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Preserved lignin structures in Miocene-aged lignite lithotypes, Bulgaria

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Abstract

Contents of preserved lignin structures in Miocene-aged lignite lithotypes were determined. Phenol aldehydes, ketones and acids released from lignin by CuO oxidative hydrolysis were separated by capillary zone electrophoresis (CZE). Products of degradation were separated and identified by GC–MS to confirm CZE data and to complement composition data. The following values for the contents of preserved lignin structures in lignite lithotypes were determined (in wt%): xylain, 8.5; humovitrain, 6.7; liptain, 6.4; and humoclarain, 3.3. Some fatty acids, negligible contents of benzene polycarboxylic acids and their hydroxy/methoxy counterparts were also observed using GC–MS.

Author Keywords: Coal; Lignin; Capillary electrophoresis

1. Introduction

The general consensus today is that the coalification process involves a selective preservation of certain resistant plant remains followed by some reorganisation of the biopolymers that survive [1]. During peatification and coalification the methoxyl groups from guaiacyl/syringyl structures are lost first via demethylation and resulting alkylated catechols are converted to alkylated phenols [2, 3, 4, 5 and 6]. At the biochemical stage hemicellulose is completely lost, cellulose content reduced, and lignin-derived structures selectively preserved. It is well documented that integral parts of lignin are incorporated into fossil organic matter, i.e. in kerogens, coals, sediments, soils, humic substances, etc. Thus the preserved lignin-derived structures provide an ideal tracer for the original inputs of vascular plant material.

Hayatsu et al. [7] studied the alkaline CuO oxidation of coals at 170–200 °C in order to estimate preserved lignin structures. They demonstrated that lignin-like polymers were incorporated into macromolecules but were still identifiable in lower rank coals. The authors concluded that: (i) the treatment resulted in hydrolytic oxidation of ether linkages in the aliphatic side chains of aromatics; (ii) did not split C–C bonds; (iii) effectively have oxidised aliphatic chains with heteroatoms; (iv) and, aromatic rings were not destroyed.

The oxidation technique allowed evaluation of the amount, quality, and stage of degradation of lignin in fossil samples. The drawbacks of the method are as follows [4]: (i) the technique is time-consuming; (ii) the microbial altered lignin monomers (catechols) are not analysed inasmuch are oxidised to low molecular fatty acids.

Hedges and Mann [8] divided the various aldehydic, ketonic, and acidic phenolic compounds produced during alkaline CuO oxidation of geological samples in three groups based on the other substituents of the aromatic nuclei interpreted to be inherited from lignin. In this way

the general vascular plant sources in paleoenvironment can be identified. The concentrations of vanillyl (V), syringyl (S) and cinnamyl (C) phenolic units were related to plant variety, lignin concentration, tissue type, and degree of fungal alternation during deposition. The data allowed compositional regions to be distinguished for: non-vascular plants; gymnosperm woods (non-flowering plants including conifers); non-woody gymnosperm tissues; angiosperms (flowering plants including hard-wood trees); non-woody angiosperms (herbs, grasses, etc.); The ratios of the concentrations for different types of phenols can be plotted in a two-dimensional diagram and regions for source assignments were defined [8]. The approach is improved and applied at different areas of organic geochemistry [1, 9 and 10].

Our ongoing research is devoted to Miocene-aged lignite lithotypes. The contents of lignin-derived phenols in products of alkaline CuO oxidation were determined by coupling with capillary zone electrophoresis (CZE). The data were quantitatively interpreted and compared with mass spectral study.

2. Experimental

Hand-picked lithotypes from Miocene lignite ‘Maritza-Iztok’, Bulgaria (Ro, 0.18%) were under study. The following macroscopically distinguished lithotypes were used: xylain (X), humovitrain (HV), liptain (L) and humoclarain (HK). In [Table 1](#) are gathered some characteristics of the samples.

