African Journal of Pure and Applied Chemistry Vol. 5(11), pp. 361-372, 10 October, 2011
Available online at http://www.academicjournals.org/AJPAC
ISSN 1996 - 0840 ©2011 Academic Journals

Full Length Research Paper

Structural study of complex lipids present in soils: Characterization of monocarboxylic acids resulting from polar lipids fraction alkaline hydrolysis

Okome Mintsa Madeleine[1]*, Nguele Jean-Calvin[1], Eba François[2], Amblès André[3] and Joffre Janie[3]

[1]Department of Chemistry-Biochemistry, Faculty of Medicine, Health Sciences University, Owendo, Gabon.
[2]Laboratory of Chemistry, Department of Sciences, École Normale Supérieure, Libreville, Gabon.
[3]Laboratory of Synthesis and Reactivity of Natural Substances, UMR–CNRS 6514, Faculty of Sciences, University of Poitiers, 40, avenue du Recteur Pineau, 86022 Poitiers-Cedex, France.

Accepted 3 August, 2011

In acid soils such as turf, the biological activity is reduced, so lipids tend to accumulate. Those lipids are made of two fractions: one fraction, named "simple lipids", directly analyzable, and the other, named "polar lipids" or "complex lipids". The structure and origin of these "polar lipids" had been investigated using chemical degradation methods. The degradation procedure consisted in an alkaline hydrolysis, where 18-crown-6 ether was used as a phase transfer catalyst. This operation yielded a large amount of acids and hydroxyl functions. Gas chromatography (GC) displayed similar distributions of monocarboxylic acids in simple lipids and in hydrolyzed polar lipids, except the eicosanoic acid which was abundant in polar lipids. Gas chromatography-mass spectrometry (GC-MS) analysis of monocarboxylic acids showed large quantities of aliphatic acids. Some hopanoic acids were also found. It seems that monocarboxylic acids were chemically linked to the polar matrix by ester group.

Key words: Polar lipids, alkaline hydrolysis, monocarboxylic acids, 18-crown-6.

INTRODUCTION

The soil is a vital environment for numerous living beings. However, its natural balance is often disturbed by various dumping such as industrial wastes or excessive pesticides, which bring about toxic effects for environment. In order to avoid those harmful outcomes, it is necessary to have a good knowledge of physical, chemical and biological properties of the soil. These properties are widely conditioned by its organic components.

Lipids are part of organic material of soils. They are from microbial (Stevenson, 1965) or plant origins (Fustec-Mathon et al., 1979; Kolattukudy, 1980; Amblès et al., 1989), and are resulting from the transformation of plant residues by microorganisms (Amblès et al., 1993). Generally, lipids rates vary in accordance with the type of soil (Albrecht, 1970; Arpino, 1973; Jambu et al., 1978), climate, vegetation and biological activity or soil characteristics. They are extracted by organic solvents and are made of simple lipids directly hydrolysable and polar lipids with complex structure, which seemingly correspond to a set of organic macromolecules resulting from simple lipids polymerization (Maggi-Churin, 1986).

We undertook this work with the aim of characterizing the structure and the origin of those polar lipids. Considering that some lipids are not directly analyzable, we resorted to using reactions of degradation. Such methods were used for kerogen structural characterization (Kribii et al., 2001). In order to identify their natural precursors, we disrupted the ester bonds using an alkaline hydrolysis reaction in the presence of crown ether, a phase transfer catalyst. Other hydrolysis catalysts were used, but the 18-crown-6 ether appeared to be the best material (Okome, 1991). This hydrolysis released carboxylic acids and alcohols. We have chosen to characterize carboxylic monoacids for they represent

*Corresponding author. E-mail: okommintsa@yahoo.fr. Tel: +241 06241175 or +241 07298535.

Table 1. Soil sample analytical characteristics.

pH(H_2O)	Clays %	Alluvium %	Sand %	C org. %	N org. %	Fe_2O_3 free %	Al_2O_3 free %	P_2O_5 %
4.3	12.5	14.4	37.3	20.3	1.5	1.0	1.1	0.14

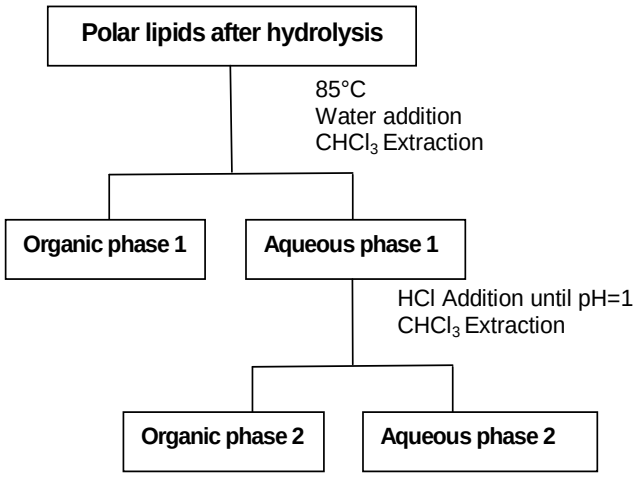

Figure 1. General Protocol of soil lipids extraction.

the most abundant family amongst hydrolysis products. Monoacids of simple lipids from same soil were analyzed for identification and comparison.

MATERIALS AND METHODS

Origins and characteristics of soil samples

Soil sample was obtained from the acid turf horizon of Millevaches plateau (Corrèze, France) between 20 and 40 cm deep. It is known to be a low-biological activity sodden soil (Table 1)

Equipment

Column chromatography

We used glass columns with two-way faucets (glass or Teflon key). For various fractionation purposes, we used 60G Merck Kieselgel silica (granules of 0.063 to 0.2 mm). For column obstruction, we used cotton and Fontainebleau sand. Silica, sand and cotton were thoroughly washed in soxhlet for 24 h, using bi-distilled chloroform and then they were vacuum-dried before being used.

Thin layer chromatography

Analyses were run on 0.25 mm thick commercial Merck plates, or on lab-made ones on which we spread 0.5 mm thick 60 G Merck silica.

Gas chromatography

Chromatography apparatuses were Packard model 427 and 438. They were equipped with a FID detector, along with an entry-divider ROS injector, a 25 mm Crompack CP^{tm} Sil5 melted silica capillary column, and a Merck D 2000 Shimadzu integrator. For analyses, temperature was selectively programmed: detector and injector (300°C); oven initial temperature (70 or 120°C); final temperature (300°C); temperature gradient (2°C/min).

Mass spectrometry (MS)

Mass spectra presented in this work were registered by two types of apparatuses: Kratos MS 25 mass spectrometer coupled with a Perkin-Elmer Sigma 3 gas chromatograph and a Data General Nova 4C computer, and a Finnigan Incos 500 spectrometer coupled with a Varian 3300 gas chromatograph and Data General computer.

Spectroscopic analyses (NMR and IR)

Proton and carbon 13 NMR spectra were measured on a Bruker Spectrospin 200 MHz WP 200 SY apparatus. IR spectra were seized thank to a Beckman Acculab 2 spectrometer.

Analytic protocol

Soil samples were dried in the open air, homogenized, ground, and then sifted at 200 µm before extraction.

