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STUDY OF HUMIC ACIDS IN VARIOUS TYPES AND KINDS OF PEATS AT KHANTY-MANSI AUTONOMOUS AREA - YGRA BY NMR ^{13}C SPECTROSCOPY

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Abstract:

This article presents the results of humic acid studies in peat resources of the Khanty-Mansi Autonomous Area - Yugra by NMR ^{13}C spectroscopy. Their differences were revealed in the distribution of carbon atoms along structural fragments which allowed to diagnose the peculiarities of the drug molecular structure, depending on the botanical composition of the original peat and its degree of decomposition. It was found that humic acids of various peats have minor differences in the content of C_{al} and C_{arom} . The resulting spectroscopic information was used for an in-depth analysis of the features concerning peat humic acid structure of various origin.

Keywords: Humic acids, NMR ^{13}C spectroscopy, The Khanty-Mansi Autonomous Area - Yugra, peat-forming plants.

1. Introduction

For two decades, the main field of NMR ^{13}C spectroscopy application for the analysis of humic acids will be the quantitative characteristic of a relative carbon content in the various structural fragments.

There are two basic problems of quantitative structural information obtaining from NMR spectra of humic acids:

1. The correct division of humic acid spectra into integrated ranges, the minimization of random errors during integration;
2. The registration of humic acid spectra under conditions ensuring the compliance with the integrated signal intensities of different types of nuclei in the NMR spectrum of their content in a sample. Currently the experts skilled in the field of natural organic material chemistry attract unique possibilities of ^{13}C NMR spectroscopy, which is the most powerful method for quantitative analysis and the study of multicomponent system structure, including humic acids [1,2]. Wide opportunities of ^{13}C NMR spectroscopy to characterize the structural features of humic acids of

various origin were demonstrated repeatedly [3,4,5,6]. An exceptional information level is conditioned by the fact that the method makes it possible to identify the groups of broadened spectrum signals the integrated intensities of which allow to calculate the content of various structural fragments in humic acid macromolecules. The comparison of labor costs for the chemical determination of functional groups with those by NMR-analysis showed that the latter is 100 times better according to analysis time, and 10 times better according to cost.

2. Subjects and Methods of Study

Peat samples were collected from the peats typical for the region during autumn period, taking into account the description of wetland systems in Western Siberia [7]. The extraction of humic acids was performed according to a previously described method [8]. ^{13}C NMR spectra were obtained at the Novosibirsk Institute of Organic Chemistry using DRX-500 spectrometer (manufactured by Bruker) at the frequency of 125.76 MHz. In order to obtain 20-50 mg of a sample 0.5 M NaOH/D₂O were dissolved in 0.5 ml and placed in a vial with an external diameter of 5 mm. In order to exclude Overhauser nuclear effect the record of ^{13}C NMR spectra was carried out with the suppression of protons in INVGATE mode. TMS was used as an external standard. Spectra were recorded with 1 second delay between pulses. The integration of ^{13}C NMR spectra was carried out in normal ranges, within which the integration of humic acid ^{13}C NMR spectra is performed, although sometimes their borders differ by 5-10 ppm. The standard relative deviation of 10 time manual integration does not exceed 3%. The botanical composition and the degree of peat decomposition were determined using modern microscopic equipment.

3. Study Results

During the determination of humic acid structural-group composition, we considered a set of fragments, the content of which is quantifiable, thereby we responded to the question about the specifics of nuclear and peripheral part of humic acid macromolecule structure.

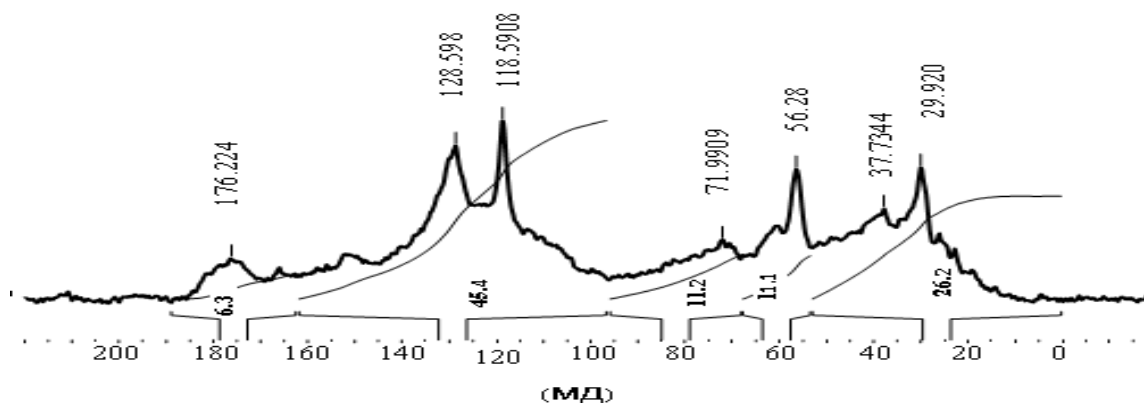


Figure 1 - ^{13}C NMR of grass transition peat humic acid.

