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Investigation of the Composition of the Ethanol Extract of Brown Coal for the Isolation of Biologically Active Substances

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Abstract

The data of the group and component composition of the ethanol extract of brown coal from the Tulgan deposit of brown coal in the South Ural basin are presented. It is shown by means of liquid chromatography, ^{13}C NMR (CPMAS) spectroscopy, FTIR spectroscopy, gas chromatography–mass spectrometry that the extract contains: saturated and unsaturated hydrocarbons, alcohols, aromatic compounds, carboxylic acids and phenols. The following biologically active substances have been identified: 1-docosene; gamma-tocopherol; octadecanoic acid; ferruginol; eicosane; sugiol; beta-amyrin, concentrated mainly in the nonsaponifying fraction of the ethanol extract.

Keywords: bitumen of brown coal, biologically active substances, component composition

INTRODUCTION

Brown coal processing is one of the most important questions within the problem of the integrated use of solid combustible fossils (SCF) to obtain a number of products for practical application [1–3]. One of the promising directions of SCF processing is the extraction of bitumen by various organic solvents. Bitumen is composed of the waxy and resinous parts [4]. The waxy part – raw fossil wax – is a valuable product, its price at the world market reaches 3000 euro per 1 t [1], and after processing increases substantially. The resinous component of bitumen is related to the class of wastes at present.

Bitumen content is different for each kind of SCF: brown coal contains 5–33 %, peat 5–28 %, black coal up to 5 % [5]. The yield and composition of bitumen depend on a number of factors: the kind of SCF under treatment, the method of extraction, temperature and duration of the process, chemical nature of the organic solvent used. Extraction with the solvents the boiling point of which is below the temperature of thermal decomposition of the organic matter of SCF allows extracting the substances without changes of their composition. This is important for the investigation of the organic composition and structure of SCF [4].

Bitumen of brown coal is the source of a number of substances and products with high practical significance [6, 7]. The presence of *n*-, *iso*- and cycloalkanes, alcohols, carboxylic acids, steroids, carbonyl compounds was established in bitumen (in wax and resin). Biologically active substances (BAS) that are present in bitumen in high con-

centrations may be extracted in the pure form and used in various areas: medicine, agriculture, cosmetic industry, *etc*. [8].

The goal of the present work was to study the composition of the ethanol extract of brown coal from the Tyulgan deposit of the Southern Urals basin for the purpose of extracting BAS.

EXPERIMENTAL

The ethanol extract was obtained from the brown coal of the Tyulgan deposit in the Southern Urals basin according to Graefe's method [9]. The scheme of the separation of the ethanol extract (EE) into the saponifiable (EES) and non-saponifiable (EEN) fractions is presented in Fig. 1. The data of technical and elemental analysis of the initial and debituminized coal are shown in Table 1.

The ethanol extract was separated into narrower fractions by means of liquid column chromatography (LC) with glass columns 500 mm long, 10 mm in diameter, filled with silica gel GOST 3956–76 (the size of silica gel grains 0.2–0.5 mm). The following eluents were used sequentially during chromatographic separation: toluene, ethylacetate, butanol, a mixture of ethanol and formic acid (1 : 1). The residual fraction was extracted with hot butanol. The yield of the fractions was controlled relying on the refractive index f the eluent used [10].