Characteristics	Lithotype			
	Xylain	Humo-vitrain	Liptain	Humo-clarain
<i>Proximate analysis</i>				
Moisture, (%)	9.8	13.9	12.7	22.9
Ash (dB)	0.8	3.4	0.4	22.8
Volatile matter, (daf)	58.8	52.8	68.1	60.5
<i>Ultimate analysis, (daf)</i>				
C	67.3	65.8	72.1	65.1
H	6.1	5.5	7.2	6.6
<i>Rock-Eval pyrolysis data</i>				
TOC (wt%)	62.3	61.1	60.7	63.9
S ₂ (mg HC/g sample)	137.7	89.4	153.1	140.9
HI (mg HC/g TOC)	221	146	252	220
T _{max} (°C)	375	374	368	394

Table 1. Characteristics of lignite lithotypes (wt%)

Rock-Eval™ pyrolysis was carried out using an Oil Show Analyser (OSA; Delsi Instrument), under standard conditions, in cycle 3 (i.e. with programmed pyrolysis from 200 to 600 °C at a rate of 30 °C/min).

Alkaline CuO oxidation was carried out in Teflon-lined steel bombs placed at 170 °C for 4 h [11]. After cooling, 2,4,5-trimethoxybenzoic acid was introduced as an internal standard.

Then lignin phenols were extracted with peroxide free diethyl ether. After solvent evaporation, lignin phenols were dissolved in methanol and kept under nitrogen in sealed vessels.

Lignin monomers separation was performed by CZE according to Maman et al. [10 and 12]. Eleven phenol compounds were separated by CZE with P/ACE 5510 system Beckman instrument equipped with a fused silica capillary (57 cm×50 µm). An UV detector was set at absorbency 214 nm through the capillary at 50 cm from the injection point. The system was supported by PC GOLD-3M40 software Beckman. After the CZE study the products of CuO oxidation were methylated by CH₂N₂ and subjected to GC and GC–MS study.

The methylated fractions were analysed on a Perkin–Elmer Autosystem XL gas chromatograph equipped with CP sil 5 CB column (0.22 mm×30 m; 0.25 µm film thickness), a flame ionisation detector (300 °C, and a split/splitless capillary injector maintained at 300 °C used in the splitless mode (valve reopened one min after injection). After 1 min hold at 50 °C the oven temperature was increase from 50 to 120 °C at 30 °C/min, and then from 120 to 300 °C at 5°/min, maintaining this final temperature for a period of 1 h.

GC–MS study is performed with a Varian 300 chromatograph connected to an ITD 800 (Finnigan Mat) by 2 m capillary interface heated to 300 °C. Other GC conditions were identical to those described earlier. Operating conditions for the ion trap were temperature 220 °C; ionisation energy 80 eV; Tscan 2 s from 50 to 500 amu. Peak identification was verified by coinjection of standard phenols, in CZE, and by comparison with NIST library data, in GC–MS.

3. Results and discussion

Lignin polymers cannot be analysed without prior chemical degradation treatment to release smaller molecules amenable to chromatographic study. Hedges and Mann [8] proposed compositional data for lignin-related phenols produced during CuO oxidation of vascular and non-vascular plant tissues to be considered as source parameters. The authors have grouped the phenols as follows:

Vanillyl phenols (**V**):

3-methoxy-4-hydroxybenzaldehyde (Vanillin)
3-methoxy-4-hydroxyacetophenone (Acetovanillone)
3-methoxy-4-hydroxybenzoic acid (Vanillic acid);

Syringyl phenols (**S**):

3,5-dimethoxy-4-hydroxybenzaldehyde (Syringe aldehyde)
3,5-dimethoxy-4-hydroxyacetophenone (Acetosyringone)
3,5-dimethoxy-4-hydroxybenzoic acid (Syringic acid);

Cinnamic acids (**C**):

3-(3-methoxy-4-hydroxyphenyl)-propenoic acid (Ferulic acid)
3-(4-hydroxyphenyl)-propenoic acid (*p*-Coumaric acid);

p-Hydroxybenzoic phenols (**H**):

4-Hydroxybenzaldehyde
4-Hydroxyacetophenone
4-Hydroxybenzoic acid;

Total lignin content, in wt%, or expressed as wt% in organic carbon (Λ), is determined as the sum of the contents of four phenolic type units. Vanillyl and syringyl compounds are derived from the oxidation of the coniferyl and sinapyl moieties of lignin, respectively. Ferulic and *p*-coumaric acids are believed to be linked to lignin polymers by ester bonds that are hydrolysed readily but not oxidised. 4-Hydroxybenzoic compounds could be partly derived from other sources than lignin and are seldom employed for correlation purposes. Typical electrophoregrams for standard mixture of the 11 phenolic compound (A) and of xylain products obtained after CuO oxidation (B) are shown in Fig. 1.

