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# Structural Characterization of Aquatic Humic Substances by NMR Spectroscopy

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## Abstract

Aquatic humic substances isolated from the Göta River (Sweden) have been investigated by $^1$H-NMR and $^{13}$C-NMR spectroscopy. The $^{13}$C-NMR study included the application of a special pulse sequence technique (DEPT). The humic substances could be characterized in terms of the occurrence of aromatic units and carboxylic groups as well as various types of methyl, methylene, and methine groups.

## Introduction

Humic substances constitute from a chemical point of view a very ill-defined group of materials [1, 2]. Aquatic humic substances could approximately be described as brown-coloured, heterogeneous, polymeric organic materials in natural waters. NMR spectroscopic methods have been found to be useful for the structural characterization of humic substances [3–6], and this paper describes NMR spectroscopic studies of aquatic humic substances, using a combination of different techniques.

Humic substances [humic acids (HA) and fulvic acids (FA)] were isolated from water of the Göta River. We have particularly focused our investigation on a fulvic acid sample (FA-1, see Experimental). The corresponding humic acid sample (HA-1) was found to have similar NMR spectral properties, but the spectra had somewhat less resolved character.

## Experimental

### *NMR spectra*

DMSO-$d_6$ was used as solvent (internal reference, TMS). Normal decoupled $^{13}$C-NMR spectra were recorded at 50.3 MHz on a Bruker WM 200 spectrometer (number of scans, 30000; pulse delay 0.5 s; pulse width, 10 $\mu$s; temperature 323 K). The DEPT (Distorsionless Enhancement by Polarization Transfer [7]) experiments were performed as described by Bardet *et al.* [8]. The DEPT experiments were generated on the Bruker WM 200 spectrometer with the Aspect 2000 pulse programmer ($D_2$, 3 ms; $D_1$, 3 s; $^1$H $\pi/2$ pulse, 33 $\mu$s). $^1$H-NMR spectra were recorded at 270 MHz on a Bruker WH 270 spectrometer (number of scans, 1000).

### *Isolation of the humic substances*

Water (27 tons) from the Göta River was passed through an anion-exchange column (Lewatit MP-500 A, $OH^-$-form). Retained compounds were eluted with 50 l of aqueous NaOH (0.01 M) and NaCl (2 M). The eluate was acidified (pH 2) and passed through a column of an adsorption resin (Amberlite XAD-7); the column was finally washed with distilled water to remove salts. The material adhered to the resin is referred to in this paper as 'humic substances'. The column was intially eluted with three bed volumes of dioxane–water (1:1) (fraction FA-1) and then with 0.01 M NaOH (fraction HA-1). The organic material in fraction HA-1 was found to be insoluble in aqueous acid (pH 1) and corresponds therefore to the humic acid portion of the humic substances, while the materials in fraction FA-1 were soluble in this solution and therefore correspond to the fulvic acid portion of the humic substances (cf. Ref. [1] and [2]).

The eluate containing fraction FA-1 was passed twice through a cation-exchange resin (Bio-Rad AG-50W × 8, $H^+$-form) to remove metal ions. The solution was lyophilized, the residue was then dissolved in 800 ml ethanol and centrifuged to remove minor amounts of insoluble compounds. The volume of the solution was reduced to 100 ml and the solution obtained was dripped into magnetically stirred ether (4 l). The precipitate formed was centrifuged off, washed several times with ether and dried *in vacuo* over $P_2O_5$. The product (15 g) is in this paper referred to as sample FA-1. The precipitate formed on acidification of fraction HA-1 was centrifuged off and dissolved in 70% ethanol. Treatment of this solution according to the procedures applied to fraction FA-1 gave a product (12 g) which in this paper is referred to as sample

HA-1. The average molecular weight of FA-1 and HA-1 is of the order of 2000 [9]. Analytical data for samples FA-1 and HA-1 can be found elsewhere [10].

*Alkaline hydrolysis of sample FA-1*

FA-1 (1 g) was dissolved in 75 ml of an aqueous solution of NaOH (2 g) and left over night (nitrogen atmosphere). The solution was extracted with 2 × 50 ml dichloromethane and passed through a cation-exchange column (Bio-Rad AG-50W × 8, $H^+$-form). The volume of the eluate was reduced to 10 ml and after addition of THF (15 ml) the solution was dripped into 500 ml ether – THF (5:1). The precipitate formed was centrifuged off, washed several times with ether, and dried *in vacuo*. Elemental composition: 51.42% C, 4.51% H, 0.97% N, 40.21% O, 2.27% S. Methoxyl content: 2.9%. Ash content: 0.02%. Carboxylic acid groups (titration): 5.35 mequiv/g.

