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FT - C13 NUCLEAR MAGNETIC RESONANCE SPECTRA OF NATURAL HUMIC SUBSTANCES

by

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Abstract:

The C13 resonance spectra of two humic acids extracted from Vertisol and Andosol soil and of a fulvic acid form Podzol soil are reported. The spectra were taken in 5 % W/W solution of the substances in 0.1 N NaOD in D_2O using the Fourier-transform-technique.

1. Introduction

The understanding of the chemical nature of humic substances is mainly derived from chemical degradation of the extracts, whereas attempts to use non destructive spectroscopic techniques have been relatively unsuccessful. Proton nuclear magnetic resonance (1), (2), (3) yields fairly resolved (3) spectra with the restriction that no protons which exchange with D_2O can be detected. Compared to proton signals, carbon-13-resonances are usually less influenced by solvent effects or the nonbonded neighbouring nuclei and therefore the carbon-13-spectra should yield more detailed information about the structure of a natural macromolecule. Also the chemical shifts are an order of magnitude larger and the spectra at natural abundance are superpositions of singlets because the protons are noise decoupled.

C-13 NMR has been used extensively to study polymeric materials and also biopolymers as proteins, nucleic acids, polysaccharides etc. (5),(6). Recently the C-13 NMR technique has been used successfully for the investigation of lignins and its model substances (7), (8), (9) and one might expect that C-13 spectra will also yield useful information for the structure of humic substances. Previously C-13 spectra (7) of a fulvic acid fraction dissolved in organic solvents are rather unsatisfying because of insufficiently developed technique. The present paper provides C-13 spectra of natural humic substances, extracted from different soils with hydrochloric acid or 0.5 n sodium hydroxide solution.

2. Experimental

The C-13 spectra were taken of 5 % W/W solutions of humic substances in 0.1 n NaOD solution. More concentrated solutions have greater viscosities, which sharply decrease the spectral resolution. The spectra are obtained by the Fourier - transform - technique, with the Varian XL - 100 - 15 spectrometer operating at 25.5 MHz, in 12 mm o.d. tubes. The spectra shown are the result of accumulating 250 blocks of 1000 transients.

The resolution of the spectra is ± 10 Hz (approximately ± 0.5 ppm). The spectrometer was locked to an external F - 19 lock. Shift values are given in the TMS scale, referenced to a coaxial internal neat TMS capillary. No attempts to correct for magnetic suspectibility effects were made.

Table 1

Chemical characterisation of the materials used.

humic substance:	humic acid	humic acid	fulvic acid
classification:			
soil	Vertisol	Andosol	Podzol
horizon	Ap_1	A_{11}	Bh
origin	Arahal(Spain)	Las Lajas (Canary I.)	Vollbüttel (Germany)
extracting reagent	0.5 N NaOH	0.5 N NaOH	0.5 N HCl
elementary composition: (dry, ash-free)			
C	57.7 % W/W	48.72	46.17
H	5.60	4.76	3.40
N	5.70	3.78	0.77
O	31.00	42.74	50.52
major oxygen-containig functional groups:			
total acidity	5.7	8.7	11.2
COOH	3.1	2.7	7.8
OH phen.	2.6	6.0	3.4
OH alcoholic	4.1	0.5	0.1
C = O total	2.8	n.d.	2.8

The characteristics of the sample are given in table 1. Methods of extraction and purification were as before (10),(11). The determination of the elementary composition and of the functional groups are also described in the literature (10), (11), (12).