Lipids extraction

Soil samples were introduced in cellulose cartridge, and twice extracted by bi-distilled chloroform through soxhlet, in order to avoid any sort of potential contamination. Chloroform was our organic solvent of reference for lipids extraction because previous experiments (Bilong, 1979) have shown that the use of chloroform would increase lipid rate and more particularly that of polar lipids, in comparison to the mixture ether of petrol (3/4) – ethyl acetate (1/4), which was more commonly used. After cooling off, the solvent was discarded by evaporation under partial vacuum (temperature lesser than 50°C) using a rotary evaporator. After extraction, we obtained directly fractionable “free” lipids. “Associated” lipids were extracted after alkaline treatment in order to free humic and fulvic acids and then acid treatment for organic and mineral matrix destruction to which they were covalently linked. Soil lipids extraction protocol was described in Figure 1.

Lipid extracts fractionation

After solvent evaporation and drying, each of both lipid sets (free and associated) was pre-fractionated using silica potash impregnated chromatography, according the Mac Carthy and Dutie Method (McCarthy and Duthie, 1962). We obtained three fractions: neutral, acid and polar. The neutral fraction was dissolved in a small volume of ether of petrol and then fractionated in silica column. The fractionation was followed by thin layer chromatography. The acidic fraction was methylated using diazomethane before being fractionated within the column. Amongst neutral and acidic fractions, we found numerous

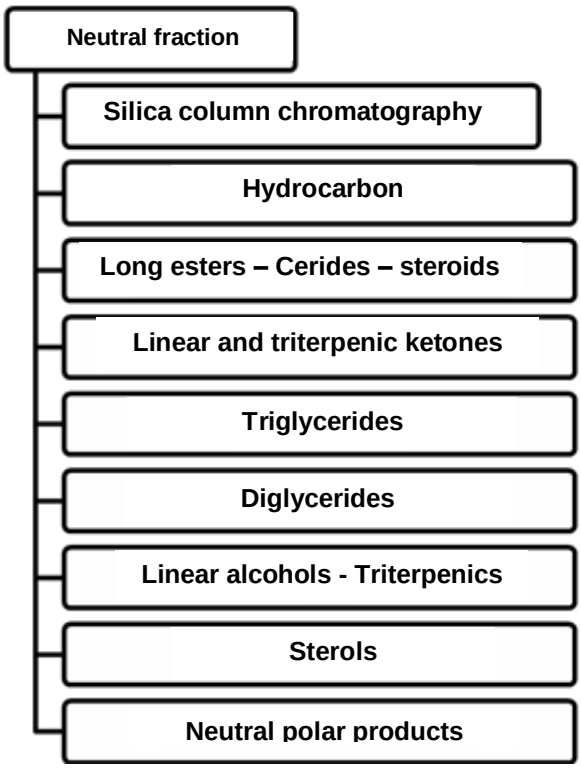

Figure 2. Neutral fraction's chromatographic analysis.

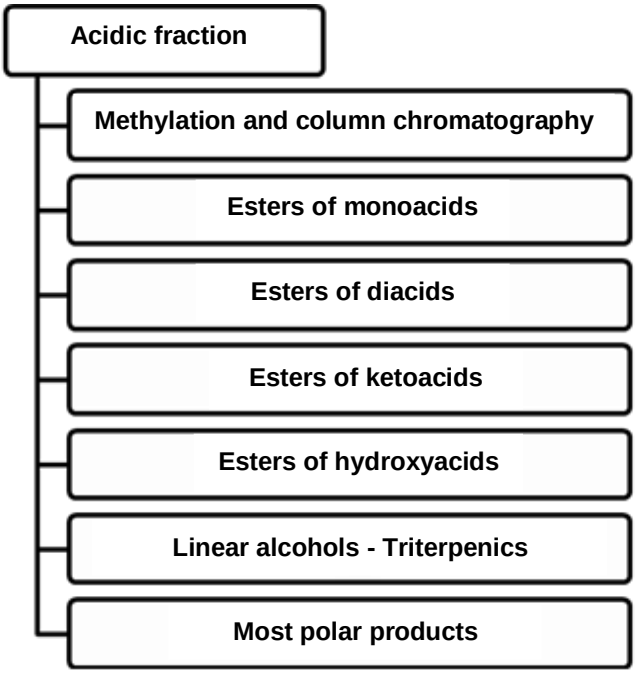

Figure 3. Acidic fraction's chromatographic analysis after methylation.

analyzable families as shown by silica column chromatography results displayed in Figures 2 and 3.

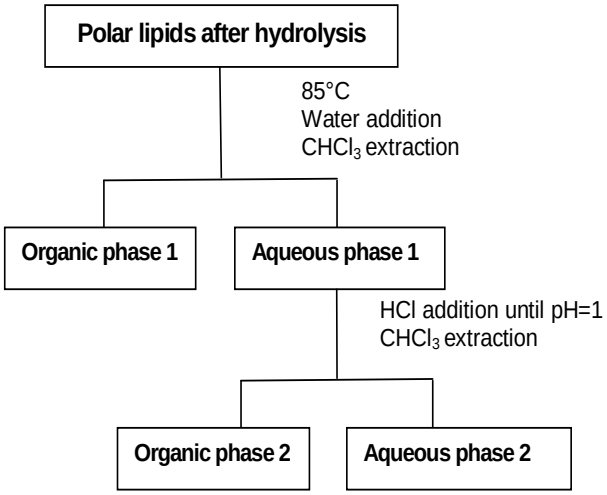

Figure 4. Hydrolysis products extraction protocol.

Complex lipids fraction obtaining

The neutral and acidic fractions' most polar lipids were those that did not migrate on the chromatographic plate in an ethyl acetate/cyclohexane mixture (40/60). They were added to the polar fraction. We then obtained the "polar lipids" fraction or "complex lipids" for which we would like to determine the structure.

Alkaline hydrolysis

Since the polar lipids structure was complex, we resorted to chain breaking reactions. We have performed an alkaline hydrolysis in the presence of a phase transfer catalyst: 1,4,7,10,13,16-hexaoxacyclooctadecane crown ether or 18-crown-6 ether. Crown ether role was to bring potassium hydroxide (KOH) in the organic phase, forming a stable complex in toluene (Dehmlow, 1983). Reactive and phase transfer catalyst were therefore within the same organic phase, then the reaction would more easily take place. The hydrolysis was performed in inert atmosphere (nitrogen), with reflux heating under agitation during 72 h. After cooling, hydrolysis products were extracted according the protocol described in Figure 4.

The organic phase 1, containing neutral and alkaline products, was obtained after alkaline extraction. After aqueous phase acidification, the extraction led to the organic phase 2 containing acidic products. First step products were separated in column and analyzed. Polar residues were hydrolyzed a second time according the same protocol.

Products analysis

After diazomethane methylation, acids resulting from the hydrolysis were fractionated using silica column chromatography and analyzed through GC-MS-coupled capillary gas chromatography.

RESULTS

Lipids extraction

After drying under dessicator and weighing lipid extracts,

Table 2. Weight results of lipids extracted and obtained polar lipids.

