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Characterization and differentiation of humic acids and fulvic acids in soils from various regions of China by nuclear magnetic resonance spectroscopy

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Abstract

Four humic acids (HAs) and four fulvic acids (FAs) isolated from soils of various regions of China were characterized by using ^{1}H NMR and cross-polarization magic angle spinning (CPMAS) ^{13}C NMR spectra, the positions of the major spectral bands of these NMR spectra were similar to each other. It was found that there are definitive compositional and structural differences between HAs and FAs from the same soil and between HAs or FAs from different soils. In particular the lime concretion fluvioaquic soil HA and FA with highest aromaticity are obviously different from others. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Humic acid; Fulvic acid; Nuclear resonance spectroscopy

1. Introduction

This paper is an extension of previous papers [1–3], where five humic acids (HAs) and five fulvic acids (FAs) were isolated from soils of various regions of China, and the thermal transformations of these soil humic substances were investigated using Fourier transform infrared (FT-IR) spectroscopy, temperature-programmed pyrolysis mass spectrometry, thermogravimetry and elemental analysis [1,2]. Two HAs and two FAs isolated from red earth soil and dark lossial soil were characterized using CPMAS ^{13}C NMR spectra and pyrolysis-capillary gas chromatograph interfaced to a mass spectrometry (Py-GC/MS) analyses [3]. The aromaticity of soil humic substances in question was clearly demonstrated and it was confirmed that aromatic units with aliphatic chain substituents were important building blocks [3]. It is generally agreed that IR spectroscopy is useful for the gross characterization and cannot effectively determine aromaticity, and that elemental analysis can only provide general information on the distribution of major constituent elements and the aromaticity, while Py-GC/MS method is destructive, hence NMR spectroscopy that distinguishes carbonaceous structures according to their chemical environment would be more valuable. Investigation of the peaks in ^{1}H NMR spectra and ^{13}C NMR spectra of HAs and FAs has revealed the details of their chemical structures [4–9].

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Schnitzer and Barton [10] were the first to use ^{1}H NMR to study the structure of soil organic matter. Recently there has been a great deal of interest in obtaining the CPMAS ^{13}C NMR spectra, because humic substances are not readily soluble in organic solvents and the CPMAS ^{13}C NMR spectra provide a quantitative measure of the aromatic, paraffinic, carboxylic acid and other groups in humic substances [6,8,9]. The aims of this paper were to characterize four HAs and four FAs in soils from different regions of China and to differentiate between humic and fulvic acids from the same soil and between humic or fulvic acids from different soils by combining both ^{1}H and ^{13}C NMR spectra.

2. Experimental

2.1. Materials

Four HAs and four FAs were the generous gifts from the Institute of Soil Science (Chinese Academy of Sciences). The red earth HA (A) and FA (B) were extracted from soil sample of Da-Ya bay of Guang Dong province near 22.5°N and 114°E. The dark lossial soil HA (C) and FA (D) were extracted from the soil sample of Hua-Jia ridge of Gansu province near 35°N and 105°E. The black soil HA (E) and FA (F) were isolated from the soil sample of Bei-Au county of Heilongjiang province near 48°N and 126°N. The lime concretion fluvioaquic soil HA (G) and FA (H) were extracted from the soil sample of Huai-Bei county of Anhui province near 34°N and 117°E. They are called samples A, B, C, D, E, F, G, and H, respectively. These soil samples were derived from the surface horizon (0–20 cm) of cultivated lands. The elemental composition, the moisture and ash contents, and the extraction procedures were described in previous papers [1,2].

2.2. Apparatus and experimental conditions

^{1}H NMR spectra were obtained with a FT-80A NMR spectrometer. Solutions for ^{1}H NMR analysis were obtained by adding 0.5 ml DMSO-d_6 to the sample (~15 mg). Chemical shifts were measured with respect to external tetramethylsilane (TMS).

CPMAS ^{13}C NMR spectra were recorded with a Bruker MSL-400 NMR spectrometer operating at the ^{13}C frequency of 100.63 MHz. The spectra were run with the following settings: 1.0 ms contact time, 4 kHz spinning speed, 1 s repetition time, scan time 1.5 h, decoupling field ~64 kHz, sample size 150 µl.