One may distinguish here the signal areas of aliphatic (0-65 ppm) and aromatic carbon atoms (90-160 ppm).

They clearly demonstrate and quantify aliphatic and aromatic carbon atoms. The integration of spectrum within specified intervals ppm allows to quantify the relative content of carbon in aliphatic and aromatic fragments (Table 1).

Table 1 – The relative content of carbon atoms in the structural fragments of humic acids, %.

R, %	Carboxyl carbon 160-200 ppm	Aromatic carbon 90-160 ppm	Polysaccharide carbon 65-90 ppm	Aliphatic carbon 0-65 ppm
Sphagnum peats				
5	9,6	29,0	9,9	51,7
10	8,9	33,0	7,9	50,3
15	12,4	38,6	9,1	42,2
20	7,5	36,2	7,4	48,9
30	8,8	38,6	5,5	47,1
35	6,9	42,0	7,9	43,2
40	7,9	43,6	8,5	39,9
55	4,7	36,3	13,3	45,7
Sedge peats				
10	6,1	44,7	2,8	46,4
15	9,6	33,2	5,8	51,5
25	1,0	4,10	6,9	4,8
30	7,2	41,0	9,3	42,5
35	8,4	40,0	5,0	47,1
40	6,9	47,0	3,5	42,6
45	10,0	44,7	5,8	39,5
50	7,5	40,1	9,1	43,3
55	7,5	40,7	5,3	46,5
Grass peats				
15	8,7	33,3	7,0	50,3
25	8,0	36,9	6,7	48,6
30	6,1	48,1	5,9	40,0
35	6,2	43,9	10,8	37,6
40	5,1	48,4	8,2	38,3
45	6,3	45,4	11,2	37,1
55	7,2	47,3	8,25	37,3
60	8,6	51,1	5,9	34,8

Grass-sphagnum peats				
10	7,7	41,2	10,4	40,7
35	9,1	39,2	9,6	42,2
65	8,9	43,5	7,4	40,2
Wood peats				
10	6,9	34,9	9,2	49,0
15	10,9	28,5	5,9	54,6
25	8,9	38,8	5,9	44,6
45	7,9	32,1	6,3	53,7
50	5,6	38,5	10,6	45,3
Wood-grass peats				
30	6,1	39,1	4,4	50,3
35	8,6	38,6	4,9	47,8
65	7,1	42,0	9,1	41,8
Peat litter (hypnum mosses)				
10	10,0	34,3	6,1	49,7

Note: R – decomposition rate, ppm – part per million

^{13}C NMR spectra are characterized by broad absorption bands which are conditioned by the overlapping of a large number signals.

The integration of ^{13}C NMR spectra in certain ppm intervals allowed to distinguish and quantify the ratios of the following macromolecule fragments: alkyl (aliphatic) carbon (0-0,65 ppm), the carbon in carbohydrates, alcohols, acetals, called polysaccharide one (65-90 ppm) for brevity, aromatic carbon (90-160 ppm) and carboxyl carbon (160-200 ppm).

These are the usual intervals, along which the integration of the ^{13}C NMR spectra of humic acids is carried out, although sometimes their borders differ by 5-10 ppm. The relative standard deviation of results concerning a 10-time manual integration does not exceed 3%.

The above-described conditions recorded ^{13}C NMR spectra of 37 humic acids. The spectrum quantitative analysis results concerning all studied humic acids presented in the table show the distribution of carbon atom along four major structural fragments: aromatic, aliphatic, carboxyl and polysaccharide carbon.

^{13}C NMR spectra clearly demonstrate and quantify aliphatic and aromatic carbon atoms ($\text{C}_{\text{aliph.}}$ and $\text{C}_{\text{arom.}}$) related to ethereal oxygen. These relations may belong to a wide variety of functional groups - phenol, alcohol, ether, ester,

anhydride, lactone and carbohydrate ones. The number of fragments $C_{\text{aliph.}}-O$ prevails a bit over the number of $C_{\text{arom.}}-O$.