Lipid pools	Lipid extracts	Polar lipids
Free lipids (mg/kg of dry soil)	18 704	12 840 (68.6%)
Associated lipids (mg/kg of dry soil)	7 380	5 807 (78.7%)

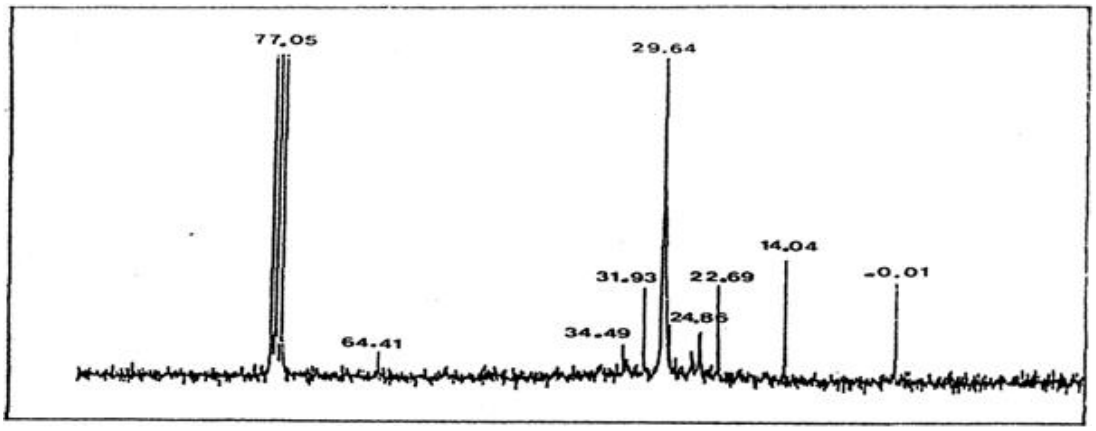

Figure 5. ^{13}C NMR Spectrum of free lipids polar fraction.

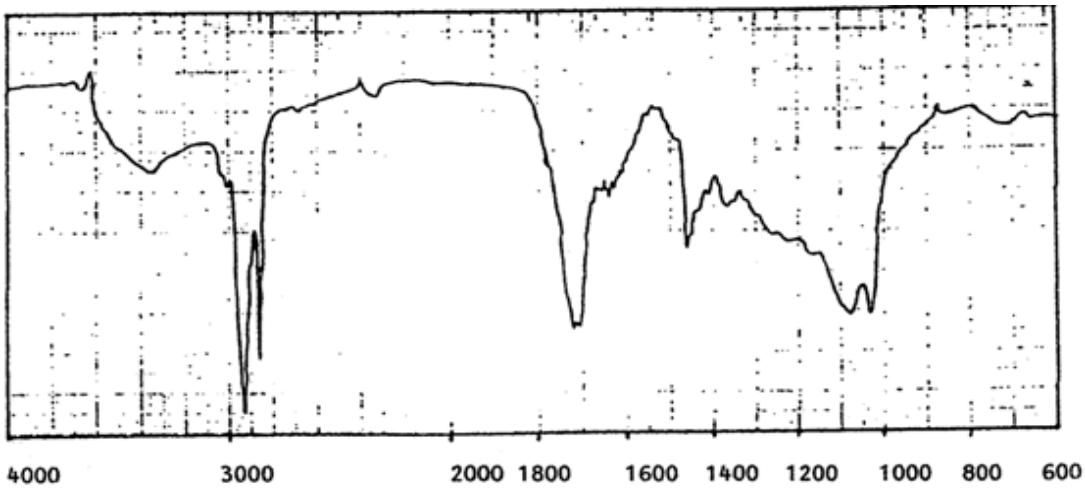

Figure 6. IR spectrum of free lipids polar fraction.

we obtained the following results. We noticed that the turf contained a higher concentration of polar lipids within “associated” lipids (78.7%) than within “free” lipids (68.6%), both lipidic fractions (free and associated) were studied separately (Table 2).

Complex lipids characterization tryout

Obtained polar lipids were under the appearance of a dark brown solid, not very soluble in methylene chloride or ether (even under heat), but they were easily soluble in chloroform or methanol. They did not migrate on silica plate chromatography with polar elution liquid containing 50% of cyclohexane and 50% of ethyl acetate. The elemental analysis has given the following results.

Carbon and hydrogen rates were less important in associated polar lipids than free ones, but on the other hand, their oxygen and nitrogen rates were lower. Free polar fractions were studied under spectroscopy: from both illustrated spectra in Figures 5 and 6, we inferred that we had non-aromatic material since nothing was disclosed under NMR ^{13}C.

The ^{13}C-NMR analyses of free lipids polar fraction

Table 3. Results of elemental polar lipids analyses.

Samples	C %	H %	N %	O %
Free polar lipids	72.39	10.16	0.27	16.96
Associated polar lipids	58.87	7.51	0.78	22.17

Table 4. Rate of different products after « free » complex lipids hydrolysis.

Products	1st step (%)	2nd step (%)	Total (%)
Monoacids	7.6	1.1	8.7
Diacids	2.7	1.4	4.1
Hydroxyacids	7.9	0.6	8.5
Alcohols	1.3	0.3	1.6
Other products	14.0	2.8	16.8
Polar residue	60.5	35.8	35.8
Total of identified products*	19.5	3.4	22.9
Mass default	6.0	58.0	24.5

*Identified products were monoacids, diacids, hydroxyacids and alcohols.

Table 5. Results of the « Associated » polar lipids hydrolysis.

Products	1st step (%)	2nd step (%)	Total (%)
Monoacids	2.3	0.5	2.8
Diacids	0.5	0.7	1.2
Hydroxyacids	3.7	0.5	4.2
Alcohols	1.4	0.1	1.5
Other products	3.8	1.2	5.0
Analyzed products	7.9	1.8	9.7

yielded the spectrum shown in Figure 5. We did the analysis of the same fraction in IR and we obtained the spectrum shown in Figure 6.

Alkaline hydrolysis

"Free" polar lipids hydrolysis

Organic phase 1 products were fractionated on the column, while those of the Organic phase 2 were fractionated after diazomethane methylation. Results of the hydrolysis were displayed in Table 3.

At the first step of hydrolysis, we obtained most of the products. The yield of second step was poor along with an important mass default probably because lighter products were freed in the course of the reaction and were lost during solvent evaporation; meanwhile others could have been dissolved in the aqueous phase. Hydroxyacids and monoacids were the most abundant products while alcohols were very much in the minority (Table 4).

Associated polar lipids hydrolysis

The yield of "associated" polar lipids was clearly lower than that of "free" ones. In the majority, we obtained hydroxyacids and monoacids, diacids and alcohols remaining a minority. We have chosen to study monoacids because they represented one of the most predominant families amongst hydrolysis products (Table 5).

Monoacids analysis

In order to better apprehend the existing relations between complex and simple lipids, we have concomitantly monoacids and simple lipids.

"Free" lipids

They represent the most abundant family amongst analyzed products resulting from « free » polar lipids

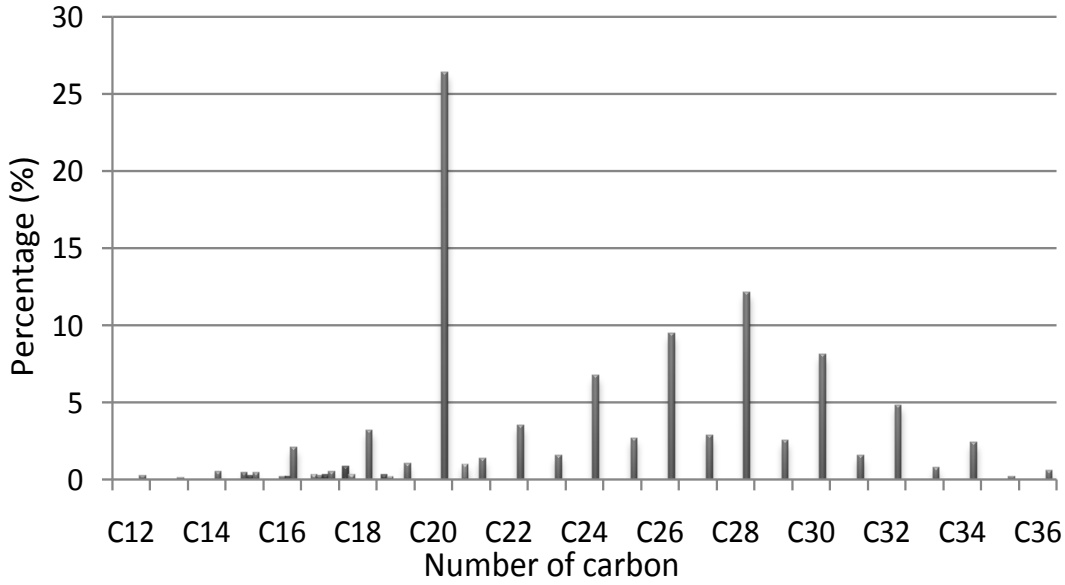

Figure 7. Histogram distribution of monoacids resulting of the first step of « free » polar lipids hydrolysis. (We separated the two histogramms).