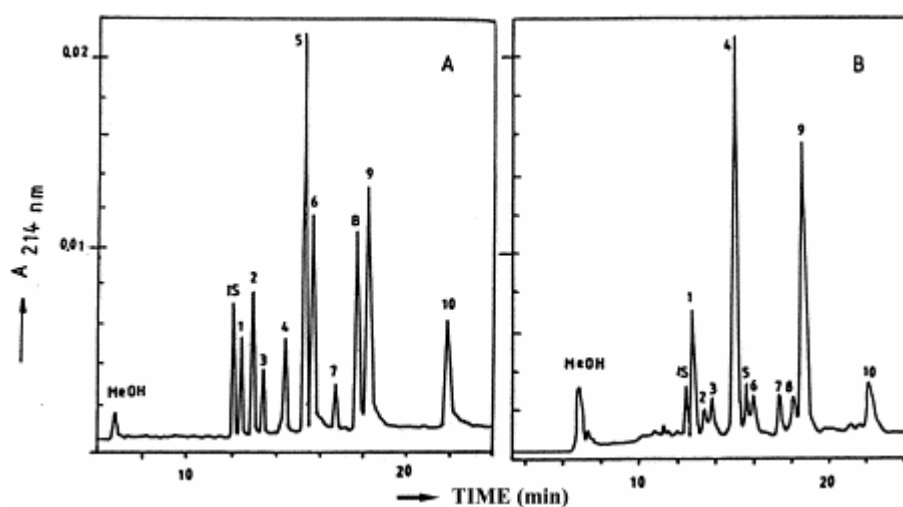


Fig. 1. Capillary zone electrophoregrams; (A) standard mixture; (B) products of CuO oxidation of xylain; Peak assignment: 1S, internal standard (2,4,5-trimethoxybenzoic acid); (1) 3-methoxy-4-hydroxyacetophenone (acetovanillone); (2) 3,5-dimethoxy-4-hydroxybenzaldehyde (syringe aldehyde); (3) 4-hydroxyacetophenone; (4) 3-methoxy-4-hydroxybenzaldehyde (vanillin); (5) 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid); (6) 3-(3-methoxy-4-hydroxyphenyl)-propenoic acid (ferulic acid); (7) 4-hydroxybenzaldehyde; (8) 3-(4-hydroxyphenyl)-propenoic acid (coumaric acid); (9) 3-methoxy-4-hydroxybenzoic acid (vanillic acid); (10) 4-hydroxybenzoic acid.

The lignin of the two most abundant classes of vascular plant, the *Gymnospermae* and the *Angiospermae*, could be distinguished according to specific position in S/V vs. C/V diagram, where S, C, V are the ratios of the syringyl, cinnamyl and vanillyl contents. In this diagram gymnosperm wood, non-woody gymnosperm tissue, angiosperm wood, and non-woody angiosperm tissues are resolved into non-overlapping areas [8].

The data for lignite lithotypes subjected to alkaline CuO oxidation are present in Table 2. The values for the lignin phenol yields are expressed as wt% coal (daf basis). The maximum value calculated in wt% organic carbon, was determined for xylain, $\Lambda=12.7$ and decreased with humification as suggested by the value $\Lambda=5.2$ wt.% organic carbon determined for humoclarain.