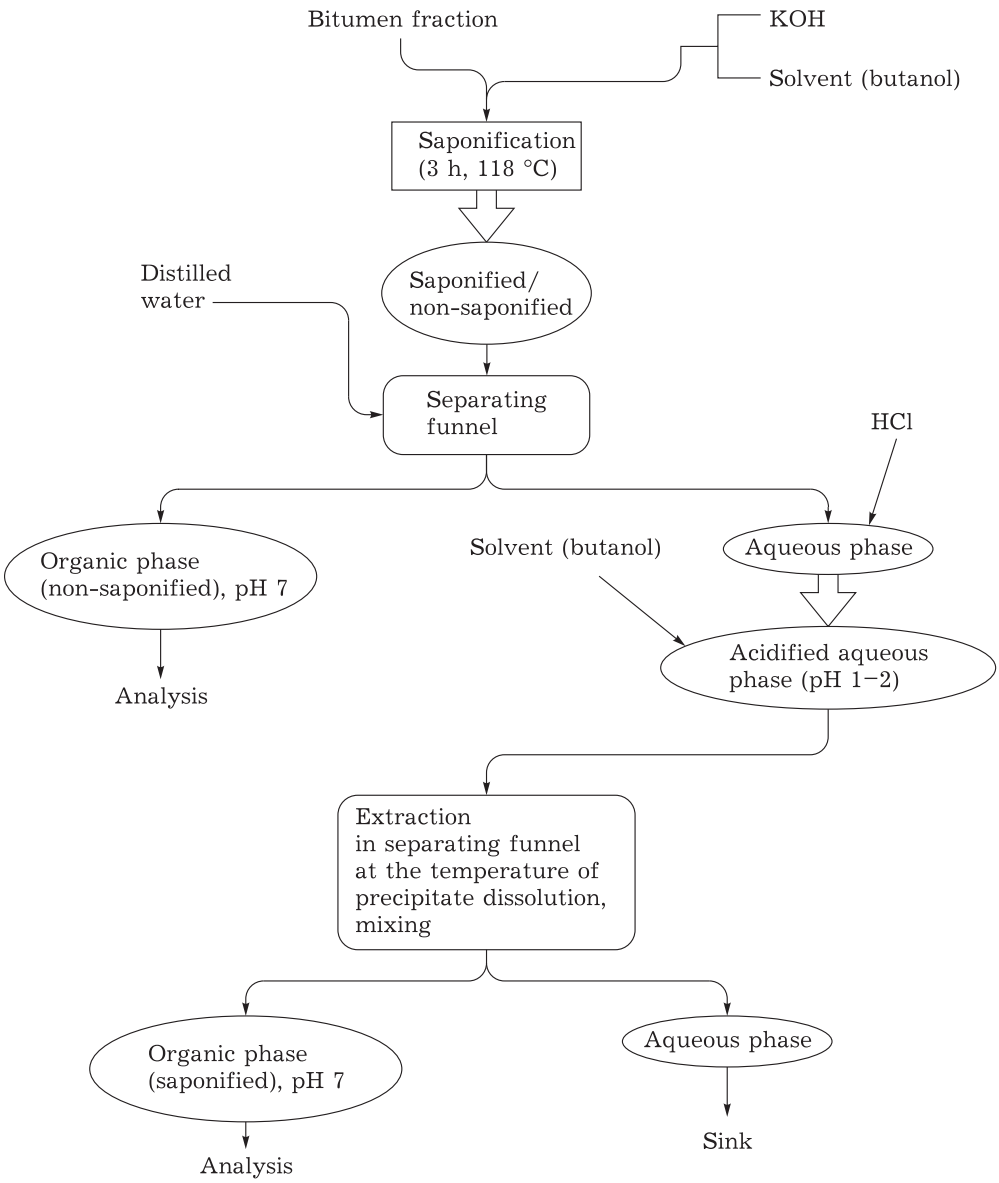

Fig. 1. Scheme of separation of ethanol extract into saponifiable and non-saponifiable fractions.

TABLE 1

Data of technical and elemental analysis of coal samples

Coal sample	W^a, %	A^d, %	V^{daf}, %	C^{daf}, %	H^{daf}, %	Atomic ratio H/C	(O + N + S), %, from difference	E^{daf}, %
Initial	9.1	21.5	65.9	57.3	6.3	1.3	36.4	12.2
Debitumized	7.5	27.0	64.4	63.6	5.9	1.1	30.5	–

Notes. 1. W^a – analytical moisture, A^d – ash content per dry sample, V^{daf} – yield of volatiles, C^{daf} – carbon content, H^{daf} – hydrogen content, E^{daf} – yield of ethanol extract, daf – dry ash-free sample. 2. Dash means the absence.

The composition of the EE of brown coal, its EES and EEN fractions and the fractions obtained by means of LC separation, was studied using the methods of IR spectroscopy (FTIR), ^{13}C NMR (CPMAS) and gas chromatography–mass spectrometry (GC-MS).

IR spectra were recorded using a Fourier spectrometer Infralyum FT-801 (Russia) with the resolution of 4 cm^{-1} accumulating 16 scans within the range 4000–550 cm^{-1} in dry KBr.