3. Results

3.1. ^{1}H NMR spectra

Fig. 1 shows the ^{1}H NMR spectra of four HAs and four FAs. Compared to the representative spectra of

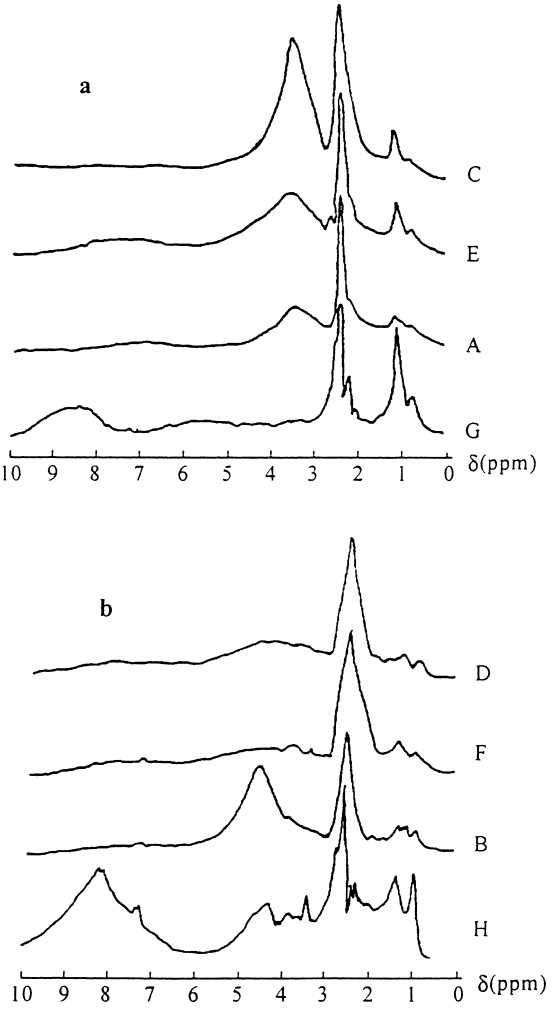

Fig. 1. The ^{1}H NMR spectra of four HAs (a) and FAs (b).

Table 1
% of total intensity

	Original spectrum		
	H^*_{Ali}	H^*_{R-O}	H^*_{Ar}
A	9	52	39
B	15	73	12
C	5	64	31
D	11	50	39
E	11	77	12
F	20	53	27
G	31	26	43
H	17	20	63

soil humic and fulvic acids [4–6,8], the spectra of Fig. 1 also consists of three broad regions at chemical shifts, 0.8–1.8 ppm (terminal methyl groups of methylene chains, protons on methyl groups of highly branched aliphatic structure, and protons on aliphatic carbons which are two or more carbons away from aromatic rings or polar functional groups), 3–6.5 ppm (protons on carbons attached to O or N heteroatoms), 6.5–10 ppm (unhindered and sterically hindered aromatic protons). Three regions will be referred to as "H^*_{Ali}" (aliphatic), "H^*_{R-O}" (attached to carbon α to oxygen groups), "H^*_{Ar}" (aromatic) regions [5]. The percentage of total intensity for each region estimated by integrating the ^{1}H NMR spectrum within each region are listed in Table 1.

3.2. *CPMAS* ^{13}C NMR *spectra*

Fig. 2 shows the CPMAS ^{13}C NMR spectra of four HAs and four FAs. It is convenient to divide the spectra into four regions at chemical shift [9], 0–50 ppm, 51–105 ppm, 106–160 ppm and 161–200 ppm, corresponding approximately to aliphatic, carbohydrate and methoxyl, aromatic, and carboxyl, amine and ester carbon. These regions will be referred to as the "aliphatic", "carbohydrate", "aromatic" and "carboxyl" regions. The ketonic C=O peaks (∼220 ppm) are weak in all spectra. The detailed assignments have been described by Malcolm [8]. The ^{13}C resonances observed and the percentage of total intensity for each region estimated by integrating the CPMAS ^{13}C NMR spectrum within each region are listed in Table 2. The total aromaticity (106–160 ppm) was calculated by expressing aromatic C as percentage of aliphatic C (0–105 ppm)+aromatic C (106–160 ppm) [11] and listed in Table 2 too.