Phenolic units	Lithotype			
	Xylain	Humo- vitrain	Liptain	Humo- clarain
<i>Yield of phenols</i>				
in wt%	8.5	6.7	6.4	3.3
Δ in wt% organic carbon	12.7	10.2	8.9	5.2
Ratio acid/aldehyde (Ad/Al)	0.67	0.64	0.67	0.87
<i>Distribution of phenolic units, (in % of total phenols)</i>				
Vanillyl units (V)	78.8	95.0	72.9	73.6
Syringyl units (S)	2.8	0.0	0.0	2.3
Cinnamyl units (C)	5.3	0.0	1.0	0.0
<i>p</i> -Hydroxyphenyl units (H)	13.1	4.9	26.1	24.0

Table 2. Yields of lignin-derived phenol and their distribution in structural types

Acid/aldehyde ratios (Ad/Al), determined on the base contents of vanillic acid/vanillin, similar for xylain, humovitrain and liptain, are approximately 0.65. The ratio magnitude sharply increased to 0.87 for humoclarain. This latter value provides an indication for fungal degradation at C α position in phenyl propane side chain, and subsequent carboxyl group formation.

Phenolic units show an unusual distribution, with a strong predominance of vanillyl units, amounting to 95% of the total lignin phenols, in the case of humovitrain (Table 2). In addition, the contents of vanillyl units for lithotypes are well correlated with the total amounts of lignin phenols, which is an indication for the same source contribution (Fig. 2). A small diversity in phenol group distributions is recognised in the xylain lithotype, where cinnamic units represented 5.3% of the total phenols.

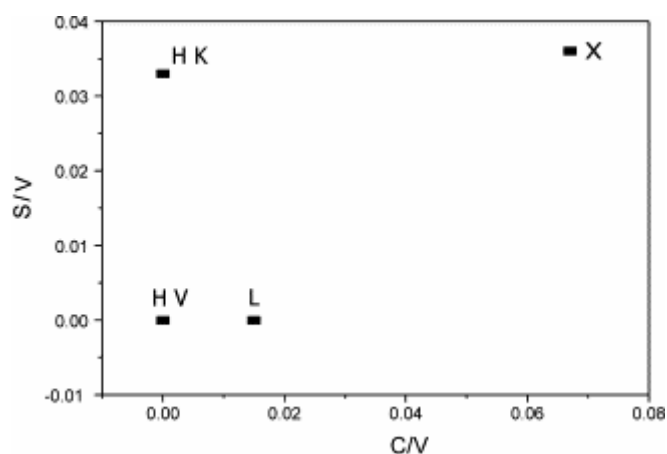


Fig. 2. Plot of lignin-derived phenols ratios in two dimensional diagram, according to Hedges and Mann [8] (X, xylain; HV, humovitrain; L, liptain; HK, humoclarain).

The distribution of phenolic units is interpreted to indicate that gymnosperm woods were predominant precursors for 'Maritza-Iztok' lignite. In the S/V vs. C/V diagram all samples under study are located near zero in Hedges and Ertel diagram [8]. This is interpreted as resulting from an approximately equal mixture of woody gymnosperm plant tissues and of non-woody material from other sources. A strong dominance of conifers in the paleoplant community preserved in the 'Maritza-Iztok' lignite is also supported by the biomarker compositions where high contents of 16 α (H)-Phyllocladane in extracts from the same lithotypes were determined [13].

Low values for S/V and C/V ratios were plotted in diagram to examine this region in detail (Fig. 3). Higher ratio calculated for xylain in comparison to the other lithotypes could be explained by the presence of non-woody angiosperm tissues, probably originating from marsh or grassland sources. The ratio C/V is a useful parameter for discrimination between woody and non-woody vascular plant tissues, since ferulic and *p*-coumaric acids only occur in the latter type of plant material. Another peculiarity is that appreciable quantities of non-woody *Gymnospermae* should have been present in liptain forming paleoplant community as far as higher than zero value was determined for C/V ratio.

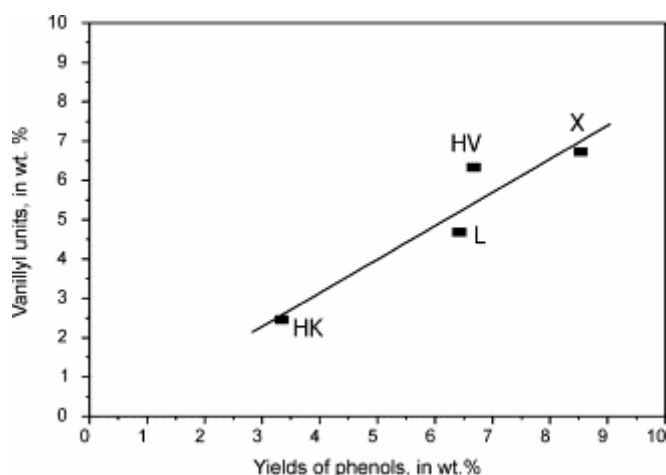


Fig. 3. Relationship between vanillyl units and total phenols produced in CuO oxidation of lignite lithotypes; (X, xylain; HV, humovitrain; L, liptain; HK, humoclarain).

