Complex organic matter present in soil structure and origin

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Organic matter, particularly humic substances and lipids, play an important role in soil processes (Stevenson, 1982). Previous studies have shown that lipid fraction from soil contains complex, macromolecular lipids which resemble a protokerogen (Amblès *et al.*, 1991). The reversible incorporation of organic molecules in macromolecular lipids by ester groups was clearly demonstrated (Amblès *et al.*, 1994). Main of our results suggest that such a tranfer may also occur in humic substances. Moreover, these biomacromolecules can partly escape biodegradation and fossilizate after deposition in aquatic or hydromorphous environments. As a matter of fact, a close relationship could be established with sedimentary organic matter. Despite their geochemical importance, the structure of soil macromolecules remains largely unknown due to their heterogeneity and extreme complexity which render structural information often difficult and controversial. Different analytical techniques must be complementarily used to avoid misinterpretations in structural determination.

In this work, the structure of macromolecular lipids, humic acids and insoluble humin present in an acidic anmoor soil sample (dystric histosol) from Plateau de Millevaches (France), where organic matter is accumulating, was studied using selective chemical degradations, pyrolysis and a new preparative TMAH thermochemolysis technique. The original soil sample contained 56% of total organic matter (TOM), based on oven-dried soil. Lipids contributed to 5% of TOM of the samples, fulvic acids to 21% and humic acids to 17%. The most important organic fraction was humin corresponding to 57% of TOM. Complex macromolecular lipids corresponded to 12% of lipids (0.6% of TOM).

Hydrolysis of humin and humic acids. Stepwise hydrolysis was applied using a toluene solution of 18-Crown-6/KOH complex. A large part (87%) of the initially insoluble humin became soluble after a first series of hydrolyses. The soluble high molecular-weight products thus obtained were still reactive when submitted again to the same conditions (2nd series of hydrolyses). Humic acids showed a lower reactivity. Linear dicarboxylic acids were produced in significant amounts from humin, they were short (mainly C_9) in the first series and long even C_{16}-C_{28} members of plant origin in the second one. Linear C_{12}-C_{32} monocarboxylic acids and alkanols were freed from humin and humic acids. The short linear fatty acids and alkanols accompanied with iso- and anteiso-C_{15} and C_{17} members denote a bacterial input while long even components, as well as various aromatic acids, originate from plant.

Alkanols appeared as important hydrolysis products from both samples, mainly freed during the first series of experiments in the case of humin. They are C_{12}-C_{30} *n*-alkanols with dominant C_{16}, C_{18}, C_{20}, C_{22}, C_{24} and C_{26} members giving evidence for an important bacterial input. The identification of the iso C_{15} member confirms such a contribution. However, the source of the dominant *n*-C_{22} alkanol observed in humin and humic acids is not well explained.

The results indicate that esterified aliphatic dicarboxylic acids are implicated in the reticulation of macromolecular chains corresponding to the matrix of humin. The matrix of humin and humic acids is substituted by alkyl chains which correspond to esterified fatty acids and alcohols, and by aromatic acids. These moieties are acting as monosubstituents. It is interesting to note that this model presents a high resemblance with the kerogen of many immature sediments.

Thermochemolysis of humin and humic acids. Humic acids and humin were also investigated in their ester and ether groups using a new preparative TMAH thermochemolysis technique which allows the treatment of high quantity of product (~2g). Preparative TMAH thermochemolysis of humin and humic acids yields various hydrocarbons (alkene/alkane doublets, sterenes, hopenes and hopanes), methyl esters of linear and branched fatty acids, linear dicarboxylic acids, ω-methoxy fatty acids and 1-methoxyalkanes.

In both samples, hydrocarbon distributions are dominated by classical pyrolytic series of C_{15} to C_{37} *n*-alk-1-ene/*n*-alkane doublets. Various steroids and triterpenoids, C_{27}, C_{29} Δ-2 sterenes, C_{29} stera-

3,5-diene (indicating a vegetal input), C_{27} hopene and C_{29}, C_{31}, C_{32} 17β(H),21β(H)-hopanes were also identified. The release upon pyrolysis of covalently bound polycyclic products from various sediments is now well documented (Chaffee *et al.*, 1983). Prist-1-ene and prist-2-ene indicate the occurrence of chemically-bound phytol and tocopherols. Sterols and stanols were released on hydrolysis but no hopanoic structures (as alcohols or acids) and no phytol. This results indicates that hopanoid and phytyl components were bound via ether linkages and released by thermochemolysis.

