STRUCTURAL DIFFERENCES BETWEEN HUMIC FRACTIONS FROM DIFFERENT SOIL TYPES AS
DETERMINED BY FT-IR AND 13 C-NMR STUDIES

R. FRÜND*, H.-D. LÜDEMANN*, F.J. GONZALEZ-VILA**, G. ALMENDROS***,
J.C. del RIO** and F. MARTIN**

*Institut für Biophysik und Physikalische Biochemie, Universität Regensburg,
 Postfach 397, D-8400 Regensburg (West Germany)

**Instituto de Resursos Naturales y Agrobiologia, (C.S.I.C.), Apartado 1052,
  41080 Sevilla (Spain)

***Instituto de Edafologia y Biologia Vegetal, (C.S.I.C.), Serrano 115,
   28006 Madrid (Spain)

SUMMARY
    Structural characteristics of fulvic and humic fractions from various Spa-
nish soils are characterized by their 13 C-NMR spectra registered under quanti-
tative acquisition conditions, and their resolution-improved FT-IR spectra
obtained by substracting the original spectra from a positive multiple of their
Laplacians.
    Although the humic samples analysed had a very different origin, the spec-
tral features do not reflect the influence of any specific soil-forming factor
upon the spectral patterns of the humic fractions from a particular soil.
    A correlation study between the most relevant peaks in the IR and NMR
spectra showed that both techniques lead to similar conclusions in terms of the
different contributions of aromatic and aliphatic moieties, but to very inde-
pendent results with regard to the oxygen-containing functional groups.
    The most significant differences between the two humic materials studied is
that carbohydrates predominate in the fulvic acids (FA's), whereas in the humic
acids (HA's) those of the aromatic carbons. The variations in the carboxyl/car-
bonyl and the aliphatic ranges are minor at best. The presence of a large
fraction of carboxyl groups bound to aromatic carbons is suggested.

INTRODUCTION
    The natural variability in vegetation cover, soil geochemistry and climate
lead to a wide diversity in the chemical processes controlling the evolution of
the soil organic matter and the formation of humic substances (ref. 1).
    The chemical structure of the humic materials is still a matter of contro-
versy, since it is to be suspected, that the drastic standard extraction proce-
dures could lead to a chemical alteration of the organic compounds. In addition,
the degradative methods usually employed in structural studies are also likely
to produce biased quantitative and qualitative results. For this reasons, the
non-destructive techniques, mainly FT-IR- and NMR-spectroscopy, are receiving
an increased attention as alternative methods of study. Thus, resolution-
enhanced FT-IR spectra obtained after computer data processing seem to give

additional valuable information concerning the composition of humic materials (ref. 2), although the 13 C-NMR in solution and in the solid state is the spectroscopic method that yielded the most useful information about the structure of humic compounds. This technique appears to be suitable to differentiate humic materials from various environments (ref. 3).

In the present study the fulvic and humic acid fractions from various Spanish soils are characterized by their resolution-enhanced FT-IR spectra and their 13 C-NMR solution spectra, registered under quantitative acquisition conditions, in order to correlate the structural information given by these non destructive technique.

MATERIALS AND METHODS

A number of field and analytical characteristics of the soil samples from which humic acids (HA) and fulvic acids (FA) were extracted are given in Table I. Methods used for extraction, separation and purification of HA's and FA's, and for functional group analysis were described previously (ref. 4,5).

TABLE I

Origin and some characteristics of Spanish soil samples.

Sample	Horizon	Depth (cm)	Soil Series	Order	Geographical origin	pH (H_2O)	C (%)
V	$A_o + A_1$	0-20	Utrera	Typic Chromoxerert	Sevilla	7.8	1.0
P	Bh	20-30	Ribadeo	Typic Haplorthod	Lugo	3.3	6.6
D	B	10-30	Donaña	Histic Humaquept	Huelva	3.6	5.1
AL	$A_o + A_1$	0-20	Las Lajas	Andic Humitropet	Canary Islands	5.4	11.3
AI	$A_o + A_1$	0-15	Izaña	Andic Humitropet	Canary Islands	4.9	17.4

Table II shows the elementary composition and functional group analysis of the samples.

The FT-IR spectra were recorded using KBr pellets (2 mg of samples per 300 mg of KBr) in a Nicolet 5DXE spectrophotometer, adding 100 interferograms at 2 cm^{-1} resolution. The resolution of the spectra were improved by a method based on techniques used for digital image processing (ref. 6), which basically consists of the subtraction of the original spectrum from a positive multiple of its Laplacian, by using a computer program developed by the authors.

