Nitrogen Compounds in the Lipids of the Recent Sediments (for the Lake Utichye-3 in Khakassia as an Example)

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Abstract

The distribution and composition of nitrogen-containing organic compounds of lipids in the recent sediments of continental origin are investigated for the Lake Utichye-3 as an example. It is established that nitrogen compounds are represented in free lipids mainly by basic and neutral components. It is shown that secondary and/or tertiary aliphatic amines and main amino acids are present in the composition of the bases.

INTRODUCTION

Solution of a number of fundamental and applied problems connected with the development of the theory of oil and gas formation, prediction of oil- and gas content of the Earth's interior and the quality of the hydrocarbon raw material is substantially dependent on the amount and profoundness of information on the composition of the initial organic matter (OM) and the main regularities of its transformation during the formation of oil- and gas-source beds.

Among a very large number of OM components, the main part in the formation of oil is played by lipids. Fossilized lipids are the initial material, the polymerization of which leads to the formation of kerogen, while destruction of kerogen in catagenesis zone results in the generation of oil compounds [1].

The results of investigations of the hydrocarbons of lipid complex are rather widely discussed in publications [2, 3]. The information about the nature of heteroorganic compounds is very scarce. The present paper deals with the nitrogen-containing components of the lipids of recent sediments. The organic compounds of nitrogen are incorporated in almost any kinds of oil. Their genesis is one of the most complicated problems of the modern theory of oil origin. Nitrogen-containing compounds of recent sediments are considered as possible sources of oil nitrogen components [4].

EXPERIMENTAL

Investigations were carried out with the lipids of sediments of the Lake Utichye-3 (Khakassia). The salt content of the lake water is 7.9 g/l. The main suppliers of the OM in the lake are green alga (*Cladophora*), animal plankton (*Gammarus lacustris*) and the terrigenous OM [5]. Judging from the conditions of sediment accumulation (aqueous-alkaline medium, contamination with hydrogen sulphide) [6], the lake is a typical representative of continental basins of reductive facies. Attention to the investigation of this type of sediments is due to the fact that during the recent years they are widely and reasonably considered as potential petroleogenetic beds [7].

The free and bound (carbonate, hydrolyzed) forms of lipids were isolated from the native

lacustrine sediments sampled in summer from the depth of 20–40 cm. The free lipids were obtained by means of cold extraction *via* percolation of the extragent through the layer of air-dry sediment at the sediment to extragent ratio equal to 1 : 3. A methanol-chloroform mixture was used as an extragent. The above system of solvents is an optimal one for isolating lipids from the recent low-maturity sediments [8]. We established that the maximal nitrogen fraction extracted is achieved at the components volume ratio in the extracting mixture equal to 1 : 1.

Then, using the procedure described in [9] the carbonates were destructed and lipids bound with them were extracted. The residue was treated with a solution of an alkali to break ester bonds according to the procedure described in [10], and hydrolyzed lipids were extracted.

In order to concentrate nitrogen compounds, we used liquid adsorption chromatography on silica gel L 100/160 at the sample to adsorbent mass ratio of 1 : 50. Non-polar compounds were desorbed with a mixture of hexane and benzene (9 : 1 by volume), polar ones with a mixture of methanol and chloroform (1 : 1 by volume).

Nitrogen bases were isolated using solvent extraction by a 0.1 M aqueous HCl solution. The product was fractionated with the help of thin-layer chromatography on standard Silufol plates in the solvent systems chosen experimentally in the presence of model compounds. The detection of separate spots was carried out with reagents prepared using the respective procedures [11].

Total nitrogen (N_{tot}) was determined by means of combustion in Pokrovskiy's reactor [12], basic nitrogen (N_{bas}) by means of non-aqueous potentiometric titration with dioxane solution of chloric acid [13] and alcohol solution of an alkali [14], respectively.

The IR spectra were recorded using a Specord (M-80) spectrometer in a chloroform solution film. Electron spectra were obtained using an Uvikon 943 spectrophotometer. The ^{13}C and ^{14}N NMR spectra were recorded using a Bruker DRX 500 spectrometer (36.13 MHz) in $CDCl_3$, chemical shifts of the signals were measured in ^{14}N spectra using dissolved molecular nitrogen as a reference, and for ^{13}C spectra – the TMS signal.

RESULTS AND DISCUSSION

The results of investigations provide evidence that the total lipid fraction of the lacustrine sediments is 1.13 % (Table 1). In this fraction, free lipids account for 53 %, carbonate and hydrolyzed ones for 34 and 13 %, respectively. Nitrogen compounds are the components of all the forms of lipids. Their highest total content (47 %) was observed in free lipids. The carbonate form exhibits 21 % and hydrolyzed form accounts for 6 % of nitrogen compounds.