The content of 2.3% of syringyl units in products of humoclarain degradation indicates the presence of deciduous trees in paleoenvironment. Since palynologic examination of lignite 'Maritza-Iztok', performed by Šiškov and Andreev [14], indicated that both *Angiospermae* and *Gymnospermae* were sources for the lignite, our study suggests that during coalification compounds syringyl units underwent preferable degradation. *Angiospermae* due to their greater susceptibility to microbial degradation, are preferably attacked during decay [5]. In coal structural study other authors came across the same observation. Erol and Olkay [15] have demonstrated that there are reduced quantities of syringyl building blocks in products of CuO degradation of Turkish lignites with similar palynologic characteristics as lignite 'Maritza-Iztok'.

Hayatsu et al. [7 and 16] have subjected lignite and higher rank coals to CuO oxidation at 200 °C for 8 h. The products of degradation lignin-derived monomers were determined. A diversity of hydroxy benzenepolycarboxylic acids and benzene carboxylic acids in prevalence with vanillic acid were the concomitant species. They failed to isolate phenyl propane units.

With the aim to provide new insight into the use of CZE data one more technique is involved in the study. Since CZE registers only charged compounds it must be admitted that some uncharged molecules were not determined. In Fig. 4 is shown GC separation of xylain CuO oxidation product and in Table 3 are gathered GC–MS identifications of peaks.

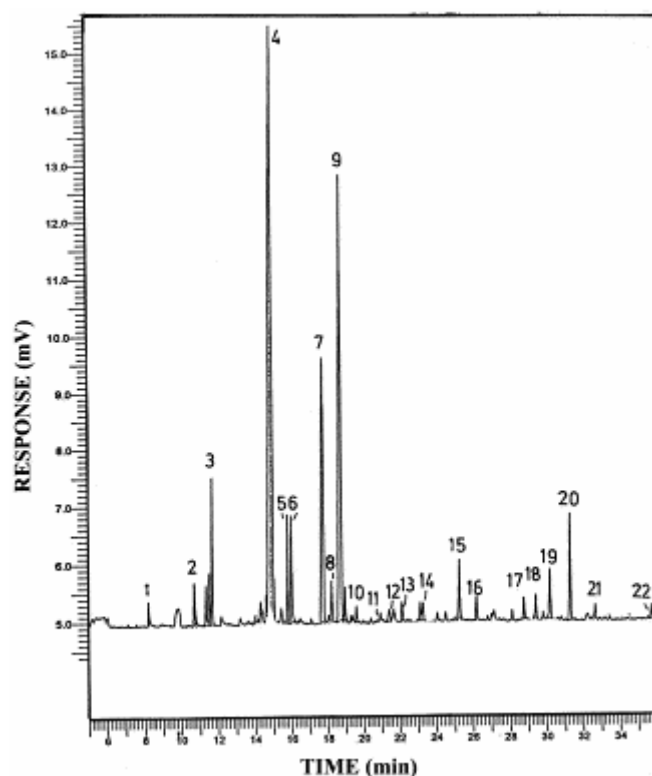


Fig. 4. GC separation of CuO oxidation products of xylain lithotype; (Peaks identification in Table 3).