TABLE II

Analytical characteristics of humic acids (HA) and fulvic acids (FA)

	C	H %*	N	O	Ash %	Total acidity	Carboxyls meq. g^{-1}*	Phenolic hydroxyls
HA-V	57.7	5.6	5.8	30.9	1.5	5.7	3.1	2.6
FA-V	39.6	3.4	2.4	54.5	2.0	8.9	7.7	1.1
HA-P	59.4	4.2	3.2	33.1	0.7	10.5	4.2	6.4
FA-P	49.6	3.3	1.2	45.9	1.3	12.6	8.6	4.0
HA-D	57.1	4.5	3.4	35.0	1.1	6.0	3.3	2.7
FA-D	31.6	4.0	n.d.	–	2.0	n.d.	n.d.	n.d.
HA-Al	48.4	4.1	2.2	45.3	4.4	8.7	2.7	6.0
FA-AL	49.1	2.3	1.8	46.8	3.0	9.8	6.8	3.0
HA-AI	52.8	5.2	5.0	37.0	1.3	n.d.	n.d.	n.d.
FA-AI	26.4	3.4	1.6	68.6	0.9	n.d.	n.d.	n.d.

*dry, ash free

The NMR measurements were taken from 150 mg dry substance dissolved in 0.5 N NaOD. All spectra were registered with a Bruker MSL 300 spectrometer at a frequency of 75.4 MHz in a 10 mm multinuclear high resolution probehead in the deuterium lock mode. The protons were inverse gated broadband decoupled in order to suppress the NOE. The spectra were referenced to a capillary filled with neat tetramethylsilane. No attempt to correct for magnetic susceptibility effects were made. The chemical shifts given are judged reliable to ± 1 ppm.

For the suppression of dead time signals and baseline curvature, the program RIDE (Ring Down Elimination) was applied (ref. 7). It yielded spectra with reasonable flat baselines to allow integration of the various groups of signals, as previously shown for NMR-spectra of humic materials from composted plant residues (ref. 8). The RIDE pulse sequence consists of:

$$90^{o}_{+x} - \Delta t - FID^{+} - Td - 180^{o}_{+y} - 90^{o}_{-x} - \Delta t - FID^{+} - Td$$

An additional 16-step phase cycling was used to correct the phase instability. T_d was set to 3.0 s, Δt to 20 ms; 12000 FID's were accumulated and a 100 Hz line broadening was applied. The spectra were quantitatively evaluated with the integration routine of the spectrometer.

RESULTS AND DISCUSSION

Fig. 1 shows original FT-IR spectra of representative samples in comparison with their respective resolution-enhanced spectra. Since the treatment applied was not too drastic (raw spectrum – 5 ∇^2), the considerable sharpening obtained for the peaks was not associated with a significant enhancement of the noise.
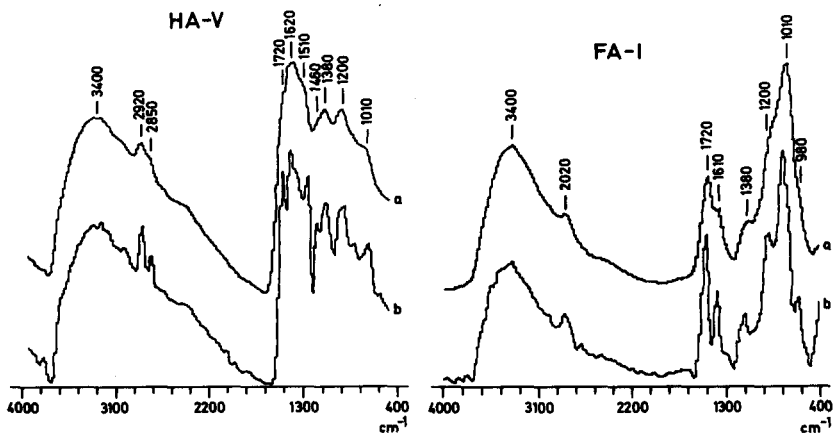
Fig. 1. FT-IR spectra of Ha-V and FA-AI (a) and their respective resolution enhanced spectra (b).

After computer treatment some shoulders were transformed into individual peaks, revealing structural features only vaguely defined in the raw spectra. Thus, in the case of the sample HA-VU the aromatic band at 1510 cm^{-1} and the carboxyl vibration at 1720 cm^{-1} became conspicuous in comparison with the broad multiple band near 1620 cm^{-1} in the raw spectrum, and the same occurred with the 1400-1000 cm^{-1} broad region in the FA-AI, which turn into a well-defined band system at 1380, 1200, 1010 and 980 cm^{-1}.