A detailed characterization of nitrogen compounds was obtained for free lipids (below referred to as the lipids), the composition of which to a greater extent depicts diagenetic processes of the OM transformation [2]. The results of potentiometric determination of the group composition of nitrogen-containing organic compounds [13] indicated that in lipids they are represented mainly by basic and neutral components (see Table 1). In the mixture of nitrogen compounds, the bases account for 18 %. Using the titration with the addition of

TABLE 1

Nitrogen content of lipids from the Lake Utichye-3

Parameter	Sediment	Lipids		
		free	carbonate	hydrolyzed
Yield, %	100.0	0.60	0.38	0.15
Content of N_{tot}, %:				
absolute	0.74	0.55	0.39	0.30
relative	100.0	47.1	21.2	6.4

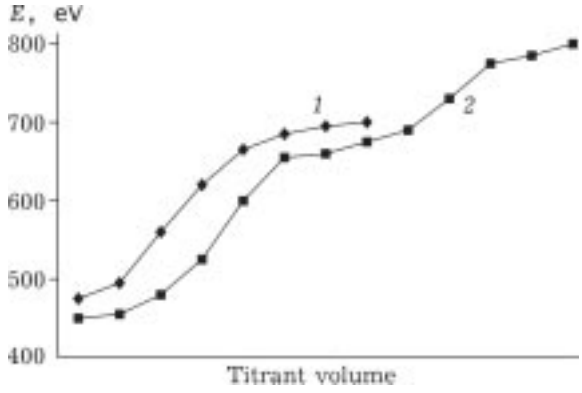

Fig. 1. Potentiometric titration of free lipids: *1* – free lipids, *2* – free lipids + diethyl amine + quinoline.

model compounds (diethyl amine, quinoline), it was established that the organic bases of lipids are strong-basic components similar to saturated amines (a sum of aliphatic and hydrogenated heterocyclic amines) (Fig. 1, curve *1*). In the mixture with diethyl amine, the bases of lipids are titrated together, while in the mixture with quinoline an additional knee corresponding to the amount of the added aromatic base appears on the titration curve (see Fig. 1, curve *2*).

Absorption bands of the associated amino group (3400–3100 cm^{-1}) are observed in the oscillation spectrum of the lipid concentrate. Neutral nitrogen compounds can include amides (with the carbonyl group of amides absorbing in the region 1689–1660 cm^{-1}). The presence of absorption at 3440 cm^{-1} corresponding to the NH group of the pyrrol ring, and a series of bands in the region 420–750 nm of the electron spectrum allow assuming that the nitrogen-containing organic compounds of lipids contain also tetrapyrrol fragments, which were previously detected in the lipids of recent sediments [15].

The results of chromatographic separation of the lipid concentrate on silica gel provide evidence that almost all the nitrogen-containing organic compounds of lipids (98 %) are polar components (Table 2). 96 % of basic nitrogenous compounds and 98 % of non-basic ones are related to the polar lipids.

Under the conditions of the performed acid extraction, low-molecular nitrogen bases are isolated [16]. These bases are the best-studied group of nitrogen-containing components of oil. However, a reliable biological predecessor has not been found yet for any of the individual compounds detected in oil.

As it follows from the data shown in Table 2, low-molecular nitrogen compounds account for 82 % of the basic compounds of the initial lipids. Judging from the character of the curves of potentiometric titration and from the data of qualitative IR spectroscopy, the isolated bases correspond to saturated amines. The presence of low-molecular aliphatic and/or hydrogenated aromatic structures containing an amino group is confirmed by the results of nuclear magnetic resonance spectroscopy. The ^{14}N NMR spectrum contains an intensive signal at 65 ppm belonging to the nitrogen atom of saturated amino compounds. Saturated structures prevail in the lipid concentrate, as evidenced by high intensity of the signals in ^{13}C NMR spectra in the strong field region (13 ppm and 25–34 ppm) and by the absence of signals characteristic of the aromatic carbon atoms (118–140 ppm).

Some compounds of the concentrate of nitrogenous bases contain a carboxyl group. This

TABLE 2

Extraction separation of polar lipids

Product	Yield, %	Content, %							N_{bas}/N_{tot}, %
		N_{tot}		N_{bas}		N_{neutr}		$COOH^-$	
		abs.	rel.	abs.	rel.	abs.	rel.		
Free lipids	100.0	0.55	100.0	0.10	100.0	0.45	100.0	–	18.2
Polar lipids	69.1	0.78	98.3	0.14	96.7	0.64	98.3	–	17.9
Concentrate of nitrogen bases	11.2	0.70	14.3	0.73	81.8	Absent	–	1.74	100.0

