Compositional study of mumiyo NOM using CHN elemental analysis and solution-state one- and two-dimensional NMR spectroscopy

Andrey I. KONSTANTINOV[a*], Yuri K. GRISHIN[a], Mikhail I. SAVINYKH[b], Irina V. PERMINOVA[a]
[a]Lomonosov Moscow State University, Department of Chemistry, Moscow, Russia. [b]Research-and-production company "Sibdal'mumiyo" Ltd, Novokuznetsk, Russia
* Tel. & Fax. No. +7-495-939-5546; E-mail: konstant@med.chem.msu.ru
Keywords Mumiyo; humus; biomolecules; CHN composition; NMR.

Abstract Thirteen mumiyo samples originated from different geographic regions were characterized with a use of elemental analysis and solution-state ^{1}H, ^{13}C and two-dimensional HSQC NMR spectroscopy. Structural NMR study demonstrated that mumiyo consists of humic-like matter, as well as low-molecular components of carbohydrate, peptide, fatty acid and aromatic nature. Compositional likeness of mumiyo organic matter and products of microbial degradation of petroleum was shown on a base of elemental analysis data.

Introduction

Native mumiyo (also known as shilajit and several less prevalent regional names) commonly used in traditional medicine is a pale-brown to black resinous matter, of characteristic balsamic odour, that seeps from rock layers of different mountain regions (Agarwal et al., 2007; Wilson et al., 2011). Comprising humic-like substances and different non-humic components it is considered to be a complex product of biotransformation of plant remains, alpine rodent faeces, or microbial transformation of mountain petroleum products (Schepetkin et al., 2002). Consequently, different biomolecules were found to be constituents of mumiyo (Agarwal et al., 2007), including fatty acids (Kiseleva et al., 1996), free and bound amino acids (Kiseleva et al., 1998), polyphenols and terpenoids (Ali et al., 2004). The purpose of this work was an integral compositional study of mumiyo originated from different geographic regions using CHN elemental analysis and NMR spectroscopy.

Materials and methods

Thirteen mumiyo samples originated from Himalaya (India), Altai (Russia and Mongolia), south Yakutia (Russia) and Central Asia (Kazakhstan, Kyrgyzstan, Uzbekistan and Afganistan) were studied. The samples were pounded, dissolved in distilled water, centrifugated and dried in a vacuum oven at 40°C. That way dried mumiyo extractions (DME) were obtained.

Elemental analysis was conducted on Euro EA CHN elemental analyzer.

^{1}H and ^{13}C solution state NMR spectra were acquired using Avance 400 spectrometer (Bruker). To exclude the Nuclear Overhauser effect while recording ^{13}C NMR spectra the pulse program INVGATE were used. A two-dimensional HSQC NMR spectrum was registered on Agilent 400-MR spectrometer. All the spectra were recorded on the DME samples dissolved in 99,9% D_2O at concentration of 80 mg/ml with a 5 mm broadband probe.

Results and Discussion

A van Krevelen diagram plotted on a base of ash-free elemental analysis data shows that mumiyo samples occupy area of chemical space between carbohydrates and peptides (Figure 1). Three averaged samples of algarites described (Uspensky et al., 1947), considered as a product of microbial transformation of paraffinic petroleum, were located in the same area. On the diagram, both mumiyo and algarites are separated from typical humic materials with the line passing through the *Carbohydrates* and *Lignins* dots.

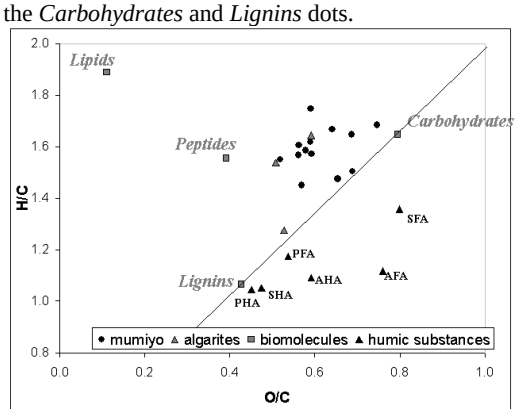

Fig. 1 Van Krevelen plot of elemental analysis data of mumiyo, compared with: biomolecules (medians) according to (Perdue and Ritchie, 2003); peat, soil and aquatic humic substances (medians) (Rice and McCarthy, 1991); and three averaged samples of algarites (water-soluble matter, without of bitumen, was analized) (Uspensky et al., 1947).

Content of nitrogen evaluated on ash-free basis for mumiyo samples were 3-10 % (wt), and the median came to 7.4 %. The median value obtained was twice as much than that of nitrogen-rich soil humic acids amounted 3.7 % (Rice and McCarthy, 1991).

Typical ^{1}H and ^{13}C NMR spectra of mumiyo shown on Figure 2 were characterized with broad overlapping bands that is characteristic for NMR spectra of humic substances, and intensive sharp signals indicating non-humic low-molecular organic components of mumiyo.