Some differences are observed between FA's when comparing their resolution enhanced FT-IR spectra. Whereas the complex system of peaks between 1300-600 cm^{-1} in the FA-V suggest a character similar to that of a strongly acidic polysaccharide-like polymer, the FA-P shows sharp bands at 1510 and 1720 cm^{-1} together with an ill-defined pattern around 1000 cm^{-1} indicating a strongly aromatic and acidic character. The other FA's presented an intermediate character between the samples described above.

The differences are lower in the case of HA's, being in general the bands due in part to aromatic structures (1620, 1510 cm^{-1} and alkyl vibrations (2920 and 1420 cm^{-1}) those that became more prominent in the processes spectra. In the HA-AI and HA-AL higher absorbance and complexity for the bands around 1000 cm^{-1} were also observed.

Fig. 2 a and b give typical examples of the fulvic and humic acid 13 C-NMR spectra. They can be considered as representative for the whole series dis-cussed in the following.

These spectra are usually analysed quantitatively by subdividing them into four major regions: Aliphatic carbons in the range from 5 to 46 ppm, carbons of carbohydrate origin from 46 to 110 ppm, aromatic carbons between 110-160 ppm, and carboxyl- and carbonyl-groups in the range from 160 to 200 ppm. The rela-

Fig. 2. 13 C-NMR spectra at
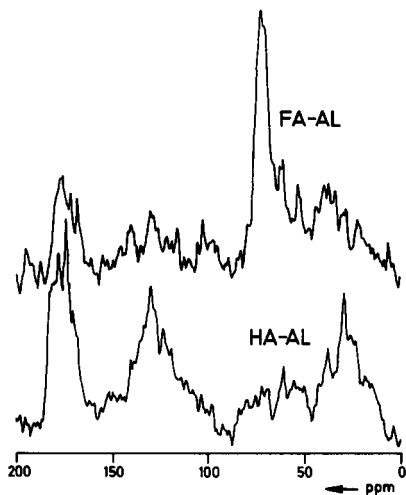75 MHz of one humic acid (HA-AL)
and one fulvic acid (FA-AL)

tive concentrations of the four classes of carbons as obtained from the integration of the spectra are given in Table III.

The most significant difference between the two series of compounds is that the structures of carbohydrate origin predominate in the FA's while the most prominent area in the HA's is that of the aromatic carbons. The variations in the carboxyl-carbonyl and the aliphatic ranges are minor at best.

The modest resolution obtainable with these complex compounds allows only a few general conclusions about the spectral details to be taken. If the spectral region between 110 and 46 ppm is to be assigned to carbohydrates, it is necessary to find sufficient intensity in the spectral range between 90 and 110 where the signals of anomeric C-1 carbons are expected. The signals in this range are usually well characterized in the FA's, while in the HA's they only appear as a shoulder at the high field side of the aromatic resonances. In all spectra analysed hitherto the whole intensi-

TABLE III

Distribution of C in HA's and FA's as determined by 13 C-NMR % of total area

Samples	Carboxyls	Aromatics	Carbohydrate derived structures	Aliphatics
chem. shifts in ppm	(200-160)	(160-110)	(110-46)	(46-5)
HA-V	16	40	18	26
HA-P	20	47	12	21
HA-D	16	37	20	28
HA-AL	28	32	18	23
HA-AI	16	35	20	30
mean values	19±9	38±9	18±6	25±4
FA-P	22	25	35	18
FA-D	14	11	48	27
FA-AL	15	15	49	22
FA-AI	24	17	36	23
mean values	19±5	17±8	42±7	22±5

ty of this range is approximately 1/6 of the total carbohydrate region, thus corroborating the assignments of the carbohydrate region, which should mostly consist of polyhexoses. A fraction of the terminal sugars has certainly its final aldo groups oxidized in the process of humification and it is thus not to be expected to find an exact stoichiometry. The values for polysaccharide carbon atoms obtained by integration of the spectra (table III) are in disagreement with the analytical determination of total sugars by the colorimetric anthrone test, which give amounts under 5 %. This indicates that chemical methods are not particularly suitable for an exact determination of carbohydrates in such complex matrices (ref. 9,10).

Another unexpected feature of all the spectra is the absence of any distinct C-methyl (around 20 ppm) and O-methyl signal (around 56 ppm). The degradation or transformation of lignocellulose starting materials is an important process contributing to the formation of humic substances and one would thus expect these signals to be present in the soluble fractions. These groups must thus have been removed in early steps of the humification process.

