^{13}C-NMR INVESTIGATION OF HUMIC AND FULVIC ACIDS OBTAINED FROM SOME TYPICAL JAPANESE SOILS

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SUMMARY

^{13}C-NMR spectra of humic acids (HAs), fulvic acids (FAs), and PVP-adsorbed and non-adsorbed fractions of FAs were measured quantitatively. The spectra indicated that more aromatic-C and less carbohydrate-C were contained in the order of HA, PVP-adsorbed fraction of FA, and PVP-non-adsorbed fraction of FA. ^{13}C-NMR spectra and the composition of carbon species also differed considerably between A-type HA and Rp-type HA obtained from an ando soil and a grey lowland soil, respectively. When compared among different types of soils, PVP-adsorbed as well as non-adsorbed fractions of FAs showed similar spectra and compositions of carbon species. Furthermore, it was suggested that saccharides in FAs were predominantly linked by 1-4 bonds.

INTRODUCTION

Structures of humic substances are not only different between humic acids (HAs) and fulvic acids (FAs) but also among the molecules which constitute HAs or FAs. When they were extracted from different soils, the difference would be even more enormous. In this study, chemical structures of HAs, FAs, and PVP-adsorbed and non-adsorbed fractions of FAs were investigated with special emphasis on the composition of carbon species using ^{13}C-NMR spectroscopy (refs. 1-2).

MATERIALS AND METHODS

Soil samples

Used soil samples were obtained from the A and A_p horizons of an ando soil (Typic Dystrandept), a brown forest soil (Typic Dystrochrept), and a grey lowland soil (Anthraquic Haplaquept). Table 1 shows several characteristics including HA and FA contents of the soil samples.

Preparation of HAs and FAs

The outline of method for preparation of HAs and FAs used was as follows. HAs and FAs were extracted from air dried soil sample (<2 mm) with 0.1 $\underline{N}$ NaOH (300 ml to 1 g soil organic carbon) by standing it under N_2 for 48 h at 25 °C. Second extraction was performed with the same volume of 0.1 $\underline{N}$ NaOH. HA was precipitated by acidifying the extract to pH 1.0 with 3 $\underline{N}$ HCl. After purification with 0.1 $\underline{M}$ HCl-0.3 $\underline{M}$ HF (1:1), it was dialyzed and freeze-dried.

FA solution was passed through the column of cation exchange resin (Dowex-50) to remove metal ions. Then a portion of FA was neutralized, concentrated and desalted by Sephadex G-10. The desalted FA was transformed into H^+-form with Dowex-50 and freeze-dried.   Another portion of FA solution was fractionated further with Polyvinylpyrrolidone (PVP, "Polyclar AT") into adsorbed and non-adsorbed fractions (ref. 3). After FA solution (pH 1.0) was passed through the column of PVP, the column was washed with 3 column volumns of pH 1 HCl and the washing solution was combined with the eluate (non-adsorbed fraction). The adsorbed fraction was eluted out with 0.1 N NaOH. PVP-adsorbed and PVP-non-adsorbed fractions were purified in the same way as unfractionated FA. Table 2 shows the yields of two fractions from FAs.

^{13}C-NMR spectroscopy

^{13}C-NMR spectra were obtained at 50.20 MHz on a JEOL JNM-FX-200 spectrometer using inverse-gated decoupling with 45°pulse, acquisition time of 0.1 sec and pulse delay time of 4.3 sec, and accumulation of 23,000-30,000 scans. Chemical shifts were referenced to external TMS or internal dioxane. Concentrations of HAs were 10 % in 0.5 $\underline{M}$ NaOD, while unfractionated FAs and their PVP-adsorbed fractions were 25-50 % in 0.5 $\underline{M}$ NaOD, and PVP-non-adsorbed fractions of FA were 20-60 % in D_2O.