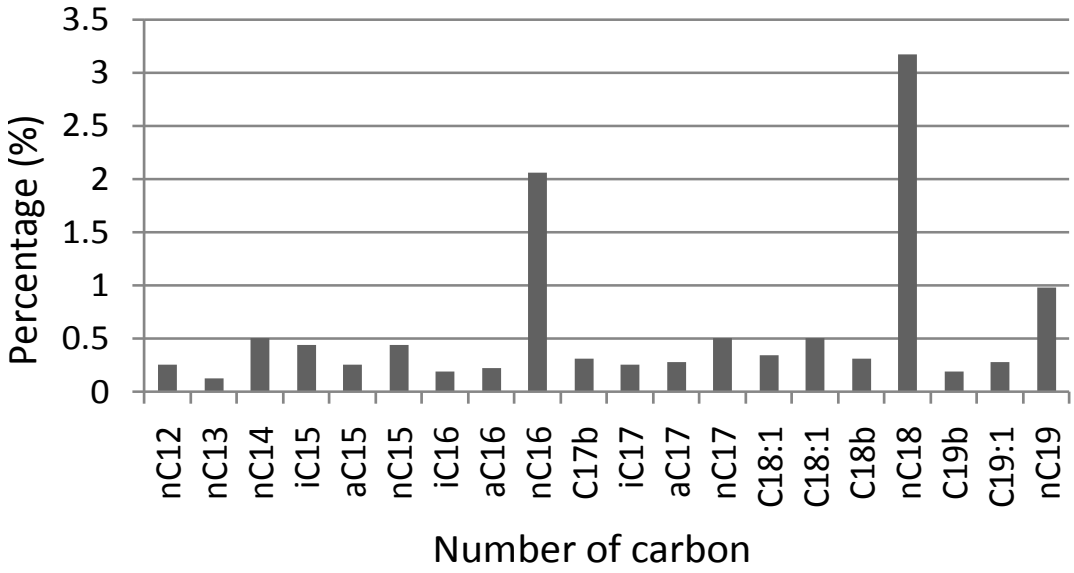

Figure 8. Histogram distribution of short-chain monoacids resulting of first step of « free » polar lipids hydrolysis.

hydrolysis. They were constituted of components whose chain length oscillate from C_{12} to C_{36}. The distribution of monoacids from the first step hydrolysis was shown in Figure 7.

As shown in Figure 8, we distinguished a set of short-chain monoacids (C_{12} to C_{19}) for which the principal ones were linear acids between C_{16} and C_{18}. We also noticed the presence of desaturated acids ($C_{18:1}$ and $C_{19:1}$) along with ramified acids such as iso- and anteiso-C_{15}.

A set of long-chain monoacids (C_{20} to C_{36}) essentially pair numbers of carbon such as nC_{26} and nC_{28} monoacid. We noticed a neat preponderance of eicosanoic acid (nC_{20}).

The outcome of the second step of hydrolysis was Analog to that of the first one, as shown on the histogram made of linear compounds, saturated with predominantly in Figure 9. Simple lipid monoacids distribution, shown on Figure 10, was different from polar ones by a larger proportion of long paired-linear carbon chain, and a lower eicosanoic acid concentration.

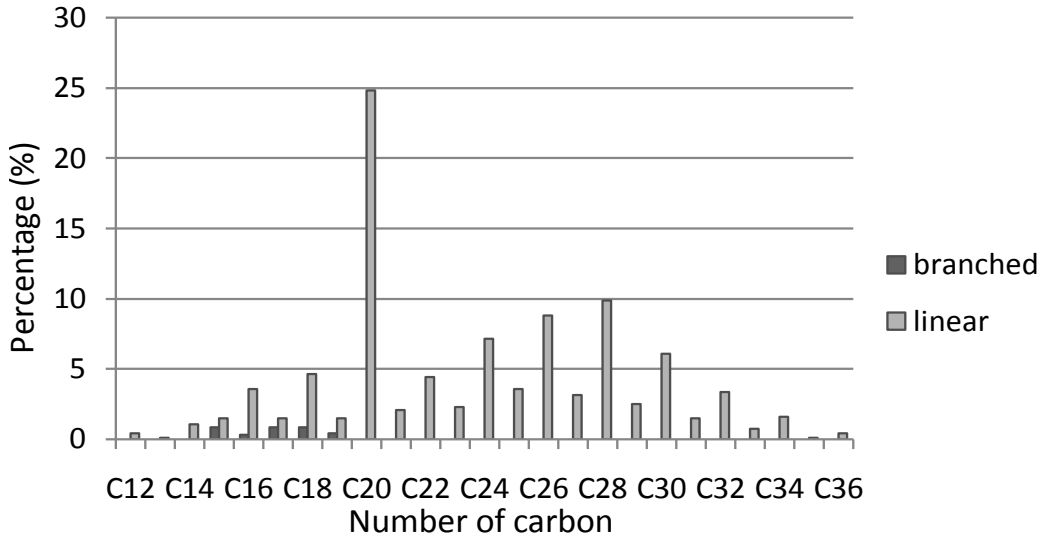

Figure 9. Histogram distribution of monoacids resulting of the second step of « free » polar lipids hydrolysis.

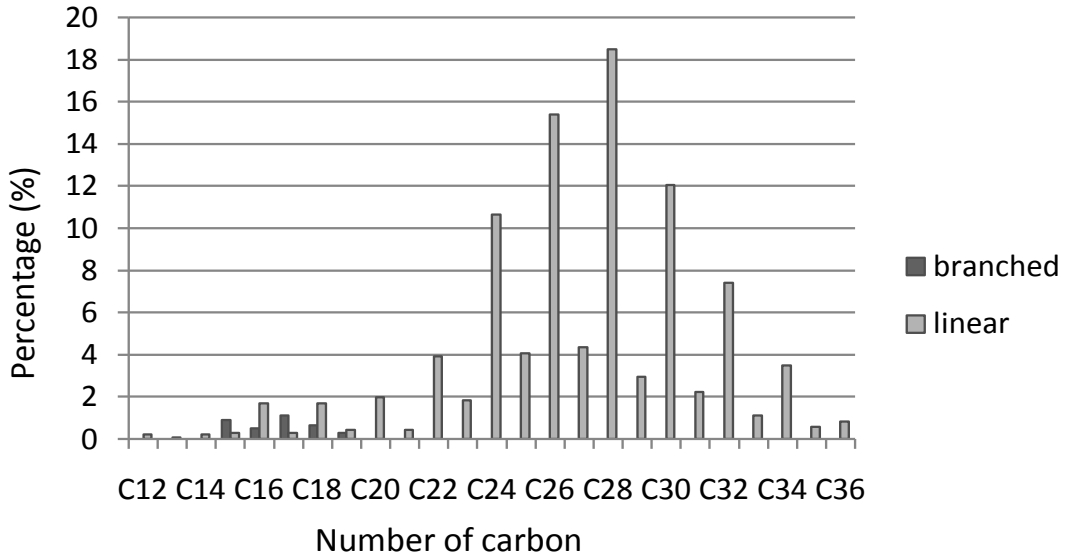

Figure 10. Histogram distribution of simple lipids monoacids.

