A novel pathway of soil organic matter formation by selective preservation of resistant straight-chain biopolymers: chemical and isotope evidence

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A novel pathway of soil organic matter formation by selective preservation of resistant straight-chain biopolymers : chemical and isotope evidence

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Abstract

A resistant soil organic residue, 'humin', has been analysed by solid-state ^{13}C-NMR and pyrolysis followed by molecular and ^{13}C determination of the pyrolysate alkane-alkene fraction. The results show the occurrence of highly aliphatic, straight-chain biopolymer material as a substantial part of soil organic matter. They confirm the hypothesis by which a part of soil organic matter can be formed by selective preservation of resistant highly aliphatic microbial polymers. This pathway comes in addition to the longstanding neogenesis hypothesis involving recondensation of small polar molecules such as amino acids, carbohydrates and phenols.

Keywords : ^{13}C of soil alkanes, NMR, resistant aliphatic biopolymer, pyrolysis of humin.

INTRODUCTION

Soil organic matter is composed of a complex mixture of living organisms, dead organic debris and anthropogenic inputs. During the decay of plants, a major part of plant carbon ($\sim 99\%$) is biodegraded and recycled to the atmosphere as CO_2. The organic matter remaining in soils is thus a carbon pool exhibiting high resistance to biodegradation. Various but minor classes of soil organic molecules including lipids, amino acids, phenols, and carbohydrates have so far been identified (Schnitzer, 1991). Indeed, despite numerous investigations, the bulk of soil organic matter is made up partly of macromolecular, brown yellow humic substances whose molecular structure is still a matter of debate (Rice and MacCarthy, 1990, de Leeuw and Hatcher, 1992). Two main hypotheses have been advanced to explain how humification takes place : the selective preservation of plant biopolymers and the polycondensation of small molecules (Bollag and Loll, 1983). The selective preservation of plant biopolymer residues should favour the accumulation of ligno-protein nuclei in humic substances. Nonetheless, ^{13}C isotope studies of various soil organic pools show that such a process should play only a minor part in the formation of humus from crop soils (Lichtfouse *et al.*, 1995). Therefore *in situ* processes are more likely to be involved :

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1) the polycondensation (or neogenesis) pathway (Maillard, 1916, 1917), and 2) a pathway proposed by Lichtfouse *et al.* (1995, 1996) which involves selective preservation of resistant aliphatic biopolymers from microbes. While the polycondensation pathway is based upon polymerisation of rather small molecules such as amino acids, phenols, carbohydrates and various oligomers from biomass decomposition products as well as from root exudates, the new proposed pathway is based upon microbial biosynthesis of highly aliphatic cell walls which are chemically and physically resistant to biodegradation.

Several investigations have already detected the presence of aliphatic carbon in soils, including humic substances and resistant residues (Hatcher *et al.*, 1981, Schulten H.-R. and Schnitzer, 1990, Kögel-Knabner *et al.*, 1992, Beyer, 1996). In a recent investigation, Lichtfouse *et al.* (1995) proposed on ^{13}C isotope grounds that soil organic matter may additionally be stored by selective preservation of resistant biopolymers from soil microorganisms. Moreover, in a recent note, we identified resistant ultralaminae (Lichtfouse *et al.*, 1996) which are structurally similar to those found in aquatic sediments (Largeau *et al.*, 1990, Derenne *et al.*, 1991), thus strengthening our view. Here, using molecular and isotope techniques, we show that a part of the resistant carbon in crop soil is indeed composed of saturated straight-chain hydrocarbons, and we discuss implications for organic matter preservation in soils.

EXPERIMENTAL
Detailed procedures are reported elsewhere (Lichtfouse *et al.*, 1996) : a sample of maize crop soil (290 g) was thoroughly extracted with $CHCl_3$-MeOH 3/1 v/v to remove free lipids, then with NaOH 0.1 M to remove fulvic and humic acids. The remaining matter (humin and minerals) was treated with HCl and HF, then extracted with $CHCl_3$-MeOH to yield 3.9 g of solid residue (31%C, 3% H, 2.5% N and 40% of uncombusted matter). Solid state ^{13}C NMR spectra of the resistant residue were recorded on a Bruker MSL 400 spectrometer at 100.6 Mhz for ^{13}C using the conventional Cross Polarization/Magic Angle Spinning (CP/MAS) technique with a contact time of 1 ms, a pulse delay of 5 s and dipolar dephasing delays T_{DD} of 0 μs, 20 μs and 40 μs. The residue was pyrolyzed for 5 min at 600°C in a gold tube under continuous argon flow. The pyrolysate was fractionated into alkane-alkene and aromatic fractions by silica gel column chromatography eluting with pentane then pentane/methylene chloride (65/35 v/v). The alkane-alkene fraction was analysed using a HP 5890 gas chromatograph coupled to a VG Autospec mass spectrometer (GC-MS). Conditions : splitless injection; helium carrier gas; 60 m x 0.2 mm capillary column coated with 0.2μm thickness methylpolysiloxane phase; oven temperature 50°C (1 min), 50°C to 140°C at 40°/min, 140°C to 320 °C at 2°/min then isothermal (60 min).; electronic ionisation 70 eV, 31-650 amu scan range. Isotopic analyses of individual *n*-alkanes from alkane-alkene fractions (3 to 4 replicates, deviation ≤ 0.3‰) were carried out under a continuous helium flow using a Hewlett Packard gas chromatograph coupled via a CuO furnace to a Finigan Mat 252 isotope ratio mass spectrometer. Isotope values were measured by comparison with CO_2, 5α-androstane and *n*-tetracontane standards. Isotopic compositions are expressed in per mil as δ^{13}C values relative to the Pee Dee Belemnite standard :
$$\delta^{13}C = [(^{13}C/^{12}Csample - {}^{13}C/^{12}Cstd)/(^{13}C/^{12}Cstd)]x10^3$$

