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Study of soil humic substances by cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance and pyrolysis-capillary gas chromatography

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Abstract

Two humic acids (HAs) and two fulvic acids (FAs) isolated from red earth soil and dark loessial soil were characterized using the cross-polarization magic angle spinning (CPMAS) ^{13}C NMR spectra and pyrolysis-capillary gas chromatography electron impact mass spectrometry (Py-GC/MS) analyses. The aromaticities of four samples were obtained from the ^{13}C NMR spectra. Up to 38 pyrolysis products were identified using a mass data base. Only 26 pyrolysis products were common to all four samples. No alkanes and only three olefins were formed, and almost all products during pyrolysis were aromatic compounds. The aromaticity of soil humic substances in question was clearly demonstrated; aromatic units with aliphatic chain substituents were important building blocks. © 1999 Published by Elsevier Science B.V.

Keywords: Soil; Humic acid; Fulvic acid; Cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance; Pyrolysis-capillary gas chromatography

1. Introduction

This paper is an extension of previous papers [1,2], where the humic acids (HAs) and 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 spectroscopy (FTIR), temperature-programmed pyrolysis mass spectrometry, thermogravimetry and elemental analysis. It was found that there is a similarity in the thermal transformation of HAs and FAs in the soils from various regions of China. The

decarboxylation is obviously the main reaction together with the dehydration reaction from 150°C to 400°C and there are clear changes of the aromatic nucleus of HAs and FAs above 400°C . Except for the mass signals m/z 18, 28, 44, and 64 representing H_2O , CO , CO_2 and SO_2 , respectively, the mass signals m/z 32, 39, 55 and 83 represent the aliphatic chains of humic substances. It is generally agreed that IR spectroscopy cannot effectively determine aromaticity, and that elemental analysis can only provide general information on aromaticity.

Recently there has been a great deal of interest in obtaining cross-polarization magic angle spinning (CPMAS) ^{13}C NMR spectra of humic substances. This interest has rapidly developed because humic

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substances are not readily soluble in organic solvents and the CPMAS ^{13}C NMR spectra provide a quantitative measurement of the aromatic, carboxylic acid and other groups in humic substances [3–5].

Pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS) involves chromatographic separation of pyrolytic products into single components using mass spectral data. The advantage of this technique is that extensive information can be derived about different compounds that are formed from pyrolysis of different components in humic substances. Bracewell and Robertson [6], Martin et al., [7,8], Schulten and Schnitzer [9,10], and Stuczynski et al., [11] have employed Py-GC/MS methods to characterize the humic substances extracted from soils. Such studies have provided evidence that this technique can give useful information on the composition of soil humic substances and confirmed that the major compounds released during pyrolysis from the humic substances are benzenes and substituted benzene.

The objectives of the present study were to measure the aromaticities of two HAs and two FAs from two soils of China by using CPMAS ^{13}C NMR spectra, to give useful information on the compositions of both HAs and FAs by Py-GC/MS techniques and to compare the aromaticities and the compositions of both HAs and both FAs.

2. Experimental

2.1. Materials

Two HAs and two FAs were the generous gifts from the Institute of Soil Science (Chinese Academy of Science). The red earth HA (REHA) and FA (REFA) were extracted from soil sample of Da–Ya bay of Guang Dong province. The dark lossial soil HA

(DLHA) and FA (DLFA) were extracted from the soil sample of Hua–Jia ridge of Gansu province. These soil samples were derived from the surface horizon (0–20 cm) of cultivated lands. The extraction procedures were described in previous papers [1,2] and the analytical characteristics are summarized in Table 1.

2.2. Methods

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 .

Py-GC/MS analyses were performed with a pyrolyzer (CXL-101, model, Najing Analysis Instruments Factory), a gas chromatograph (HP5840 A model, HP company, USA) and a mass spectrometer (VG ZAB–HS model, VG Company, UK). Samples of 1 mg were placed in quartz sample holders and pyrolyzed at 650°C for 10 s. The pyrolytic products were separated on a HP40B fused silica column (30 m \times 0.25 mm i.d. 0.25 μm) using a split mode injector (split: 1/80) and a temperature ramp of 50–250°C at 4°C min^{-1} . The products were detected with following settings: ion source and interface temperature 190°C, 70 eV electron impact ionization energy, resolving power 1000.