Peak no.	RT (min)	(%)	Structure	Molecular mass
1	8.22	0.43	4-Hydroxybenzoic acid, methyl ester	152
2	10.72	1.00	3-Methoxy-4-hydroxybenzaldehyde (vanillin)	152
3	11.52	1.04	4-Methoxybenzoic acid, methyl ester	166
4	14.90	39.17	3,4-Dimethoxybenzaldehyde (4-methoxy vanillin)	166
5	15.80	2.39	3-Methoxy-4-hydroxybenzoic acid, methyl ester (Vanillic acid, methyl ester)	182
6	16.01	3.13	Artefact (ionol)	220
7	17.77	12.70	3,4-Dimethoxyacetophenone (4-methoxy acetovanillone)	180
8	18.24	1.27	1-(3,4-Dimethoxyphenyl)-propanone	194
9	18.80	21.55	3,4-Dimethoxybenzoic acid, methyl ester (Veratric acid, methyl ester)	196
10	19.61	0.40	hydroxybenzenedicarboxylic acid, dimethyl esters	210
11	21.47	0.50	3-(3-Methoxy-4-hydroxyphenyl)-propenoic acid (Ferulic acid)	194
12	22.16	0.55	3-(3-Methoxy-4-hydroxyphenyl)-propenoic acid, methyl ester (Ferulic acid, methyl ester)	208
13	23.16	0.49	Hydroxybenzenedicarboxylic acid, dimethyl esters	210
14	23.30	0.56	3,4,5- Trimethoxybenzoic acid, methyl ester (4-methoxy syringic acid, methyl ester)	226
15	25.27	1.67	Internal standard (2,4,5-trimethoxybenzoic acid, methyl ester)	226
16	26.21	0.67	Methoxybenzenedicarboxylic acid, dimethyl esters	224
17	28.78	0.61	3-(3,5-Dimethoxy-4-hydroxyphenyl)-propenoic acid, methyl ester (Sinapic acid, methyl ester)	238
18	29.43	0.64	Benzoic acid, <i>o</i> -(<i>o</i> -Toluic), methyl ester	254
19	30.21	1.44	3-(3,4,5-Trimethoxyphenyl)-propenoic acid, methyl ester (4-methoxysinapic acid, methyl ester)	252
20	31.31	2.94	Pollution (phthalate)	
21	32.69	0.39	<i>n</i> -C ₁₆ fatty acid, methyl ester	284
22	35.76	0.40	<i>n</i> -C ₁₈ fatty acid, methyl ester	312

Table 3. GC–MS identification of products of CuO oxidation of xylain lithotype

The GC–MS data strongly supported CZE data in several ways:

- (i) The main products of alkaline CuO oxidation were phenolic compounds structurally related to lignin with a prevalence of vanillyl structures.
- (ii) Hydroxy benzenedicarboxylic acids are present in negligible quantities. Polycarboxylic benzoic acids and naphthalene structures were not recognised.
- (iii) *n*-Fatty acids in the range *n*-C₁₂ to *n*-C₁₈, especially *n*-C₁₆ and *n*-C₁₈, were identified in humoclarain sample.
- (iv) Phenylpropane units were present at trace levels, but supply useful information for non-woody contribution.

Certain small peaks represented artefacts, alkylbenzenes (SIM *m/z* 91) and alkane homologues (SIM *m/z* 57) maximising at *n*-C₂₉. These compounds could be referred to bitumens inasmuch lithotypes were not preliminary extracted and could not be considered as lignin related species.

4. Conclusions

The alkaline CuO oxidation treatment of lignite at 170 °C for 4 h, coupled with CZE identification, is a precise procedure and could be recommended as a mild, reproducible and sensitive technique for evaluation of preserved lignin structures in immature coals. The following values for the contents of preserved lignin related structures in lignite ‘Maritza-East’ were determined, in wt%: xylain, 8.5; humovitrain, 6.7; liptain, 6.4; and, humoclarain, 3.3.

The oxidative degradation of lithotypes results in lignin monomers with strong dominance of vanillyl phenols. The negligible contents of benzenepolycarboxylic acids, their hydroxy/methoxy counterparts, and the presence of preserved phenylpropane units indicate that the treatment predominantly splits β-O-4 bonds in lignins. The presence of coumaric and ferulic acids, evidenced by the two techniques, indicates non-woody tissue contribution to coal formation.

The data suggest a paleoplant community with *Gymnospermae* domination and minor non-woody plant contribution. With the advance of coalification process syringyl units were gradually removed and in humovitrain lithotype highly pure vanillic acid building units compose the coal organic matter.

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