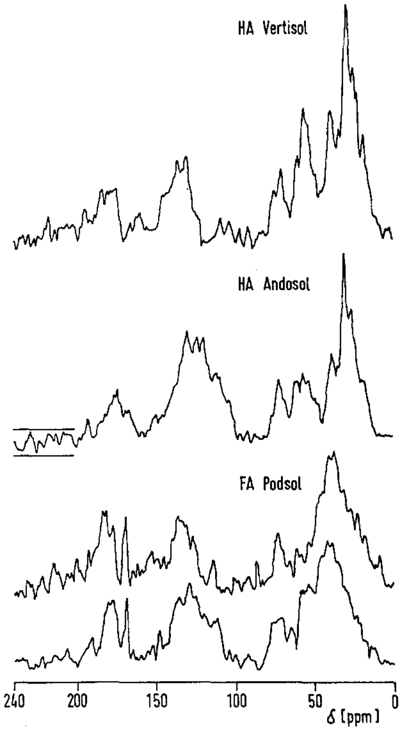

Fig. 1: C-13 NMR spectra of humic acids (HA) and fulvic acid (FA) extracted from different soils. The second spectrum from the bottom was taken two days after preparation of the solutions and provides a measure of reproducibility. Intensities in arbitrary units. δ is the chemical shift in ppm on the TMS scale. The approximate base line noise is indicated by two horizontal bars in the third spectrum from the bottom.

3. Results and Discussion

The spectra are given in fig. 1, where maximal baseline noise is indicated. For the Podzol fulvic acid, the spectrum war rerun after 2 days (second curve from bottom in fig. 1) to show the sort of reproducibility to be expected. There may be considerable more detail in the spectra than is evident in fig. 1, but this is masked by the base line noise. The centers of the peaks are

C-13 chemical shifts of humic substances and assignments from related materials.

$-\delta$	HA Vertisol	HA Andosol	FA Podzol	lignins(6)	amino acids and peptide(4)	ketones ets. (4),(5)
200	195 vw		200			C = 0 ketones
190		193	193	C = 0 side chains		
180	180 m	173	180		COOH groups	C = 0 quinone
170			170			
160	160 vw			aromatic rings		
150						
140	135 s		135	olefinic carbons	aromatic and heteroaromatic rings	
130	130 s	125				
120			114			
110	109 104					
100	97 92					
90						
80	75 m	75	73	CH_2	CH_2	carbohydrates
70						alcoholics
60	59 s	59	55			
50				OCH_3	C_α	
40	38 m	36	38	aliphatic	C_β C_γ,C_δ and methyl	
30	28 s	27				
20						
10						

listed in table 2. Possible assignments for the peaks as suggested from results given in the literature for related substances are indicated in table 2.

The chemical shifts of the humic substances can be assumed to be accurate to ± (5-10) ppm only, due to the high molecular weight, the high viscosity of the solution and the interaction of the carbon atoms with unpaired electrons. Likewise the observed peaks are fairly wide. In complex substances like humic acids, one has also to consider that the molecule might consist of rigid and mobile regions, in which only the carbon-13 nuclei of the flexible parts have sufficiently long transversal relaxation times so as to be observable in a high resolution spectrum. Thus it is hazardous to conclude that the intensities of the signals in different samples correspond linearly to absolute concentrations of certain classes of carbon atoms, or that the absence of a certain signal means the absence of a specific chemical structure.

All spectra can be roughly divided into three parts. Between 160-200 ppm the resonance of cabonyl groups is observed. In the case of the Podzol fulvic acid the well resolved peak at 170 ppm is perhaps due to carboxylic carbon atoms in accordance with the relatively high content of carboxylic groups given in table 1. Aromatic carbons are located between 100-160 ppm. The peaks in this region are similar in intensity and position for all samples. In the third region from 10-100 ppm the aliphatic carbon atoms are observed. The intensities of the bands between 50-70 ppm decrease from Vertisol over Andosol to Podzol. The same decrease is found for the nitrogen content (table 1). Thus these bands may originate from amino acids. The sharp band at 28 ppm is due to aliphatic side chains. In the case of Podzol these chains may be less mobile compared to other samples and thus are broadened.

The results presented here show the applicability of the C-13 NMR method for structural investigations of the humic substances. Additional results were obtained for fungal humic acid like pigments (13). The resolution of the spectra obtained so far is restricted by the signal to noise ratio. Since more highly concentrated solution would not improve the spectra, higher magnetic fields or larger diameter probes have to be used for higher resolution. A more detailed spectral assignment will come from C-13 spectra of suitable model substances dissolved in NaOD solutions.

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