The pyrolytic products were identified by the comparison of their mass spectra to the EPA/NIH mass spectral library [12].

3. Results

3.1. ^{13}C NMR spectra

Fig. 1(a) and (b) shows the CPMAS ^{13}C NMR of REHA, DLHA (a) and REFA, DLFA (b) samples. It is

Table 1
Analytical and ^{13}C NMR characteristics

Type of HA or FA	Analytical characteristics (on a moisture- and ash- free basis)						^{13}C NMR characteristics (chemical shift ppm) % of total				
	%C	%H	%N	%O	%S	C/H	0–50	51–105	106–160	161–200	Aromaticity
REHA	55.51	5.21	5.29	33.05	0.94	0.96	15	30	32	23	41
REFA	54.68	4.73	3.72	35.90	0.97	0.98	10	41	28	21	35
DLHA	60.44	3.53	4.22	31.31	0.50	1.43	15	21	47	17	57
DLFA	50.15	4.42	5.38	39.56	0.49	0.95	16	28	19	37	30

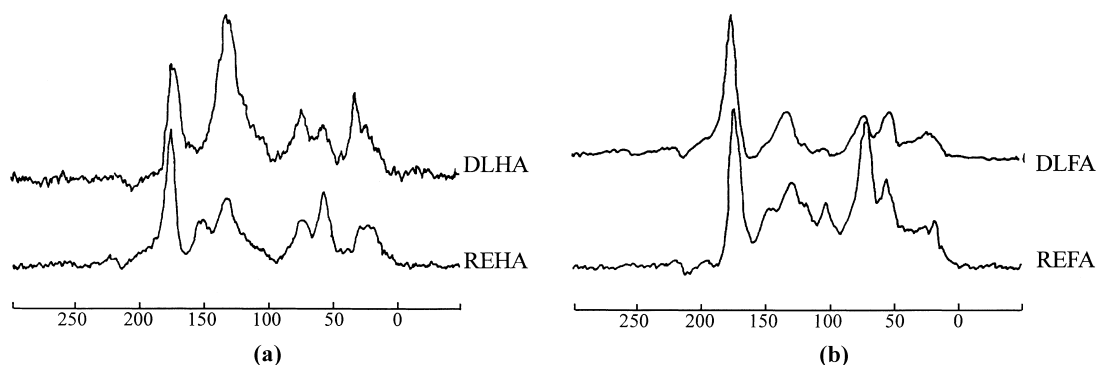


Fig. 1. CPMAS ^{13}C NMR spectra of both HAs and both FAs.

convenient to divide the spectra into four chemical shift regions [5], 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 “aliphatic”, “carbohydrate”, “aromatic” and “carboxyl” regions. The detailed assignments have been described by Malcolm [4]. The percentage of total intensity for each region is estimated by integrating the CPMAS ^{13}C NMR spectrum within each region and the total aromaticities calculated by expressing aromatic C as percentage of aliphatic C (0–105 ppm)+aromatic C (106–160 ppm) are listed in Table 1 too.

3.2. Py-GC/MS analyses

The total ion pyrograms of the four samples are displayed in Fig. 2. In Table 2 the mass to charge ratios, m/z , and identifications of the peaks during pyrolysis of the four samples are summarized.

As shown in Table 2, almost all products during pyrolysis were aromatic compounds except for CO_2 and three olefines. In addition, no alkanes were produced [6–11]. Of the 38 products of REHA and REFA, only 26 products correspond to those of DLHA and DLFA. A comparison of the products between both HAs and both FAs indicates that no clear distinction can be made.

4. Discussion

From the information described above, it is likely that most products of the four samples were derived

from aromatic units within both HAs and both FAs, and that aromatic units with aliphatic chain substituents are important building blocks of the soil humic substances in question.

Although there was no clear distinction between the elemental compositions and the aromaticities of red earth soil humic substances and dark loessial soil humic substances, as shown in Table 1, a clear distinction between the products during pyrolysis of red earth soil humic substances and dark loessial soil humic substances is demonstrated in Table 2. In other words, the building blocks of soil humic substances are strongly dependent on the sources but not on the fractionation of the soil humic substances. Though pyrolysis products of a humic substances depend on the source and the experimental conditions, it was found that for the same soil, the pyrolysis products of the HA fraction was hardly different from those of the FA fraction. The absence of alkanes in the products from the four samples may be attributed to the high pyrolysis temperature 650°C . The common presence of three olefines in the products of the four samples suggests similar mechanisms of olefines formation and/or substances containing similar chemical structures with aliphatic chain substituents.