RESULTS AND DISCUSSIONS

^{13}C-NMR spectra were divided into three regions : aliphatic-C (0-110 ppm), aromatic- and olefinic-C (110-165 ppm) and carbonyl-C (165-216ppm) (ref. 4).

TABLE 1

Characteristics of used soil samples.

Soils	texture	pH (H_2O)	T-C (%)	T-N (%)	C/N	HA-C (% to Total C)	FA-C
Inogashira(ando soil)	LiC	4.2	16.7	1.04	16	30.5	21.1
Dando(brown forest soil)	LiC	4.7	6.2	0.34	18	11.1	23.4
Nagano(grey lowland soil)	LiC	5.4	1.5	0.14	11	7.9	6.2

TABLE 2

Yields of PVP-adsorbed and non-adsorbed fractions from
FAs (carbon basis).

FAs	PVP-adsorbed fr.	PVP-non-adsorbed fr.	recovery
Inogashira	36.5(%)	61.8(%)	98.3(%)
Dando	51.7	44.7	96.4
Nagano	29.7	71.7	101.4

PVP-non-adsorbed
fr.

TMS

dioxane

unfractionated
FA

PVP-adsorbed
fr.

TMS

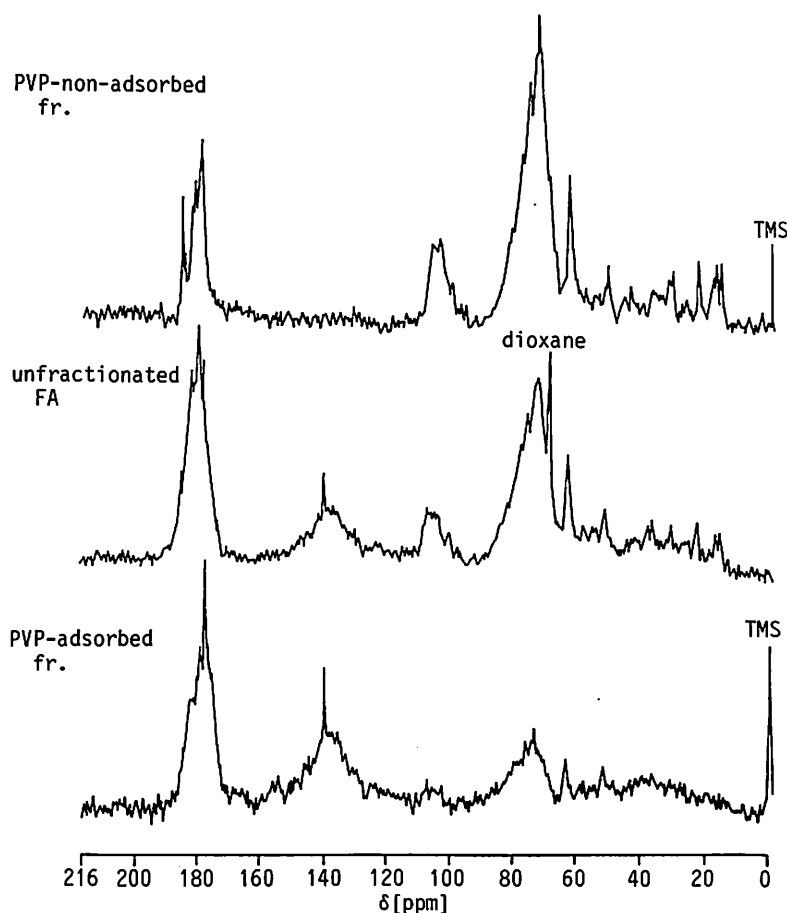216 200   180   160   140   120 100   80   60   40   20   0

δ[ppm]

Fig. 1. ^{13}C-NMR spectra of Inogashira FA and its PVP-adsorbed and non-adsorbed
fractions.