"*Associated" lipids*

Monoacids represented 2.8% of hydrolysis products. After hydroxyacids, they were the most abundant. Their chromatogram, which was identical for both steps, was revealed on Figure 11.

The distribution was different from that of free lipids. On the other hand, we recorded numerous branched iso- and anteiso-compounds. We also identified branched monoacids whose mass spectra revealed a methylated carbon-10 structure. Mass spectra of the compound C_{17} is shown in Figure 12.

We mostly obtained a set of long-chain, linear, saturated and paired-numbers of carbon monoacids stretching from C_{20} to C_{34}. We have identified, using GC-MS, four hopanoic acids: C_{30}, C_{31}, C_{32}, and C_{33} (respectively A, B, C and D in the chromatogram shown on Figure 11) and traces of a diterpenic acid: dehydroabietic acid. The mass spectra of their methylic ester are shown on Figure 13 (compound A); Figure 14 (compound B), and Figure 16 (dehydroabietic acid).

The chromatogram of "Associated" simple lipids monoacids, presented in Figure 17, displays a distribution resembling those resulting from complex lipids hydrolysis.

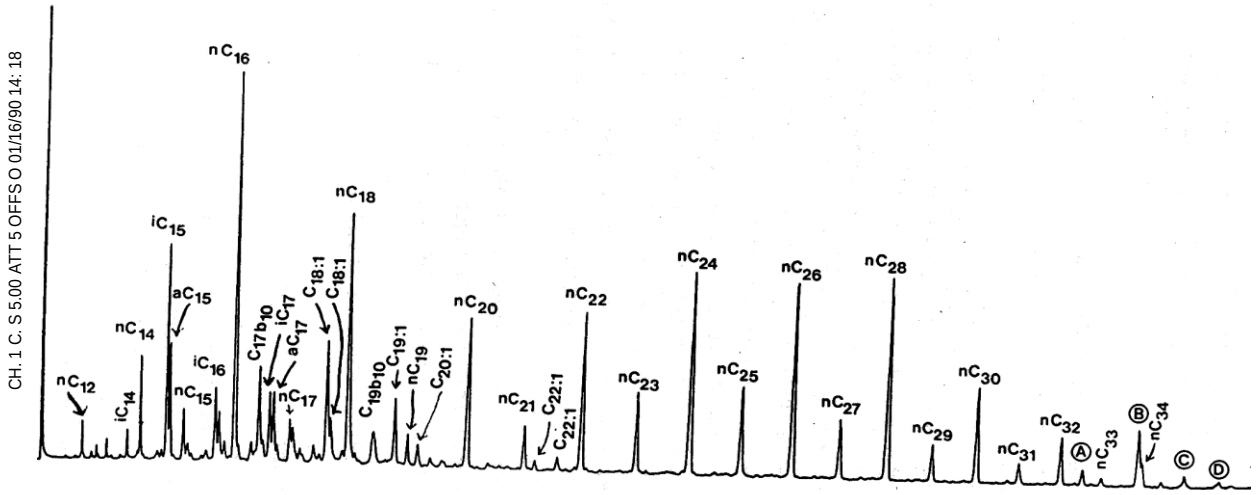

Figure 11. Chromatogram of monoacids resulting of the « associated » polar lipids hydrolysis.

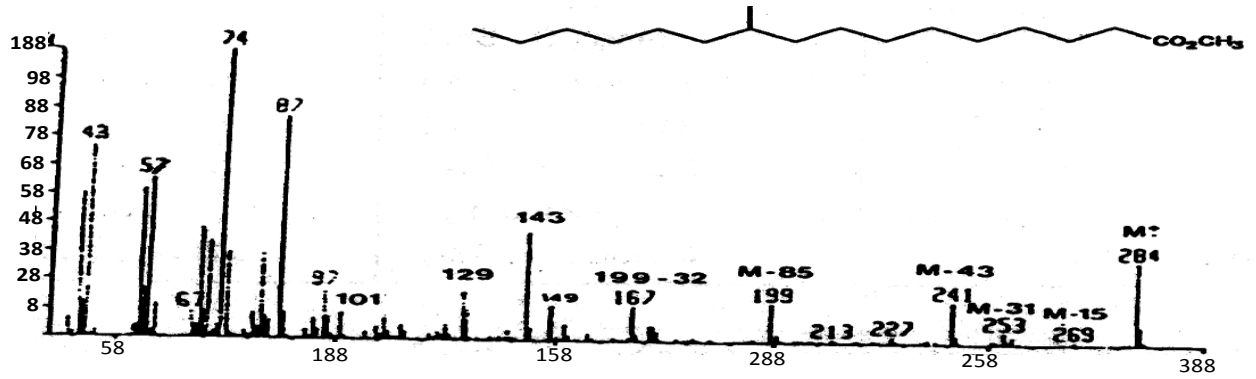

Figure 12. Mass spectrum of methyl-10-methylhexadecanoate (C_{17b10}).

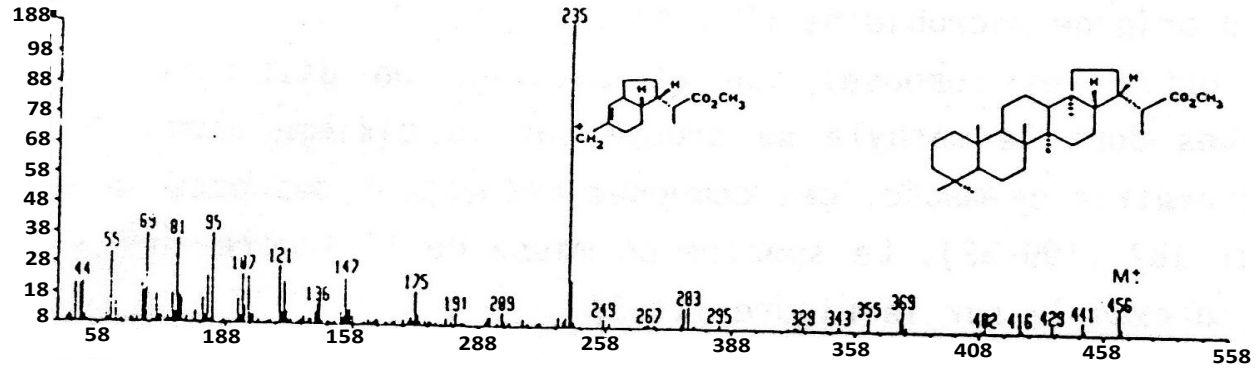

Figure 13. The mass spectrum of 17β-H-hopanoic acid (C_{30}) methylic ester.