The high-resolution NMR spectra in the solid were recorded with a Bruker Physik AG WP-200 instrument (Germany) at the frequency of 75 MHz using a standard procedure of cross polarization with the suppression of proton signals and with rotation at the magic angle (CPMAS). Accumulation of 1024 scans was carried out at room temperature. The chemical shift was measured with respect to the signal of tetramethyl silane.

The GC-MS analysis was carried out with an Agilent 6890N chromatograph (the USA) with mass-selective detector Agilent 5973: capillary column HP-5ms; evaporator temperature 290 °C; solvent evaporation 4 min; flow separation 50 : 1; the rate of carrier gas helium 1 mL/min; the volume of sample for analysis 5.0 μL; programmable column temperature rise from 50 °C with the exposure for 3 min to 280 °C with the rate of 5 °C /min; exposure at 280 °C for 60 min. The content of individual compounds was recorded on the basis of the full ionic current. Identification of the component composition of the samples under study was carried out using the NIST-8 and Wiley spectral libraries. For chromatographic separation, bitumen fractions were additionally etherified with butanol.

RESULTS AND DISCUSSION

The IR spectra of the samples of the EE of brown coal, its fractions EES and EEN are shown in Fig. 2. The assignment of absorption bands in the IR spectra was carried out on the basis of published data [11–15]. It was established that EE is a complex multicomponent mixture of substances: alkanes, alcohols, phenols, unsaturated hydrocarbons, carboxylic acids, esters of unbranched saturated carboxylic acids, and a small amount of aromatic compounds. The EES fraction is represented by the substances of the groups of

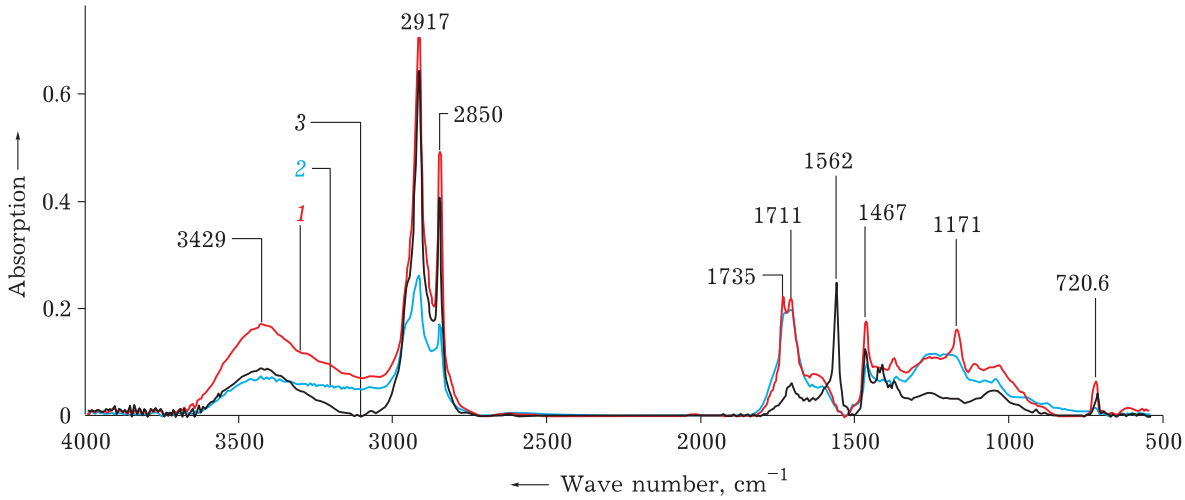

Fig. 2. IR spectra of ethanol extract (*1*) of brown coal and its saponifiable (*2*) and non-saponifiable (*3*) fractions.

TABLE 2

Integral intensities of the signals from functional groups over the ^{13}C NMR spectra (CPMAS) of extracts, %

Sample	Spectral regions of functional groups, ppm							f_a	f_{al}
	220–187	187–165	165–145	145–108	108–90	90–48	48–5		
	C=O	COOH	C_{ar-O}	C_{ar}	$C_{O-alk-O}$	C_{alk-O}	C_{alk}		
EE	2.3	2.9	1.9	6.9	2.6	11.6	70.9	8.8	85.2
EES	2.9	3.9	2.7	9.1	2.7	13.8	64.2	11.8	80.7
EEN	1.6	1.8	1.3	4.8	2.2	9.8	77.9	6.1	90.0

Note. f_a – degree of aromaticity, f_{al} – degree of aliphaticity, C – carbon atoms of corresponding functional groups.

alkanes and carboxylic acids. Alcohols present in the EES are the products of ester hydrolysis proceeding under the conditions of saponification. Alkanes and alcohols dominate in EEN. Carboxylic acids present in this fraction are the products of the decomposition of the esters of natural origin.