Furthermore, the range of 60–110 ppm was differentiated as carbohydrate-C region
from aliphatic-C region (ref. 5). Aliphatic-C region and aromatic-C region were
separated at 110 ppm in this study, because the peaks of anomeric-C in FAs
(maximum near 104 ppm) were extended over to 110 ppm. The composition of carbon
species was obtained by calculating the relative percentage of the area of each
carbon region to the total area.

(1)Comparison of each FA fraction

     Fig. 1 shows the ^{13}C-NMR spectra of Inogashira FA and its PVP-adsorbed and
non-adsorbed fractions. ^{13}C-NMR spectra of adsorbed fractions were largely
different from those of non-adsorbed fractions and the large difference in their
chemical compositions was indicated. The peaks of aromatic-C were observed

TABLE 3

The composition of carbon species in each FA fraction from ^{13}C-NMR spectra.

FA fractions	Aliphatic-C (0-60 ppm)	Carbo-hydrate-C (60-110 ppm)	Aromatic & olefinic-C (110-165 ppm)	Carbonyl-C (165-216 ppm)
unfractionated FA				
Inogashira	17.6(%)	38.6(%)	16.9(%)	26.9(%)
Dando	24.1	29.5	17.7	28.7
Nagano	17.1	41.5	17.0	24.4
PVP-non-adsorbed fr.				
Inogashira	18.6(%)	53.0(%)	6.9(%)	21.5(%)
Dando	24.0	43.4	10.0	22.6
Nagano	21.7	45.4	11.1	21.8
PVP-adsorbed fr.				
Inogashira	18.7(%)	21.6(%)	28.9(%)	30.8(%)
Dando	21.6	20.5	25.0	32.9
Nagano	20.4	19.4	30.0	30.2

almost exclusively in adsorbed fractions, while signals of carbohydrate-C were more eminent in non-adsorbed fractions.

Table 3 shows the composition of carbon species in each FA fraction. In Fig. 1, the broad band in 10-48 ppm assigned to alkanes not substituted by hetero atoms, was observed only in the spectrum of adsorbed fraction, while the small sharp peaks in this region were observed only in non-adsorbed fraction. The signal at 51-52 ppm assigned to methoxyl-C was commonly found in the spectra of both fractions. These were also observed in other soils. Therefore, saturated aliphatic structures of these two FA fractions were considered to be different in quality, though their relative contents in these fractions were very similar (<2.5 % deviation) in all three soils as showen in Table 3. Only the signal at 51-52 ppm assigned to methoxyl-C was commonly found in the spectra of both fractions.

A sharp peak at 63 ppm (C_6 of hexose or C_5 of pentose), a large and broad band in 78-86 ppm (C_2-C_5 of hexose or C_2-C_4 of pentose) and a band in 95-110 ppm (C_1 of hexose and pentose) were observed in the carbohydrate-C region of all PVP-non-adsorbed fractions. According to Ishizuka (ref. 5), chemical shifts of hexose C_6 and pentose C_5 in glycoside bonds (64-68 ppm) differ from those in a free form (61-64 ppm). Likewise, chemical shifts of C_1 were separated according to the type of bond into 100-105 ppm (C_1-C_4) and 95-100 ppm (C_1-C_6, C_1-C_2 and C_1-OH). Saccharides contained in FAs were considered to be linked mainly by 1-4 bonds, because signals of C_1 had the maximum near 104 ppm and the chemical shift of C_6 were 63 ppm.