In the range of 90-160 ppm the signals are present characteristic for aromatic carbon atoms ($C_{\text{arom.}}$) in condensed systems which are quite well presented for all samples of humic acids.

The aromaticity of humic acids, isolated from the peats Khanty-Mansi Autonomous Area - Yugra, is quite high. It was found that the method of humic acid extraction makes a significant impact not only on the functional group content. It is reflected on the nuclear, i.e. aromatic moiety of a humic acid. Depending on the nature of the alkali used for the extraction the humic acid spectra demonstrate the changes in the distribution of resonance signal intensities concerning aromatic carbon atoms. When a humic acid is extracted by a weaker base (NH_4OH) the decrease of $C_{\text{arom.}}$ is detected, and at the extraction of $NaOH$ humic acid contains more alkyl moieties and the total aromaticity is higher as well as the number of cycles in its molecule.

The highest content of $C_{\text{arom.}}$ (51.1%) is observed in the buck-bean lowland peats ($R = 60\%$), and the lowest content of $C_{\text{arom.}}$ (23.7%) in the sphagnum top peat ($R = 0\%$). The average value of $C_{\text{arom.}}$ content in humic acids of Khanty-Mansi Autonomous Area - Yugra makes 43%.

The comparison of results obtained by NMR ^{13}C spectroscopy method and the elemental analysis conducted by us leads to the same conclusions about the peculiarities of the peat humic acid studied samples [9,10] (Fig. 2).

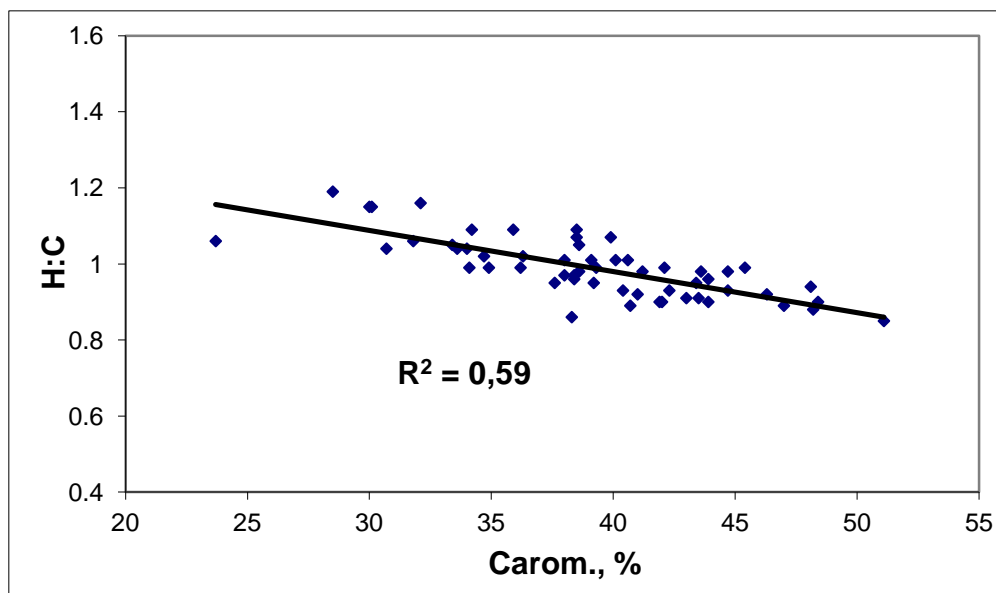


Figure 2 – The relation of atomic ratios H:C and ^{13}C NMR spectroscopy of peat humic acids.

The aliphatic portion of ^{13}C NMR spectra in the range from 0 to 65 ppm contains well resolved signals indicating the presence of branched aliphatic structures. The samples of sphagnum humic acids contain more resolved signals in this part of the spectrum than grass peat humic acids. Wood peats have average values.

Table 1 and Figure 3 demonstrate that the humic acids of various types and kinds of peats have the following pattern:

with a consistent increase of $C_{\text{arom.}}$ percentage content, the reduction of $C_{\text{aliph.}}$ content takes place in the same, but decreasing sequence. Figure 3 shows that the overall correlation between aromatic and aliphatic content for this natural object is quite high and makes $\approx 0,8$.