Monoacids resulting from “associated” polar lipids hydrolysis were unlike those produced with « free » polar lipids by a higher concentration of short compounds (< C_{20}), a normal rate of eicosanoic acid and by the presence of hopanoic acids. Distribution of those monoacids is equivalent to their homologous within the corresponding simple lipids fraction. Like the “free” lipids, we would encompass that “associated” lipids could be

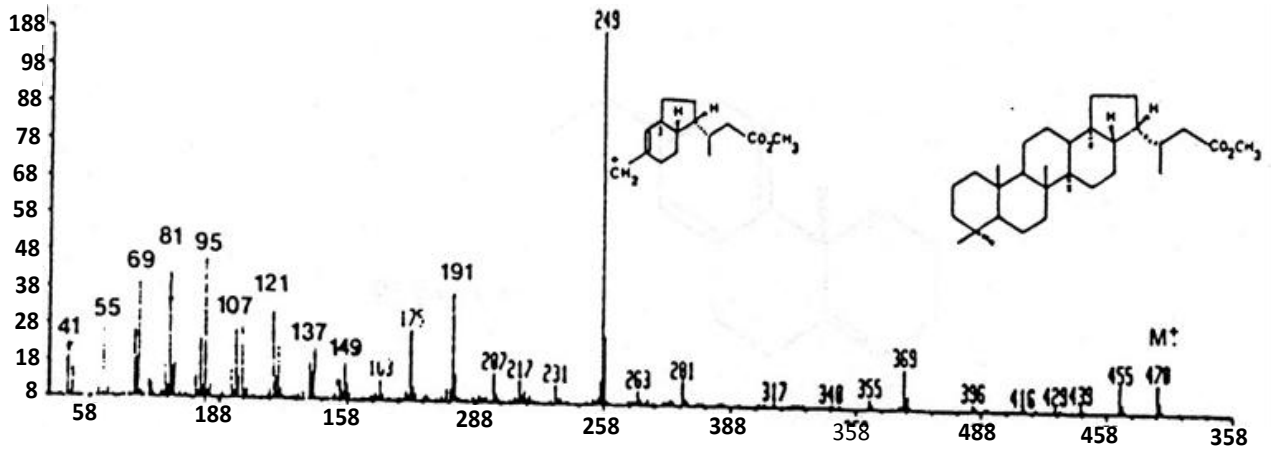

Figure 14. The mass spectrum of 17β-H-hopanoic acid (C31) methylic ester.

incorporated in the polar matrix.

DISCUSSION

The acid turf horizon of plateau de Millevaches, the soil of our study, contains about 70 to 80% of polar lipids (Table2). We have chosen that type of soil because it appeared that most of "free" and "associated" lipids were "complex lipids". An independent and parallel study realized on a podzol, under the same conditions, has shown a lower rate of extracted lipids (43.5% of free polar lipids) comparatively to that of the anmoor (68.6% of polar lipids) (Okome Mintsa, 1991).

Carbon and hydrogen rates were less important in associated polar lipids than free ones (Table 3), but on the other hand, their oxygen and nitrogen rates were lower. Free polar fractions were studied under spectroscopy: from both illustrated spectra in Figures 5 and 6, we inferred that we had non-aromatic material since nothing was disclosed under NMR ^{13}C. We noticed a 64.41 ppm peak which was probably resulting from carbon atoms with O-CH_2- groups (Hammon et al., 1998). On the other hand, we could disclose the non-aromatic nature of this complex mixture through the absence of a peak between 110 and 160 ppm, which was confirmed by IR analysis whose spectrum displayed around 3030, 1500, along with 1600 and at less than 900 cm^{-1} (Hammon et al., 1998; Allinger et al., 1982).

The large band seen around 3200 cm^{-1} had shown the presence of OH group (Allinger et al., 1982). Interestingly, previous data (Mahieu et al., 1999) supported our hypothesis by showing that ^{13}C CPMAS-NMR yielded different peaks according the nature of chemical groups among which the carbon was pertaining. They disclosed that depending chemical shift regions, alkyl groups had between 0 to 50 ppm peaks, whereas O-alkyls had 50 to 110 ppm; 110 to 160 for aromatics and 160 to 200 for carbonyls.

Following the same interest, other teams have also shown that ^{13}C-NMR spectroscopy could help to assess the interaction of soil organic matter with aluminium and allophane. They found a peak at 179 ppm assigned to carboxylic groups (Parfitt et al., 1999). Alkaline hydrolysis with crown-ether was performed according two steps; the second one could complete the first in case of inaccessibility of certain ester bounds.

At the first step of hydrolysis, we obtained most of the products. The yield of second step was poor along with an important mass default probably because lighter products were freed in the course of the reaction and were lost during solvent evaporation; meanwhile others could have been dissolved in the aqueous phase.

Hydroxyacids and monoacids were the most abundant products while alcohols were very much in the minority. The yield of "associated" polar lipids was clearly lower than that of "free" ones (Table 5). In the majority, we obtained hydroxyacids and monoacids, diacids and alcohols remaining a minority. We have chosen to study monoacids because they represented one of the most predominant families amongst hydrolysis products.

Monoacids distribution displayed a neat preponderance of eicosanoic acid (nC_{20}) (Figures 7 and 9). We also found that acid in this soil's cerides and triglycerides. The relevant acid could result from their enzymatic hydrolysis (Magnoux, 1982; Amblès et al., 1985), followed by a recombination amongst complex lipids.

Simple lipid monoacids distribution, shown in Figure 10, was different from polar ones by a larger proportion of long paired-linear carbon chain, and a lower eicosanoic acid concentration. Long-chain, linear, paired-carbon number and saturated monoacids are from plant origin (Martin and Jupiler, 1970; Tulloch, 1976; Stevenson, 1982), while short-chain, branched and desaturated were from microbial origin (Boon et al., 1977; Volkman et al., 1980). Surprisingly, apart from the eicosanoic acid (nC_{20}) we noticed that fatty monoacids freed by hydrolysis such as "free" complex lipids were close to simple monoacids.

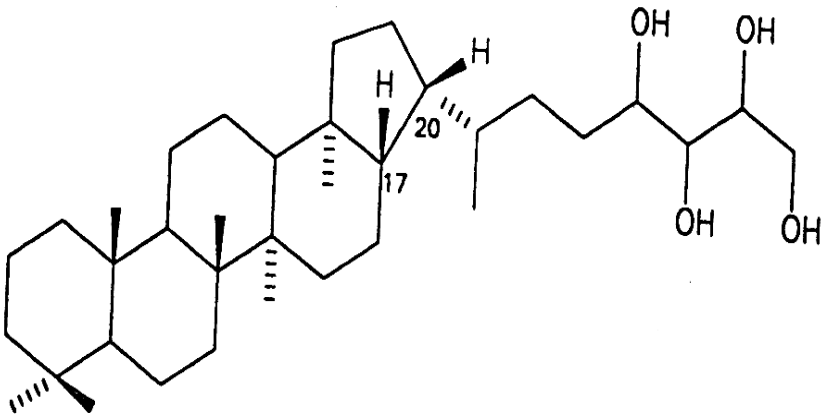

Figure 15. Chemical structure of bacteriohopanetetrol.