^{1}H and HSQC NMR signals obtained were attributed to fatty acids, carbohydrates, low-molecular aromatic compounds, and nitrogenous compounds (such as amino acids). The ^{13}C NMR spectra recorded also allowed to identify in the structure of mumiyo (poly)phenols as well as an abundance of carboxyl groups and peptide bonds.

Integration of the ^{1}H and ^{13}C NMR spectra of mumiyo according to specific intervals of chemical shift provides the amounts of carbon and non-exchangeable hydrogen (Table 1). The samples

studied were characterized with 25-30% of hydrogen attributed to carbohydrate and aliphatic nitrogenous structures and 16-25% of aromatic hydrogen. High content of alkyl long-chain protons (CH_n) amounted 30-40%, as compared with hydrogen of aliphatic units in α-position to sp^2 carbon atoms (α-CH_n) amounted 14-20%, indicates low biodegradation degree of mumiyo organic matter. Portions of carbon distributed among alkyl (CH_n), carbohydrate along with aliphatic nitrogenous (CH_n-O,N) and aromatic (C_{AR}) structures were approximately equal amounting about 20-26%. Parts of phenolic carbon as well as carboxylic and amide (peptide) structures came to 7-10% and 11-15% correspondingly.

Table 1 Distribution of carbon and non-exchangeable hydrogen among the main structural groups of mumiyo samples (quartiles of 25% and 75% and medians) as determined from their ^{13}C and ^{1}H NMR spectra.

	Range of $\delta(^{13}C)$, ppm						
	0-50	50-90	90-110	110-145	145-165	165-185	185-220
	Structural units						
	CH_n	CH_n-O,N	OCO	C_{AR}	C_{AR}-O,N	(C=O)-O,N	C=O
	Proportion of carbon in structural units, %						
$Q_{25\%}$	19.8	20.8	3.5	21.9	6.9	11.4	3.0
Median	24.0	21.9	4.6	24.5	8.3	12.4	3.7
$Q_{75\%}$	26.5	24.0	5.3	25.6	10.1	14.5	4.5

	Range of $\delta(^{1}H)$, ppm			
	0-1.9	1.9-3.2	3.2-4.6	6.0-11.0
	Structural units			
	CH_n	α-CH_n	CH_nO,N	H_{AR}
	Proportion of non-exchangeable hydrogen in structural units, %			
$Q_{25\%}$	30.5	14.1	25.5	16.2
Median	32.5	14.7	28.9	22.4
$Q_{75\%}$	41.4	19.7	29.9	25.0

Thus, NMR spectroscopic study of mumiyo originated from different geographic regions displayed that water-extractable organic matter of mumiyo is a complex mixture of humic-like substances and biomolecules, such as carbohydrates, peptides, polyphenols, lipids and carboxylic acids, representing microbial, plant and animal metabolites. Elemental compositional analysis demonstrated that mumiyo organic matter, as compared to humic substances, is characterized with essentially higher nitrogen content. Elemental compositional likeness of mumiyo organic matter and products of microbial degradation of petroleum (algarites) was shown. The letter suggests that initial role of bioconversion of petroleum in mumiyo formation processes is to be considered.

We thank Prof. G.N. Pilipenko and Dr G.N. Vladimirov for their help in mumiyo samples collection.

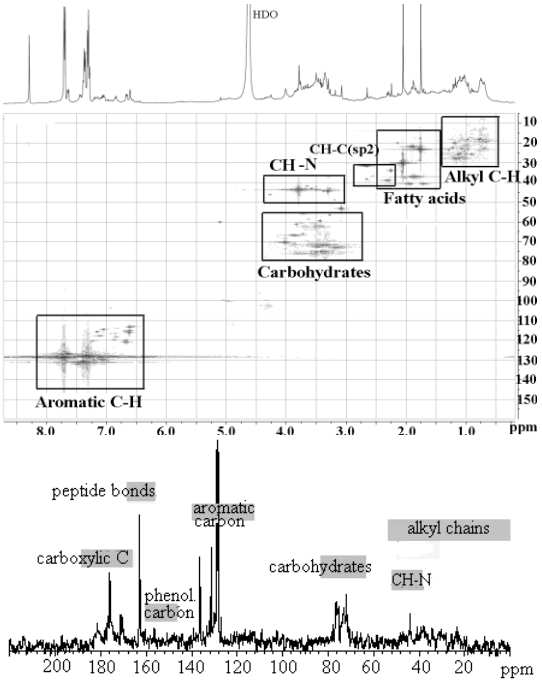

Fig. 2 ^{1}H (top), two-dimensional {^{1}H, ^{13}C} HSQC, and ^{13}C (bottom) NMR spectra of mumiyo. Ascriptions of spectral signals are indicated.

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