RESULTS AND DISCUSSION

A resistant organic residue was isolated from a maize crop soil by removal of lipids, humic acids, fulvic acids, and minerals. The solid state ^{13}C-NMR of this residue exhibits a relatively high signal at 32 ppm which shows the occurrence of aliphatic, saturated carbon (Fig. 1). The dephasing experiment shows a strong decrease in the 32 ppm peak, which indicates that the saturated carbon is mainly composed of secondary (R-CH$_2$-R') and tertiary carbon (R-CH-R'R'') with few methyl groups (15 ppm) and few quaternary carbons. This finding suggests the occurrence of straight-chain skeletons with few branched and cyclic alkane skeletons, and is in agreement with IR analyses which give peaks at 2850 and 2920 cm^{-1} (unpublished data). The other main NMR peaks have been tentatively assigned to α carbons of C-O or C-N functions (56 ppm), protonated C-O or C-N functions (73 ppm), glycosidic C-O (104 ppm), olefinic and/or aromatic C=C (120 ppm), phenols (148 ppm), and quaternary ester or amide C (173 ppm).
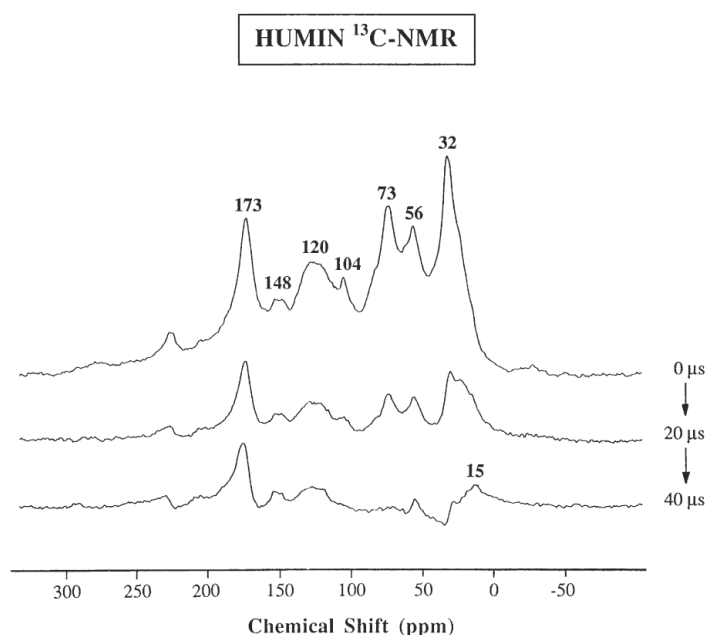

Figure 1. Solid-state ^{13}C-NMR spectra of a resistant organic residue from a maize crop soil. Note the major peak at 32 ppm showing the occurrence of saturated carbon. The dipolar dephasing experiments with delays of 20 μs and 40 μs show that this saturated carbon is mainly composed of secondary (R-CH2-R') and tertiary carbon (R-CH-R'R'').

GC-MS analyses of the alkane-alkene fraction from the pyrolysate residue show a major contribution of linear alkanes and alk-1-enes along with very few branched and cyclic molecules (Figure 2). The hydrocarbons can be classified into two main categories. First, *n*-alkanes extending from C$_{27}$ to C$_{33}$ with a strong odd predominance, which are typical plant wax products (e.g. Lichtfouse *et al.*, 1997). These alkanes have somehow been trapped in the organic network despite exhaustive CHCl$_3$-MeOH extraction of the residue prior to pyrolysis. Nonetheless, this finding is in agreement with the occurrence of bonded alkanes and fatty acids in humic substances (Schnitzer and Neyroud, 1975), and further suggests that this physical mode of sequestration of apolar molecules may play a role in the preservation of soil organic matter. Second, *n*-alkane/*n*-alkene doublets extending from C$_{10}$ to C$_{25}$, are present with no odd/even predominance, which are 'true' cleavage products of the macromolecular soil matrix. Such a

