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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 <sup>13</sup>C-NMR and pyrolysis followed by molecular and <sup>13</sup>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 : <sup>13</sup>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 (~ 99%) is biodegraded and recycled to the atmosphere as CO<sub>2</sub>. 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, <sup>13</sup>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  $^{13}\text{C}$  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  $\text{CHCl}_3$ -MeOH 3/1 v/v to remove free lipids, then with NaOH 0.1 M to remove fulvic and humic acids. The remaining matter (humins and minerals) was treated with HCl and HF, then extracted with  $\text{CHCl}_3$ -MeOH to yield 3.9 g of solid residue (31% C, 3% H, 2.5% N and 40% of uncombusted matter). Solid state  $^{13}\text{C}$  NMR spectra of the resistant residue were recorded on a Bruker MSL 400 spectrometer at 100.6 Mhz for  $^{13}\text{C}$  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  $T_{\text{DD}}$  of 0  $\mu\text{s}$ , 20  $\mu\text{s}$  and 40  $\mu\text{s}$ . 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 0.2 $\mu\text{m}$  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  $\leq 0.3\%$ ) 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  $\text{CO}_2$ , 5 $\alpha$ -androstane and *n*-tetracontane standards. Isotopic compositions are expressed in per mil as  $\delta^{13}\text{C}$  values relative to the Pee Dee Belemnite standard :

$$\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{std}})/({}^{13}\text{C}/{}^{12}\text{C}_{\text{std}})] \times 10^3$$

## 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  $^{13}\text{C}$ -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 ( $\text{R-CH}_2\text{-R}'$ ) and tertiary carbon ( $\text{R-CH-R}'\text{R}''$ ) 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  $\text{cm}^{-1}$  (unpublished data). The other main NMR peaks have been tentatively assigned to  $\alpha$  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).

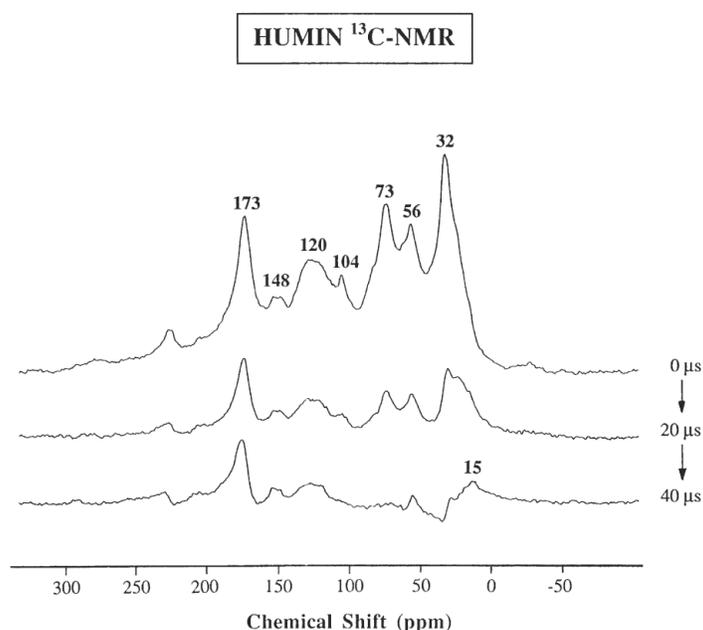


Figure 1. Solid-state  $^{13}\text{C}$ -NMR spectra of a resistant organic residue from a maize crop soil. Note the major peak at 32 ppm showing the occurrence of saturated carbon. The dipolar dephasing experiments with delays of 20  $\mu\text{s}$  and 40  $\mu\text{s}$  show that this saturated carbon is mainly composed of secondary ( $\text{R-CH}_2\text{-R}'$ ) and tertiary carbon ( $\text{R-CH-R}'\text{R}''$ ).

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  $\text{C}_{27}$  to  $\text{C}_{33}$  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  $\text{CHCl}_3$ -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  $\text{C}_{10}$  to  $\text{C}_{25}$ , 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  $^{13}\text{C}$ -NMR data, these findings show clearly that a part of resistant organic matter from soil is made of straight-chain saturated hydrocarbons.

