Insights into the Composition of Humin from an Irish Grassland Soil

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1. INTRODUCTION

Humin is defined as the fraction of the humic substances (HS) that is insoluble in aqueous solution at any pH value (1). Because of its insolubility it has been the least studied of all the humic fractions (2). Humin typically represents more than 50% of the organic carbon (C) in a soil, and is regarded as the residual organic C that remains after extraction of the HAs and FAs (3). Based on data from solid-state ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, it has been concluded that a repeating aliphatic structural unit, possibly attributable to branched and cross-linked algal or microbial lipids, are common to both sediment and soil humin samples (4). Humin becomes enriched in these aliphatic substances because of their selective preservation during organic diagenesis (2). To investigate the composition of humin, The Clonakilty soil, a well drained Brown Podzolic soil in grassland lysimeter studies at the Teagasc Soils Research Centre, Johnstown Castle, Co. Wexford, Ireland and was used in the present study.

2. MATERIALS AND METHODS

Soil samples were H^+-exchanged and exhaustively extracted with 0.1 M NaOH, at pH 7.0, then with NaOH solution adjusted to pH 10.6, then at pH 12.6, then with 6 M urea in the 0.1 M solution. Extracts were pressure filtered (0.2 μm membrane) at each pH, and sediment on the filter was returned for extraction by the next solvent in the series. The fine clays retained on the filter after extraction with the 6M urea system were collected, dialysed, and freeze dried. To isolate the humin organic C, the residue was treated with 10% HF to dissolve the aluminosilicates. Some samples were further extracted with a DMSO-concentrated H_2SO_4 (94% DMSO: 6% H_2SO_4) mixture to isolate a humin fraction following extraction in aqueous base and in the basic urea medium. Solid-state ^{13}C NMR spectroscopy experiments were carried out at ^{13}C and ^{1}H frequencies of 100.5 and 400.0 MHz, respectively. Typical cross-polarization times of 1 ms, acquisition times of 13 ms,

and recycle delays of 500 ms were used. For Variable Amplitude Cross-Polarization (VACP) experiments a Magic-Angle Spinning (MAS) frequencies of 13 kHz was used. Bloch Decay experiments were carried out while spinning at 13 kHz with a recycle delay of 1 s and a sweep width of 27 kHz.

3. RESULTS AND DISCUSSION

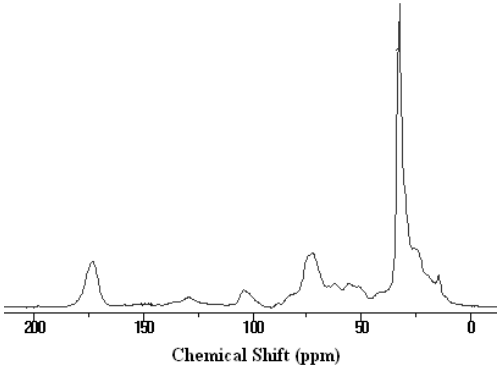

Figure 1. ^{13}C CP-MAS NMR spectrum of Humin isolated from the 0-10 cm depth while spinning at 13 kHz, with a cross-polarization time of 1 ms, acquisition time of 13 ms and a recycle delay of 500 ms.

Fine clay was isolated from soil at the 0-10 cm depth after exhaustive extraction with NaOH followed by NaOH and Urea. The organic matter in the isolated fine clay is classified as humin as it was found to be insoluble in aqueous media at any pH. Figure 1 shows the spectrum for 0-10 cm depth humin. In aerobic soils, the spectra of humin show the presence of polysaccharides and aromatic structures most likely derived from the lignin of vascular plants. However, another major component of humin contains paraffinic carbons and is thought to be derived from algal or microbial sources. A major peak in the humin spectra is that of aliphatic carbons at 30 ppm. Cutin and cutan from plant cuticles are considered to be major contributors to this region. These structures may well be the major contributors to the carboxyl or amide groups (peak at 175 ppm) (2).