distribution, with very few branched and cyclic species is typical of kerogens and resistant aliphatic biopolymers (Behar *et al.*, 1984, Derenne *et al.*, 1988, 1991, 1992). Together with ^{13}C-NMR data, these findings show clearly that a part of resistant organic matter from soil is made of straight-chain saturated hydrocarbons.
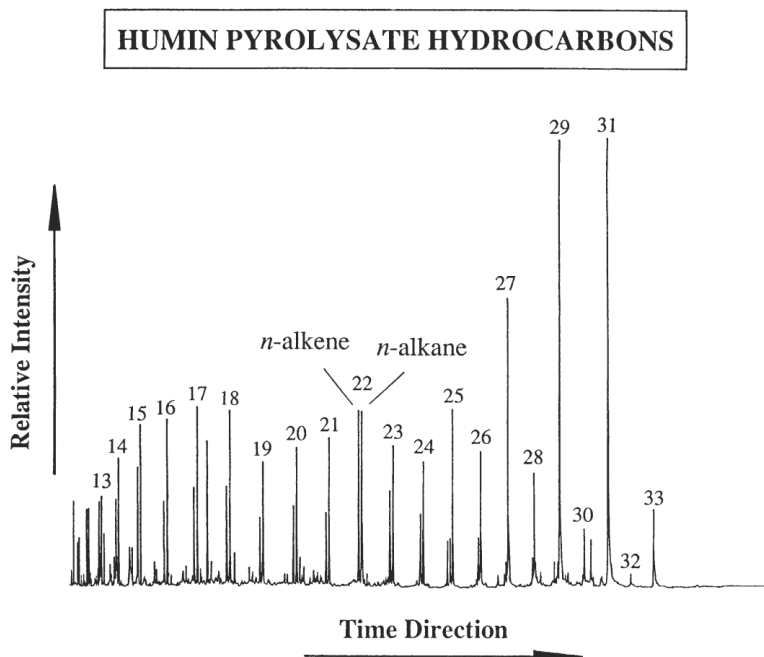

Figure 2. GC-MS total ion current of the alkane-alkene fraction of the soil organic residue pyrolysate. Note the occurrence of C_{10}-C_{25} alkane-alkene doublets, typical of straight-chain macromolecular material.

The δ^{13}C values of *n*-alkanes and *n*-alkenes from the soil residue pyrolysate are reported in Fig. 3. The ^{13}C-depleted values for the C_{29} and C_{31} *n*-alkanes are in agreement with the derivation of these compounds from plant waxes of maize and wheat grown previously at the site (Lichtfouse, 1997). The C_{27} *n*-alkane derives also from plant waxes, despite a ^{13}C enrichment which can be explained by a higher quantitative input of this molecule from maize waxes (Lichtfouse *et al.*, 1994). In contrast, C_{15}-C_{19} *n*-alkenes are significantly enriched in ^{13}C relative to *n*-alkane homologues. Furthermore, the δ^{13}C values of C_{15}-C_{19} *n*-alkenes are closer to the δ^{13}C value of the total organic carbon. Such an isotopic feature gives two pieces of information. First, it shows that these cleavage products are more representative of the bulk carbon than the longer chain *n*-alkanes. Second, it indicates that the carbon source of the organisms synthesizing the *n*-alkene precursors is enriched in ^{13}C. Therefore, this latter observation confirms our previous hypothesis on the formation of a substantial part of soil organic matter by 'preservation of resistant aliphatic biopolymers from microorganisms consuming ^{13}C-enriched plant polysaccharides' (Lichtfouse *et al.*, 1995). This novel, additional pathway contrasts with the current hypothesis involving recondensation of rather small molecules such as amino acids, phenols, carbohydrates and various oligomers. Nonetheless, a recent investigation on archaeological plant remains has brought some molecular evidence for the Maillard (or browning) reaction in buried organic matter (Evershed *et al.*, 1997). Further work is now in progress to evaluate the significance of these two processes in various fossil material.
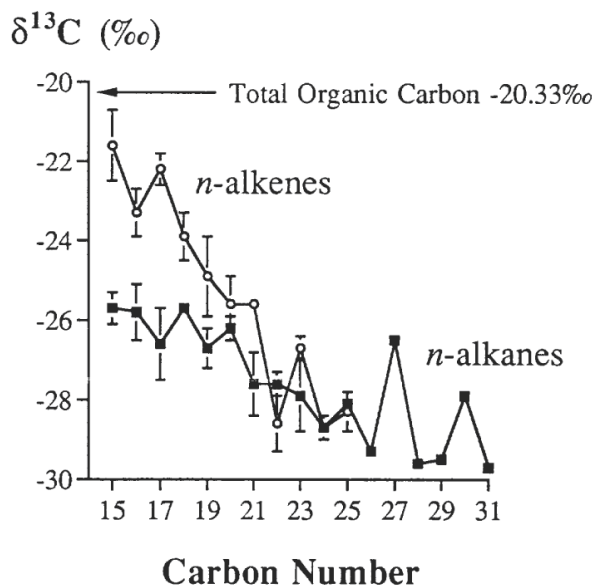
Figure 3. δ¹³C values of *n*-alkanes and *n*-alkenes from the pyrolysate of a soil residue. Note the ¹³C enrichment in the short-chain *n*-alkenes

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