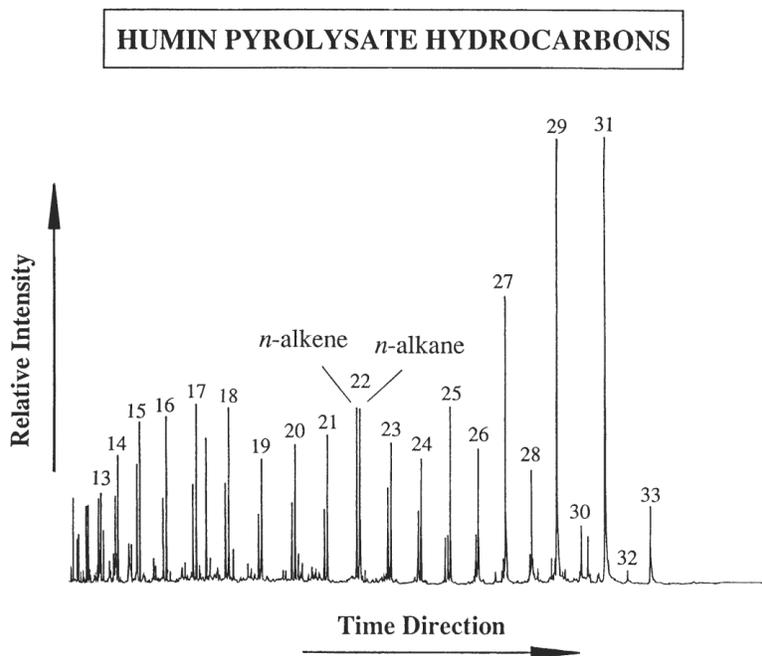


Figure 2. GC-MS total ion current of the alkane-alkene fraction of the soil organic residue pyrolysate. Note the occurrence of  $\text{C}_{10}$ - $\text{C}_{25}$  alkane-alkene doublets, typical of straight-chain macromolecular material.

The  $\delta^{13}\text{C}$  values of *n*-alkanes and *n*-alkenes from the soil residue pyrolysate are reported in Fig. 3. The  $^{13}\text{C}$ -depleted values for the  $\text{C}_{29}$  and  $\text{C}_{31}$  *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  $\text{C}_{27}$  *n*-alkane derives also from plant waxes, despite a  $^{13}\text{C}$  enrichment which can be explained by a higher quantitative input of this molecule from maize waxes (Lichtfouse *et al.*, 1994). In contrast,  $\text{C}_{15}$ - $\text{C}_{19}$  *n*-alkenes are significantly enriched in  $^{13}\text{C}$  relative to *n*-alkane homologues. Furthermore, the  $\delta^{13}\text{C}$  values of  $\text{C}_{15}$ - $\text{C}_{19}$  *n*-alkenes are closer to the  $\delta^{13}\text{C}$  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  $^{13}\text{C}$ . 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  $^{13}\text{C}$ -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.

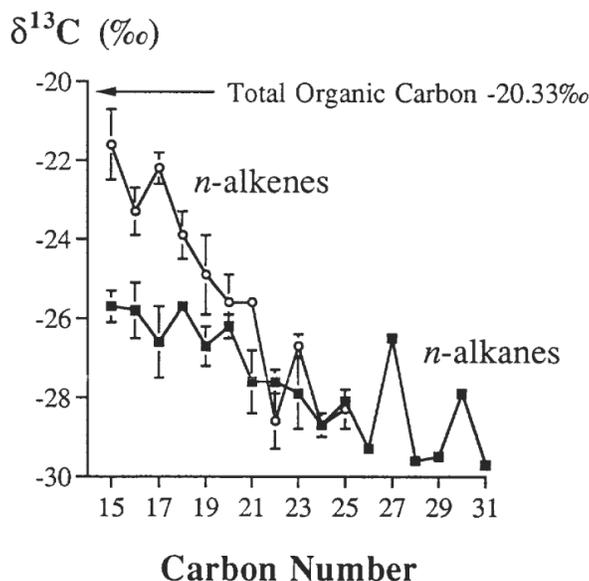


Figure 3.  $\delta^{13}\text{C}$  values of *n*-alkanes and *n*-alkenes from the pyrolysate of a soil residue. Note the  $^{13}\text{C}$  enrichment in the short-chain *n*-alkenes