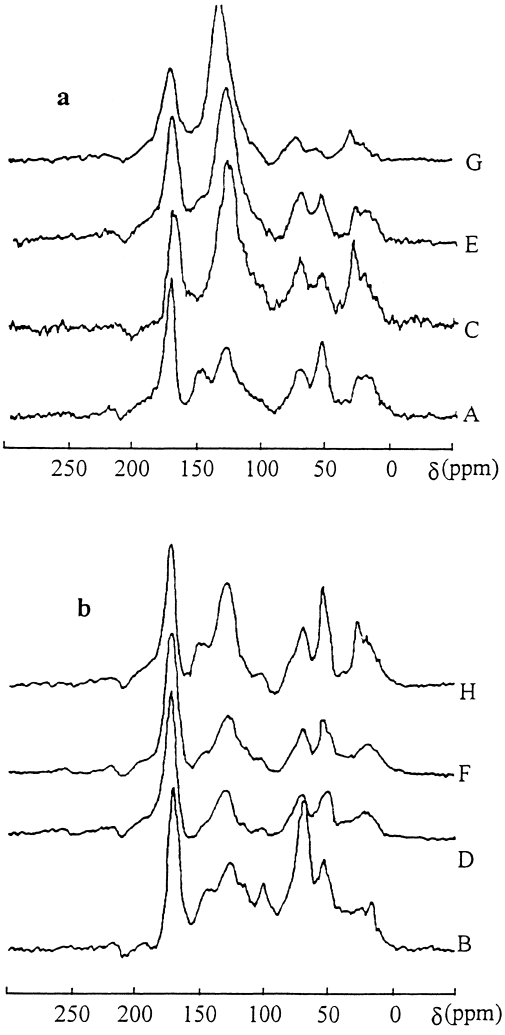

Fig. 2. The CPMAS ^{13}C NMR spectra of four of HAs (a) and FAs (b).

4. Discussion

As shown in Fig. 1, in all ^{1}H NMR spectra of four HAs and four FAs, the positions of the major spectral bands are similar to each other, and also similar to the ^{1}H NMR spectra of humic substances obtained by previous authors [4–6]. The remarkable similarity in the positions is due to the fact that all four HAs and

Table 2
^{13}C resonance, relative intensity (%) and total aromaticity

Sample		0–50 ppm Aliphatic region	51–105 ppm Carbohydrate region	106–160 ppm Aromatic region	161–200 ppm Carboxyl region	Total aromaticity (%)
A	Observed resonance	22	56, 73	130, 149	174	41
	%	15	30	32	23	
B	Observed resonance	17	56, 71, 102	129	173	35
	%	10	41	28	21	
C	Observed resonance	30	57, 73	120	171	57
	%	15	21	47	17	
D	Observed resonance	23	52, 72, 105	133	175	30
	%	16	28	19	37	
E	Observed resonance	29	56, 72	130	172	66
	%	9	16	48	27	
F	Observed resonance	23	56, 71	131	175	41
	%	14	24	27	35	
G	Observed resonance	30	56, 72	130	172	85
	%	5	6	61	27	
H	Observed resonance	30, 23	56, 71	130, 150	174	42
	%	18	28	33	21	

FAs are composed of very similar functional groups. Comparison of ^{1}H NMR spectra of different samples examined here reveals that they differ mainly from variation in relative intensities. As shown in Table 1, except for the sample B, the content of aromatic protons (H^{*}_{Ar}) for each sample is larger than that of aliphatic protons (H^{*}_{Ali}). Except for the samples G and H, the content of H^{*}_{Ali} of HA for each soil is less than that of the corresponding FA, and also except for samples A and B, the content of H^{*}_{Ar} of HA for each soil is less than the corresponding FA. Except for samples A and B, the content of H^{*}_{R-O} of HA for each soil is larger than that of the corresponding FA. The lime concretion fluvioaquic soil HA and FA have highest values of H^{*}_{Ar} and lowest values of H^{*}_{R-O}.

In the H^{*}_{Ali} region, the centres of aliphatic peaks are 0.85 and 1.21 (1.22, 1.24) ppm. However, for sample B, the peak at 1.21 splitted up into two weak peaks with equal intensity. In the H^{*}_{Ali} region, the signal at 0.85 ppm is the methyl ^{1}H resonance, the signal at 1.2 ppm is the resonance of methylene protons. As shown in Fig. 1(a), the peaks at 1.2 ppm of four HAs are obviously higher than those at 0.85 ppm, while in Fig. 1(b), the intensities of both signals at 0.85 and 1.2 ppm are close to each other. It appears that the aliphatic chain substituents in HAs are longer than those in FAs.