## Results

*Ethoxylation*

In the course of the work it was found that the use of ethanol in the purification procedures (see Experimental) resulted in partial ethoxylation of the carboxylic groups in the sample. Thus the signals at $\delta$ 14.0 and 60.3 in the $^{13}$C-NMR spectrum of FA-1 (Fig. 1) are due to ethyl ester groups (cf. Table I). These peaks were lacking in the $^{13}$C-NMR spectrum of the product (sample FAA-1) obtained on alkaline hydrolysis of sample FA-1; the spectrum did not differ in other respects from the one given in Fig. 1. Our results suggest that there is a general risk for esterification when ethanol (or any other lower alcohol) is used [1, 2] in the fractionation of humic substances.

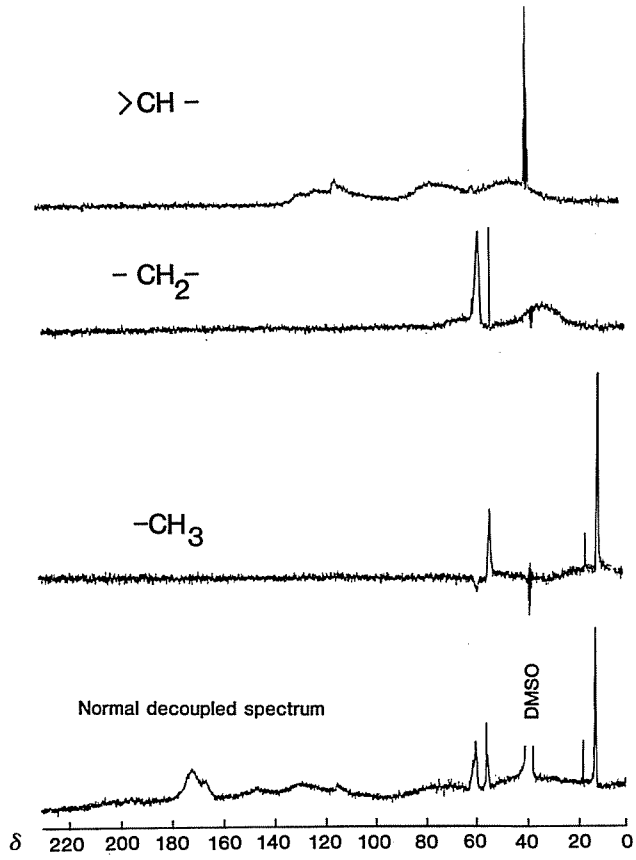


*Fig. 1* $^{13}$C-NMR spectra of a fulvic acid (sample FA-1) from the Göta River (solvent, DMSO-$d_6$). Spectra showing separately signals from methyl, methylene and methine groups were obtained by a DEPT experiment.

Table I. *Assignment of signals in $^{13}$C-NMR spectra of a fulvic acid (sample FA-1) from the Göta River (Fig. 1). Several peaks are broad and have irregular shapes; $\delta$ values given always refer to the highest point of the peak.*

| $\delta$ Value (ppm) | Assignment |
|---|---|
| 14.0 (narrow)[a] | $CH_3$– in $C_2H_5OCO$– |
| 15 (very broad) | $CH_3$– in alkyl groups |
| 18.5 (sharp) | $CH_3$– in ethanol (contaminant) |
| 35.5 | –$CH_2$–CO– or –$CH_2$– in hydrocarbon structures |
| 39.4 | DMSO (solvent) |
| 45.9 | aliphatic >CH–, >CH–CO– |
| 55.6 | Ar–O–$CH_3$ |
| 56.1 (sharp) | –$CH_2$– in ethanol (contaminant) |
| 60.3[a] | –$CH_2$– in $C_2H_5OCO$– |
| 65 (broad) | –$CH_2$–O– |
| 75.2 | >CH–O– |
| 114.5, 122.2, 128.0 | Aromatic >CH– |
| 147 | Quaternary, unsaturated carbon |
| 167 | Ar–COOH |
| 172 | R–COOH |

[a] This peak was absent in the spectrum of alkali treated FA-1 (sample FAA-1), see text.