In agreement with Hatcher et al. (ref. 11) the carboxyl content measured by NMR more closely approximated total acidities rather than carboxyl contents measured by titration. This fact has been discussed by several authors, and it has been suggested that the discrepancy might indicate the presence of other groups in the carboxyl region (ref. 12). On the other hand we found that the contents of carboxyl groups are similar in HA's and FA's, in disagreement with the chemical data. A possible explanation for this might be that the higher reactivity of the FA would be in part due to their low MW and the surface distribution of COOH groups, whereas in the HA's, an important fraction of the less reactive COOH groups might be localized in the interior of the polymer.

The quantitative evaluation of the peak areas in the NMR spectra are shown in the stick diagram in Fig. 3.

The determination of correlation indices between the magnitude of the IR and NMR peaks showed a very significant ($p < 0.99$) negative correlations between the absorbance at 1510 cm^{-1} and the alkyl peaks at 43, 62 and 77 ppm, and positive with the NMR bands in the aromatic region (125 and 150 ppm). Also, as expected, there were very significant correlations between the absorbance at 2920 cm^{-1} and the bands in the aliphatic region of the NMR spectra (43 and 48 ppm). This good agreement between NMR and IR spectra was, however, not observed for the oxygen-containing functional groups. The intensity of the 1720 cm^{-1} band was not correlated at a significant level with any of the NMR bands. On the other hand the areas of the aliphatic regions and C=O groups in the NMR spectra were not significantly different between both groups of substances ($p < 0.95$) in contrast with the very different proportions of aromatic carbons and polysaccharide-derived carbons.
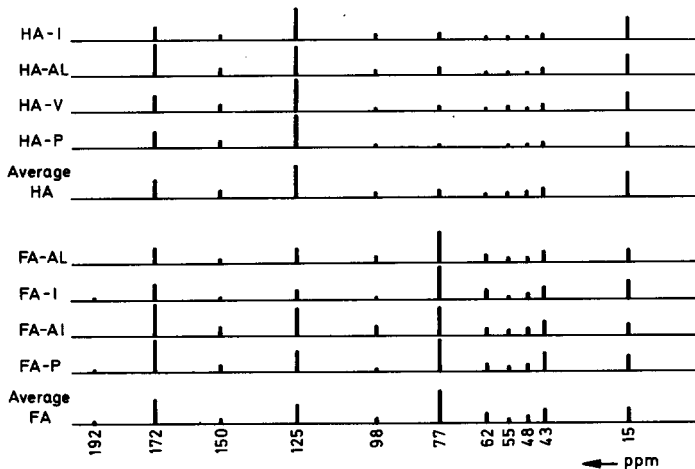
Fig. 3. Stick diagram of the 13 C-NMR spectra obtained for the various humic and fulvic acids.

The five soils analysed here have a very different origin, and it is not to be expected, that a quantitative analysis of the 13 C-NMR data can reveal any specific influence of the mineral composition, vegetation cover or any other soil-forming factor upon the development and characteristics of the organic fractions. However, when comparing the ratios between the four classes of structural groups some interesting features deserving further investigation emerge. The quotients between the intensities of the aromatic- and the carbohydrate-, resp. the aliphatic- and the carboxyl-signals are compiled in Table IV.

TABLE IV

Ratios of the distributions given in Table III.

Sample	% Carboxyl	Aromatic / Carboxyl	Carbohydrate / Carboxyl	Aliphatic / Carboxyl
HA-V	16	2.5	1.1	1.6
HA-D	16	2.3	1.3	1.8
HA-AI	16	2.2	1.3	1.9
HA-P	20	2.4	0.6	1.1
HA-AL	28	1.1	0.6	0.8
FA-D	14	0.8	3.4	1.9
FA-AL	15	1.0	3.3	1.5
FA-P	22	1.1	1.6	0.8
FA-AI	24	0.7	1.5	1.0

The two groups of humic substances are arranged in the order of increasing carboxyl concentration. With the exception of HA-AL, which has an unusually large carboxyl intensity, the aromatic/carboxyl ratio appears to be constant in each group and thus independent of the total carboxyl concentration. This could

indicate that a large fraction of the carboxyl groups is bound to aromatic carbons, although this latter possibility has been recently questioned by Wilson et al. (ref.13). The ratios of carbohydrates/carboxyl and aliphatic/carboxyl decrease significant with increasing concentration. The most probable explanation for this finding is that in the natural oxidative decomposition these groups or moieties of the native mixture of macromolecules are oxidized faster than the aromatic parts and thus disappear first from the starting material.

Considering the small number of samples studied hitherto it appears premature to attempt a more detailed discussion, the data however show that a more systematic study of various organic fractions of the soil might yield very interesting and general conclusions about the formation of humic material.

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