The distributions of fatty acid methyl esters (FAMEs) were close in both samples with a strong even-over-odd carbon number predominance, maxima at $C_{16:0}$ and $C_{24:0}$ and presence of unsaturated $C_{16:1}$, $C_{18:1}$ components. Branched iso - and anteiso-C_{15} and C_{17} FAMEs, typical of bacterial activity were detected in minor amounts. As these acids are similar in distributions with the fatty acids released by hydrolysis of humin and humic acids, it can be concluded that a great part of FAMEs from thermochemolysis were obviously released after ester bond cleavage. The esterified fatty acids present in humin and humic acids are of plant or bacterial origin (Grasset, 1997).

α,ω-Dimethylesters from TMAH thermochemolysis were identified in the range C_8-C_{28}. In humin, the C_9 member was major accompanied with even C_{16}-C_{26} components. In humic acids, C_{22} member was dominant. As FAMEs, they originate from ester bond cleavage. Sources of long-chain members are higher plant cutin and suberin (Kolattukudy, 1976) or microbial ω oxidation of fatty acids. The C_9 member originates probably from previous oxidation of Δ-9 fatty acid precursors (Amblès *et al.*, 1994).

Most of the identified aromatics bear oxygenated chemical groups, principally methyl ester and methoxy groups. There is an obvious relationship with monomeric subunits of lignin polymers. These observations are consistent with the inferred contribution of plants in such humic structures.

Three series of methylated hydroxyacids (as esters) were identified in the thermochemolysis products from humin: 2-methoxy FAMes (bacterial input), (ω-1)-methoxy FAMe of C_{28} acid, and even ω-methoxy FAMes from waxes, cutins and suberins. No hydroxy acids were released on hydrolysis of the same humin and humic acids. It probably indicates that the hydroxyl group of such molecules was not initially bound to the macromolecular network through an ester linkage but through an ether bond. In humin, a series of 1-methoxyalkanes, corresponding to methylated *n*-alkanols, ranging from C_{14} to C_{30} (max. C_{22}) with exclusively even carbon numbers was found. A similar distribution was observed for esterified *n*-alkanols. It indicates that 1-methoxyalkanes formed by thermochemolysis were mainly alkanols bound by ester groups in humin structure. Some of them can be bound to the matrix by ether linkages.

Ether bond cleavage with iodhydric acid. Humin and humic acids were treated with iodhydric acid for a confirmation of the presence of alkyl chains bound by ether groups in their structure. Alkyl iodides were transformed into propionate derivatives (with cesium propionate) which give a highly characteristic m/z = 75 ion on mass spectrometry. Alkylpropionates obtained from humin and humic acids present a strong even over odd carbon predominance, particularly in humin. Branched iso and anteiso C_{15} (iC_{15}, aC_{15}) members are also present. This result confirms that some of the 1-methoxyalkanes formed on thermochemolysis were initially alkyl chains bound to humin and humin acids matrix by ether groups.

Complex macromolecular lipids. The structure of complex macromolecular lipids was studied using flash pyrolysis and off-line preparative pyrolysis. Series of fatty acids, (ω-1)-ketoacids, (ω-1)-ketoalkanols, α,ω-dicarboxylic acids, 2- and ω-hydroxyacids, aliphatic and steroidal ketones were identified. They are of plant or microbial origin. All the results confirm the high aliphaticity of the complex lipid fraction and the important role of dicarboxylic acids and hydroxy acids in the reticulation of alkyl chains in the matrix (Amblès *et al.*, 1991). The importance of ether groups in macromolecular lipids is now studied.

As a conclusion, the detailed study of degradation products from soluble complex lipids, partly soluble humic acids and completly insoluble humin shows that important differences exist between these forms of organic matter. Lignin contribution is much more pronounced in humic acids than in humin. The structure of humin is very heterogeneous with an important cellulose contribution. Complex lipids are highly aliphatic. There are some analogies between complex lipids and the aliphatic part of humin. Our results indicate that lignin and lipidic polymers contribute highly to the formation of complex, macromolecular organic matter in soil. Ester and ether groups are noticeably involved in the insolubilisation process of organic matter: the reticulation of moieties originating from microbiological metabolism or inherited from higher plants is partly assumed by these chemical groups. Most of our results give evidence for a Selective Preservation pathway occurring in the studied acidic soil where organic matter is accumulating.