Proteinaceous substances would give rise to resonances in the 0-50 ppm region of ^{13}C NMR spectra, but usually the aliphatic signals of protein are centered more closely toward 20, 40, and 50 ppm than the 30 ppm resonance (2). Humin contains significantly less carboxyl than the corresponding HA isolate (not shown). There is little indication for lignin-like structures in the spectra because of the absence of significant peaks at 56 ppm and at 150 ppm for methoxyl and O-aromatic carbons, respectively.

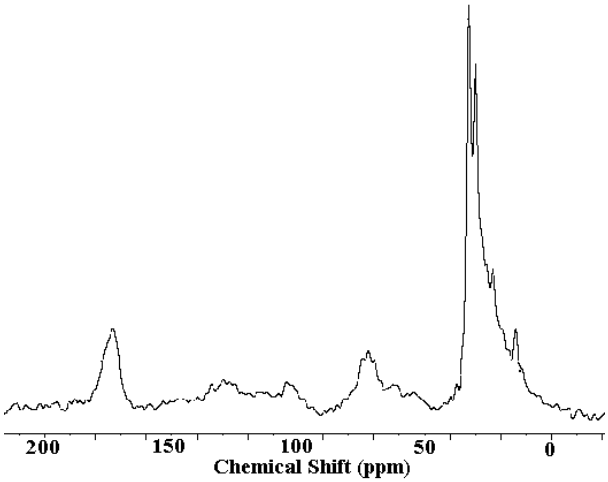

Figure 2. ^{13}C Bloch Decay NMR spectrum of Humin isolated from the 0-10 cm depth while spinning at 13 kHz with a recycle delay of 1 s and a sweep width of 27 kHz.

Figure 2 shows the Bloch decay ^{13}C NMR spectrum for the 0-10 cm depth humin. Again the dominance of aliphatic signals is evident. The spectrum shows an aliphatic region characterized by a small peak at 14 ppm, from CH_3, and a large peak for main-chain CH_2 (30 ppm). It can be seen that the main-chain CH_2 groups are composed of both amorphous chains (29 ppm) and crystalline chains (32 ppm) (5)

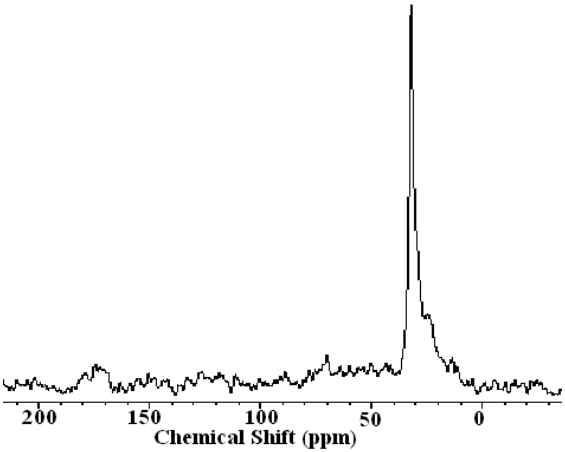

Figure 3. ^{13}C CP-MAS NMR spectrum of the DMSO/H_2SO_4 extract of Humin while spinning at 13 kHz, with a cross-polarization time of 1 ms, acquisition time of 13 ms and a recycle delay of 500 ms.

A DMSO-concentrated H_2SO_4 mixture has been employed to isolate a humin fraction following extraction in aqueous base and in the basic urea medium (6). It was shown that $\geq$ 70% of the traditional humin fraction could be solubilised. The spectrum of the dried extract can be seen in Figure 3. It contains mainly aliphatic signals with some very minor contributions from carbohydrates and carboxyl signals.

4. CONCLUSIONS

The humin samples studied contain contributions from aliphatic, carbohydrate, and little aromatic and carboxyl resonance. The strong aliphatic resonance would support the theory of the "selective preservation" of the more aliphatic compounds. It is necessary to structurally define the paraffinic components of humin and to ascertain whether these are truly biomolecules or are rapidly transformed products of decomposition of vegetal matter (2). Data will be shown to highlight the contribution of cutin/cutan to the humin fraction. Further work needs to be done on this, in addition to analyses of the organic matter in the lower depths, to determine the contribution of biomolecules to humin.

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