*$^{13}$C-NMR spectral analysis of sample FA-1*

Fig. 1 includes a normal decoupled $^{13}$C-NMR spectrum of sample FA-1 and spectra of the same sample obtained by the DEPT technique which show separately signals from methyl, methylene and methine groups. Peak positions and interpretations are given in Table I. It is evident from Fig. 1 that the use of the DEPT technique dramatically improves the possibilities of $^{13}$C-NMR spectral analysis of humic substances. The normal decoupled spectrum includes a few very small signals at $\delta$ values above 180, suggesting the presence of minor amounts of carbonyl groups in the sample.

*$^1$H-NMR spectral analysis of fulvic acid FAA-1*

The $^1$H-NMR spectrum of sample FAA-1 is given in Fig. 2. For peak positions and interpretations, see Table II. The spectrum exhibits significant signals from hydrocarbon-related alkyl groups in the sample, largely because such groups are rich in hydrogen. The triplet at $\delta$ 7.08 ($J$ = 49 Hz) (Fig. 2) is

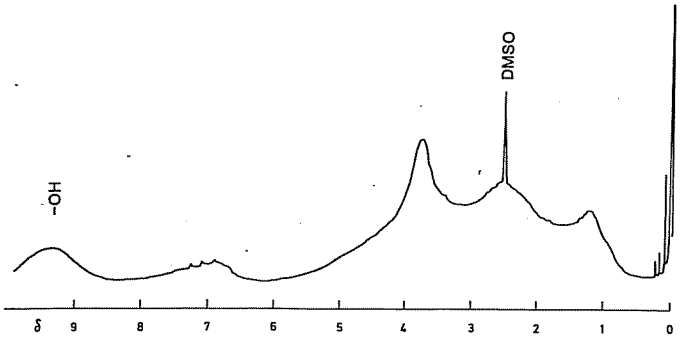


*Fig. 2.* $^1$H-NMR spectrum of a fulvic acid from the Göta River (sample FAA-1; solvent, DMSO-$d_6$).

Table II. *Assignment of signals in the $^1$H-NMR spectrum of the fulvic acid sample FAA-1 from the Göta River (Fig. 2). The peaks are broad and have irregular shapes; δ values given always refer to the highest point of the peak. (Various types of aliphatic methine groups should contribute to the signals in the range δ 1.5–5.0.)*

| δ values (ppm) | Assignment |
|---|---|
| 1.20 | $CH_3$–, –$CH_2$– (in alkyl groups) |
| 2.5 | –$CH_2$–CO–, Ar–$CH_2$– |
| 2.53 | DMSO (solvent) |
| 3.71 | Ar–O–$CH_3$, –$CH_2$–O– |
| ≈7 | Aromatic protons |
| 7.08 (triplet, $J = 49$ Hz) | Protons on nitrogen in ammonium and amine salts |
| 9.2 | –OH |

typical for the coupling of hydrogen in ammonium or amine salts with nitrogen [11] and demonstrates the presence of small amounts of such groupings in sample FAA-1. It is noteworthy that this signal was absent in the corresponding spectrum of sample HA-1.

## Discussion

Some additional structural information regarding the sample FA-1 can be derived from a comparison of $^1$H-NMR and $^{13}$C-NMR spectroscopic data. Peaks which can be attributed to aromatic units are present in both the $^1$H-NMR and the $^{13}$C-NMR spectrum (Tables I and II). Since the $^1$H-NMR spectrum suggests the presence of relatively small amounts of hydrocarbon-related alkyl groups (Fig. 2), the signals at δ 35.5 and 45.9 in the $^{13}$C-NMR spectrum are most likely due to methylene and methine groups adjacent to carboxylic acid groups; this is in agreement with the rather strong and broad peak at about δ 2.5 in the $^1$H-NMR spectrum (cf. Tables I and II).

Most of the signals in the $^1$H-NMR spectrum as well as in the $^{13}$C-NMR spectrum are very broad. The reason for this is most likely complexity of the sample (cf. Ref. [12]). Since the molecular weight is rather low (≈ 2000) [9], line-broadening effects related to the polymeric nature of the sample probably are of minor importance. This is in accordance with the fact that spectra run at different temperatures had essentially the same shape with the exception of the hydroxyl peak in the $^1$H-NMR spectra.

## Acknowledgements

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