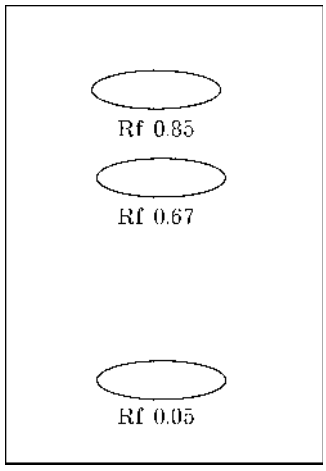

Fig. 2. Thin layer chromatography of the concentrate of nitrogen bases.

is confirmed by the data of potentiometric determination of $COOH^-$ (see Table 2) and qualitative IR and ^{13}C NMR spectroscopy. The absorption related to the carbonyl group of acids (1710 cm^{-1}) manifests itself in the IR spectrum of the concentrate; a signal at 178 ppm characteristic of the carbon atoms of the carbonyl group is observed in ^{13}C NMR spectrum.

Separation of nitrogen bases by means of thin layer chromatography in hexane – chloroform system (1 : 3 by volume) allowed obtaining three fractions differing in chromatographic mobility (Rf) (Fig. 2). The qualitative IR spectroscopy did not reveal differences in the composition of the fractions obtained. They are characterized by an intensive absorption of amino groups (3400–3100, 1660 cm^{-1}) and the carbonyl group of acids (1710 cm^{-1}) [11]. The application of iodine vapour as a universal detector showed that only two fractions – Rf 0.05 and Rf 0.67 – contain the substances of lipid nature [11]. The compounds of the fraction characterized by the highest chromatographic mobility (Rf 0.85) may have been co-extracted during the isolation of the lipid complex from the sediment and may be of the protein nature. A strong connection between the lipid and protein substances was marked in [4]. The development of the fractions of lipid components (Rf 0.05, Rf 0.67) with the Dragendorf reagent confirms that they contain amino compounds. This is indicated by the orange colour of the corresponding chromatographic zones [11]. The absence of characteristic colouring of the zones after development with ninhydrin proves that the structures with secondary and/or tertiary amino groups are predominant among the amino compounds [11]. The electron spectra of the lipid fractions in ethanol contain absorption bands at 200–250 and 250–300 nm (Fig. 3). After dilution in alcohol, the band at 200–250 nm shifts to shorter wavelengths. This is shown in Fig. 4 where the second derivatives of the spectra of the fraction with Rf 0.05 recorded after dilution are represented. The disappearance of the band at 247 nm is likely to point to the destruction of possible intermolecular interactions in which the compounds of these fractions participate.

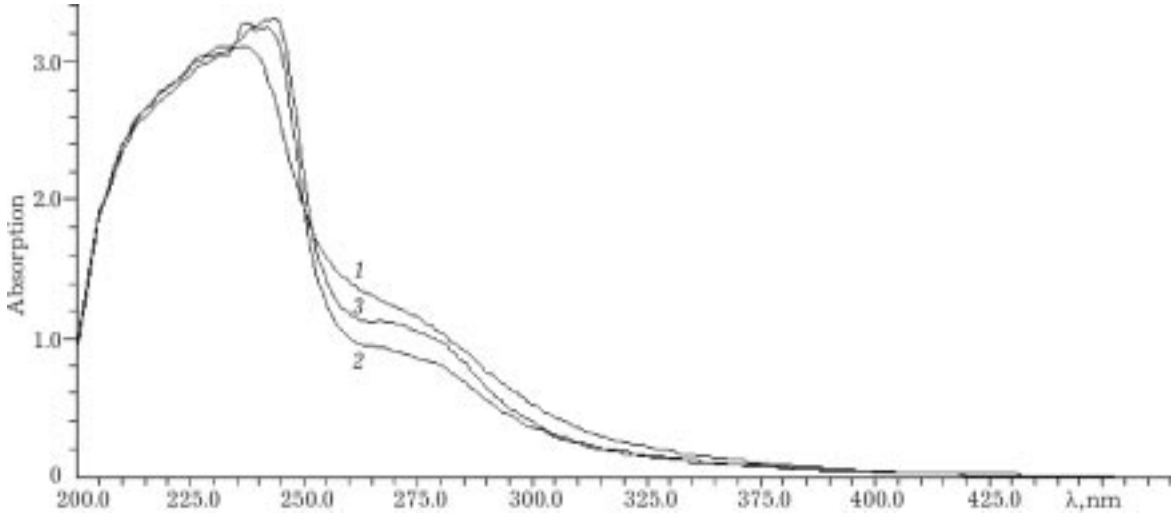

Fig. 3. Electron spectra of lipid fractions of nitrogenous bases: *1* – fraction Rf 0.85; *2* – fraction Rf 0.67; *3* – fraction Rf 0.05.