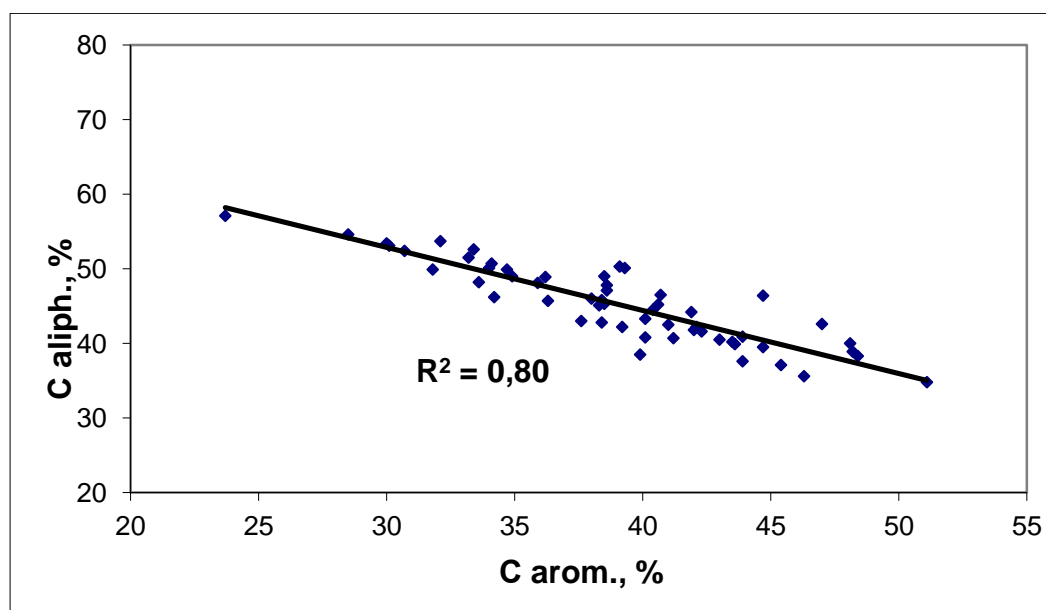


Figure 3 - Correlation of $C_{\text{arom.}}$ and $C_{\text{aliph.}}$ content in the humic acids of Khanty-Mansi Autonomous Area - Yugra peats according to ^{13}C NMR results.

The same dependence is clearly seen separately in botanical groups. It is the most pronounced among sedge peats.

In decreasing order the correlation of $C_{\text{al.}}$ and $C_{\text{arom.}}$ is the following one: moss peat (0.99) > grass peat (0.94) > grass-sphagnum peat (0.93) > wood-grass peat (0.87) > sphagnum peat (0.80) > sedge peat (0.29).

The quantitative processing of ^{13}C NMR spectra also allowed to receive the data on the carbon content in ester and carbonyl groups of aldehydes and ketones. It was established that the carbon content of COO groups which may be incorporated into various functional groups - carboxylic, ester, lactone and, to a lesser extent, anhydride ones ranges from 7 to 11% of the original one. In our studies, the excess of these limits is observed and a wider range is revealed: from 4.7% - sphagnum fuscum peat, sphagnum peat ($R = 55\%$) to 12.4% (sphagnum peat moss ($R = 15\%$)). The lowest and the highest carboxyl carbon content is observed in sphagnum peat samples (Table 1).

The carbohydrate complex in the peat humic acids is represented by water-soluble, easily hydrolyzable and hardly hydrolyzable components which make 7 - 63% of organic mass totally.

Soil carbohydrates attract a special attention of researchers due to their relatively high distribution in different types of soil and the established opinion about the possibility of these components involvement in the development of humic acid molecules [11].

Peat carbohydrates have a low biochemical resistance, so their content in peat should be significantly decreased with the increase of decomposition degree. In our studies, this pattern is not observed. Apparently, this is conditioned by the specificity of peat formation ecological conditions on this territory, where an excess moistening is present due to the fact that there is a lot of rainfall which can't be evaporated fully, HTC (hydrothermal coefficient) > 1 or the imperfection of the method for the determination of these parameters, as opposed to the determination of $C_{\text{arom.}}$ and $C_{\text{aliph.}}$ content. Polysaccharide carbon content varies in our samples from 2.8% (lowland sedge peat, $R = 10\%$) up to 13.3% (sphagnum fuscum peat, sphagnum peat, $R = 55\%$). The increase of peat decomposition degree in our studies does not reduce the polysaccharide carbon content.

