopolymers*. Wiley-VCH. v.1 pp. 325-348.

Bechtel, A., Sachsenhofer, R.F., Zdravkov, A., Kostova, I., Gratzer, R. 2005. Influence of floralassemblage, facies and diagenesis on petrography and organic geochemistry of the Eocene Bourgas coal and the Miocene Maritza-East lignite (Bulgaria). *Organic Geochemistry* 36, 1498-1522.

Cranwell, P.A. 1982. Lipids of aquatic sediments and sedimenting particules. *Prog. Lipid Res.* 21, 271-308.

Deport, C., Lemée, L., Amblès, A. 2006. Comparison between humic substances from soils and peats using TMAH and TEAAc thermochemolysis. *Organic Geochemistry* 37, 649-664.

Grasset, L., Amblès, A. 1998. Structural study of soil humic acids and humin using a new preparative thermochemolysis technique. *J. Anal. and Applied Pyrolysis* 47, 1-12.

Grasset, L., Guignard, C., Amblès, A. 2002. Free and esterified aliphatic carboxylic acids in humin and humic acids from peat sample as revealed by pyrolysis with TMAH or TEAAc. *Organic Geochemistry* 33, 181-188

Guignard, C., Lemée, L., Amblès, A. 2005. Lipid constituents of peat humic acids and humin. Dis-tinction from directly extractable bitumen components using TMAH and TEAAc thermochemolysis. *Organic Geochemistry* 36, 287-297.

Hashimoto N., Aoyama T., Shiori T. 1981. New methods and reagents in organic synthesis. 14. A simple efficient preparation of methyl esters with trimethylsilyl diazomethane (TMSCHN2) and its application to gas chromatographic analysis of fatty acids. *Chemical Pharmaceutical Bulletin* 29, 1475-1478.

Kolattukudy, P.E. 1976. Chemistry and biochemistry of natural waxes, Elsevier, Amsterdam.

Kolattukudy, P.E. 1980. Biopolyester membranes of plants: cutin and suberin. *Science* 208, 990-1000.

Lehtonen, K., Hänninen, K., Ketola, M. 2001. Structurally bound lipids in peat humic acids. *Organic Geochemistry* 32, 33-43.

McCarthy, R.D., Duthie, A.H. 1962. A rapid quantitative method for the separation of free fatty acids from other lipids. *J. of Lipid Res.* 2, 117-119.

Oros, D.R., Standley, L.J., Chen, X., Simoneit, B.R.T. 1999. Epicuticular wax composition of predominant conifers of western North America. *Z. Naturforsch.* 54c, 17-24.

Šiškov, G.D. 1997. Bulgarian low rank coals: geology and petrology. In: Gayer, R. and Pešek, J. (eds), *Eur. Coal Geology and Technol. Geol. Soc. Spec. Publ.* 125, 141-148.

Stefanova, M., Velinova, D., Marinov, S.P., Nikolova, K. 1993. The composition of lignite humic acids. *Fuel* 72, 681-684.

Stefanova, M., Magnier, C., Velinova, D. 1995. Biomarker assemblage of some Miocene-aged Bulgarian lignite lithotypes. *Organic Geochemistry* 21, 1067-1084.

Stefanova, M., Oros, D.R., Otto, A., Simoneit, B.R.T. 2002. Polar aromatic biomarkers in the Miocene Maritza-East lignite, Bulgaria. *Organic Geochemistry,* 33, 1079-1091.

Válkova, D., Grasset, L., Amblès, A. 2009. Molecular compounds generated by RuO_4 oxidation and preparative off line thermochemolysys of lignite humic acids from South Moravia: implication for molecular structure. *Fuel* 88, 2113-2121.

Wang, T.-G., Simoneit, B.R.T. 1990. Organic geochemistry and coal petrology of Tertiary brown coal. 2. Biomarker assemblage and significance. *Fuel* 69, 12-20.