A homologous series of n -alkyl benzene in the products of the four sample was observed only up to $n=3$, while benzene substituted by longer alkyl chains ($n>4$) was missing. In addition to saturated alkyl derivatives, styrene, methyl styrene and divinylbenzene as unsaturated aliphatic substituents were pyrolysis products of four samples. The contribution of alkyl benzenes to the chemical structure of humic substances was emphasized by Schulten and Schnitzer [13].

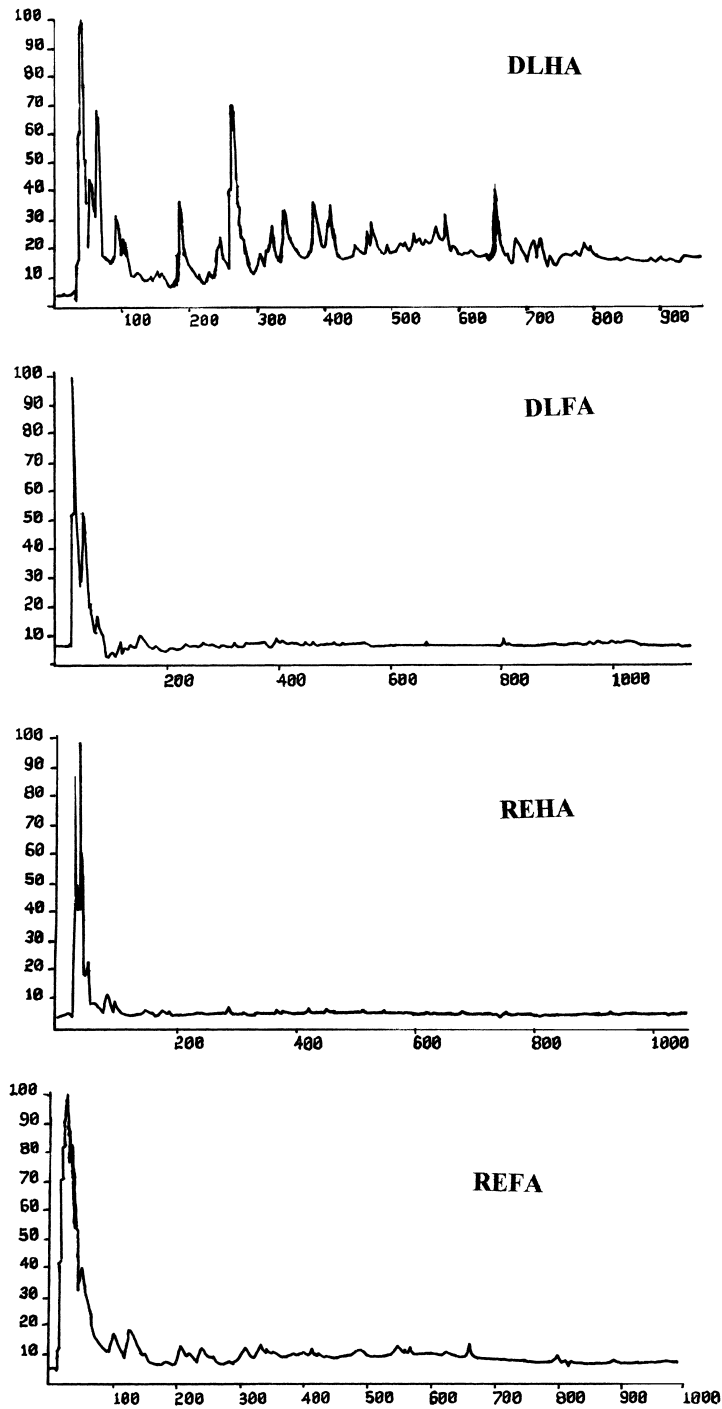


Fig. 2. Py-GC/MS total ion pyrograms of both HAs and both FAs.