The integral intensities of the signals obtained by means of ^{13}C NMR spectroscopy (CPMAS) for the EE of bitumen, its fractions EES and EEN are presented in Table 2. The signal assignment was made on the basis of published sources [14, 15]. According to the data obtained, the EE sample is a mixture of alkanes, acids, alcohols and aromatic compounds.

The compositions of EES and EEN fractions according to the results of the analysis of IR and NMR spectra are represented by similar groups of compounds. In the NMR spectrum of the EES sample, a decrease in the integral signal intensity in the region corresponding to alkanes and an increase in the integral intensities of the signals from acids, phenols and alcohols in comparison with the spectrum of the EE sample are observed. The major part of aromatic compounds present in EE during the separation pass into the EES fraction.

For the EEN sample, an increase in the integral intensity of the signal in the region of alkanes is observed, while the integral intensities of signals in the regions corresponding to other groups decrease.

According to the data of ^{13}C NMR spectroscopy [16], the structural group parameters for the samples under investigation EE, EES, and EEN were calculated: the degree of aromaticity (f_a) and the degree of aliphaticity (f_{al}), %: $f_a = C_{ar-O} + C_{ar}$ и $f_{al} = C_{O-ar-O} + C_{alk-O} + C_{alk}$ (see Table 2), where C means carbon atoms of functional groups, ar is aryl group and alk is alkyl group.

Calculations showed (see Table 2) that aliphatic compounds dominate in EE, EES and EEN samples.

The group and component composition of EE, EES and EEN fractions was studied by means of GC-MS (Table 3). According to the data obtained, the compounds that are present in EE in the highest amounts are alcohols, alkenes, acids, sterols, phenols and terpenes.

The use of LC is efficient for bitumen separation. The separation of EE allowed us obtaining narrower fractions of substances that were then examined in more detail by means of GC-MS. The group assignment of the substances detected in the resulting fractions is shown in Table 4. In the fractions eluted with toluene the most completely extracted substances were those detected previously and concentrated in EEN, among which there are *n*-tetracosanol-1; begenyl alcohol; γ-sitosterol; sugiol; 13-hydroxy-14-isopropylpodocarpa-5,8,11,13-tetraene-7-on, *etc*. Ethylacetate eluates the fraction in which the prevailing com-

TABLE 3

Group composition of the ethanol extract (EE), its saponifiable (EES) and non-saponifiable (EEN) parts for the compounds with the coincidence in NIST databases above 70 %, obtained by means of GC-MS, %

Sample	Groups of substances											
	1	2	3	4	5	6	7	8	9	10	11	12
EE	0.3	3.6	1.4	1.0	0.4	7.0	1.3	1.2	1.4	–	0.1	–
EES	1.2	1.5	5.4	0.9	1.4	0.2	–	0.8	0.2	–	0.8	0.9
EEN	1.6	4.7	2.5	1.8	0.4	13.8	1.0	1.5	3.5	0.3	–	1.1

Note. 1 – alkanes, 2 – alkenes, 3 – carboxylic acids represented by their butyl esters, 4 – ketones, 5 – natural esters, 6 – alcohols, 7 – sterols, 8 – phenols, 9 – terpenes, 10 – aldehydes, 11 – amines, 12 – other. Dash means that the group of substances was not detected in the sample.