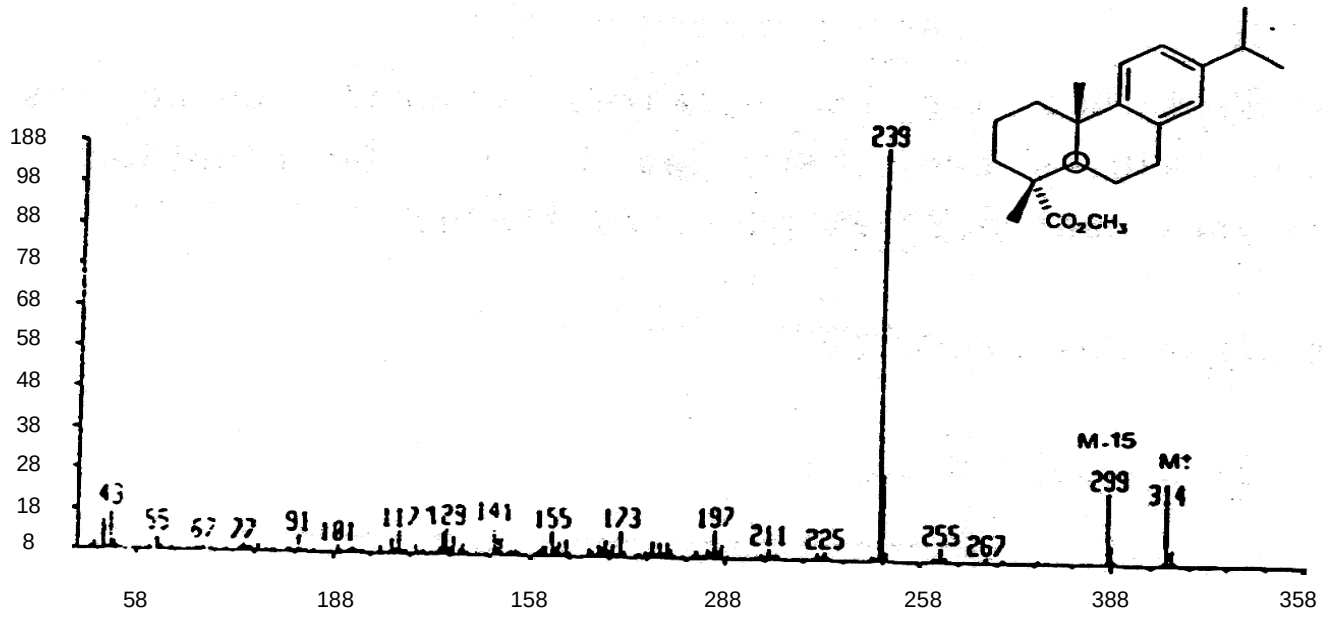

Figure 16. Mass spectrum of methyle dehydroabietate.

It seemed that simple lipids would have incorporated complex soil organic lipids.

The distribution was different from that of free lipids. Nevertheless, we distinguished two modes: a short mode, comporting in most cases: linear, saturated and predominantly paired-numbers of carbon monoacids stretching from 12 to 19 (nC_{16} was the major one). They were from microbial origin (Parker, 1969; Boon, 1977). On the other hand, we recorded numerous branched iso- and anteiso- compounds. We also identified branched monoacids whose mass spectra revealed a methylated carbon-10 structure (Figure 12).

Hopanoic acids were triterpenic acids often found in the form of acids or hydrocarbons within sedimentary organic material (Ensminger et al., 1973; Tulloch, 1976; Ourisson et al., 1979; Stevenson, 1982). In conformity with previous studies, we hypothesized that microorganisms were the main source of hopanes (Förster et al., 1973; Rohmer, 1975; Ourisson et al., 1984; Rohmer and Ourisson, 1984), with bacteriohopanetetrol as a putative precursor (Ensminger et al., 1973; Förster et al., 1973; Rohmer and Ourisson, 1976, 1977; Dastillung et al., 1980). The chemical structure of the bacteriohopanetetrol is shown in Figure 15.

Dehydroabietic acid is a diterpenic acid which is abundantly found in forest soils and in recent sea sediments (Simoneit, 1977, 1979). The chromatogram of "Associated" simple lipids monoacids, presented in Figure 17, displays a distribution resembling those resulting from complex lipids hydrolysis. Distribution of monoacids resulting from "associated" polar lipids hydrolysis is equivalent to their homologous within the

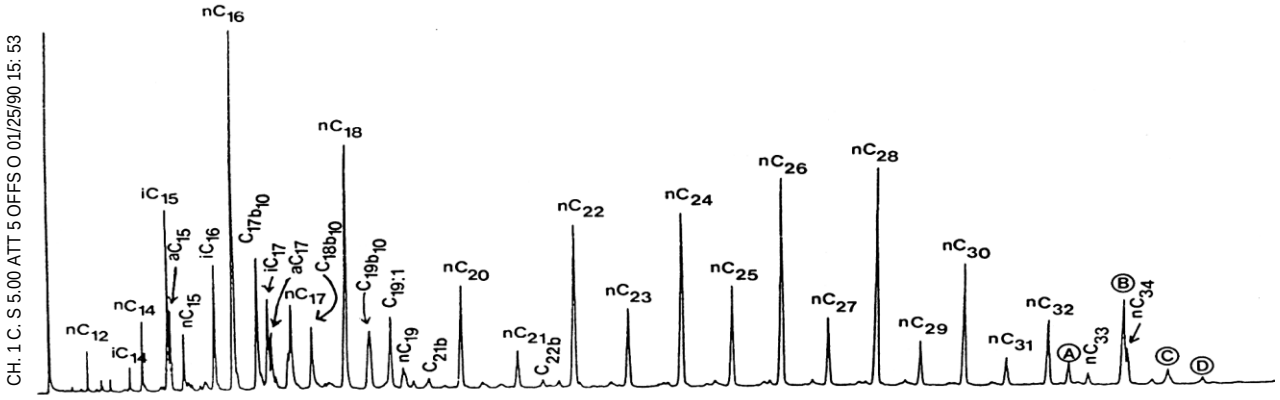

Figure 17. Chromatogram of Monoacids from "Associated" simple lipids.

corresponding simple lipids fraction (Figure 17). Like the "free" lipids, we would encompass that "associated" lipids could be incorporated in the polar matrix.

Conclusion

Polar lipids represented an important fraction of soil organic material. It had been shown that those lipids could never be directly analyzed (Amblès et al., 1985). Alkaline hydrolysis in the presence of a phase transfer catalyst had helped to free numerous molecules bearing carboxyl and hydroxyl functions, which indicated thepresence of an important number of ester bonds in the polar lipidic fraction of this soil. Some analogous methods of structure characterization have been used for kerogen characterization (Kribii et al., 2001).

"Free" complex lipids hydrolysis had yielded more products than that of "associated" ones. It seemed that in the anmoor, hydrolysable functions of "free" polar lipid were more important than the "associated" lipids. Nevertheless, the same classes of lipids were found but under different proportions.

The analysis of monoacids resulting from alkaline hydrolysis in the presence of crown ether revealed the presence of aliphatic chains, linked to the macromolecular network by ester bonds. This study has shown that simple molecules, through the oxidation process, could have been incorporated into the soil's polar matrix. This strong statement had also been demonstrated in a complementary study (Amblès et al., 1993). There must be a subtle equilibrium between simple and complex lipids. Supplementary investigation methods could allow determining the nature of other possible bonds existing between the free lipids and the soil's polar matrix.

REFERENCES

Albrecht P (1970). Study of the organic components of the sedimentary sequences of Logbaba and Messel. Digénétic transformations. Memory of the geological map of Alsace and Lorraine, No. 32.