Table 2
Products formed by pyrolysis

M ⁺	Assigned compound	REHA	DLHA	REFA	DLFA	Code
44	Carbon dioxide	+	+	+	+	124-38-9
56	2-Butene	+	+	+	+	107-01-7
70	1-Pentene	+	+	+	+	009-67-1
78	Benzene	+	+	+	+	71-43-2
96	1H-Pyrazole, 3,5-dimethyl	+	-	+	+	67-51-6
79	Pyridine	+	+	+	+	110-86-1
92	Toluene	+	+	+	+	108-88-3
81	1H-Pyrrole, 2-methyl	+	-	+	-	636-41-9
106	Xylene	+	+	+	+	95-47-6
104	Styrene	+	+	+	+	100-42-5
95	1H-Pyrrole, 2,5-dimethyl	+	-	+	-	600-28-2
107	Dimethyl pyridine (or ethyl pyridine)	+	-	+	-	620-02-0
120	Trimethyl benzene (or propylbenzene)	+	+	+	+	95-63-8
103	Benzonitrile	+	+	-	-	00-00-2
118	Methylstyrene (or benzene, (1-methylethenyl)-)	+	+	+	+	100-80-1
94	Phenol	+	+	+	+	108-95-2
117	1H-Indole	+	-	+	-	120-72-9
116	1H-Indene	+	-	+	-	95-13-6
108	Cresol	+	-	+	-	106-44-5
107	Toluidine	+	+	+	+	100-46-9
182	1-Tridecene	+	+	+	+	2437-56-1
117	Tolunitrile	+	+	+	+	140-29-4
130	Benzene diethenyl	+	+	+	+	1321-74-0
148	Methylene difuran	-	-	+	+	1197-40-6
122	Dimethylphenol	+	-	+	+	105-67-9
128	Naphthalene	+	+	+	+	91-20-3
142	Methylnaphthalene	+	+	+	+	90-12-0
158	Trimethyl-allene	+	+	+	+	29555-07-5
154	Biphenyl	+	+	+	+	83-32-9
131	Methylindole	+	+	+	+	3420-02-8
156	Dimethylnaphthalene	+	+	+	+	569-41-5
268	Dimethyl-octylnaphthalene	+	+	+	+	55000-54-9
168	Diphenylmethyl	+	+	+	+	643-93-6
170	Trimethylnaphthalene	+	+	+	+	3031-08-1
182	Dibenzofuran, 4-methyl	+	-	+	+	7320-53-8
186	Pyrazolo[5,1-c][1,2,4]benzotriazin-8-ol	+	+	+	+	14394-47-9
214	Benzene, (1,1'-chloro-1,2-ethenediyl)bis-	+	-	+	-	1460-06-6
246	Benzene-(1,3,3-trimethylnonyl)	+	+	+	+	54986213-44-6

In contrast to aliphatic substituents of benzene, aliphatic substituents of N-heterocyclic rings such as dimethylpyrolyzole, methypyrrole, dimethylpyrrole, dimethyl piperidine and indole were only observed in the products of red earth soil HA and FA.

The mass spectra with molecular ions at m/z 128, 170 and 268 in the products of four samples indicated that naphthalene and aliphatic substituents of naphthalene were formed in all four samples. In contrast to aliphatic substituents of benzene, naphthalene was substituted by longer aliphatic

chains. Hence the cleavage mechanisms of aliphatic chains from benzene and naphthalene seemed to be different.

Though phenol was observed in the products of four samples, cresol and dimethyl phenol were missing in the products of dark lossial soil HA and FA.

For furans, methylene difuran was only observed in the products of both FAs. On the contrary, benzonitrile was only observed in the products of both HAs, while toluonitrile was detected in the products of the four samples.

The aromaticities of the four samples ranged from 30% to 57%. Though it was found difficult to express the ^{13}C NMR information in the terms of chemical structure, combining the identification of a great number of aromatic compounds in the products during pyrolysis and the fact that the aromaticities ranged from 30% to 57%, it appears to be possible to estimate the average length of aliphatic chains attached to an aromatic ring. A chemical structure consisting of six carbon atoms in an aromatic ring to which an aliphatic chain of five carbons is attached has an aromaticity of 55%. To obtain aromaticity of 30%, we need to attach to the ring an aliphatic chain of 14 carbons. For the four samples in question, the average lengths of aliphatic chain attached to an aromatic ring ranged from 5 to 14 carbons. This conclusion was substantiated by the pyrolysis products in Table 2.

Finally, the results and discussion presented in this paper show that combining Py-GC/MS with ^{13}C NMR spectroscopy is a valuable method for structural studies on humic substances.

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