TABLE 4

Group composition of the ethanol extract for substances with coincidence in the NIST database above 70 % obtained by means of LC and GC-MS, %

Eluent	Groups of substances in EE								
	1	2	3	4	5	6	7	8	9
Toluene (fraction 1)	–	17.3	–	33.0	4.4	13.4	–	–	31.9
Toluene (fraction 2)	–	54.1	–	37.5	–	–	–	–	8.4
Ethylacetate	–	61.8	–	–	–	–	3.6	9.8	24.8
Butanol	–	80.7	6.4	–	–	–	–	–	12.9
Ethanol	–	99.9	–	–	–	–	–	–	0.1
Ethanol + formic acid (1 : 1)	–	32.8	–	–	–	–	–	–	67.2
Butanol (T = 118 °C)	2.1	50.7	1.2	11.1	–	–	–	–	34.9

Note. 1 – alkanes, 2 – carboxylic acids in the form of butyl esters, 3 – natural esters, 4 – alcohols, 5 – sterols, 6 – terpenes, 7 – ethers, 8 – amines, 9 – non-identified compounds. Dash means that the group of substances was not detected in the sample.

pounds are esters formed as a result of the esterification of carboxylic acids with butanol during preliminary treatment of the samples. Among these compounds, there are: butyl ester of hexadecanic acid; butyl ester of tetracosanic acid; butyl ester of octacosanic acid. The substances belonging to the classes of amines and ethers were detected in small amounts. The fraction eluted with butanol is composed of the substances detected previously in EES and EEN. Prevailing substances are those belonging to the class of carboxylic acids: butyl ester of hexadecanic acid; butyl-9-octadecenoate; butyl ester of hexacosanic acid butyl ester of octacosanic acid, *etc*. Ethanol eluates the fraction composed by 99.9 % of carboxylic acids, represented by butyl esters among which the relative content of butyl-9-octadecenoate was 71.9 %. The highest relative content in the fraction eluted with a mixture of ethanol and formic acid (1 : 1) is that of the butyl esters of tetracosanic (12.9 %)

TABLE 5

Biologically active substances detected in the fractions of ethanol extract by means of GC-MS

Substance title [17]	Content in sample, %	Coincidence with NIST, %
Saponifiable fraction		
Eicosane	0.2	94
1-Docosene	0.9	95
Butyl ester of octadecanic acid	0.3	95
Dibutyl ester of decadionic acid	0.6	91
Butyl ester of hexadecanic acid	0.6	99
γ-Tocopherol	0.2	87
Ferrutinol	0.2	90
Sugiol	0.3	93
Non-saponifiable fraction		
Ferruginol	0.7	87
Butyl stearate	0.3	96
n-Butyllaurate	0.2	98
Butyl ester of hexadecanic acid	0.7	99
Butylmyristate	0.4	99
Ceryl alcohol	4.0	99
Lignoceryl acid	4.5	99
n-Tetracosanol-1	0.3	93
β-Amirine	0.4	92
Sugiol	0.7	95
14-Isopropylpodocarpa-8,11,13-triene-7β,13-diol + totarol	0.9	90

and hexacosanic acid (11.1 %). Hot butanol eluates the fraction composed of the substances that were previously detected in the saponifying and non-saponifying components of EE, which have the following relative content: the ester of octacosanic acid (11.6 %); the butyl ester of tetracosanic acid (6.42 %); the butyl ester of hexacosanic acid (7 %); butyl-9-tetradecenoate (7.9 %); the butyl ester of triacontanic acid (8.4 %).

Some BAS were identified in the fractions of EE, EES, EEN; these compounds are present in the samples in substantial concentrations (Table 5).

CONCLUSION

Ethanol extract is a complex multicomponent mixture composed of free and substituted alkanes, unsaturated hydrocarbons, alcohols, a small amount of aromatic compounds, acids and phenols. Aliphatic substances dominate in EE samples and its saponifiable and non-saponifiable fractions. The EES fraction is represented by carboxylic acids, esters, the fragments of the organic mass acid in nature, phenols and alcohols – the products of ester hydrolysis under the conditions of saponification. The resulting EEN fraction is mainly composed of alkanes, alcohols and the neutral fragments of the organic mass.

The applied column LC is efficient for the separation of bitumen into narrower fractions. Ethanol was used to eluate the fraction composed by 99.9 % of carboxylic acids in the form of butyl esters, with the relative content of butyl-9-octadecenoate 71.9 %.

BAS identified in the bitumen extracted with ethanol from the brown coal of the Tyulgan deposit include: 1-docosene; γ-tocopherol; butyl ester of octadecanic acid; ferruginol; eicosane; sugiol; β-amyrine; butyl stearate; ceryl alcohol; *n*-butyllaurate; *n*-tetracosanol-1. The major part of BAS is concentrated in the EEN fraction.

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