Humic acids of various types and kinds of peats were divided into groups for a detailed assessment and $C_{\text{arom.}}$ content were compared with the values of the atomic relations C:H. The correlation of atomic ratios H:C, defined by us earlier and $C_{\text{ar.}}$ is the following one among humic acids in decreasing sequence: wood and grass peats (0.99) $>$ hypnum peats (0.98) $>$ grass peats (0.76) $>$ wood peats (0.70) $>$ grass-sphagnum peats (0.57) $>$ sphagnum peats (0.51) $>$ sedge peats (0.36).

A weak correlation between all parameters is present in sedge peats, which is conditioned probably by a large variety of sedge plant names.

Then, moving to further particular characteristics, we also grouped humic acids according to the peat decomposition degree used for these acids extraction (Table. 2).

According to ^{13}C NMR results it was stated the lowest development and "maturity" are presented basically by the same humic acids of the same botanical composition and decomposition degree which were determined by us earlier in the elemental analysis. This shows an acceptable compatibility of CNH and ^{13}C NMR methods.

Table 2 - The results of ^{13}C NMR spectra study concerning the humic acids of Middle Ob peats, grouped according to the peat decomposition degree.

Peat types	Humic acids			
	Carboxyl carbon 160-200 ppm	Aromatic carbon 90-160 ppm	Polysaccharide carbon 65-90 ppm	Aliphatic carbon 0-65 ppm
Peat humic acids with $R = 5\%$				
Sphagnum peats*	9,6*	30,0*	9,9*	51,7*
Peat humic acids with $R = 10\%$				

Hypnum, sphagnum, wood*	8,43*	32,3*	7,8*	51,6*
Hypnum, sedge, sphagnum, grass-sphagnum	9,16	37,5	6,3	47,0

Peat humic acids with R = 15%

Sphagnum, pine-suffruticous, grass,	10,6*	34,2*	8,1*	47,1*
Sedge, grass, sphagnum	8,7	35,8	6,8	48,2

Peat humic acids with R = 20-25%

Sedge *	1,0*	4,10*	0,9*	4,8*
Woody, grass, sphagnum, woody	8,26	37,5	6,48	47,0

Peat humic acids with R = 30-35%

Sphagnum, grass, sedge, woody - grass, sphagnum, grass - sphagnum	7,13	41,3	6,7	44,7
Sedge, grass - sphagnum	8,7*	38,7*	7,9*	44,7*

Peat humic acids with R = 40-45%

Grass, sphagnum, sedge, woody	7,4	43,5	7,3	41,9
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Peat humic acids with R = 50-55%

Sedge, woody, sphagnum, grass	6,6	42,7	9,1	42,6
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Peat humic acids with R = 60-65%

Woody - grass, grass - sphagnum, grass	8,0	44,4	7,8	39,7
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Note: * – less «mature» humic acids.

4. Conclusions

Humic acids obtained from the peats with a particular botanical composition have the lowest development ("maturity") of a molecular structure. These include five samples which contain 95-100% of sphagnum mosses raw in original peat composition, two samples of hypnum green mosses, wood and wood suffruticous peats with a significant predominance of wood in peat and two samples which are composed of a large number of peat-forming plants (13-14 plants are included in peat composition at approximately equal quantities).

5. Summary

In all groups of humic acids, distributed according to decomposition degree, the same correlation is observed well as in the distribution according to botanical composition: with the increase of $C_{arom.}$ content, $C_{aliph.}$ content decreases,

and vice versa, regardless of peat botanical composition and original peat decomposition degree. The correlation coefficients for the groups distributed according to decomposition degree range from $\approx 0,7$ to 1,0. Definite aliphatic carbon content data, defined by ^{13}C NMR spectrometer, is less consistent with conducted earlier physical-chemical research methods than the percentage of the aromatic carbon content as an aromatic part is the main characteristic, obviously, the basis on which the macromolecules of humic acids are developed. This is possible due to the chemical shifts of ^{13}C NMR spectra according to aliphatic elements in the range of 0-65 ppm, because it is a very diverse one. The content of $\text{C}_{\text{arom.}}$ ranges from 23.7 to 51.1%. The average value of $\text{C}_{\text{arom.}}$ content in humic acids of Middle Ob peats makes 43%.

According to nuclear magnetic resonance data, humic acids of Khanty-Mansi Autonomous Area - Yugra peats differ significantly by quantitative content of structural fragments in molecules which depend on the botanical composition and the degree of original peat decomposition.

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