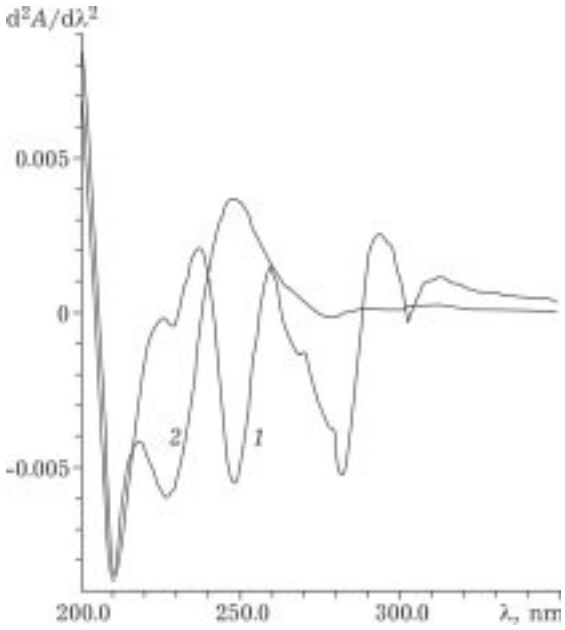

Fig. 4. Graphic presentation of the second derivative of spectra of Rf 0.05 fraction for the sample concentration *C*, g/ml: *1* – 0.001, *2* – 0.0007.

It follows from the analysis of the difference spectra that the concentration of these compounds is higher in the sample with Rf 0.05 than in the sample with Rf 0.67. This is confirmed by higher intensity of the bands related to $\pi - \pi^*$ and $n - \pi^*$ transitions in the compounds which exhibit the lowest chromatographic mobility (Fig. 5).

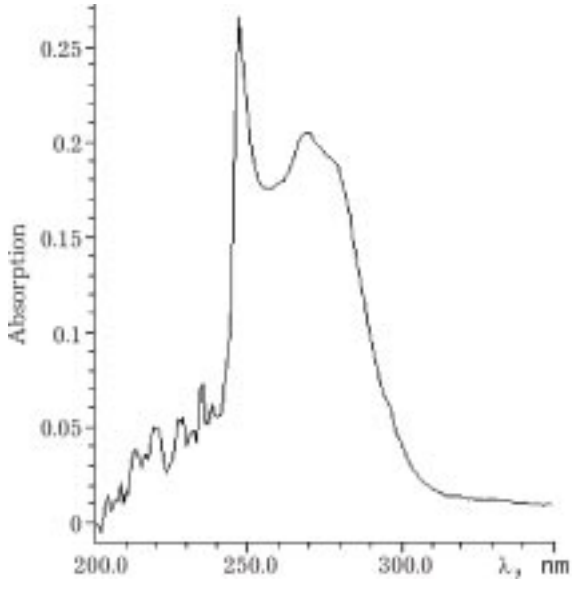

Fig. 5. Difference spectrum of Rf 0.05 fraction in alcohol.

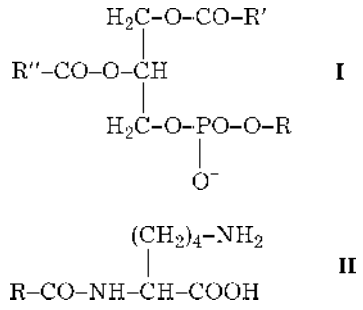

Fig 6. Natural sources of nitrogen in the lipids of the recent sediments.

Thus, the results of our investigation allow us to assume that the bases of lipid fractions are saturated amines and basic amino acids. Aliphatic structures should be predominant among the amines. Under the conditions of sediment formation (aqueous alkaline medium), aliphatic amines and amino acids can be formed from phospholipids **I** and amino acids **II** of the lipid complex of the initial OM (Fig. 6).

The presence of aliphatic amines was not established in oils recovered in West Siberia. The analysis of literature data and the results of our own investigations indicate that the nitrogen bases of oil occurring in this region are represented by the aromatic heterocyclic structures [17]. It is natural to assume that primary and secondary amines of the oil-source matter, being reactive, were undergoing further transformations.

CONCLUSIONS

It was established as a result of investigations that the nitrogen compounds of lipids of the sediment of continental genesis are distributed over free and bound forms. Free lipids are characterized by the maximal content of these compounds. They are represented by basic and neutral components.

The larger part of nitrogen bases of the lipids is comprised by low-molecular components. They include secondary and/or tertiary aliphatic amines, as well as basic amino acids. Phospholipids and amino acids of the lipid complex of the initial organic matter can participate in the formation of these nitrogen compounds. Primary and/or secondary amines were not detected in West-Siberian oils. Therefore,

the structure of these compounds was not inherited. They undergo substantial changes during the formation of oil systems. Amides and tetrapyrrol pigments are identified among the neutral nitrogen components.

The results obtained are the basis for experiments modelling the processes involved in the formation of nitrogen-containing compounds of oil.

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