In the H^{*}_{R-O} region, for four HAs, the peaks are situated in the 3–4 ppm range, while for four FAs, the peaks are situated in the 4–6.3 ppm range. These results are in accordance with the fact that the content of H^{*}_{R-O} of HA for each soil is larger than that of the corresponding FA except for samples A and B. The broadness of these peaks can be attributed to the large variety of O substituted compounds in these samples.

In the H^{*}_{Ar} region, a striking feature of spectra of samples G and H is the strong resonance at 8.1 ppm which arise from polycyclic aromatics due to sterically hindered periportions [5]. On the contrary, relatively weak signals are present in this region of spectra of samples A–F suggesting that polycyclic aromatics do not comprise a major fraction of the aromatic carbon. For samples A–F, aromatic rings are probably mainly monocyclic instead. Based on the discussion mentioned above, it may be concluded that the lime concretion fluvioaquic soil HA and FA (samples G and H) do not exhibit an ordinary behaviour.

As shown in Fig. 2, the positions of major spectral bands of four HAs and four FAs are similar to each other and are also similar to the CPMAS ^{13}C NMR spectra of humic substances obtained by previous authors [8,9,12,13], prominent signals are from the aromatic and carboxyl carbons. Comparison of

CPMAS ^{13}C NMR spectra of samples A–G examined here reveals that they differ mainly from variation in relative intensities.

The aliphatic region includes the peak maxima 29, 30 and 22, 23, 17 ppm, the former peaks can be assigned to terminal methyl groups, and the latter peaks to the methylene carbon. The carbohydrate region are dominated by maxima at 56 and 71, 72, 73 ppm. The former can be mainly assigned to aliphatic esters and ethers, methoxyl, ethoxyl, and latters to carbon in CH (OH) groups, ring carbon of polysaccharides, ether bonded aliphatic carbon. The aromatic region is very strong with maxima at 130, 131, and 133 ppm which are assigned mainly to unsubstituted and alkyl substituted aromatic carbon. In addition, the spectra of four FAs exhibit a weak peak near 100 ppm which is mainly assigned to carbon singly bonded to two oxygen atoms, anomeric carbon in polysaccharides, acetal or ketal. The spectra of samples A, B and H exhibit a weak peak near 150 ppm which is mainly assigned to aromatic carbon substituted by O or N, aromatic ether, phenol and aromatic amines. None of the other spectra has an obvious peak at this position, despite the fact that phenol and dimethylphenol as pyrolysis products were detected from samples C and D in the previous paper [3]. Carboxyl signals, including largely carboxyl carbons and some ester occur from 162 to 190 ppm with a sharp maxima at 169, 171, 172, 173, 174, 175 ppm. The difference between four HAs and four FAs is clearly shown in aromatic region of their spectra, and the relative intensity of aromatic regions of HA for each soil is larger than that of corresponding FA. On the contrary, the relative intensity of carbohydrate region of HA for each soil is less than that of corresponding FA. Sample G has the highest total aromaticity of all four HAs, and sample H has the highest total aromaticity of the four FAs. Compared with the representative soil HAs and FAs [6], the total aromaticities 85% and 42% for samples G and H do not exhibit normal behaviour.

The final conclusions are as follows:

1. The positions of major spectral bands of these samples from various regions from a subtropical zone to a boreal zone are similar to each other suggesting that all four HAs and four FAs are composed of very similar building blocks regardless of climatic soil conditions.
2. There are definitive compositional and structural differences between the four HAs and four FAs. Humic acids clearly contain structures having a relatively large proportion of aromatic carbons, a relatively low proportion of carbohydrate carbons, a relatively low proportion of aliphatic protons.
3. The lime concretion fluvioaquic soil HA and FA differ from others in their aromaticities and the high aromaticity may be related to the soil forming conditions including the alternation of wet and dry climate, low and flat terrain, parent material with abundant bases and heavy texture.
4. Also there are definitive compositional differences between the four FAs or HAs in various soils from different regions, including great variabilities in aromaticity and relative intensities of peaks.

These conclusions, obviously, are tentative because they are based on only four soils from various regions of China.

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