## REFERENCES

- Behar F., Pelet R. and Roucache J. (1984) Geochemistry of asphaltenes. *Organic Geochemistry* **6**, 587-595.
- Beyer L. (1996) The chemical composition of soil organic matter in classical humic compound fractions and in bulk samples, a review. *Zeitschrift für Pflanzenernährung und Bodenkunde* **159**, 527-539.
- Bollag J.-M. and Loll M. J. (1983) Incorporation of xenobiotics into soil humus. *Experientia* **39**, 1221-1231.
- Derenne S., Largeau C., Casadevall E. and Connan J. (1988) Comparison of torbanites of various origins and evolutionary stages. Bacterial contribution to their formation. Cause of the lack of botryococcane in bitumens. *Organic Geochemistry* **12**, 43-59.
- Derenne S., Largeau C., Casadevall E., Berkaloff C. and Rousseau B. (1991) Chemical evidence of kerogen formation in source rocks and oil shales via selective preservation of thin resistant outer walls of microalgae : origin of ultralaminae. *Geochimica et Cosmochimica Acta* **55**, 1041-1150.
- Derenne S., Metzger P., Largeau C., Van Bergen P. F., Gatellier J. P., Sinninghe Damsté J. S., De Leeuw J. W. and Berkaloff C. (1992) Similar morphological and chemical variations of *Gloeocapsomorpha prisca* in Ordovician sediments and cultured *Botryococcus braunii* as a response to changes in salinity. *Organic Geochemistry* **19**, 299-313.
- Evershed R. P., Bland H. E., van Bergen P. F., Carter J. F., Horton M. C. and Rowley-Conwy P. A. (1997) Volatile compounds in archaeological plant remains and the Maillard reaction during decay of organic matter. *Science* **278**, 432-433.

Hatcher P. G., Maciel G. E. and Dennis L. W. (1981) Aliphatic structure of humic acids; a clue to their origin. *Organic Geochemistry* **3**, 43-48.

Kögel-Knabner I., De Leeuw J. W. and Hatcher P. G. (1992) Nature and distribution of alkyl carbon in forest soil profiles : implications for the origin and humification of aliphatic biomacromolecules. *The Science of the Total Environment* **117/118**, 175-185.

Largeau C., Derenne S., Casadevall E., Berkaloff C., Corolleur M., Lugardon B., Raynaud J. F. and Connan J. (1990) Occurrence and origin of 'ultralaminar' structures in 'amorphous' kerogens of various source rocks and oil shales. *Organic Geochemistry* **16**, 889-895.

de Leeuw J. W. and Hatcher P. G. (1992) Letter to the Editors, responding to A Chemical Structure for Humic Substances by H.-R. Schulten, B. Plage and M. Schnitzer. *Naturwissenschaften* **79**, 330-331.

Lichtfouse E. (1997) Heterogeneous turnover of molecular organic substances from crop soils as revealed by  $^{13}\text{C}$  labeling at natural abundance with *Zea mays*. *Naturwissenschaften* **84**, 23-25.

Lichtfouse E., Elbisser B., Balesdent J., Mariotti A. and Bardoux G. (1994) Isotope and molecular evidence for direct input of maize leaf wax *n*-alkanes into crop soils. *Organic Geochemistry* **22**, 349-351.

Lichtfouse E., Dou S., Girardin C., Grably M., Balesdent J., Behar F. and Vandenbroucke M. (1995) Unexpected  $^{13}\text{C}$ -enrichment of organic components from wheat crop soils : evidence for the *in situ* origin of soil organic matter. *Organic Geochemistry* **23**, 865-868.

Lichtfouse E., Chenu C. and Baudin F. (1996) Resistant ultralaminae in soils. *Organic Geochemistry* **25**, 263-265.

Lichtfouse E., Bardoux G., Mariotti A., Balesdent J., Ballentine D. C. and Macko S. A. (1997) Molecular,  $^{13}\text{C}$ , and  $^{14}\text{C}$  evidence for the allochthonous and ancient origin of  $\text{C}_{16}$ - $\text{C}_{18}$  *n*-alkanes in modern soils. *Geochimica et Cosmochimica Acta* **61**, 1891-1898.

Maillard L. C. (1916) Synthèse des matières humiques par action des acides aminés sur les sucres réducteurs. In *Annales de Chimie*, eds A. Haller and Ch. Moureu, Vol. V, pp. 258-317. Masson, Paris.

Maillard L. C. (1917) Identité des matières humiques de synthèse avec les matières humiques naturelles. In *Annales de Chimie*, eds A. Haller and Ch. Moureu, Vol. VII, pp. 113-152. Masson, Paris.

Rice J. A. and MacCarthy P. (1990) A model of humin. *Environmental Science and Technology* **24**, 1875-1877.

Schnitzer M. (1991) Soil organic matter, the next 75 years. *Soil Science* **151**, 41-58.

Schnitzer M. and Neyroud (1975) Alkanes and fatty acids in humic substances. *Fuel* **54**, 17-19.

Schulten H.-R. and Schnitzer M. (1990) Aliphatics in soil organic matter in fine-clay fractions. *Soil Science Society of America Journal* **54**, 98-105.

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