The composition of carbon species in PVP-adsorbed fractions was very similar among the used three soils, and differences in the relative contents of each carbon species were less than 5 %. For example, the relative contents of carbo-
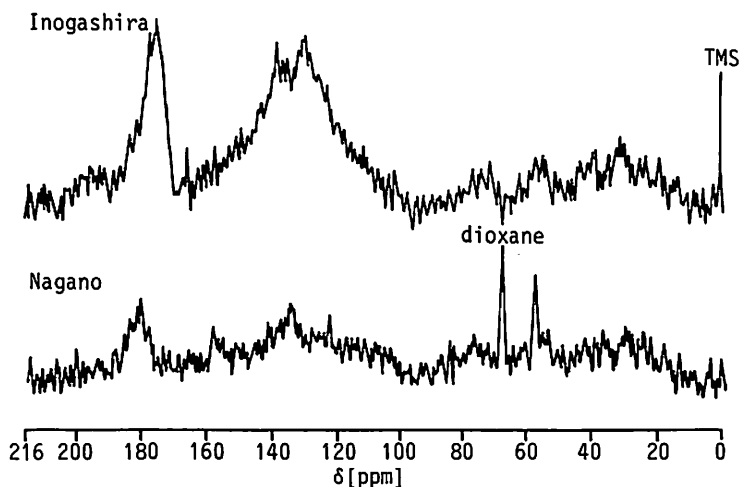
Fig. 2. ^{13}C-NMR spectra of Inogashira HA (A-type) and Nagano HA (Rp-type).

nyl-C were as high as 30-33 % in all samples and those of carbohydrate-C were at the range of 19-22 %.

The composition of carbon species in PVP-non-adsorbed fractions was also similar among the three samples, although the difference among them was larger than those in the adsorbed fractions, especially with respect to the relative content of carbohydrate-C.

(2) Comparison of A-type HA and Rp-type HA

Fig. 2 shows the ^{13}C-NMR spectra of Inogashira HA and Nagano HA. Inogashira HA obtained from an ando soil was classified into A-type while Nagano HA from a grey lowland soil into Rp-type according to Kumada et al (ref. 6). Various properties of HAs changed from Rp-type via B-type to A-type (refs. 7). ^{13}C-NMR spectra of HAs also differed remarkably between A-type and Rp-type HAs. Relative intensities of the peaks of aromatic-C and carboxyl-C were larger in Inogashira A-type HA. On the other hand, peaks of methoxyl-C (57 ppm) and phenolic- or aryl ether-C (158 ppm) were more eminent in Nagano Rp-type HA.

TABLE 4

The composition of carbon species in HAs from ^{13}C-NMR spectra.

HAs	Aliphatic-C (0-60 ppm)	Carbo-hydrate-C (60-110 ppm)	Aromatic & olefinic-C (110-165 ppm)	Carbonyl-C (165-216 ppm)
Inogashira(A-type)	18.8(%)	10.9(%)	44.7(%)	25.7(%)
Nagano(Rp-type)	25.4	19.1	35.2	20.3

Table 4 shows the compositions of carbon species in the two HAs. The relative content of aromatic- plus olefinic-C was 45 % in Inogashira HA and 35 % in Nagano HA, indicating that aromaticity was higher in the A-type HA. The more distinguished signal of phenolic-C in Nagano HA may be due to the higher content of lignin-like structure (ref. 8) as well as the simpler composition of aromatic structure in this HA than that in Inogashira HA.

(3) Comparison of HA and PVP-adsorbed fraction of FA

When ^{13}C-NMR spectra of HA and PVP-adsorbed fraction of FA obtained from Inogashira soil were compared, the peak of aromatic-C in A-type HA was broader but higher and its relative content was 1.6 times larger than that in PVP-adsorbed fraction of FA. In contrast, the peaks of carbohydrate-C in PVP-adsorbed fraction were more intense and its relative content was 2 times larger than that in HA. The difference in relative contents of carbonyl-C was not so large between two fractions (Figs. 1, 2 and Tables 3, 4). On the other hand, in Nagano soil, ^{13}C-NMR spectra of Rp-type HA and PVP-adsorbed fraction of FA considerably resembled each other, and the relative contents of aromatic-C and carbohydrate-C were similar. However, the relative content of carbonyl-C in HA was two-thirds of that in PVP-adsorbed fraction of FA. These results indicated that structural relationship between HA and FA differed according to the type of soil.

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