Allinger NL, Cava MP, De Jongh DC, Johnson CR, Lebel NA, Stevens CL (1983). Organic chemistry. Applications. McGraw-Hill Ed., 3: 990-991.

Amblès A, Magnoux P, Jambu P, Jacquesy R, Fustec E (1989). Effects of the addition of bentonite on the hydrocarbon fraction of a podzol soil (A1) horizon. J. Soil Sci., 40: 685-694.

Amblès A, Jacquesy R, Jambu P, Joffre J, Fustec E (1985). Influence of iron on the dynamics of lipids in hydromorphic acidic environment. Agrochimica, 24(2, 3, 4): 199-209.

Amblès A, Jacquesy JC, Jambu P, Mayoungou P, Okome-Mintsa M, Hita C, Parlanti E (1993). High molecular weight lipids present in soil. Nature and origin. Org. Geochem. (K. Øygard, Ed.): 668-671.

Amblès. A, Magnoux P, Jambu P, Jacquesy R, Fustec E (1989). Effects of addition of bentonite on the hydrocarbon fraction of a podzol soil (A1 horizon). J. Soil Sci., 40: 685-694.

Arpino P (1973). Lipids from the Eocene lakes sediments. Geological science thesis, Strasbourg.

Bilong P (1979). Evolution of the compounds humic and lipid of the soils of the moors of the Plateau de Millevaches. Doctorate thesis, University of Poitiers, p. 113.

Boon JJ, De Leeuw JWD, Hoek JH (1977). Significiance and taxonomic value of iso and anteiso monoenoic fatty acids and branched β-hydroxyacids in Desulfovibrio Desulfuricans. J. Bacteriol., 129(3): 1183-1191.

Dastillung M, Albrecht P, Ourisson G (1980). Aliphatic and polycyclic alcohols in sediments. Alcohols derived from the hopane and 3-methyl hopane. J. Chem. Res., (M): 2353-2374.

Dastillung M, Albrecht P, Ourisson G (1980). Aliphatic and polycyclic ketones in sediments C_{27} - C_{35} ketones and aldehydes of hopane series. J. Chem. Res., (S): 166-167, (M): 2325-2352.

Dehmlow EV, Dehmlow SS (1983). Phase transfert catalysis, Verlag Chimie, p. 386.

Ensminger A, Van Dorsselaer A, Spyckerelle C, Albrecht P, Ourisson G (1973). Pentacyclic triterpenes of the hopane type as ubisquistous geochemical markers: Origin and significance. Advances in organic geochemistry. Edition Tissot B and Biehner F. Technip 1974, pp. 245-260.

Förster HJ, Biemann K, Haig WG, Tattrie NH, Colvin JR (1973). The structure of novel C_{35} pentacyclic terpenes from Acetobacter-xylium. Biochem. J., 135: 133-143.

Fustec-Mathon E, Jambu P, Bilong P, Amblès A, Jacquesy R (1979). Inter-relationships between lipids and iron in soils. Influence of the addition of iron A1 podzol horizon. International symposia of the CNRS n° 303. Organo-mineral migration in temperate soils.

Hamon M, Pellerin F, Guernet M, Mahuzier G (1998). Analytical chemistry. Spectral methods and organic analysis. Masson Ed, 3: 179.

Jambu P, Fustec E, Jacquesy R (1978). Lipids of soil: nature, origin, evolution, properties. Soil science. Bulletin de l'A.F.E.S., 4: 229-240.

Kolattukudy PE (1980) cutin, suberin and waxes. The biochemistry in plants. Lipids, structures and functions. P.K. stumpf Ed., 4: 571-645.
Kribii A, Lemée B, Chaouch A, Amblès A (2001). Structural study of the Moroccan Timahdit (Y-layer) oil shale kerogen using chemical degradations. Fuel, 80: 681-691.
Maggi-Churin R (1986). Organic matter of soils. Structural study of the polar lipid fraction. Doctorate thesis, University of Poitiers, p. 63.
Magnoux P (1982). Study of the influence of inflow of clay on the composition of the lipid fraction of a deficient soil. Doctorate thesis, University of Poitiers, 115 p.
Mahieu N, Powlson DS, Randall EW (1999). Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. Soil Sci. Soc. Am. J., 63: 307-319.
Martin JT, Juniper BE (1970). The cuticles of plants. Edward Arnold, London, 347 p.
McCarthy RD, Duthie AH (1962). A rapid method for the separation of free fatty acids from other lipids. J. lipids Res., 3: 177-179.
Okome Mintsa M (1991). Study of complex lipids in acidic soils: structure and origin. Doctorate thesis, University of Poitiers, 189 p.
Ourisson G, Albrecht P, Rohmer M (1979). The hopanoids. Paleochemistry and biochemistry of a group of natural product. Pure Appl. Chem., 51: 709-729.
Ourisson G, Albrecht P, Rohmer M (1984). The microbial origin of fossil fuels. Pour la Science. 84: 55-56.
Parfitt RL, Yuan G, Theng BKG (1999). A ^{13}C-NMR study of the interactions of soil organic matter with aluminium and allophone in podzols. Eur. J. Soil Sci., 50: 695-700.
Parker PL (1969). Fatty acids and alcohols. Org. Geochem. Methods and results (Eglinton G. and Murphy M. T. Ed.). Spring Verlag.
Rohmer M (1975). Triterpenoids of prokariots. Science doctorate thesis, University Louis Pasteur of Strasbourg.
Rohmer M, Ourisson G (1976). Structure of the bactériohopanetétrols of acetobacter-xylium. Tetrahedron lett., 40: 3633-3636.
Rohmer M, Ourisson G (1977). Derived from the bacteriohopane: structural variation and distribution. Tetrahedron lett., 40: 3637-3640.
Rohmer M. Bouvier-Nave P, Ourisson G (1984). Distribution of hopanoid triterpenes in prokaryotes. J. Gen. Microbiol., 130: 1137-1150.
Simoneit BRT (1979). Eolian particulates from oceanic and rural areas. Their lipids fulvic and humic acids and residual carbon. In Advances in Organic Geochemistry. Physics and chemistry of the Earth, 12: 343-352.
Simoneit BRT (1977). Diterpenoid compounds and other lipids in deep-sea sediments and their geochemical significance. Geochim. Cosmochim. Acta., 41: 463-476.
Sljivar Brosic S, Saban M, Amblès, Vitorovic D (1987). Newly discovered constituents of the bitumen from Aleskinac (Yougoslavia) shale. Isoprenoids and hopanoic acids. J. Serb. Chem. Soc., 52(4): 187-195.
Stevenson JF (1982). Soil lipids in humus chemistry. Genesis, composition, reactions. A. Wiley Interscience Publication John Wiley and Sons Inc. Stevenson JF (1966). Lipids in soils. Am. Oil Chem. Soc., 43(4): 203-210.
Tulloch AP (1976). Epicuticular wax of Agropyron Smithili leaves. Phytochemistry, 15(7): 1153-1156.
Van Dorsselaer A, Ensminger A, Spyckerelle C, Dastillung M, Sieskind O, Arpino P, Albrecht P, Ourisson G (1974). Degraded and extended hopane derivatives (C_{27} to C_{35}) as ubiquitous geochemical markers. Tetrahedron lett., 14: 1349-1352.
Volkman JK, John RB, Gillan FT, Perry GJ, Bavor HJJr (1980). Microbial lipids of an intertidal sediments I. Fatty acids and hydrocarbons. Geochim. Cosmochim. Acta., 44: 1133-1134.